NETAJI SUBHAS OPEN UNIVERSITY

STUDY MATERIAL

POST GRADUATE ZOOLOGY

Paper : 7

Group : A

Developmental Biology

[illegible]

PREFACE

In the auricular structure introduced by this University for students of Post- Graduate degree programme, the opportunity to pursue Post-Graduate course in Subject introduced by this University is equally ava.. ble to all learners. Instead of being guided by any presumption about ability level, it would perhaps stand to reason if receptivity of a learner is judged in the course of the learning process. That would be entirely in keeping with the objectives of open education which does not believe in artificial differentiation.

Keeping this in view, study materials of the Post-Graduate level in different subjects are being prepared on the basis of a well laid-out syllabus. The course structure combines the best elements in the approved syllabi of Central and State Universities in respective subjects. It has been so designed as to be upgradable with the addition of new information as well as results of fresh thinking and analysis.

The accepted methodology of distance education has been followed in the preparation of these study materials. Co-operation in every form of experienced scholars is indispensable for a work of this kind. We, therefore, owe an enormous debt of gratitude to everyone whose tireless efforts went into the writing, editing and devising of a proper lay-out of the materials. Practically speaking, their role amounts to an involvement in invisible teaching. For, whoever makes use of these study materials would virtually derive the benefit of learning under their collective care without each being seen by the other.

The more a learner would seriously pursue these study materials the easier it will be for him or her to reach out to larger horizons of a subject. Care has also been taken to make the language lucid and presentation attractive so mat they may be rated as quality self-learning materials. If anything remains still obscure or difficult to follow, arrangements are there to come to terms with them through the counselling sessions regularly available at the network of study centres set up by the University.

Needless to add, a great deal of these efforts is still experimental—in fact, pioneering in certain areas. Naturally, there is every possibility of some lapse or deficiency here and there. However, these do admit of rectification and further improvement in due course. On the whole, therefore, these study materials are expected to evoke wider appreciation the more they receive serious attention of all concerned.

Professor (Dr.) Subha Sankar Sarkar
Vice-Chancellor

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POST GRADUATE ZOOLOGY

[M.Sc]

PAPER : GROUP

PGZO - 7 : A

	Writer	Editor
Unit 1	Prof. Dipak Ray	Prof. Rupendu Ray
Unit 2	Prof. Rupendu Ray	Dr. Bibhas Guha
Unit 3	Prof. Dipak Ray	Prof. Rupendu Ray
Unit 4	Prof. Rupendu Ray	Dr. Bibhas Guha
Unit 5	Prof. C.R. Sahu	Prof. Rupendu Ray
Unit 6	Prof. C.R. Sahu	Do
Unit 7	Prof. C.R. Sahu	Do
Unit 8	Prof. C.R. Sahu	Do
Unit 9	Prof. C.R. Sahu	Do
Unit 10	Prof. Dipak Ray	Do
Unit 11	Prof. Dipak Ray	Do

NETAJI SUBHAS OPEN UNIVERSITY

PGZO - 7
Developmental Biology and Endocrinology, Cell & Tissue structure, Function

Group A

Developmental Biology

Unit 1 ❐ Differentiation of primordial germ cell and structure of mature gamate in *Drosophila*, Role of poleplasm, influence of oskar gene, effect of grand childness mutation

Structure

1.1 Germ cells

Gametogenesis is the process by which the sperm and the egg are formed from primordial germ cells that provide the continuty of life between generations. In many animals such as insects, round worms and the vertebrates, there is a clear and early separation of germ cells, from somatic cell types. In several animal phyla these divisions are not well established. In these species such as cnidarians, flat worms and tunicates somatic cells can readily become germ cell, even in adult organism the zooids, buds and the polyps of many invertebrate phyla testify the ability of somactic cells to give rise to new individuals.

In this chapter we will discuss the process of origin of germ cells and the process of differentiation and the factors that regulate the differentiation of germ cell in the context of *Drosophila.*

A. Germ cell migration in *Drosophila*

The organisms where there is an established germ line, the germ cell donot arises within the gonad itself. Rather, their precursor primordial germ cells (PGC) migrate into the developing gonads, the first step in gametogenesis. Then, involves forming the PGC and in the genital ridge as the gonad is forming.

The *Drosophila* germ cells arise form the posterior pole of the zygote. The first step is passive phase wherein the germ cells are displaced by the movement of embryonic cells during gastrulation. The differentiation of the endoderm triggers active amoeboid movement in the primordial germ cells, they travel through the gut endothelium and migrate to the mesoderm. The PGCs then split into 2 groups, each of which become associated with the developing gonadal primordium.

The migration of the PGCs from endoderm to mesoderm is dependent on the

expression of the gene called *wunen*. The product of *wunen* gene is a protein which is expressed in the endoderm immidiately before PGC migration and it repels the PGCs. A mutant of their gene shows the PGCs wonder randomly instead of their normal fate within the developing gonad (Fig. 1.1).

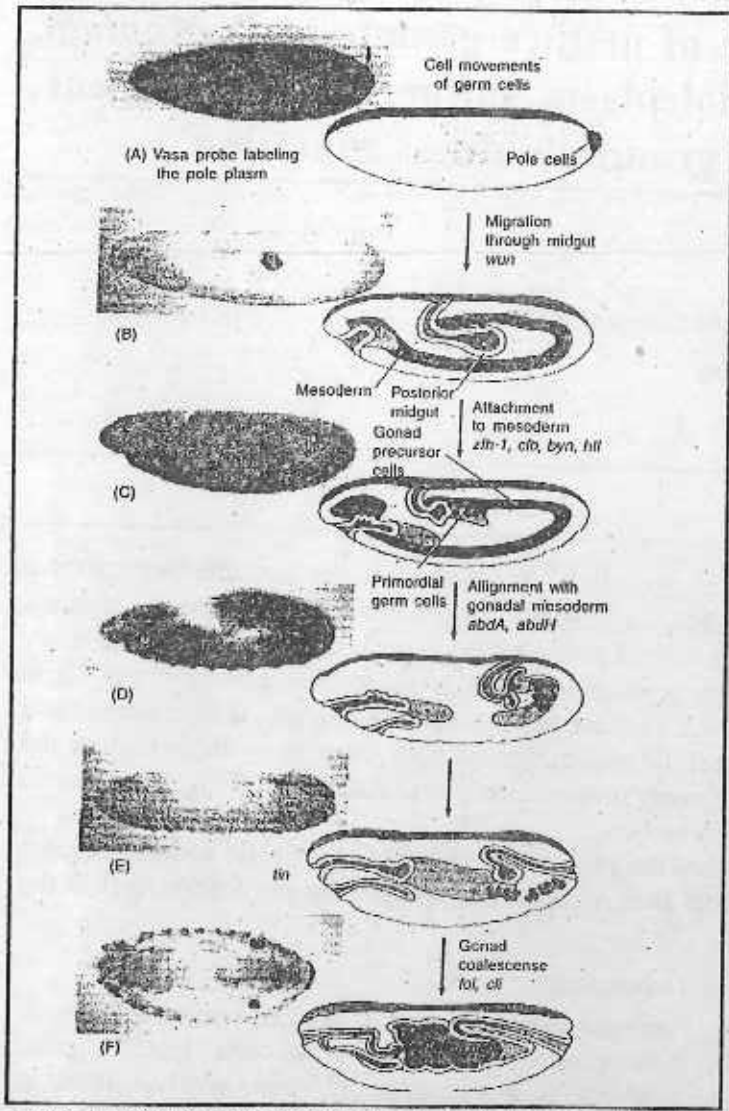


Fig 1.1 : Migration of germ cells in the *Drosophila* embryo. The left column shows the germ plasm as stained by antibodies to Vasa, a protein component of the germ plasm (D has been counterstained with antibodies to Engrailed protein to show the segmentation, and E and F are dorsal views.) The right column diagrams the movements of the germ cells. (A) The germ cells originate from the pole plasm at the posterior end of the egg. (B) Passive movements cary the PGCs into the posterior midgut. (C) The PGCs move through the endoderm and into the caudal visceral mesoderm by diapedesis. The *wunen (wun)* gene product expressed in the caudal mesoderm attracts them. (D-F) The movements of the mesoderm bring the PGCs into the region of the tenth through twelfth segments, where the mesoderm coelesces around them to form the gonads. (Photographs from Warrior et al. 1994, courtesy of R. Warrior ; diagrams after Howard 1998.)

B. Production of sperm or egg

The PGCs that migrating into the gonad are bipotential and can be differentiated into sperm or ova, depending on their gonadal environment. This fate of PGCs into the gonads has been observed in a number of animals including eligans, house fly, mouse and other animals.

In *Drosophila* the germ cells are instructed to differentiate either into sperm or eggs by the gonad cells. Female gonad cell make a product that is received by germ cells and which activates a series of proteins whose activity is critical for the early transcription of the germ cell sxl gene. The proper X-chromosome : autosome (A) ratio is also needed. By these mechanism the fliés get to make eggs while the XY flies make sperm.

C. Spermatogenesis

Spermatogenesis is the process of production of sperm from primordial germ cells. The primordial germ cells arrive at the genital ridge of male embryos then they become incorporated into the sex cord. They remain there until maturity and the PGC differentiated into spermatogenic cells. They remain associated with Sertoli cells and the Sertoli cells nourish and protect the developing sperm cells.

The spermatogenic cells divide mitotically to give rise a clone of sper-matogonia.

At maturity sper-matogonial cells divide to generate the primary spar-matocytes that enter into meiosis.

Each primary sperma-tocyte undergoes the 1st meiotic division to yield a pair of secondary sparmatocyte which complete the 2nd division of meiosis. The haploid cells are called spermatids.

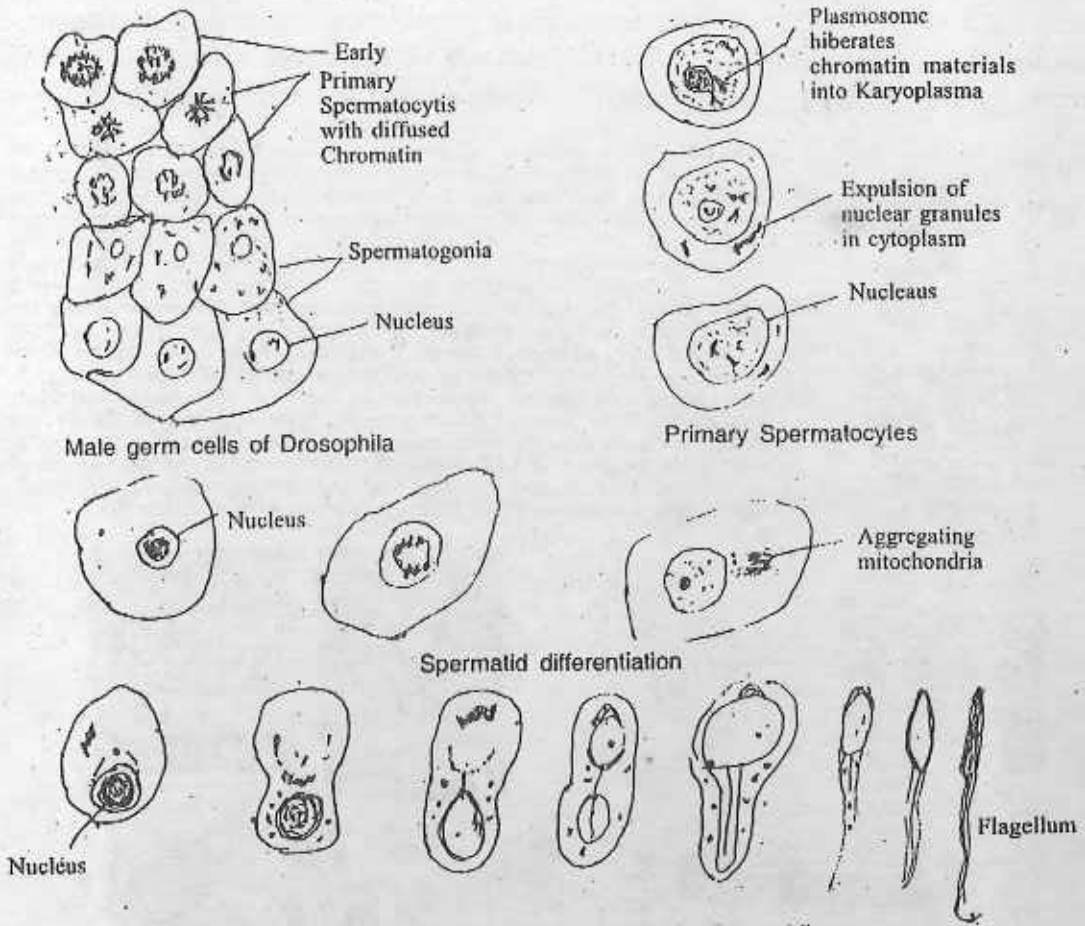


Fig 1.2 : Stages of Spermiogenesis in *Drosophila*

A haploid spermatid undergoes maturation by a process called spermiogenesis or spermateliosis.

During spermiogenesis spermatids undergo several morphological changes viz. the formation of acrosome from Golgi apparatus, condensation of neclear apparatus, formation of flagellum from centriole and extrudation of the remaining cyloplasm as cyloplasmic droplets.

Spermiogenesis is completed into production of mature male sex cells or sperm.

D. Genetic events during spermatogenesis

Like formation of PGC, their migration and localization in the gonad and the process of spermatogenesis is under the control of several genes. It has been shown that like mammalian DAZ gene the *Drosophila* gene RB97D and BOULE are both essential for spermatogenesis. Spermatogonia degenerate in male flies, deficient in RB97D, while the germ cells of male flies lacking the BOULE gene do not enter meiosis. Similarly ROUGHEX gene transcribe by premeiotic *Drosophila* spramatogonia control the number of meiotic division. Males lacking functional copy of ROUGHEX gene, undergo an extra meiotic metaphase in addition to the normal one. Increase in

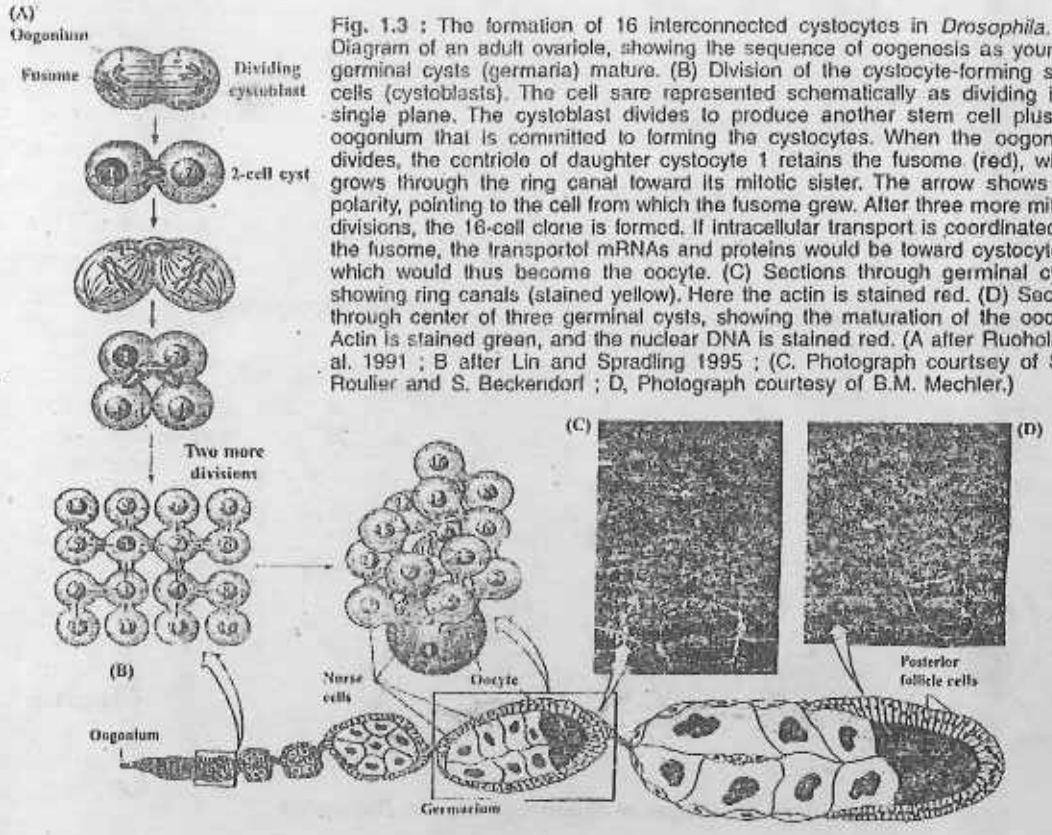


Fig. 1.3 : The formation of 16 interconnected cystocytes in *Drosophila*. (A) Diagram of an adult ovariole, showing the sequence of oogenesis as younger germinal cysts (germaria) mature. (B) Division of the cystocyte-forming stem cells (cystoblasts). The cell sare represented schematically as dividing in a single plane. The cystoblast divides to produce another stem cell plus an oogonium that is committed to forming the cystocytes. When the oogonium divides, the centriole of daughter cystocyte 1 retains the fusome (red), which grows through the ring canal toward its mitotic sister. The arrow shows the polarity, pointing to the cell from which the fusome grew. After three more mitotic divisions, the 16-cell clone is formed. If intracellular transport is coordinated by the fusome, the transportol mRNAs and proteins would be toward cystocyte 1, which would thus become the oocyte. (C) Sections through germinal cysts showing ring canals (stained yellow). Here the actin is stained red. (D) Section through center of three germinal cysts, showing the maturation of the oocyte. Actin is stained green, and the nuclear DNA is stained red. (A after Ruohola et al. 1991 ; B after Lin and Spradling 1995 ; (C. Photograph courtsey of S.E. Roulier and S. Beckendorf ; D, Photograph courtesy of B.M. Mechler.)

the concentration of ROUGHE gene result in the failure to executed meiosis II (Fig. 1.2).

During sparmatogenesis some sperm specific genes are transcribed. In *Drosophila melanogaster* the sperm specific genes transcribed is for β2 tubulin. This isoform of β2 tubulin is seen only during sparmatogenesis and is responsible for formation of meiotic spindles, the axonym and the microtubules. It is to be noted that a sperm axonym in *Drosophila* is a large undertaking. The sperm tail is 2mm long as long as the entire male fly. The sperm of related species of *D. biphorka* is approximately 20 times longer than the flies producing them.

E. Oogenesis

Oogenesis, a process of differentiation of the ovum differs from sparmatogenesis in several ways. The gamete produced by spermatogenesis is essentially a motile nucleus whereas gamate formed by oogenesis contains all the factors needed to initiate and maintain metabolism and development, in addition to forming a haploid nucleus. Oogenesis also builds up to store of cyloplasmic factors such as enzymes, mRNAs, organelles and metabolic substances. The sperm differentiate for motility, the oocyte develops a remarkable complete complex cytoplasm (Fig. 1.3).

In *Drosophila* and other insects the Oogenesis takes place by meroistic type of oogenesis. In meroistic oogenesis cytoplasmic connection remains between the cells produced by the organism. In *Drosophila*, each oogonium divides 4 times to produce a clone of 16 cells connected to each other through ring canals. These interconnected cells are called cystocytes and involves a highly order array of cell divisions. Only these 2 cells having 4 interconnections are capable of developing into oocyte and of these two, only one becomes egg. The other begins meiosis but does not complete it. Thus only one of the cystocytes become an ovum and all the other cells become nurse cells.

F. Transport of RNA from nurse cell to oocyte

The oocytes of meroistic insects do not pass through a transcriptionally active stage have no lampbrush chromosome. Autoradiography suggests that RNA synthesis is largely confined to nurse cells and that RNA made by these cells is actively transcript in oocyte of cytoplasm. When the egg chamber of a house fly is incubated in radioactive cytosine, the nuclei of nurse cells show intense labelling. When the labelling is stopped and the nurse cell is incubated for 5 more hours in non-radioactive medium, the RNA is seen entirely in the oocyte of nurse cells. Oogenesis takes place in only 12 days so that the nurse cells are metabolically active this time. As polytene chromosome present in *Drosophila* their enhanced RNA synthesis activity in nurse cells is attributed to a mechanism in which all the products of 15 nurse cells are known to pass ribosomal and massenger RNAs into the oocyte *cytoplasm* as a result

enhance proteins along with ribosomes are aggrigated in the anterior end of oocyte cytoplasm. Such a condition is called polyribosome or polysome.

1.2 Spermatogenesis in *Drosophila*

The detailed study on *Drosophila* spermatogenesis is difficult to unravel. Their chromosome number being only eight but their size and behaviour are somehow advantageous to study in the critical periods of spermatogenesis. The classical cytological study in this aspect was made as early as in 1929 by La Cellule, Metz in 1926 and Huettmer in 1930. They used routine fixatives and stains as former-alcohol-acitic acid and hematoxylin. Later, molecular and genetic studies of *Drosophila* spermatogenesis were done by Henning in 1996, Hales and Fuller in 1997 and Tates in 1971.

The gonads are the only organs that retain their identity throughout the entire development. The testes can be found from early larva to mature fly.

In earlier stage, they are located in the posterior third of the larva, and are first spherical and later ovoid in shape. At pupal stage, they begin to elongate, coiling and twisting and at the time of emergence, they are fully grown and elongate tubular organs.

Germ cells

The early testes of the very early larva contain only *spermatogonia*. They are small rounded cells, The testis attain an ovoid shape when the larva increases in size, and the growth period of spermatocytes begins followed by meiosis. Larvae, on way to become pupae, contain all the stages of spermotogenic germ cells excepting mature spermo. Pupal larger testes contain more spermatocytes and almost mature spermatozon. The testis of adult fly is almost filled with mature sperms.

The structure of the testis is unique in lacking well-defined cysts as formed in other insects. Certain stages of spermatogenesis have a tendency to be grouped together and such groups shrunk away from other groups of germ cells in different stages. But in later stages, larvae and pupa become intermingted with all the types of germ cells.

Spermatogonia and primary spermatocytes

Spermatogonia are comparatively smaller in size with heavily stained chromatin. When the testis elongates and increases in size, the spermatogonia are restricted to a small part in the distal end of the testis and continue to remain there of the adult fly. Dipteran homologous chromosomes have a tendency to occur in pairs in the gonodial divisions but in *Drosophila* male, these are seldom found.

By slight gradations, spermatogonia develop into spermatocytes by gradual

dissolution of the chromatin of spermatogonia as they enter growth period. In the spermatogonia chromatin is heavier and mere condensed. Whereas, in the early spermatocytes, the chromatin becomes loose and flocculent with a round vesicular body appearing in the nucleus.

The large plasmosome in the nucleus of early spormatocyte gradually disintegrates when the primary spermatocyte is ready to enter leptotene stage and now nucleouls also disappears.

Synapsis and diakinasis

Previous observations on their dipteraus have indicated that meiotic stages as leptotene, pachytene diplotene are observe and not usually found in males and chromosome pairing is also absent. But in Drosophila such conditions do not appear. All pre-dialinetic stages are extremely short in duration. The condensation of chromosomes is absent except in late diakinetic stage. The chromosomes conjugates in pairs but crossing over is absent in male. The partial synopsis occurs and is swiftly followed by diakinesis. The pairing is soon followed by tetrad formation.

After this stage, chromosomes intract into masses to assemble into four independent groups in every spermatocyte.

Subsequently, the nucleus elongates, its membrane disappears, the asters are formed and the chromosomes enter the 1st maturation division with XY combination lagging to appear at one side of the other *tetrads* of *dyads* which move to their respective poles.

Spermatials : After second maturation division, four spermatids are produced, which are small round germ cells and the small nucleus of such spermatids increases rapidly in size prior to its transformation.

Mitochondria : In the growing spermatocyte, mitochondria are situtated as a spheroid mass in localised area in the cytoplasm near the nucleus.

During diakinesis the large mitochondrial mass moves toward nucleus surronding it in the form of a ring. At 1st meiosis. it divided into two masses that move to respective poles.

During the second meiosis, the mitochondria surround the entire spindle and divide again into two equal masses in the formation of spermatids.

Spormiogenesis : Spermatial centriole is single and is located close to nuclear membrane. Later, when axial filament grows out from ,the centriole, it is becomes double by proximal division. Nucleus is greatly elongated and narrowed to from the head in spermatozoa. During this progressive transformation of the spermatid, the fibrillar dictyosomes disappear gradually and are replaced by ring-like structures.

The acroblast moves toward opposite side of the nucleus. The two separate masses eventually unite, forming a cap on the nucleus and forms the ascrosome of the sperms.

"Nebenkern" : The **Nebenkern** is a remarkable mitochondrial formation in the spirm of insect as *Drosophila.* Just after meiosis is completed mitochrondia of the spermatid are collected on one side of the haploid nucleus and fuse together into *two giant aggregates.* These aggregates this wrap around one another to produce the spherical Nebenkern. Fuller, 1993 and Tates, 1971revealed EM studies of nebenkern that it resembles an *onion slice* and this early stage of spermiogenesis is called "onion stage". When their flagellum elongates, the two mitochondrial regions of the Nebenkern unfold and elongate down the sides of the axonen (Fig 1.4).

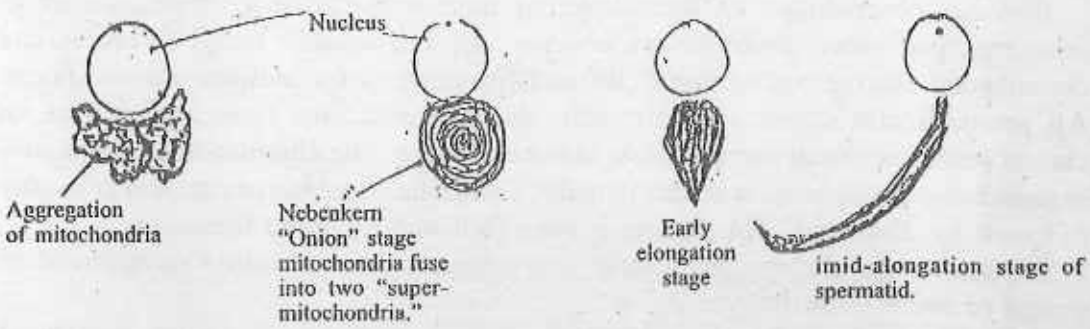


Fig 1.4 : Nebenkern formation in *Drosophila*

"Fuzzy Onions"

The mediator of mitochondrial fusion that regulates Nebenkern formation, is the product of the *fuzzy onionsgene* and appears to be a trans-membrane GTPase associated with the mitochondria during the time of fusion. Male flies with the mutation of fuzzy onion are sterile. They have defective Nebenkern due to the fact that mitochondria have failed to fuse into two giant "supermitochondria".

Hales and Fuller (1997) isolated and sequenced the wild-type allele of *Fuzzy onions* and showed that its product was a GTPase with transmembrane.

Using antibodies against the fuzzy onions GTPase, they showed that this protein is associated with mitochondria at the time of its fusion. It appears on the mitochondria just prior to fusion during the last stages of meiosis II and disappears soon after mitochondrial elogation.

Molecular and genetic aspects of *Drosophila* spermatogenesis

Hennig (1996) revealed that in Drosophila the regulation of sex determination of germ cells occurs relatively independent of that of somatic cells. The paternal and maternal genomes during the germ cell development receive different pattern of imprinting which results in a specific expression pattern of parental genes in the embryo.

During meiosis prophase, differentiation of male germ cells is initiated by re-organisation of chromatin, which leads to a higher packagin of chromatin required to accommodate the genoine in the small sperm head.

The genomic activity during spermatogenesis is found in cells upto the first meiotic division while the actual morphogenetic processes occur post miotically.

From the genetic point of view, it is to note that is *Drosophila* the number of genes giving male sterile phenotypes if mutated,is unexpectedly large. It is possible to assume that pleiotropic effects of many genes may affect sperm development. Another remarkable observation is that the no. of genes specifically and exclusively active during made from cell development is very small. Many genes required for sperm development. Another remarkable observation is that the no. of genes specifically and exclusively active during male germ cell development is very small. Many genes required for sperm development.' We also required for other cellular differentiation pathway. As for required for other cellular differentiation pathway. As for example, *Muscular myosin heavy chain,* though is expressed in testis (Miedema et al 1995), its mutation results embryonic or larval lethality.

Chromatin constitution in *Drosophila* male germ line

During sperm development the nuclear proteins undergo a transition resulting substitution of the normal chromosomal protein by another protein. There are 2 imp. aspects : (1) The normal *histone H1* absent in all shapes of spermatogenesis excepting stem cells. (2) During early postmeiotic development, the *chromatin* passes through a cycle of *condensation and decondensation* before it is finally packed and condensed into the sperm head.

This indicates that transcription is either very slow or fully absent in postmeiotic cells. The condensation-decondensation cycle is therefore related to the rearrangement of chromosomal proteins.

Normally, histone mRNAs are not polydenylated but polyadenylated variant forms of the mRNA for histone genes costing for the histones H2B, H3 & H4 exist in testis (Kremer, 1991). Both genes, **H3.3A** and **H3.3B** are expressed in testes and also in other tissues. However, H3.3A is more transcribed in testis while H3.3B is more strongly expressed in somatic cells.

Structure and function of Y-chromosomal male fertility gene

In 1961, Meyer et al. discovered that in Drosophila the activity of Y-chromosomal male fertility genes in the primary spermatocyte is accompanied by the formation of large lampbrush loops. These are the only genes in Drosophila forming such chromosomal structures contrary to amphibian oocyte.

Only few genes in Drosophila are active is the male germ line as : (i) B2-Tubulin (Kamphues et al. 1979), (ii) a group of seven genes of unknown function (Schafer

c.t. al. 1986), (iii) a history H5-like gene (Russel & Kaiser 1993), (iv)the stellage locus (Livak, 1990), (v) Janus B (Yanicostas, et al. 1989), (vi) a no. of Y-chromosomal fertility genes (Henning, 1988).

The nature of proteins bound to the Y-chromosomal lampbrush loops has been explored (Henmig 1996), one of the proteins, called tzf (testis zine finger appears as a typical nucleic acid binding protein, comparable to some *Transcription factors.*

Other genes active during *Drosophila* spermatogenesis

Henning (1996) identified several gene active in the male germ line. Two genes are single-copy gene functioning in somatic tissues. Some other genes have pleiotrop effects in Drosophila male germ line leading to male sterility one is the gene for the *Laminin B2 chain* (Wanpetel, 1992), and the other gene codes for the *muscular myosin heavy chain (mMHC)* (Miedema et al. 1995).

The *Laminin B2 gene* is expressed in the extracellular matrix of the testis envelope and also expressed in spermatocytes at the RNA level and its protein is expressed at the ultrastructural level of axoneme and the elongation of the nucleus.

The mMHC gene is transcribed in primary spermatocytes during meiotic prophase. The mMHC protein is found within differentiating Nebenkern derivatives. The myosin molecule may be constituents of the percrystalline material found in the tail of mature sperm. This protein may also control its final position in sperm alogation.

Both genes, laminin B2 and mMHC. Thus confirm that genes important in somatic tissues are also important in sperm differentiation.

1.3 Role of poleplasm

In *Drosophila* PGCs forms as a group of cells (Pole cells) at the posterior pole of the cellularizing blastoderm region. These nuclei migrate into the posterior region at the 9th nuclear division, and they become surrounded by the *Poleplasm,* a complex

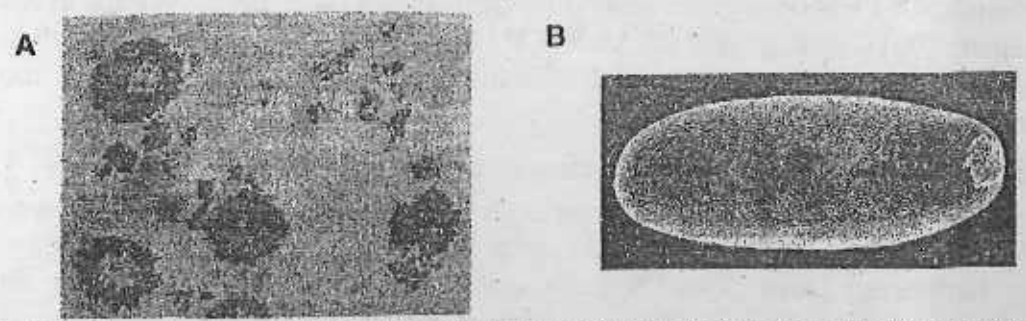


Fig 1.5 : The pole plasm of *Drosophila.* (A) Electron micrograph of polar granules from particulate fraction of *Drosophila* pole cells. (B) scanning electron micrograph of a *Drosophila* embryo just prior to completion of cleavage. The pole cells can be seen at the right of this picture. (Photograph courtesty of A.P. Mahowald.)

collection of mitochondria, fibrills and polar granules. If the pole cells nuclei are prevented from reaching the pole plasm, no germ cells will be made (Fig.1.5).

Nature has provided confirmation of the importance of both the poleplasm and its polar granules. One of the components of the poleplasm is the mRNA of the germ-cell-less gene (gcl). This gene was discovered by Jongens and his collegues (1992) when they mutated *Drosophila* and screened for females who did not have 'Grand offspring'. They assumed that if a female did not place functional poleplasm in her eggs, she could still have offspring, but those offspring would be sterile. This wild-type gcl gene is transcribed in the nurse cell of the fly's ovary, and its mRNA is transported into the egg. Once inside the egg, it is transported to the posterior most portion and resides within what will become the poleplasm. The gcl-encoded protein appears to enter the nucleus, and it is essential for pole cell production. Flies with mutations of this gene lack germ cells (Fig. 1.6).

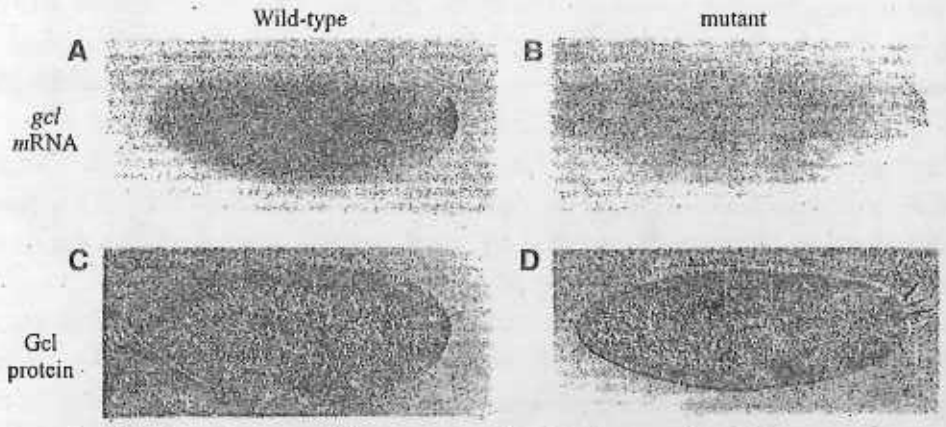


Fig 1.6 : Localization of *germ cell-less* gene products in the posterior of the egg and embryo. (A, B) The gel mRNA can be sen in the posterior pole of early-cleavage embryos produced by wild-type females (A), but not in embryos produced by *gcl*-deficient mutant females (B). (C,D) The protein encoded by the *gcl* gene can be detected in the germ cells at the cellular blastoderm stage of embryos produced by wild-type females (C), but not in embryos from mutant females (D). (From Jongens et al. 1992 ; (Photograph courtesy of T.A. Jongens.)

The posterior end of the egg is known to develop into sperm or egg cell. The posterior blastoderm cells are called poleplasm. The role of polar cytoplasm in *Drosophila melanogaster* in gamete formation can be established by different sets of experiments—

(a) The poleplasm can be stained specifically by using flourecent dyes and autoradiographic techniques. The results show that posterior blastoderm ie. polar cytoplasm has special staining specificity. Such syncytial blastoderm with posterior cells develops into normal flies.

(b) Irradiation of the end of the Syncytial blastoderm causes sterility in the fly that develops.

(c) The contents of the posterior end of a cellular preblastoderm can be removed and injected into an irradiated egg. Progametic cells can be found in the anterior end and the fly that develops is fertile.

1.4 Oskar gene

Cytoplasmic localization of mRNAs

Information required to start a fertilized animal egg on the path toward formation of an enbryo is laid down within the oocyte during oogenesis. We will briefly consider the fruit fly, whose egg, larval and adult stages are illustrated in figure.

The development of the anterior—posterior (head-abdomen) axis of a larval fly and subsequent adult is foreshadowed by localization of specific mRNAs along this same axis in the oocyte. For example mRNAs transcribed from the bicoid gene become preferentially localized at the anterior end of the oocyte, while mRNAs transcribed from the oscar gene become localized at the opposite end.

The mRNAs are subsequently translated at the site of localization. The protein encoded by bicoid mRNA plays a critical role in the development of the head and thorax, where as the protein encoded by oskar mRNA is required for the formation of germ cells, which develop at the posterior end of the larva.

The information that governs the cytoplasmic localization of a mRNA resides in the 3′ UTR (untranslated region) of either the bicoid or oskar mRNA. When the foreign gene is transcribed during oogenesis, the mRNA becomes localized in the site determined the 3′ UTR. Localization of mRNA is mediated by specific proteins that recognize localization sequences (called Zip codes) in this region of mRNA.

Microtubules, and the motor proteins that use them as tracks, play a key role in transporting mRNA containing particles to particular locations. The localization of oskar mRNAs in a fruit fly oocyte, for example, is disrupted by agents such as colchicine that depolymerize microtubules and by mutations that alter the activity of the Kinesis I motor protein.

Unit 2 ❐ Composition of semen, seminal protein and accessary reproductive structure of mammals

Structure

2.1 Semen

Semen or seminal fluid is the important organic substance which the male contributes during reproductive events the copulation. It is the product of the entire male reproductive system and is composed of *spermatozoa* and the *seminal plasmae.*

The Seminal plasma is a fluid and lymph like substance which contains many enzymes, nourishment in the form of fructose and proteins and contain those chemical molecules which protect the spermatozoa from other enviromental hazards. In many mammals, the semen tends to coagulate after its discharge from the penis. In the mouse, rat, gnineapegs, opossum etc. the semen coagualates into a second mass, The *vaginal plug,* once it reaches the vagina if the female. Coagulation of the semen also occurs in man, pig etc. but not in dog, bull and many other mammals. Human semen coagualtes immediately after discharge but liquifies a short time afterward due to the activity of two enzymes viz., *fibrinogenase* and *fibrinolysin,* both of which are synthesized in prostate gland. The liquification frees the sperm to make their long journey to the ova.

2.2 Composition of semen

During the process of ejaculation, sperm passes through the ejaculatory ducts and mixes with fluids from the seminal vesicles, the prostate, and the bulbourethral glands to form the semen. The seminal vesicles produce a yellowish viscous fluid rich in fructose and other substances that makes up about 70% of the human semen. The prostatic secretion, influenced by dihydrotestosterone, is a thin fluid containing

proteolytic enzymes, citric acid, acid phosphatase and lipids. The bulbourethral glands secrete a clear secretion into the lumen of the urethra to lubricate it.

Sertoli cells, which nurture and support developing spermatocytes, secrete a fluid into seminiferous tubules that helps transport of sperms to the genital ducts.

Seminal plasma of human contains a complex range of organic and inorganic constituents.

The components and contributions of semen are as follows :

Gland	Approximate%	Description
testes	2-5%	Approximately 200 to 500-million spermatozoa (also called *sperm* or *spermatozoons),* produced in the testes, are released per ejaculation.
seminal vesicle	65-75%	amino acids, citrate, enzymes, flavins, fructose (the main energy source of sperm cells, which rely entirely on sugars from the seminal plasma for energy), phosphorylcholine, prostaglandins (involved in suppressing an immune response by the female against the foreign semen), proteins, vitamin C.
prostate	25-30%	acid phosphatase, citric acid, fibrinolysin, prostate specific antigen, proteolytic enzymes, zinc (serves to help to stablize the DNA-containing chromatin in the sperm cells. A zinc deficiency may result in lowered fertility because of increased sperm fragility. Zinc deficiency can also adversely affect spermatogenesis).
bulbourethral glands	< 1%	galactose, mucus (serve to increase the mobility of sperm cells in the vagina and cervix by creating a less viscous channel for the sperm cells to swim through, and preventing their diffusion out of the semen. Contributes to the cohesive jelly-like texture of semen), pre-ejaculate, sialic acid.

A 1992 World Health Organization report described normal human semen as having a volume of 2 ml or greater, pH of 7.2 to 8.0, sperm concentration of 20×10^6 spermatozoa/ml or more, sperm count of 40×10^6 spermatozoa per ejaculate or more, and motility of 50% or more with forward progression (catagories a and b) of 25% or more with rapid progression (category a) within 60 minutes of ejaculation.

2.3 Semen protein

Semenogelin (Sg), the major protein of the human semen congulum, is present at high concentrations in seminal fluid vesicle secretions. It is degraded by the prostate specific antigen (PSA) to generate peptides of various biological activities. That were found on and inside spermotozoa. Lamirande et al (2009) experimentally proved that at concentrate of 0.1 to 1.0 mg/ml. Sg did not affect sperm motility but completely prevented capicitation induced by foetal cord serum ultrafiltrate; a partial inhibition of capacitation was noted with 0·03 mg Sg/ml.

Ribonuclease (RNase), which has as high as iso-electric point (PI-9.7) as Sg. (PI-9.5), also prevented sperm capacitation and O_2-related chemiluminscence but to a lower extent. Semenogelin is a potential scavenger for O_2-, but probably also affects the sperm oxidase. Spermatozoa rapidly processed Sg and a high proportion of Sg can be degraded after 15 minutes of incubation. The resulting polypeptide patterns were reminiscent of those obtained with PSA as a proteolytic enzyme. Therefore, semenogelin, the main protein of semen coagulum, inhibits human sperm capacitation by interfering with the superoxide anion generated during this process.

2.4 Accessory reproductive structures

More complex male accessory reproductive organs are found in those vertibrates where gamate union occurs within the protective structures of the maternal body (higher vertebrates). These are as follows :

Vasa efferentia and Vas deferens : The mature spermatozoa are collected by vasa efferintia which convey them to the main reproductive duct, the vas deferens.

Epididymis : The anterior portion of vas deferens becomes greatly lengthened, twisted or convoluted and highly coiled to form a compact structure called epididymis. The epididymis remains situtated at one cephalic end of a testis and it is a place of physiological maturation of sperm and also a place of storage of mature sperms.

Urethra and seminal vesicles : The posterior portion of each vas deferens remains less contorted and finally empties into the urinogenital sinus which is the male *urethra.* In same animals, vas deferens gives out some pouch-like, hollow glands called *seminal vesicles.* In most vertebrates, the seminal vesicle act as sperm storage organs during breeding season. In mammals it act as secretory glands which produce

a mucoid material containing structure and other nutrients and also large quantities of prostaglandins and fibrinogen.

Prostate gland : In metatherian and eutherian mammals, there are some more glands such as prostate glands and mucous glands. In man, prostate glands secrete a thin, milky, alkaline fluid containing citric acid, calciums, acid phosphatase, a clotting enzyme and profibrolysin.

Intromittent organ : When fertilization is internal, the male vertebrate usually develops intromittent or copulatory organs for introducing sperm into the reproductive tract of the female. Intromittent organs are particularly characteristics of reptiles and mammals. The intromittent organs of reptiles and mammals are of two types—paired hemipenis and penis. Snakes and lizards have a pair of stubby, grouped, sac-like hemipenes lying in pockets under the skin beside the cloaoa. These can be everted during sperm transfer. Male mammals exhibit a unpaired erectile penis.

Unit 3 ❐ *in vitro* and *in vivo* capacitation of mammalian sperm and role of fertilizin and ZP protein in fertilization

Structure

3.1 Contact and recognition between the gametes

Fertilization is the process whereby the sperm and the egg are fused together to begin the creation of a new individual whose genome is derived from both parents. It is a process, antithesis to meiotic division in respect of chromosomal numbers and number of genes in sexually reproducing organism.

Fertilization accomplishes two separate functions. The first function of fertilization is to transmit genes from parent to offspring and the second is to initiate in the egg cytoplasm, those reactions that permits development to proceed.

Although the details of fertilization vary from species to species, conception generally focused on four major events. In our discussion the content area will primarily be restricted to mammalian system and occasional references would be cited from other sources.

A complex dialogue exists between egg and sperm. The egg activates the sperm metabolism that is essential for fertilization and in turn the sperm reciprocates by activating the egg metabolism needed for the onset of development.

The structure of the gametes (the sperm and the egg) is organized in such a form that a sperm of a species structurally fits with the egg architecture. This fittings is not merely dependent on structural contributes but also on their chemical affinity.

The interaction by sperm and egg generally proceeds according to five basic steps– –

(i) Chemoattraction of the sperm to the egg by soluble molecules secreted by the egg.

(ii) The exocytosis of the acrosomal vesicle to release its enzymes.

(iii) The binding of sperm to the extracellular envelops of the egg.

(iv) Passage of the sperm through the extracellular envelope

(v) Fusion of egg and sperm cell membrane followed by gametic nuclei.

3.2 Sperm attraction

Species specific sperm attraction has been documented in numerous species including cnidarians, echinoderms, molluscs, urochordates, and vertebrates. In many species, sperm are attracted toward egg of their species by chemotaxis following a gradient of a chemical secreted by the egg. Miller (1978) first demonstrated that the eggs of cnidarians ***Orthopyxis caliculata*** not only secrete a chemostatic factor but also regulate the timing of its release. Miller, in his experiment, demonstrated that when sperm were added to oocytes that have not yet completed their second meiotic division, there was no attraction of sperm to egg. However, after the second meiotic division was finished and the eggs were ready to be fertilized, the sperm migrated towards them.

The mechanisms of chemotaxis differ among species and the chemotactic molecules are different in closely related species. One chemotactic molecule, a 14-amino acid peptide called *resact* has been isolted from the egg jelly of Sea urchin *Arbacia punctulata.* Resact is specific for *A. punctulata* and does not attract sperm of other species. *A. punctulata* sperm have receptors in their cell membranes, that binds resact and can swim up a concentration gradient of this compound until they reach the egg. The moleculer mechanism controlling the chemotaxis has recently been worked out. In Figure 6 the sperm chemotaxis in mammalian species has been summarized.

3.3 The acrosome reaction

A second interaction between sperm and egg is the acrosome reaction. In most marine invertebrates the acrosome reaction has two components—

(i) The fusion of the acrosomal vesicles with the sperm cell membrane in which an exocytosis that results in release of the content of the acrosome vehicle.

(ii) The extension of acrosomal process.

Summers and Hylander (1974) have extensively studied the events of acrosomal reactions in sea urchin *Stongylocentrotus purpuratus* in which the events have been sequentially summerized below (Fig. 3.1) :

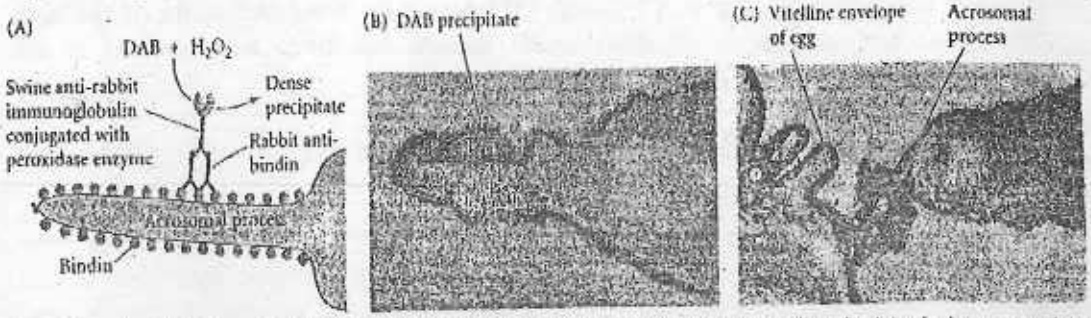


Fig. 3.1 : Localization of bindin on theacrosomal process. (A) Immuno-chemical technique used to localize bindin. Rabbit antibody was made to the bindin protein, and this antibody was incubated with sperm that had undergone the acrosome reaction. If bindin were present, the rabbit antibody would remain bound to the sperm. After any un-bound antibody was washed off, the sperm were treated with swine antibody that had been covalently linked to peroxidase enzymes. The swine antibody bound to the rabbit antibody, placing peroxidase molecules wherever bindin was present. Peroxidase catalyzes the formation of a dark precipitate from diaminobenzidine (DAB) and hydrogen peroxide. Thus, this percipitate formed only where bindin was present. (B) Localization of bindin to the acrosomal process after the acrosome reaction (33,200×). (C) Localization of bindin to the acrosomal process at the junction of the sperm and the egg. (B and C from Moy and Vacquier 1979 ; phtograph courtsey of V.D. Vacquier.)

Similarly in mammalian form, acrosomal reaction has been studied in golden hamster. Miezel (1984) has shown that during acrosomal reaction sperm cell membrane

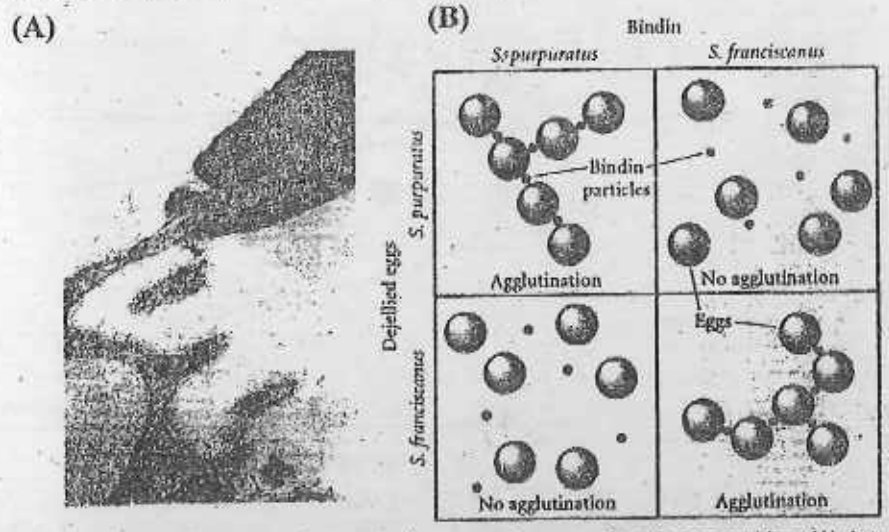


Fig. 3.2 : Species-specific binding of acrosomal process to egg surface in sea urchins. (A) Actual contact of a sea urchin sperm acrosomal process with an egg microvillus. (B) In vitro model of species-specific binding. The agglutinationof dejellied eggs by bindin was measured by adding bindin aggregates to a plastic well containing a suspension of eggs. After 2-5 minutes of gentle shaking, the wells were photographed. Each bindin bound to and agglutinated only eggs from its own species. (A from Epel 1977, photograph courtesy of F.D. Collins and D. Epel ; B based on photographs of Glabe and Vacquier 1977.)

and acrosomal membrane swell up and fusion between sperm cell membrane and adjuscent acrosomal membrane takes up at the tip of sperm nucleus and gradually glide down across the sperm membrane. The acrosomal reaction in mammalian form is intensely controlled by a protein called ZP3 reside on zona pellucida of the egg.

The moleculer mechanism of acrosomal reaction has been summarized in the following diagram (Fig. 3:2).

3.4 ZP proteins and binding of sperm to the extracellular envelop of the egg

Before the mammalian sperm can find the oocyte, its membrane first bind to and penetrate the egg's zona pellucida. The zona pellucida in mammals play a role analogous to that of vitelline envelope of invertebrates. However, the zona pellucida is more thicker and more dense structure than the vitelline envelope. The binding of sperm to zona pellucida is relatively species specific but not that of absolute level or category.

Zona pellucida is made of three major glycoproteins—ZP1, ZP2 and ZP3 [also called as zona proteins 1, 2 and 3]. Sperm binding to zona pellucida have been reviewed typically and the studies revealed that zona proteins interact with sperm in a sequencial way closely resembling an antigen-antibody reaction. However, it should be kept in mind that zona pellucida proteins are not only the exclusive architect of

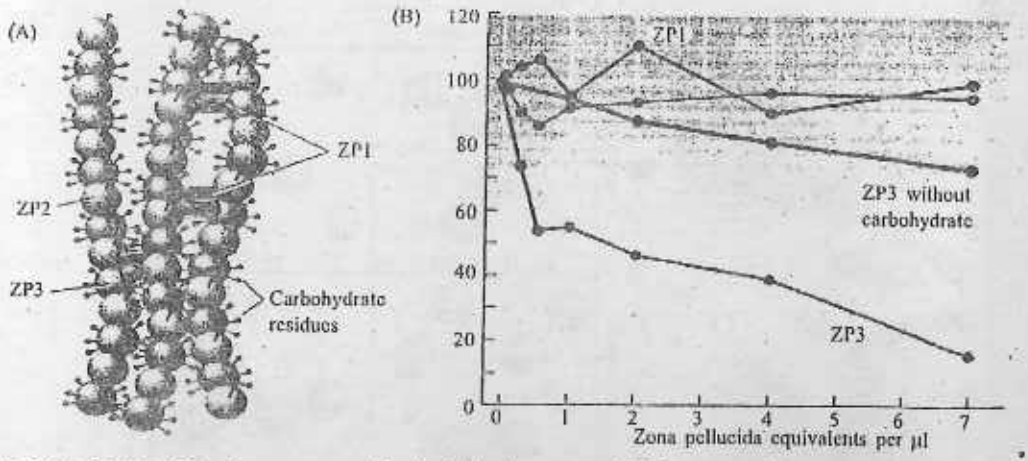


Fig 3.3 : Mouse ZP3, the zona protein that binds sperm. (A) Diagram of the fibrillar structure of the mouse zona pellucida. The major strands of the zona are composed of repeating dimers of proteins ZP2 and ZP3. These stands are occasionlly crosslinked by ZP1, forming a meshlike network. (B) Inhibition assay showing the specific decrease of mouse sperm binding to zonae pellucidae when sperm and zonaewere first indubated with increasingly large amounts of the glycoprotein ZP3. The importance of the carbohydrate portion of ZP3 is also indicated by this graph. (A after Wassarman 1989 ; B after Bleil and Wassarman 1980 and Florman and Wassarman 1985.)

gamete attraction or binding. Sperm specific proteins that express only in mammalian sperm during its maturation phase in epididymis facilitates egg-sperm attraction and binding. An inhibitor which blocks the expression of sperm specific protein in different parts of epididymis results in failure in fertilization process and to some extent infertility in man (Ray and Maiti, 1980) (Fig. 3.3).

3.5 Passage of the sperm through the extracellular envelop

The acrosome reaction releases engymes exocytotically. These proteolytic enzymes digest the egg protective coating, allowing the sperm to reach and fuse with the egg cell membrane.

The passage of sperm through egg envelope has been studied extensively in different organisms and the mechanism has been outlined as shown in the following diagram.

3.6 Fusion of egg and sperm cell membrane followed by gametic nuclei

The mammalian sperm that finally enters the egg carries its genetic contribution in a haploid pronucleus. In mammals, the process of pronuclear migration takes about 12 hours, compared with less than 1 hour in the sea urchin.

The mammalian sperm enterd almost tangentially to the surface of the egg rather than approaching perpendicularly and it fuses with numerous microvilli (Fig 3.4).

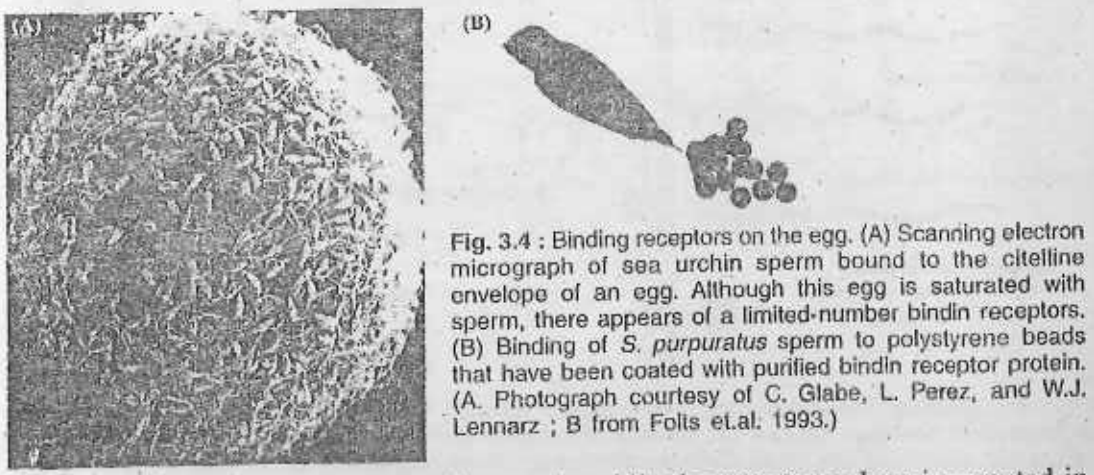


Fig. 3.4 : Binding receptors on the egg. (A) Scanning electron micrograph of sea urchin sperm bound to the citelline envelope of an egg. Although this egg is saturated with sperm, there appears of a limited-number bindin receptors. (B) Binding of *S. purpuratus* sperm to polystyrene beads that have been coated with purified bindin receptor protein. (A. Photograph courtesy of C. Glabe, L. Perez, and W.J. Lennarz ; B from Folts et.al. 1993.)

The mammalian sperms enters the oocyte while the oocyte nucleus is arrested in metaphase of its 2nd meiotic division. Through a series of chemical reactions the sperm pronucleus fuses with egg pronucleus. The division is actually contributed by microtubules joined the 2 pronuclei and enable them to migrate toward one another.

Upon meeting the two nuclear envelop breakdown. However, instead of producing

a common zygote nucleus as found in sea urchins, the chromatin condenses into chromosomes that orient themselves. Thus a true diploid nucleus in mammal is not seen first in the zygote but in the two cell stage.

Each sperm brings into the egg not only its pronucleus but also its mitochondria, its centriole and a small amount of cytoplasm. The sperm mitochondria and their DNA are degraded in the egg cytoplasm while the sperm centriole that survives during the process act as the organizing agent for making the new mitotic spindle.

Thus, the event of fertilization (Fig. 3.5) physically can be summerized as follows.

1. Fertilization accomplishes two separate activities and two separate activition i.e. sex and reproduction.
2. The events of fertilization substages are—
   (i) contact and recognision between sperm and egg.
   (ii) Regulation of sperm entry into the egg.
   (iii) Fusion of genetic material from the two gametes.
   (iv) Activation of egg metabolism to start development.

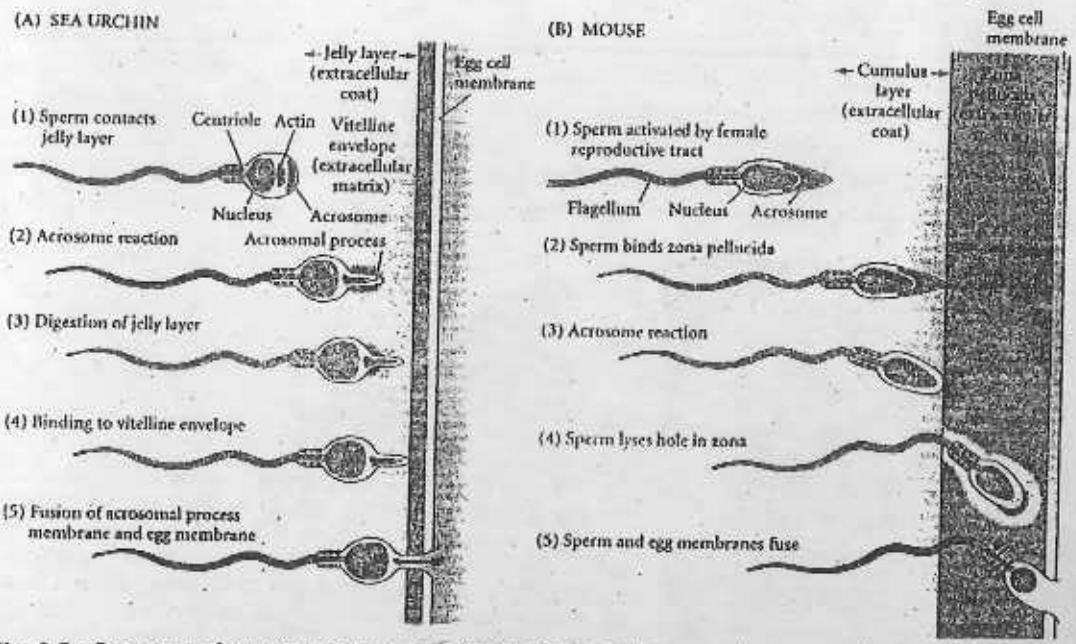


Fig. 3.5 : Summary of events leading to the fusion of egg and sperm plasma membrane in (A) the sea urchin and (B) the mouse. (A) Sea urchin fertilization is external. (1) The sperm is chemotactically attracted to and activated by the egg. (2, 3) Contact with the egg jelly triggers the acrosome reaction, allowing the acrosomal process to form and release proteolytic enzymes. (4) The sperm adheres to the vitelline envelope and lyses a hole in it. (5) The sperm adheres to the egg plasma membrane and fuses with it. The sperm pronucleus can now enter the egg cytoplasm. (B) Mammalian fertilization is internal. (1) The contents of the female reproductive tract capacitate, attract, and activate the sperm. (2) The acrosome-intact sperm binds to the zona pellucida, which is thicker than the vitelline envelope of sea urchins. (3) The acrosome reaction occurs on the zona pellucida. (4) The sperm digests a hole in the zona pellucida. (5) The sperm adhres to the egg, and their plasma membranes fuse.

3. When fertilization takes places, the egg secretes diffusible molecules that attract and activates the sperm.

4. Species specific chemotactic molecules secreted by the egg which attract sperm to enable fertilization.

5. The acrosome reaction releases enzymes exocytotically and such proteolytic enzymes digest the egg protective coating, allowing sperm to reach and fuse with the egg cell membrane.

6. Fusion between sperm and egg is mediated by protein molecules.

7. The fusion of sperm and egg results in the activation of crutial metabolic reactions within the egg.

8. Genetic material is carried in a male and female pro-nucleus which migrate toward each other.

9. Fertilization takes place either internally (as in mammals) or externally (as in sea urcnin, cnidarians etc.)

3.7 Capacitation of sperm in mammals

The female reproductive tract in mammals is not a passive conduct through which sperm reach but a highly specialized tissues that actively regulates the transport and maturity of both gamets. Both the male and female gametes utilize a combination of

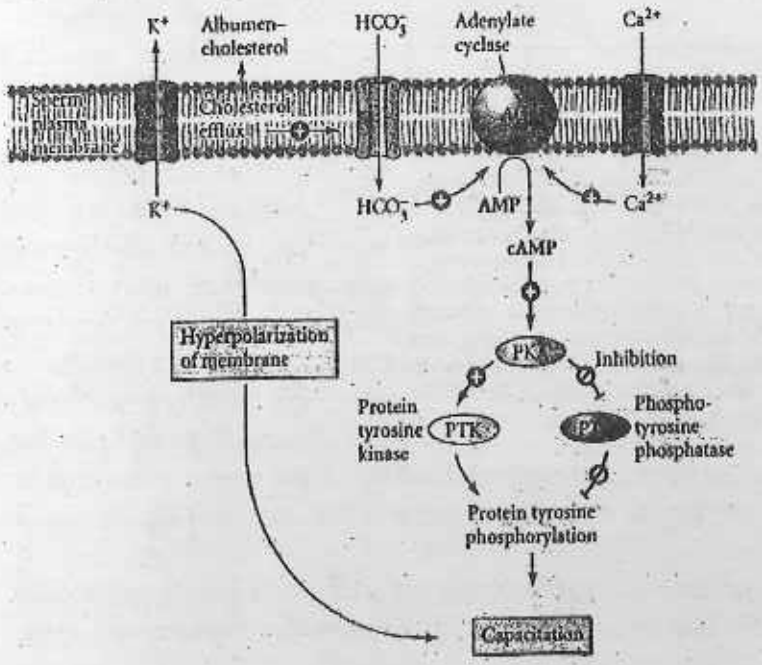


Fig. 3.6 : Hypothetical model for mammalian sperm capacitation. The efflux of potassium (whose cause we do not know) results in a change in the resting potential of the sperm cell membrane. The removal of cholesterol by albumin stimulates ion channels that enable calcium and bicarbonate ions to enter the sperm. These ions promote the activity of adenylate cyclase, which makes cAMP from AMP. The rise in cAMP activates protein kinase A, causing it to activate the protein tyrosine kinases (while inactivating the protein phosphateses). The kinases phosphorylate proteins that are essential for capacitation. (After Visconti and Kopf 1998.)

small scale biochemical interactions and large scale physical propulsion to get to the ampullae, the region of the oviduct where fertilization takes place.

Similarly, the sperm after its formation within the testis are released into epididymal part and migrate through differentially and selectively for maturation.

Newly ejaculated mammalian sperm are unable to undergo the acrocrome reaction until they have resided for sometime in the female reproductive tract the set of physiological changes by which the sperm become competent to fertilize the egg is called ***capacitation***. The sperm, that are not capacitated are 'held up' in the cumulus matrix and are unable to reach the egg.

The requirements for capacitation very from species to species. Capacitation can be **mismicker** in vitro by incubating sperm in a tissue culture medium containing calcium ions, bicarbonate and serum albumin or in fluid taken from the oviducts. The role of oviduct in capacitation has attracted the attention of the reproductive biologist to solve out the cases of infertility due to contribution of inactivated or ghost sperm from the male contributors. It has been found that it is compulsory for the sperm to reach the ampullae where they first acquire the competence for the fertilization. Subsequent competence achieved in the oviduct where small molecules enriched the sperm to encounter the external surface protectively covered by several membranes and envelopes.

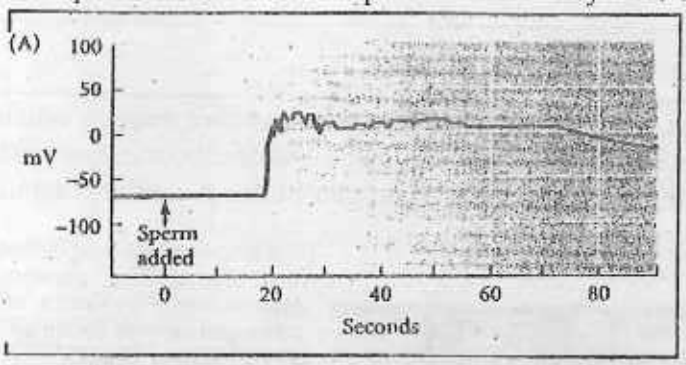


Fig. 3.7 : Membrane potential of sea urchin eggs before and after fertilisation. (A) Before the addition of sperm, the potential difference across the egg plasma membrane is about –70 mV. Within 1-3 seconds after the fertilizing sperm contacts the egg, the potential shifts in a positive direction. (B,C) *Lytechinus* eggs photographed during first cleavage. (B) Control eggs developing in 490 mM Na+. (C) Polyspermy in eggs fertilized in similarly high concentrations of sperm in 120mM Na+ (choline was substituted for sodium). (D) Table showing the rise of polyspermy with decreasing sodium ion concentration. (From Jaffe 1980 ; photographs courtsy of L.A. Jaffe).

There may be an important connection between sperm translocation and capacitation. Smith (1998), has documented that before entering the ampullae of the oviduct the uncapacitated sperm bind actively to the membranes of the oviduct cells in the isthmus part. This binding is temporary and appears to be broken when the sperm become capacitated through temporary binding of the sperm with ampullar cells is extremely significant for the expansion of sperm life span and its survival in the oviduct canal.

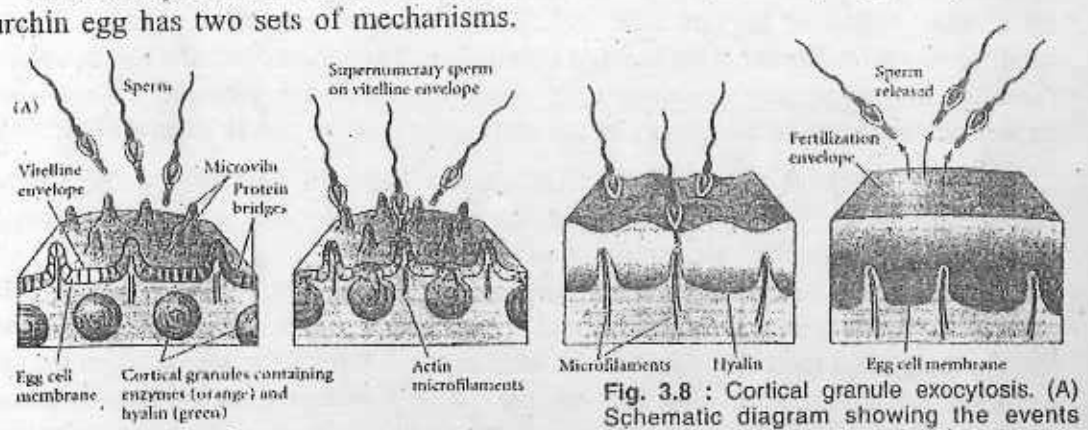
The moleculer mechanism that take place during capacitation is poorly understood. But very recently a model has been proposed to explain the mammalian sperm

capacitation, the diagram explains the mechanism of sperm capacitation (Fig. 3.6 & 3.7) as follows :

3.8 Polyspermy

In most animals, any sperm that enters the egg can provide a haploid nucleus & a centroile to the egg. But in normal, only one sperm enters the egg & a haploid sperm nucleus & a haploid egg nucleus combine to form diploid nucleus of fertilized egg & thus restores the chromosome number appropriate for the species. This event is called *monospermy*. However, a set of multiple sperm may enter the egg called *polyspermy* leads to disastrous consequences in most organisms. In nature and in experimental conditions such events have been studied and the mechanism by which the polyspermy is prevented has been worked out.

Different species have evolved various mechanism to prevent polyspermy. The sea urchin egg has two sets of mechanisms.



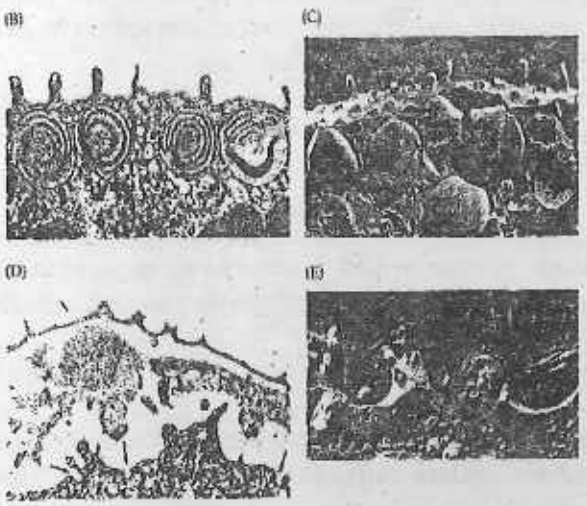


Fig. 3.8 : Cortical granule exocytosis. (A) Schematic diagram showing the events leading to teh formation of the fertilization envelope and the hyaline layer. As cortical granules undergo exocytosis, they release proteases that cleave the proteins linking the vitelline envelope to the cell membrane. Mucopolysaccharides released by the cortical granules form an osmotic gradient, thereby causing water to enter and swell the space between the vitelline envelope and the plasma membrane. Ohter enzymes released from the cortical granules harden the vitelline envelope (now the fertilization envelope) and release sperm bound to it. (B,C) Transmission and scanning electron micrographs of the same region of a recently fertilized egg, showing the raising of the fertilization envelope and the points at which the cortical granules have fused with the plasma membrane of the egg (arrows in D). (A after Austin 1965; B-E from Chandler and Heuser 1979, photographs courtsey of D.E. Chandler.)

A) Fast block to polyspermy : Accomplishing an electric change in the egg cell memebrane in a very fast reaction & mechanism is known as *the fast block to polyspermy.* A fast block to polyspermy is achieved by changing the electric potential of egg membrane. The egg membrane provides a selective barrier between the egg cytoplasm & outside environment, so that the ion concentration within the egg differs greatly from those of its surrounding. This concentration difference is especially significant for sodium & potassium ions. Sea water has a particularly high sodium ion concentration whereas egg cytoplasm contain relatively small Na+. The reverse is true for K+. This condition is maintained by the cell membrane which inhibits entry of Na+ into the oocyte & prevents K+ to leaking out into the environment. At this stage, resting membrane potential is generally about seventy millivolt (expressed as –70 mv) because the inside of the cell is negetively charged with respect to the exterior). Within 1 to 3 seconds after binding of sperm with egg cell membrane the resting potential shifted to a positive level, i.e. about +20 mv. This change is caused by a small influx of Na^+ into the egg. This change in electrical gradiant inhibits binding of sperm further with the egg membrane. Thereby no further sperm entry is possible through the egg membrane. The result of membrane potential change under experimental condition using eggs of sea urchin has been shown in diagram (Fig. 3.8).

B) Slow block of polyspermy : Unlike fast block to polyspermy the another mechanism which brings about prevention of further entry of sperm is known as *cortical granule reaction* or as *slow block to polyspermy.*

Beneath the sea urchin egg cell membrane, there are about 15,000 cortical granules, each about 1 mm in diameter, are found. Upon sperm enjoy these cortical granules fuse with egg cell membrane & release their controls into the space between the cell membrane & vitelline envelop fibrous mat. Several proteins are released by these cortical granules exocytosis which ultimately accumulate on the fibrous mat forming a secondary barrier. This cortical reaction is essential to prevent polyspermy & is achieved very rapidly within the perview of prevention of polyspermy.

The detailed mechanism of cortical granule reaction has been summarized in the following diagram.

3.9 Molecular mechanism of egg activation

Although fertilization is depicted as merely the means to merge two haploid nuclei but actually it is the event that initiate the activation of metabolic processes in the egg cytoplasm required for initiation of development.

Sea urchin is used as a classical model to study the events that undergo sequentially and in some, hand to hand.

Contact or fusion between sea urchin sperm & egg activates the two major blocks to polyspermy. The fast block initiated by sodium influx into the cell and the slow block initiated by the intracellular release of Ca^{++}.

The release of sodium as stated earlier is responsible for cortical grannule reaction and is also responsible for the reentry of the egg into the cell cyle and activation of egg protein synthesis.

Calcium release activates a whole series of metabolic reaction that initiates embroynic development such as the activation of NAD$^+$ kinase which converts NAD$^+$ to NADP, a key process for supply of oxygen in the developing organism.

Epel, (1980) has summarised the molecular mechanism of activation of egg metabolism in sea urchin which is presented below (Fig. 3.9 and 3.10).

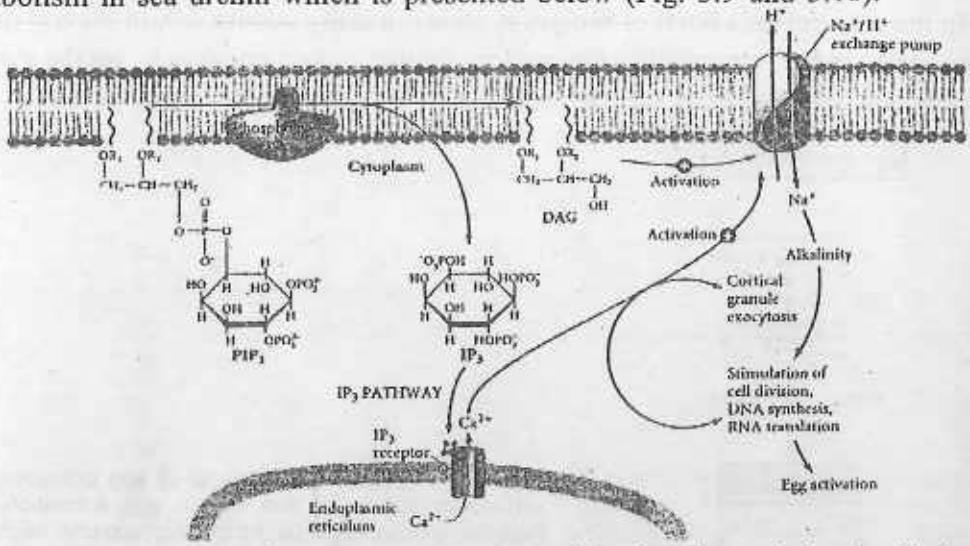


Fig. 3.9 : The roles of inositol phosphates in releasing calcium from the endoplasmic reticulum and the initiation of development. Phospholipase C splits PIP_2 into IP_3 and DAG. The IP_3 releases calcium from the endosplasmic reticulu, and the DAG, with assistance from the released Ca^{2+}, activates the sodium-hydrogen exchange pump in the membrane.

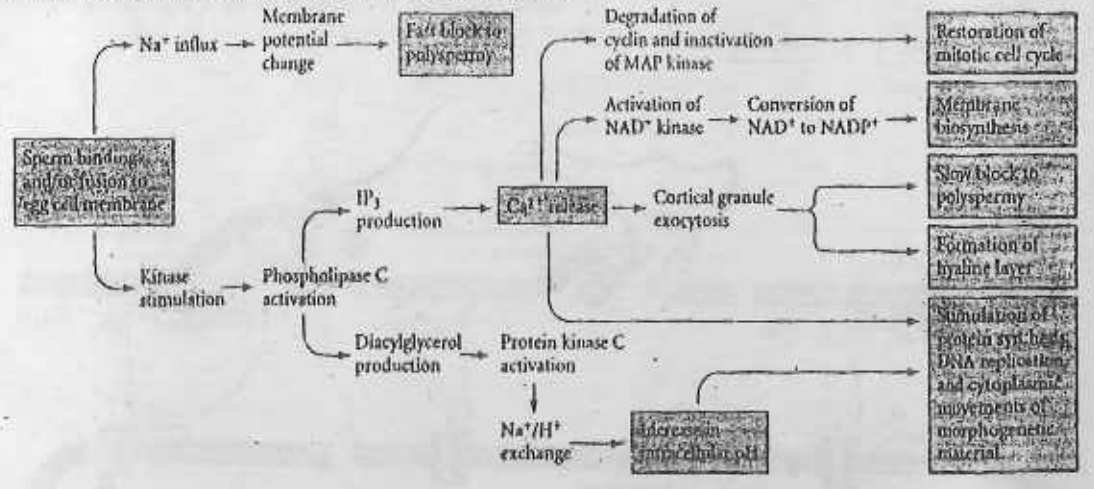


Fig. 3.10 : Model of egg activation in the sea wreliz (after Epel 1980)

On the other hand, the late responses of fertilization includes the activation of new burst of DNA & protein synthesis. The fusion of egg and sperm causes the intracellular pH to increase due to the production of diacylglycerol. This rise in intracellular pH begins with a second influx of Na^+ ions which causes 1:1 exchange between Na^+ from the sea water and H^+ from the egg. The loss of H^+ causes the pH of the egg to rise which consequently increases Ca^{++} elevation and together to stimulate new DNA synthesis.

In the sea urchin, a burst of protein synthesis usually occurs within several minutes after sperm entry. Interestingly this protein synthesis does not depend on the synthesis of new mRNAs, rather it utilizes mRNAs present in the oocyte cytoplasm.

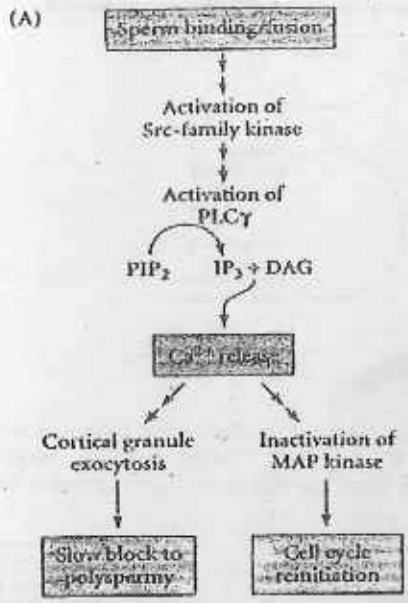
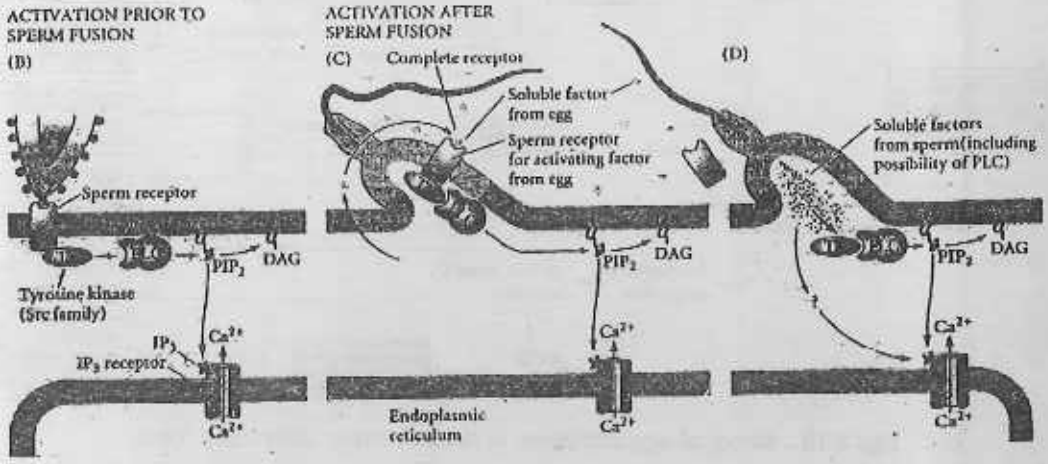


Fig. 3.11 : Possible mechanisms of egg activation. (A) A schematic outline of sea urchin egg activation. (B-D) Possible mechanisms by which this scheme might be a accomplished. (B) the bindin receptor activates a cytoplasmic Src kinase. (C) An activated Src kinase or PLC in the sperm plasma membrane activates the egg pathways. (D) Calcium release and egg activation by activated PLC from the sperm or by a substance from the sperm that activates egg PLC.



The mRNAs present in the egg cytoplasm in non-translated form meant to encode proteins such as listones, tubulish, actins & morphogenetic factors that are used during early development.

One mechanism for this global rise for the translation of messages stored in the oocyte appeares to be the release of inhibitors which musked the encoded RNAs. The removal of these mask proteins triggered by synthesis of activator proteins which degrades mask proteins such as 4E binding protein which ancode cyclin B. The cyclin B protein combines with Cdk 1 cyclin to create mitosis promoting factor (MPF) which is required to initiate cell division.

Thus fertilization activates pathways that target the translational inhibitory proteins for degradation and the newly accessible 5′ end of the mRNA can interact with those proteins that allow the messages to be translated. One of those mRNAs encodes a protein (CP) critical for cell division. In such a manner, fertilization can initiate mitosis and the sea urchin can begin to from a multi-cellular organism.

In recent years the molecular mechanism of egg activation has been further revisited. The mechanism suggests that sperm contact & fusion activates a special kind of protein called G protein which in turn in a cascade manner activates a series of protein channels and ultimately release Ca^{++} which is responsible for cortical grannule exocytosis and inactivation of MAP kinsese (Fig. 3.11).

3.10 *Invitro* fertilization

Infertility i.e. the inability to achieve or sustain pregnancy is not a disease in the usual sense of the word. It is not a symptom nor a condition that prevents the physical well being of the infertile individual or couple. However, since the desire to have children can be exceptionally strong for biological and social reasons, the search for alternative ways to have child is a pivotal issue in clinical research.

In vitro fertilization (IVF) is an assisted reproductive technology in which oocytes and sperms retrieved from the male and female partners and placed together in a petridish, where fertilization can take place. After the fertilized eggs have began dividing, they are transferred into the female partner uterus, where implantation and embryonic development can occur as in a typical pregnancy.

IVF was developed in the early 1970s and the first IVF baby Louise Brown was born in England in 1978. Since then the number of IVF procedures perform in each year has increased and their success rate has improved.

The IVF procedure has 4 basic steps—

Step-1 : Ovarian stimulation and monitering : In this step oocytes are stimulated and women are injected with gonadotropins or anti oestrogens over a period of days or weeks in order to hyperstimulate the ovaries to produce mature oocytes.

Step-2 : Egg retrieval : Once the follicle has matured (but not yet ruptured), the physician retrieves as many oocytes as possible. This is done surgically, guiding an aspiration pipette to each mature follicle and sucking up the oocyte. Once recovered, these oocytes that are mature and healthy are transferred to a sterile container to await fertilization in the laboratory.

Step-3 : Fertilization : A semen sample is collected from the male partner approximately 2 hours before the female partner's oocytes are retrieved. These sperm are processed by a procedure called sperm washing. Sperm washing capacitates the sperm and selects only the healthiest and most active sperm in the sample. The selected sperm are placed in a petridish with the oocytes, and the gametes are incubated at body temperature. In general, each oocyte is incubated for 12-18 hours with 50,000-100,000 motile sperm. If fertilization is successful, the eggs will begin to divide. The success rate for achieving fertilization in this way is between 50 to 70 percent.

Step-4 : Embryo transfer : Embryo transfer is not complicated and can be performed without anesthesia or surgery. The procedure is usually done 3 days after egg retrieval and fertilization. The healthy embroyos (those that have divided well and now contain 6-8 cells). The embryos are sucked into a tubular catheter and then transferred via the catheter direcetly to the uterus. Normal implantation and maturation of at least one embryo (Fig. 3.12) is required to achieve pregnancy.

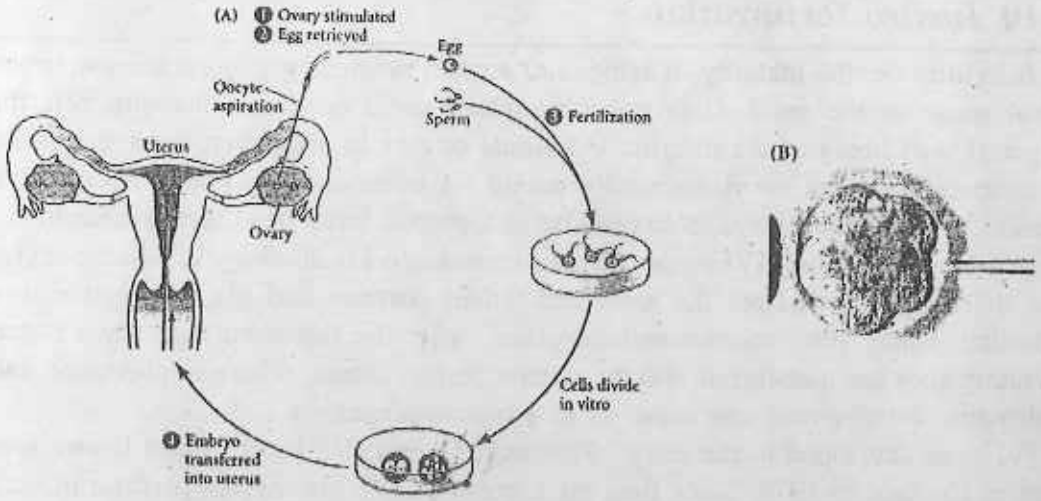


Fig. 3.12 : In vitro fertilization. (A) The IVF process can be divided into four basic steps : (1) ovarian stimulation, (2) egg retrieval ; (3) fertilization and (4) transfer of the embryo into the uterus. (B) Assisted hatching, whereby a hole is poked in the zona pellucida, is a procedure to help ensure the embryo implants in the uterus. (B, photograph courtesy of The Institute for Reproductive Medicine and Science of St. Barnabas, Livingstone, NJ.)

3.11 Success rate and complications : limitation of IVF

Inspite of it's novelty IVF is not free from ethical or surgical limitations. The success rate till today is 31 couples out of every 100 who try one retrieval with IVF and likely to achieve pregnancy and delivery. However, the success rate, drops to 25% or low according to the age of the mother. After 40 years of age the success rate is less than 5%. This decline may be due to the declining viability of eggs as women advance in age.

Another serious limitation is the rate of multiple births. It has been statistically proven that when three embryos were transferred, the multiple birth rate was 46% for women aged 20-24. The rate was 39% for women aged 40-44, when seven or more embroys were transferred. Therefore, the multiple birth poise severe health hazards to mother along with malformations, infant death, premature delivery, low birth rate etc. Moreover, multiple birth rate may increase the incidence of diseases (Fig. 3.13) like high blood pressure, diabetic etc.

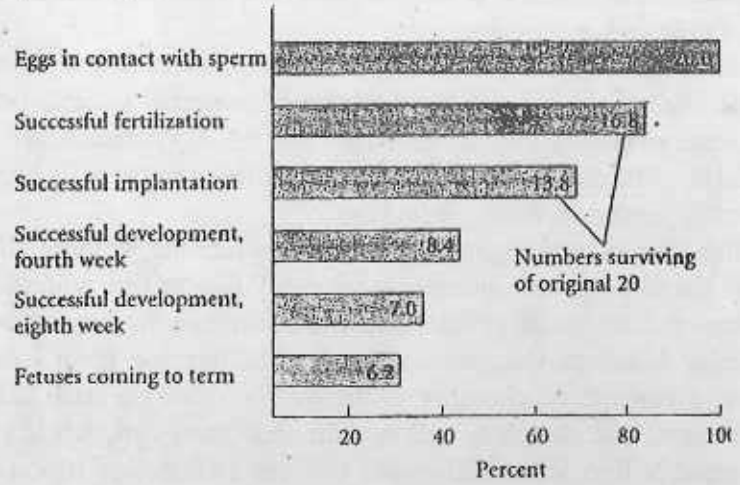


Fig. 3.13 : The fate of 20 hypotheitcal human eggs in the United States and western Europe. Under normal conditions, only 6.2, or fewer, of the original 20 eggs would be expected to develop successfully to term. (After Volpe 1987).

The wide spread practice of IVF has given rise numerous ethical and legal concerns over the safety of the techniques as well as availability of the technique to the greater mass of people. The economic inequality that occurs when only a portion of the population has financial access to a medical technology consider as a gift of medical wonder.

Unit 4 ❐ Role of nurse cell and follicular cell in yolk production in *Drosophila*

Structure

4.1 Nurse cell

The *Nurse cells* are found only in invertebrates such as annelids, cnidarians, molluscs and insects. They are originated from the same organiser that give rise to the oocyte. During the original divisions in the ovary, at some points a differential division separates cells destined to become oocytes from their sisters which develop into *nurse cells.*

The oocytes of *Drosophila* lack lampbrush chromosomes and do not synthesize RNA. In it the nurse cells are the chief source of maternal genetic information and ribosomes. Gene amplification mechanisms for RNA synthesis are found in the genomes of nurse cells, due to which the chromosomes of nurse cells became polytanic and metabolically active in RNA synthesis.

Thus, during oocyte vitellogenesis of *Drosophila,* the volume of a nurse cell nucleus, nucleoli and cytoplasm doubles once every four to five hours. As *Drosophila* nurse cells develop, they retain cytoplasmic connections with the oocyte forming ring canals, bridges or fusomes. Oocytes and nurse cells develop from stem cells within a germinarium, a constricted chamber in the ovaris. A single stem cell undergoes a differential division, one daughter cell remain as a stem cell, while the other cells ooginium, completes four mitotic divisions to form a cluster of 16 cystocytes one of which becomes an oocyte and next 15 become nurse cells. The follicle cells, surround all the cystocytes.

Functions : The principal role of nurse cells are :

1) Supply oocyte nutrient reserve during its growth.

2) acts as selective barrier between vascular system and the oocyte, transporting ascented molecules (Yolk precursers) into oocyte cytoplasm.

3) synsnsize accessory egg membrane.

4.2 Follicle cells

The follicle cells are the accessory cells of vertebrates and invertebrates. In

vertebrates, These cells are derived from the germinal epithelium and become organised as a single layer of epithelial cells surrouding the developing oocyte. Though they may synthesise same substances for oocyte storage. They act more as a selective barrier. Mediating transfer of materials (eg. yolk proteins) entering the oocyte from the blood stream, have to pass through the membranes of the follicle cells on the way.

To facilitate this transfer processes. These are thousands of cytoplasmic processes

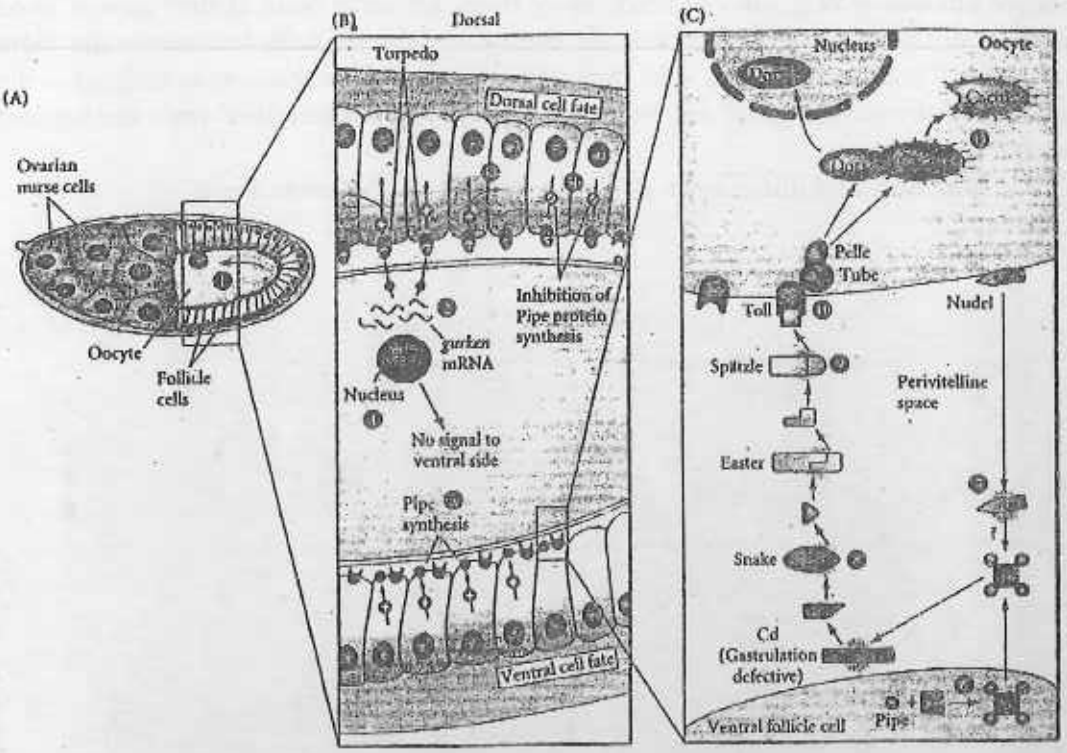


Fig. 4.1 :

(1) Oocyte nucleus travels to anterior dorsal side of cocyte. It synthesizes *gurken* mRNA, which remians between the nucleus and the follicle cells.
(2) *gurken* messages are translated. The Gurken protein is received by Torpedo proteins during mid-oogenesis.
(3a) Torpedo signal causes follicle cells to differntiate to a dorsal morphology.
(3b) Synthesis of pipe protein is inhibited in dorsal follicle cells.
(4) Gurken protein does not diffuse to ventral side.
(5) Ventral follicle cells synthesize Pipe protein.
(6) In ventral follicle cells, Pipe completes the modification of an unknown factor (x).
(7) Nudel and factor (x) interact to split the Gastrulation-deficient (Gd) protein.
(8) The activated Gd protein splits the Snake protein, and the activated Snake protein cleaves the Easter protein.
(9) The activated Easter protein splits Spatzle ; activated sparzle binds to Toll receptor protein.
(10) Toll activation activates Tube and Pelle, which phosphorylate the Cactus protein. Cactus is degraded, releasing it form Dorsal.
(11) Dorsal protein enters the nucleus and ventralize the cell.

called *microvilli,* reading out from the surfaces of book the oocytes and the follicle cells. In contrast with nurse cells, follicle cells do not arise from oogonia, are not in direct cytoplasmic communication with the oocyte and generally transport rather than synthesize oocyte—bound materials (Fig. 4.1).

In *Drosophila,* the follicular epithelium surrounding the developing oocyte is initially symmetrical but this symmetrical is broken by a signal from the oocyte nucleus. The oocyte nucleus is originally located, away from the nurse cells. It then moves to an anterior dorsal position and signals the overlying follicles cells to become the more columnar dorsal follicle cells. The dorsohizing signed from the oocyte nucleus is the product of the *gurken gene,* the only gene known to be transcribed from the haploid ooctye nucleus.

The function of follicle cells is similar to that of the nurse cells.

Unit 5 ❐ Teratogenesis – genetic & induced by drug thalidomide

Structure

5.1 Introduction

Teratology is the study of abnormalities of physiological development. It is often thought of as the study of **birth defects**, but it is much broader than that, taking in other developmental stages, such as **puberty**; and other life forms, such as plants. The term stems from the **Greek** (téras, **genitive** - tératos), meaning *monster*, or *marvel* and - lógos, meaning *speech* or, more loosely, *the study of*.

Teratology meaning *monster*, or *marvel* and as early as 17th century referred to a discourse on prodigies and marvels, of anything so extraordinary as to seem abnormal. In the 19th century, it acquired a meaning closer related to biological deformities, mostly in the field of botany. Currently, its most instrumental meaning is that of the medical study of teratogenesis, congenital malformations or grossly deformed individuals.

Teratogen & Teratogenesis : Any agent that can disturb the development of an embryo or foetus. Teratogens may cause a birth defect in the child. Or a teratogen may halt the pregnancy outright. Thus abnormal development or formation of a terata (individuals' shows gross deviation from the normal due to congenital malformations) is called teratogenesis. Since the development of a normal phenotype requires both a normal genotype and a favorable environment, therefore the teratogenesis can be due to either abnormal genotype or the environment.

5.2 Objectives

This unit of the module of Developmental Biology is meant for the study of teratogenesis that gives a concise idea about how the terata delopes. Finally after completion of the topic, the reader shall be able to understand the

- Basic idea of teratology
- How teratogenesis is related with genes
- How drugs involved in teratology
- Different types of drugs and their interaction
- Mechanism of teratogenesis and
- Wilson's 6 principle

5.3 Genetic teratogenesis

Genetic teratogenesis in human beings

Abnormal genes may be inherited from one or both parents and they may be dominant or recessive. In the majority of the badly affected individuals, however, there is no Mandelian pattern of inheritance. The abnormalities merely occur more frequently among relatives than in general population. In those occurring among relatives, there may be complex interplay between several genes.

Deformities due to abnormal dominant genes are rare. In most of these, the skeleton is affected and the deformities include **achondroplasia** i.e. insufficient growth of long bones, **arachnodactyly** i.e. abnormally long hand and foot bones , **craniocleidal dysostasis** i.e. absence of rudimentary development of clavicle and abnormal shape of skull, and **osteogenesis imperfecta** i.e. incomplete development or hypoplasia of osteoid tissue and collagen, resulting in bone fracture. Such conditions will appear in 50% of the offspring of the affected parent.

Abnormal recessive genes do not fid phenotypic expression unless inherited from both parents. In such cases the abnormality may be found in 25 % of the offspring. Ex. Cystic fibrosis, Sprengel's deformity of the shoulder etc.

5.4 Genetic teratogenesis in animals

1. **Gene-phene relationship** : Several different gene can cause the same terata, though not necessarily by the same route. For example, there are more than twenty genes which affect eye color in *Drosophila melanogaster.* The mutants causing the same defect may be either recessive or dominant. For example, in fowl the trait of rumplessness i.e. absence of tail is controlled by either recessive or dominant gene.

In some cases the same mutation may behave as a recessive or a dominant depending on the genetic background. Thus, in mice the fused gene i.e. fusion or absence of ribs and/ or absence of tail is dominant in *Mus musculus musculus* but recessive in *Mus musculus bactrianus.*

The proportion of affected individuals in a population and degree of effect of mutant genes are dependent on both genetic and environmental factors. For example fowl carrying **rumpless gene** can be selectively bred to produce a 'normal' tail phenotype. Similarly in *Drosophila* carrying **Bar eye gene,** the size of the eye and the number of facets in the eye decreases by about 100 facets during development.

2. **Autophene, allophene and pleiotropy :** Not all genetic terata are the result of intrinsic action of genes in the affected tissues.

i) **Creeper mutation** (cp/cp) in fowl affects the limbs forming abnormally short limbs known as **Phocomelia** and the small eyes called **microphthalmia;** the embryo does not survive till hatching. Transplants of the cp/cp limb rudiments in the normal hosts produce phocomelia limbs. However transplants of cp/cp eye rudiments in the normal hosts produce normal eyes. Therefore the cp gene I intrinsically affects the eye development. This is known as **autophene** but only indirectly affects the eye development and that is known as **allophone.**

ii) Multiple effects of one gene are **pleiotropy.** Any given gene mutation essentially affects the production or structure of one transcribed RNA molecule. The translation product of this RNA (i.e. mRNA), the defective protein, may ultimately result in various defects due to correlation of various biochemical reactions in the body e.g. death in rat due to gray lethal mutant gene and sickle cell anemia in human beings.

5.5 Teratogenesis due to drug (Thalidomide)

Few reports on the drug

Thalidomide was a drug, which after years of extensive animal tests, was first marketed as an over-the-counter sedative: it came to be used by pregnant women in many countries during the late 1950s and early 1960s as a treatment for morning sickness. In fact, before being marketed, the danger signs had already appeared during the 1950s at the University Clinic at Bonn. Thalidomide had been tested on 140 children, seven of whom were less than a year old. Forty children, most of whom had brain damage, had been given the drug for up to nine weeks. The parents were not asked for their permission, nor were they informed that their children were being treated with an entirely new sedative. Doses used were 11 to 20 times higher than

the recommended dose for adults. Half the children were mentally disturbed or had brain damage.

Other children also received Thalidomide in the same high dosage. One child had a circulatory collapse, one died from a congenital heart defect, a three-month-old baby died from heart failure, a twentyone-month-old baby temporarily lost her vision. The doctor responsible stopped using the drug when he heard that his medical colleagues had similar experiences with Thalidomide (Although twelve years would elapse before Thalidomide was withdrawn from the market).

In 1955, one year before the commencement of the marketing of Thalidomide in its various formulae, three physicians, along with a Professor Kloos, took part in a symposium arranged by Chemie Grünenthal at which they reported to the company unsatisfactory experiences with Thalidomide. However, these were ignored. In 1956 the pharmaceutical companies (then) SmithKline and French (now SmithKline Beecham) revealed that even when used in very high doses Thalidomide could not induce sleep in mice. When administered at doses 50 times larger than that claimed by Chemie Grünenthal to be 'sleep inducing' this company could still not achieve the hypnotic effect in animals that it had on humans. Nor when given 650 times the dose effective in humans. This was substantiated and confirmed at the thalidomide trial by pharmaceutical Companies Richardson-Merrel and Ciba.

In November 1956 and October 1957, Thalidomide was marketed in Germany by Chemie Grünenthal. In 1957, after launching Contergan (Thalidomide) in West Germany, reports began to appear regarding peripheral neuritis which revealed thalidomide's toxic effects on the nervous system of the user.

Such a suspicion was suggestive enough to cause Dr. Frances Kelsey, the Medical Officer of the American Food and Drug Administration, to reject the pharmaceutical company's application to market Kevadon (Thalidomide) in the United States, because among other reasons, she was not satisfied that the drug would be safe to take during pregnancy. In pregnancy and during the lactation period the female organism is under great strain. Sleeplessness, unrest and tension are constant complaints. The administration of a sedative and a hypnotic that will hurt neither mother nor child is often necessary.

The potential danger to new drugs to the fetuses was exemplified by unrelated drug thalidomide. Thalidomide is a mild sedative (tranquilizer) that was prescribed for use in pregnancy in many European countries in the late 1950's. In 1961 the German scientist Lenz reported a possible connection between this drug and an increase frequency of a human congenital abnormality of the limbs known as Amelia, where there are no limbs and the closely related phocomelia where there is no development of long bones of limbs and flipper-like hands or feet attached directly

to the trunk. A daily intake of thalidomide for one week during early pregnancy was sufficient to induce limb effect. In 1959-1961 thousands of babies in West Germany and hundreds in other countries such as Japan, were born with partial or complete absence of limbs or limbs with defects (Fig. 5.1). This has led to extreme caution in the introduction of new drugs for commercial distribution, for the pregnant mothers. Since they may cause irreparable harm to human embryos.

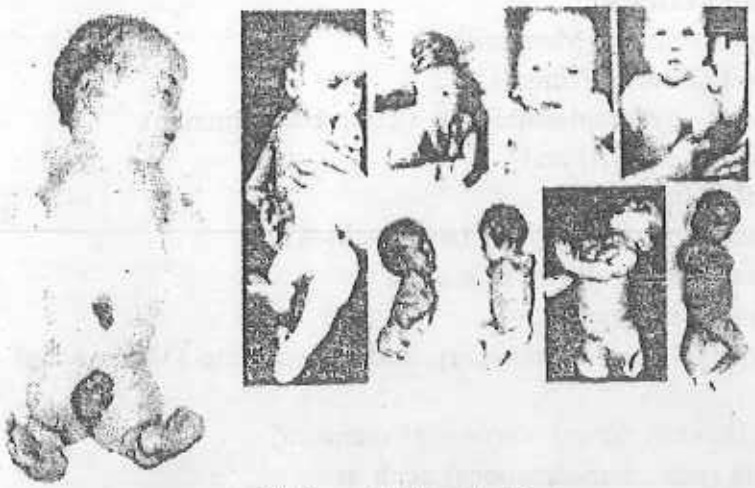


Fig 5.1 : Victims : Thalidomide infants

Teratogenicity tests have particular problems which make the results even more difficult to extrapolate to humans than other animal tests. In addition to the usual variation in metabolism, excretion, distribution and absorption which can exist between species, there are also differences in placental structure, function and biochemistry. Foetal and placental metabolism, and the handling of foreign compounds, are different in different species, and the use of several species does not necessarily overcome the problem. The difficulties are highlighted by aspirin, a proven teratogen in rats, mice, guinea-pigs, cats, dogs and monkeys, yet despite many years of extensive use by pregnant women, it has not been linked to any kind of characteristics malformation.

An unexpected finding was that the mouse and rat were resistant, the rabbit and hamster variably responded, and certain strains of primates were sensitive to thalidomide developmental toxicity. Different strains of the same species of animals were also found to have highly variable sensitivity to thalidomide.

Teratogenic drugs : A teratogen is an agent that can disturb the development of the embryo or fetus. Teratogens halt the pregnancy or produce a congenital malformation (a birth defect). Classes of teratogens include radiation, maternal infections, chemicals, and drugs.

Drugs that are capable of acting as teratogens include :

- ACE (angiotensin converting enzyme) inhibitors such as:
  - benazepril (Lotensin),

- captopril (Capoten),
- enalapril (Vasotec),
- fosinopril sodium (Monopril),
- lisinopril (Zestril, Prinivil),
- lisinopril + hydrochlorothiazide (Zestoretic, Prinzide),
- quinapril (Accupril) and
- ramipril (Altace).

- Acne medication isotretinoin (Accutane, Retin-A).
- Alcohol ingested chronically or in binges.
- Androgens (male hormones).
- Antibiotics tetracycline (Achromycin), and doxycycline (Vibramycin), and streptomycin.
- Anticoagulant (blood-thinner) warfarin (Coumadin).
- Anticonvulsants (seizure medications) such as:
  - phenytoin (Dilatin),
  - valproic acid (Depakene, Valprotate),
  - trimethadione (Tridione),
  - paramethadione (Paradione), and
  - carbamazepine (Tegretol).
- Anti-depressant drug lithium (Eskalith, Lithob).
- Antimetabolite/anticancer drugs methotrexate (Rheumatrex) and aminopterin.
- Antirheumatic agent and metal-binder (chelator) penicillamine (Ciprimene, Depen).
- Antithyroid drugs such as:
  - thiouracil/propylthiouracil and
  - carbimazole/methimazole.
- Cocaine..
- DES (diethylstilbestrol), a hormone.
- Thalidomide (Thalomid) which was approved by the FDA for the treatment of a complication of leprosy (erythema nodosum leprosum).

Antimitotic drugs :

It might be thought that antimitotic drugs used in cancer therapy would be especially harmful to the rapidly growing embryo.. However only aminopetrin (a folic acid antagonist) has proved to be teratogenic in man. This chemical has been used in order in man. This chemical has been used in order to bring about abortion. When it fails to induce abortion, the offspring is likely to show multiple malformations.

Other drugs :

1. Quinine ingested by a pregnant mother can cause deafness and alcohol cause physical and mental retardation in the infant.
2. Teratogenic effect of certain other drugs such as busulphan (for leukemia) and chlorambucil (for Hodgkin's disease) have also been reported.
3. Of the antibiotics, only tetracycline may give rise to an anomaly. When these drugs are administered during the period of enamel formation (for teeth), they may produce yellowing of deciduous teeth.
4. Epileptic mothers taking anticonvulsant drugs such as phenytoin and barbiturates are about three times as likely as normal mothers to give birth to malformed babies.
5. And in some cases such harmless drugs such as caffeine and aspirin have teratogenic effects at very low doses.
6. The teratogenic effect of lysergic acid diethylamide (LSD) is unproven. Some studies have implicated LSD in the appearance of defects in the hands and feet of offspring whose mother's have taken this drug either before or during pregnancy.

5.6 Hormones

None of the hrmones has been implicated in teratogenesis following their administration to the pregnant women. However in mice and rabbits , cortisone is known to induce cleft palate in the offspring. It also causes cleft palate in human fetuses but in less percentage.

Side-effects and drug interactions

Teratogenicity is thalidomide's most severe toxicity, and it is labeled as pregnancy category X. It is lipid soluble and readily crosses the placenta, so it should never be taken by pregnant women or those who could become pregnant. Even one dose of a 50 mg capsule can cause severe birth defects. The teratogenic risk is highest during the critical period, which is days 20-40 of gestation or days 35-50 after the last menstrual period.

The risk of additional, potentially severe birth defects outside the critical period is unknown but may be significant. Therefore, women should not use this drug any time during pregnancy. Phocomelia is a very common birth defect seen with thalidomide use. It is characterized by defective, shortened limbs resulting in flipper hands and feet (Fig. 5.1). In more severe cases, the complete absence of limbs can occur. Additionally, the fetus can develop external ear abnormalities, hypoplastic or

completely absent bones, facial palsy, eye abnormalities, and gastrointestinal and genitourinary tract malformations. Approximately 40 percent of exposed fetuses die at or shortly after birth, with bowel atresia being the most common cause of death.

5.7 Mechanism for teratogenicity

There are over 24 different proposed mechanisms for the teratogenicity. Currently, the most widely held theories involve either thalidomide's anti-angiogenic effect or its direct toxic effect on the segmental sensory neurons, with resulting limb deformities. The McCredie-McBride hypothesis states that neural tissue normally has an inductive effect on the development of the limb, and because neural tissue is damaged by thalidomide, the limb bud subsequently becomes malformed.

Teratogenic effects of thalidomide

- fetal limb growth retardation (arms,legs,hands,feet)
- ingrown genitalia
- absence of lung
- partial/total loss of hearing or sight
- malformed digestive tract, heart, kidney
- stillborn infant

Teratogenesis review

Birth defects are known to occur in 3-5% of all newborns. They are the leading cause of infant mortality in the United States, accounting for more than 20% of all infant deaths. Seven to ten percent of all children will require extensive medical care to diagnose or treat a birth defect. And although significant progress has been made in identifying etiologic causes of some birth defects, approximately 65% have no known or identifiable cause.

It was previously believed that the mammalian embryo developed in the impervious uterus of the mother, protected from all extrinsic factors. However, after the thalidomide disaster of the 1960s, it became apparent and more accepted that the developing embryo could be highly vulnerable to certain environmental agents that have negligible or non-toxic effects to adult individuals.

Wilson's 6 principles.

Along with this new awareness of the in utero vulnerability of the developing mammalian embryo came the development and refinement of *The Six Principles of Teratology* which are still applied today. These principles of teratology were put forth by Jim Wilson in 1959 and in his monograph *Environment and Birth Defects*. These principles guide the study and understanding of teratogenic agents and their effects on developing organisms :

1. Susceptibility to teratogenesis depends on the genotype of the conceptus and the manner in which this interacts with adverse environmental factors.
2. Susceptibility to teratogenesis varies with the developmental stage at the time of exposure to an adverse influence. There are critical periods of susceptibility to agents and organ systems affected by these agents.
3. Teratogenic agents act in specific ways on developing cells and tissues to initiate sequences of abnormal developmental events.
4. The access of adverse influences to developing tissues depends on the nature of the influence. Several factors affect the ability of a teratogen to contact a developing conceptus, such as the nature of the agent itself, route and degree of maternal exposure, rate of placental transfer and systemic absorption, and composition of the maternal and embryonic/fetal genotypes.
5. There are four manifestations of deviant development (Death, Malformation, Growth Retardation and Functional Defect).
6. Manifestations of deviant development increase in frequency and degree as dosage increases from the No Observable Adverse Effect Level (NOAEL) to a dose producing 100% Lethality (LD100).

Studies designed to test the teratogenic potential of environmental agents use animal model systems (e.g., rat, mouse, rabbit, dog, and monkey). Early teratologists exposed pregnant animals to environmental agents and observed the fetuses for gross visceral and skeletal abnormalities. While this is still part of the teratological evaluation procedures today, the field of Teratology is moving to a more molecular level, seeking the mechanism(s) of action by which these agents act. Genetically modified mice are commonly used for this purpose. In addition, pregnancy registries are large, prospective studies that monitor exposures women receive during their pregnancies and record the outcome of their births. These studies provide information about possible risks of medications or other exposures in human pregnancies.

Understanding how a **teratogen** causes its effect is not only important in preventing congenital abnormalities but also has the potential for developing new therapeutic drugs safe for use with pregnant women.

5.8 Suggested reading

1. Stirling DI. Thalidomide and its impact in dermatology. Semin in Cutan Med Surg. 1988;17(4):231-42.
2. Stephens TD. Proposed mechanisms of action in thalidomide embryopathy. Teratology. 1988;38:229-39.
3. McBride WG. Thalidomide embryopathy. Teratology. 1977;16(1):79-82.

Unit 6 ❐ Immunocontraception – an overview

Structure

6.1 Introduction

Although several different choices and approaches are available for contraception in women, the choices for men are currently limited to condoms and vasectomy. Male hormonal contraceptives developed over the past several years have now advanced to clinical trials, and the outcome of these studies may determine whether the suppression of sperm production through androgen regulation can become a realistic product. Immunocontraception, an alternative non-hormonal method, has been studied for many years, with the major emphasis on immunization of females to prevent pregnancy or fertilization.

6.2 Objectives

This specialized section of the unit of the module of Developmental Biology gives a clear picture of the idea of Immunocontraception. When the reader finishes this particular unit one will be able to understand

- What is contraception ?
- Basic differences between contraception and immunocontraception
- Different proteins involved in immunocontraception
- An overview of immunocontraception

6.3 What is contraception ?

Contraception is the prevention of fertilization without destroying fertility by natural,

mechanical or chemical means. In other words, a method or system which allows intercourse and yet prevents conception is called contraceptive method. This contraception may be temporary when the effect of preventing pregnancy lasts, but the fertility returns immediately or within a few months of its discontinuation.

6.4 What is immunocontraception ?

Immunity = Body defense mechanisms

Contraception = Protection against unplanned pregnancy

Immunocontraception= The use of body defense mechanisms to provide protection against an unplanned pregnancy.

6.5 Immunocontraception differs from contraception

Immunocontraception is a birth control method that uses the body's immune response to prevent pregnancy. It is used to control populations of wild animals (e.g. white-tailed deer) or feral animals (e.g. mustangs), because it is more humane than culling, and cheaper and less labor-intensive than spaying or castrating animals. It is not popular for domestic animals and is not used in humans.

One drug often used for immunocontraception is porcine zona pellucida or PZP. It is made from the zona pellucida of pigs. It is similar enough to that of other animals that a female animal vaccinated with PZP will produce antibodies against her own oocytes, which prevent fertilization.

6.6 Proteins in immunocontraception

Glycoproteins in ZP1, 2, and 3 are targets for immunocontraception.

In non-mammalian animals, the zona pellucida (called vitelline layer) plays an important role in preventing breeding of different species, especially in species that fertilize outside of the body (e.g. fish).

The zona pellucida is commonly used to control wildlife population problems by immunocontraception. When the zona pellucida of one animal species is injected into the bloodstream of another, it results in sterility of the second species due to immune response. This effect can be temporary or permanent, depending on the method used. In New Jersey, Porcine zona pellucida is used to keep deer populations low, and this process is commonly referred to as "spay-vac".

Overall picture :

Immunocontraception is a birth control method that uses the body's immune

response to prevent pregnancy. The Humane Society of the United States continues to lead development of this emerging technology, which offers a humane means of controlling animal populations in situations where it is necessary and appropriate to do so.

Immunocontraception is one of youngest branch of immunology. Investigations on immunocontraception field endure in last hundred years due to revolutionary advancement was made with apparition of genetic, molecular biology and reproductive immunology.

6.7 Advancement in immunocontraception

Antigenic basis of the first contraception vaccine was whole cells or tissue extracts, so that the most important antigen of the vaccines was not been precisely defined. In last twenty years, the concept of immunocontraception was established on the one-antigen or one-epitopes based vaccines. There are several advancement of immunocontraception relating classical approach in problems of contraception. The advancement refers to the comfort, prices, efficacy, complications, and possibility nonselective acting on animal populations. Classical contraception is inapplicable for treatment of animal population without engaged many of competent persons which can provide the procedure. To that effect, contraception vaccination is revolutionary procedure. This possibility comes as results of development in technology of recombinant DNA and creating a new microorganisms, which might express certain antigens. Live microorganisms like antigenic basis of contraception vaccine enable possibility for epidemic immunization whole population of animals. At the same time, this model of the immunization adapted for people, lead in epidemic model of the immunization with characteristics of biological weapon.

6.8 Examples of bio-control approach

- Interfering with fertilization
- Preventing development of embryo
- Preventing development of the reproductive system
- Interfering with lactation

Unit 7 ❐ Role of thyroxin in metamorphosis in amphibians

Structure

7.1 Introduction

Many animals complete their embryonic development without coming to resemble a young adult. Instead the embryo in these species forms a larva, a transition during which the animal is free-living but sexually immature. Many classes of the different invertebrate phyla, even some vertebrates, e.g. amphibians form larvae. The animals thus possess a free-living larval stage interposed between the stages of embryo and adult. The change from larva to adult can include a radical reorganization, of both body plan and physiology, called metamorphosis.

Amphibian metamorphosis is a complex process regulated by a number of external (environmental) and internal (hormonal) processes. The transformation from larval to adult form in amphibians provides excellent models for developmental biologists examining tissue and cell differentiation and morphogenesis. Metamorphosis is also an excellent model for endocrinologists because most of the changes during larval development and metamorphosis are under the direct influence of hormones. The metamorphic hormones (the thyroid hormones, and steroids) also function by altering gene expression; thus metamorphosis is an excellent tool for examining gene regulation and hormone-regulated gene expression. Because metamorphic rates are determined by various environmental changes that are translated into hormonal changes, with the hormones functioning at the molecular level, metamorphosis is also an excellent model for integrative studies.

7.2 Objectives

This particular unit of Developmental Biology gives a concise idea about the

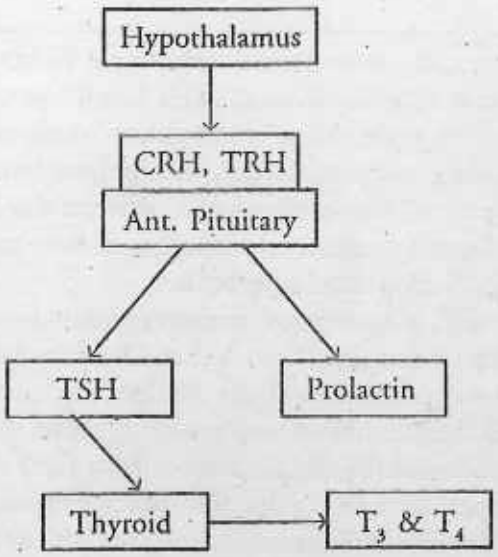
metamorphosis of first class of vertebrates to conquer the land, and most present-day amphibians still return to the water to reproduce. When the topic will be covered entirly, the reader will be able to understand

- metamorphic changes
- Hormonal action
- Control of metamorphosis by thyroid hormone

7.3 Definition of metamorphosis

Metamorphosis is a postembryonic extension of the developmental potential and involves a dramatic change in habit, habitat, morphology, physiology and behavior of larva so that it is transformed into the adult having entirely different habitat and structure.

Hormonal control of Amphibian metamorphosis :



7.4 Some experiments and their results

The control of metamorphosis by thyroid hormones were demonstrated first by Gudernatsch in 1912 who discovered that tadpoles metamorphosed prematurely when fed powdered horse thyroid gland . Allen (1916) found that when he removed or destroyed the thyroid rudiment of early tadpoles, the larvae never metamorphosed, but grew into giant tadpoles. Subsequent studies by Saxen et.al, 1957; Hanken & Hall 1988 showed that steps of anuran metamorphosis are regulated by increasing

amounts of thyroid hormone. Some events (development of limbs) occur early, while other events (regression of tail, remodeling of the intestine) occur later, after the thyroid hormones have reached higher concentrations. This experiment gave rise to a **threshold model.** Suggesting different events of metamorphosis are triggered by different concentration of thyroid hormones.

Metamorphic changes :

The metamorphic changes of amphibian development are brought about by

(1) the secretion of the hormone thyroxine (T_4) into the blood. By the thyroid gland.

(2) The conversion of T_4 into a more active hormone , tri-iodothyronine (T_3) by the target tissues and

(3) The degradation of T_3 in the target tissue.

T_3 binds to the nuclear thyroid hormone receptors (TRs) with much higher affinity than does T_4 and causes them to become transcriptional activators of gene expression. Thus the levels of T3 and TRs in the target tissues are essential for producing the metamorphic response in each tissue.

TRH in action :

The hypothalamic hormone that stimulates TSH release in mammals is thyrotropin releasing hormone (TRH). TRH is active in stimulating TSH release in frogs only when they are post metamorphic. In tadpoles effecting hormone driving TSH release is corticosterone releasing hormone (CRH), which also stimulates the release of adrenocorticotropic hormone (ACTH). ACTH stimulates the adrenal cortex to secrete corticosteroids. These adrenal steroids have been shown to regulate, at least in part, the production of enzymes that convert T_4 to T_3 in target tissues. Recent evidence indicates that thyroid hormones stimulate the pituitary to produce another hormone, prolactin that also plays a part in regulating some aspects of metamorphosis.

In short, during the stages leading up to metamorphic climax, there is an increase in TSHG, T_3 ,T_4 and prolactin, the very high levels of T_3 & T_4 then act on the hypothalamus as part of negative feedback loop to lower TSH and perhaps CRH production to levels appropriate for juveniles.

Prolactin antagonizes :

Prolactin has different effects in different vertebrates. In experiment when high levels of mammalian prolactin injected into tadpoles showed slower metamorphic changes. This means that prolactin in the frog antagonizes the actions of T_3 & T_4. Because it interferes with the formation of T_4 & T_3 receptors.

Thyroid hormone action is tissue specific :

A single hormone T_3 , initiates many changes. For example muscles in the developing limbs are stimulated to increase in size and to differentiate, while muscles in the tail are caused to wither and disappear. Some of the changes induced by T_3 are the following :

Organ/System	Changes from larva to adult
Movement	Tail fins to legs
Respiration	Gills and skin to lungs
Nutrition	Diet herbivore to carnivore
Gut	Lengthy to short
Skull and mouth	Extensive morphological changes
Nitrogen excretion	Ammonia to urea
Skin	Epidermis thin to stratified
Mucous glands	none to numerous

It is probably fair to say that every tissue organ system is affected by this hormone. Not only are there dramatic morphological changes during metamorphosis, some fundamental metabolic machinery also gets retooled.

7.5 Time of metamorphosis regulated by hormone levels

A principal factor is the concentration of thyroid hormones, whose action is modified in some target tissues by corticosteroids from the adrenal glands and by prolactin. Thyroid hormones concentrations increase during the progressive changes of metamorphosis, as do levels of corticosteroids and prolactin. Several experiments in which the levels of these hormones have been controlled to some extend clearly show that metamorphic events are induced by different levels of thyroid hormones. Shortening of the intestine and growth of the hind limbs occur at very low thyroxin levels, while tail regression occurs only at much higher levels.

These kinds of results support the idea that each of the different local responses to the hormones has a threshold concentration. Until the hormone reaches or surpasses its threshold, the local response will not occur. While this model seems reasonable, it does not tell us much about the mechanisms involved.

7.6 Regulation of molecular events by thyroid hormones

A) Control of protein synthesis

Liver cells undergo important metabolic changes during metamorphosis. For Ex.

The production of urea depends upon enzymes of the arginine – ornithine cycle and these are low or absent in young tadpole liver but are actively synthesized during metamorphosis.

B) Control of differentiation

i) Stem cells continuously generate the RBC of tadpole; in low levels of thyroxin. they express larval hemoglobin gene. As the thyroxin level in stem cell's proliferation is stimulated and new population of erythroblasts are diverted into pathways of adult hemoglobin synthesis. This shows that the action at the transcription level "selecting" a specific genetic programme by depressing adult hemoglobin gene.

ii) Like wise synthesis of enzymes needed for regression of tail, gut and gills during metamorphosis depends upon thyroxin stimulation. Histolysis of tail tissues is brought about by the action of variety of hydrolytic enzymes (e.g. cathepsin, collagenase and phosphatase) which are synthesized to 200 times their original levels before the tail is resorbed.

7.7 Regulation by hormones receptors

All hormones –including T_3, T_4 and prolactin – act by way of receptor proteins. The concentration and the properties of particular receptor types in a given tissue determine, at least in part, the response. The earliest response to thyroid hormones is an increase in transcription of thyroid hormone receptor genes (TR genes), of which there are at least two. Thus, T_3 induces greater levels of its receptor, a positive feedback loop that stimulates increased amounts of its receptor and thus the potential for an increased sensitivity to that hormone.

TR proteins belong to the same class as ecdysone receptors, e.g. the steroid hormone receptor super family. As with ecdysone, these receptors work as heterodimers, in which each TR protein is joined with a molecule from a class of retinoic acid receptors called RXR (retinoic acid –like receptor). There is some evidence that prolactin serves to decrease expression of TR genes, which may explain in part why prolactin counteracts some actions of thyroid hormones. It seems likely that local tissue-specific responses and the regulation of hormone sensitivity are mainly due to the levels of TR proteins, particular TR family members involved, level(s) and type(s) of RXR, and probably other accessory proteins that confer transcriptional specificity. We know that T_3 stimulates transcription of some genes, such as the gene for adult globin, while decreeing transcription of others. Similarly, we know that steroid hormone receptor members posses the kind of specificity capable of turning on some genes and turning off others.

Unit 8 ❐ Role of juvenile hormone & ecdysone in insect metamorphosis

Structure

8.1 Introduction

While the hormonal control of vertebrate development is of great interest for understanding our own sexuality, the molecular mechanisms of hormone action have been analyzed most successfully for the insect molting hormone ecdysone. Study of this hormone is advantageous because of i) it is steroid hormone. Its target action is straightforward, ii) effects of ecdysone can be seen directly on the puffing pattern of the polytene chromosome of Dipterans larvae and iii) Drosophila mutants facilitated the molecular analysis of ecdysone action.

Insect metamorphosis is a special form of molting. Usually molting comprises mainly the casting off of an old cuticle and the acquisition of a new one. This change of cuticle is necessary for growth and metamorphosis to occur because the fully sclerotized cuticle is rigid and does not expand.

8.2 Objectives

The unit of this module of Developmental Biology covers the basic concept of role of hormones in insect metamorphosis. This unit will assist you to develop an idea about the control of molting and metamorphosis of insect. When the topic will be covered finally, you will understand

- What metamorphosis is
- How metamorphosis is controlled
- Hormonal circuits during molting
- Molecular biology of 20 hydroxy ecdysone

8.3 Definition of metamorphosis

Metamorphosis is a biological process by which an animal physically develops after birth or hatching, involving a conspicuous and relatively abrupt change in the animal's form or structure through cell growth and differentiation. Some insects, amphibians, molluscs, crustaceans, cnidarians, echinoderms and tunicates undergo metamorphosis, which is usually (but not always) accompanied by a change of habitat or behaviour.

8.4 Molting—an essential part of insect development

In order for the insect to grow in size, the restrained of this exoskeleton must be removed. All insect larvae shed their cuticle, usually more than once, in process called **molting**. The stage of larval development between the molts is often called **instars**. Insect species vary in number of molts. The interval between the two molts is known as **stadium**.

Some insects, like grasshoppers produce an embryo that look approximately like their adult forms, smaller and not fully differentiated. This kind of larval development, called **hemimetabolus**, molting allows for an increase in size and further differentiation of the different tissues.

In many other insects, however, including *Drosophila*, the larvae appears to be quite different from the adult. After a number of larval molts, in which the larvae increases in size, the larva constructs an external cuticle called a puparium; once in its puparium, the larva is called a **pupa**. The adult insect emerges from puparium. This kind of development is called **holometabolus**.

8.5 Hormonal circuits in molting

The sequence of events of molting cycles and metamorphosis of insects has been found under precise hormonal control. Certain stimuli associated with the state of nourishment cause the neurosecretary cells of the brain to discharge the 'brain hormone', which is carried by neurosecretary cell axons to the corpora cardiaca (insect endocrine glands), from where it is released into the blood. This hormone then stimulates the thoracic glands to produce a hormone which causes the epithelial cells to begin the processes which lead to molting.

The metamorphosis of insects appears to be regulated by effector hormones controlled by neurosecretory peptide hormones in the brain. The molting process is initiated in the brain, where neurosecretory cells release prothoracicotropic hormone

(PTTH) in response to neural, hormonal, or environmental factors. PTTH is a family of peptide hormones with a molecular weight of approximately 40,000, and it stimulates the production of ecdysone by the prothoracic gland. Ecdysone, however, is not an active hormone, but a prohormone that must be converted into an active form. This conversion is accomplished by a heme-containing oxidase in the mitochondria and microsomes of peripheral tissues such as the fat body. Here the ecdysone is changed to the active hormone 20-hydroxyecdysone.

Each molt is occasioned by one or more pulses of 20-hydroxyecdysone. For a molt from a larva, the first pulse produces a small rise in the hydroxyecdysone concentration in the larval hemolymph (blood) and elicits a change in cellular commitment. The second, large pulse of hydroxyecdysone initiates the differentiation events associated with molting. The hydroxyecdysone produced by these pulses commits and stimulates the epidermal cells to synthesize enzymes that digest and recycle the components of the cuticle. In some cases, environmental conditions can control molting, as in the case of the silkworm moth *Hyalophora cecropia*. Here, PTTH secretion ceases after the pupa has formed. The pupa remains in this suspended state, called diapause, throughout the winter. If not exposed to cold weather, diapause lasts indefinitely. Once exposed to two weeks of cold, however, the pupa can molt when returned to a warmer temperature.

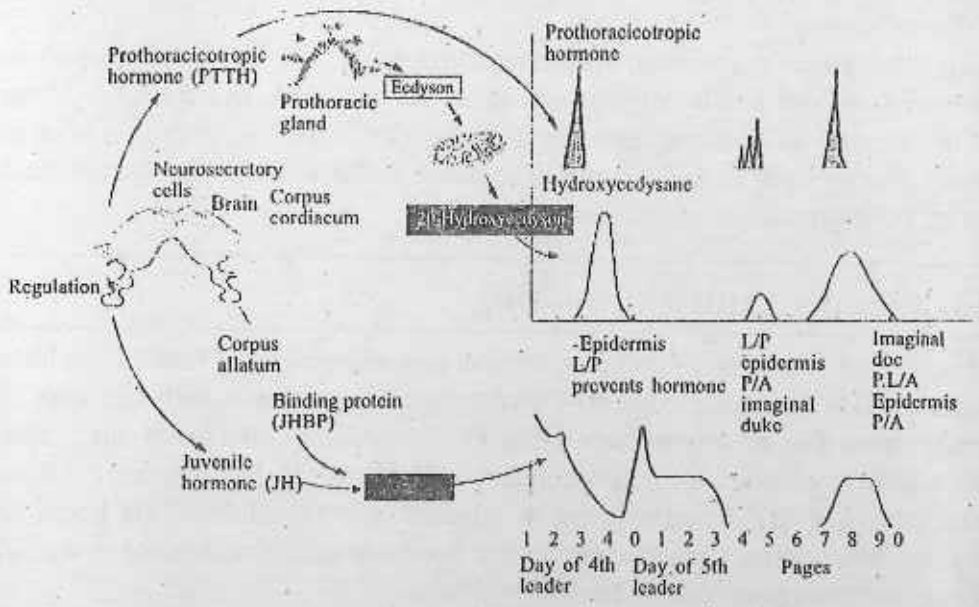


Fig. 8.1 Schematic diagram illustrating the control of molting and metamorphosis in the tobacco hornworm moth. There appear to be critical sensitive periods when the presence or absence of JH determines whether a tissue is retained at the same stage or changes to a more mature state. Different tissues have different sensitive periods. (After Nijhout 1994.)

The second major effector hormone in insect development is **juvenile hormone** (JH). JH is secreted by the corpora allata. The secretory cells of the corpora allata are active during larval molts but are inactive during the metamorphic molt. This hormone is responsible for preventing metamorphosis. As long as JH is present, the hydroxyecdysone-stimulated molts result in a new larval instar. In the last larval instar, the medial nerve from the brain to the corpora allata inhibits the gland from producing juvenile hormone, and there is a simultaneous increase in the body's ability to degrade existing JH. Both these mechanisms cause JH levels to drop below a critical threshold value. This triggers the release of PTTH from the brain. PTTH, in turn, stimulates the prothoracic glands to secrete a small amount of ecdysone. The resulting hydroxyecdysone, in the absence of JH, commits the cells to pupal development. Larval-specific mRNAs are not replaced, and new mRNAs are synthesized whose protein products inhibit the transcription of the larval messages. After the second ecysone pulse, new pupal-specific gene products are synthesized, and the subsequent molt shifts the organism from larva to pupa. It appears, then, that the first ecdysone pulse during the last larval instar triggers the processes that inactivate the larva-specific genes and prepare the pupa-specific genes to be transcribed. The second ecdysone pulse transcribes the pupa-specific genes and initiates the molt.

From the 1950s until recently, it had been thought that the type of molt was determined by the juvenile hormone titre at the time of the ecdysone pulses. High levels of JH induced larvae, intermediate levels of JH produced pupae, while low levels of JH produced adults (see Piepho 1951). However, when the titre of JH could actually be determined, it was found that it fluctuated during the final instar period, having specific peaks and troughs. Metamorphosis is not correlated with or caused by a progressive decline in JH activity. The control of metamorphosis appears more complex (Figure 8.1).

As shown in Figure 8.2, in the tobacco hornworm moth *Manduca sexta*, there are specific times when different cells are sensitive to juvenile hormone. As a general rule, if JH is present during a JH-sensitive period, the current developmental state is maintained, whereas if JH is absent during that period, this tissue will progress to a more mature developmental state. The onset and duration of the JH-sensitive period appears to be an autonomous state of the cell and is not controlled by hormones (Nijhout, 1994). (It has been hypothesized that this may be a time when JH receptors are available in these tissues). In each larval instars, there is a period where the presence of JH prevents the larval epidermis from transforming into pupal epidermis. If JH is present, the epidermis continues to be pupal, if JH is absent, it becomes pupal. During the penultimate instar larva, JH titers are able to retain the epidermis in its larval condition. During the last instars, there are two windows of JH sensitivity.

The first is for the epidermis. At this time, though, ecdysone levels have dropped significantly. Thus, the epidermis will be transformed from larval epidermis to pupal epidermis. The second JH sensitive period concerns the imaginal disc tissue. At this time, however, the JH titer has risen again, so that the imaginal discs are not instructed to evert and differentiate. The molt transforms the larva into a pupa (Nijhout and Wheeler, 1982). The next time the ecdysone pulses occur, no JH is seen during the critical periods. The epidermis transforms from pupal to adult, and the imaginal discs are allowed to evert and differentiate. Injection of JH into the pupa at this time can cause it to molt again into a second pupa (Williams, 1959).

8.6 Role of ecdysone : the molecular biology of 20-hydroxyecdysone activity

Ecdysone receptors : 20-hydroxyecdysone cannot bind to DNA by itself.20 E first binds to nuclear receptors. These proteins, called ecdysone receptors (EcRs), are almost identical structure to the thyroid hormone receptors of amphibians. An EcR protein forms an active molecule by pairing with an Ultraspiracle (Usp) protein. In the absence of the hormone-bound EcR, the Usp protein binds to the ecdysone-responsive genes and inhibits their transcription. This inhibition is converted into activation when the ecdysone receptor binds to the Usp.

Although there is only one gene or EcR, the EcR mRNA transcript can be spliced in at least three different ways to form three distinct proteins. All three EcR proteins have the same domains for 20 E and DNA binding, but they differ in their amino -terminal domains. The type of EcR in a cell may inform the cell how to act when it receives a hormonal signal. is therefore possible that the different receptors activate different sets of genes when they bind 20E.

Binding of 20-hydroxyecdysone to DNA: During molting and metamorphosis, certain regions of the polytene chromosomes of *Drosophila* puff out in the cells of certain organs at certain times. These chromosome puffs represent areas when DNA is being actively transcribed. Moreover these organ specific patterns of chromosome puffing can be reproduced by culturing larval tissue and adding hormones to the medium or by adding hydroxyecdysone to an earlier stage larva. When 20 E is added to larval salivary glands, certain puffs are produced and others regress. Puffing is mediated by the binding of hydroxyecdysone at specific places on the cromosomre.

8.7 Molts are driven by ecdysone production

Insect molting and metamorphosis are controlled by two effector hormones: the steroid 20 hydroxyecdysone (20E) and the lipid juvenile hormone (JH). 20

hydroxyecdysone initiates and coordinates each molt and regulates the changes in gene expression that occur during metamorphosis.

The molting process is initiated in the brain, where neurosecretary cells release prothoracotropic hormone (PTTH) in response to neural, hormonal or environmental signals. PTTH is a peptide hormone with a molecular weight of approx. 40,000 and it stimulates the production of ecdysone by the prothoracic gland. Ecdysone is modified in peripheral tissues to become the active molting hormone 20E. Each molt is initiated by one or more pulses of 20E. PTTH secretion is regulated by both environmental and autonomous signals, produce waves of ecdysone production. As the first wave ends, during the first instar, a small pike in ecdysone concentration occurs, soon followed by a more intense wave of ecdysone release. Stimulated by the hormone, the epithelial cells of the body surface withdraw from the cuticle and produce a molting fluid containing proenzymes that after activation will digest the old cuticle. The epithelium then generates a new cuticle. Because it is distensible, the new cuticle expands as the larva grows until this cuticle, too, becomes hard and inelastic, and the processes repeated again. During the latter portion of the third instar in *Drosophila,* a spike in ecdysone levels again begins the process of molting.

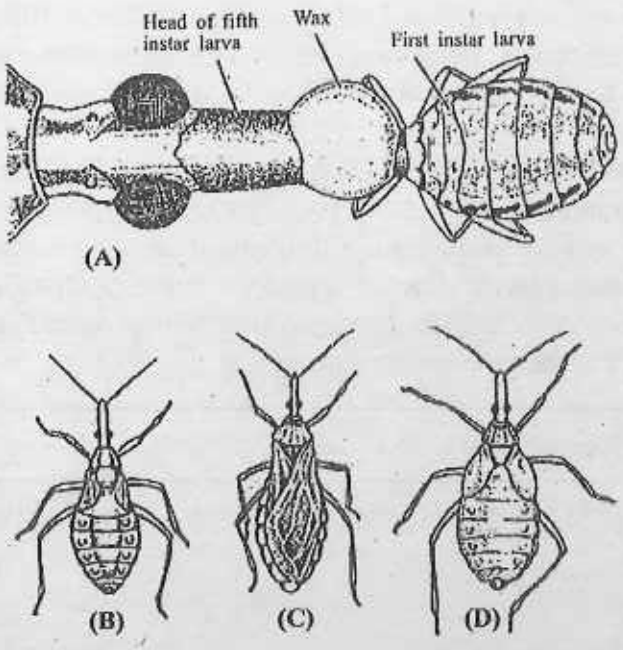


Figure 8.2 Demonstration of hormonal control of insect metamorphosis. (A) Technique of producing precocious "adult" from first instar larva of *Rhodnius* by fusing it to the head of a molting fifth instar larva. (B) Normal fifth instar larva of *Rhodnius.* (C) Normal adult *Rhodnius.* (D) "Sixth instar larva" produced when corpora allata from a fourth instar lava were implanted into the abdomen of a fifth instar larva. (After Wigglesworth 1939).

About JH :

It is the corpora allata, and the juvenile hormone produced by them that determine whether the result of a molt will simply be an increase in larval size, or pupation and metamorphosis. Removing the corpora allata surgically during the second instar will cause the next molt to undergo pupation, one fall instar early. On the other hand, implanting an actively secreting corpus allatum into a late third- instar larva may in the next molt result in a giant larva than a pupa. Puparium formation and pupation are initiated when an ecdysone wave occurs during very low levels of JH, or even in its absence. Recent measurements show that some JH is present in late third-insar larvae, and that the precise timing of JH release and the presence or absence of active JH receptors are also involved in regulating pupation.

8.8 Experiments of metamorphosis

Control of insect metamorphosis was shown by the dramatic experiments of Wigglesworth (1934), who studied *Rhodnius prolixus*, a blood-sucking bug that has five instars before undergoing a striking metamorphosis. When a first-instar larva of *Rhodnius* was decapitated and fused to a molting fifth-instar larva, the minute first instar developed the cuticle, body structure, and genitalia of the adult. This showed that blood-borne hormones are responsible for the induction of metamorphosis (Figure 8.2).

Wigglesworth also showed that the corpora allata, near the insect brain, produces a hormone that counteracts this tendency to undergo metamorphosis (Figure 1D). If the corpora allata was removed from a third-instar larva, the next molt turned the larva into a precocious adult. Conversely, if the corpora allata from fourth-instar larvae were implanted into fifth-instar larvae, these larvae would molt into extremely large "sixth-instar" larvae rather than into adults.

8.9 Suggested reading

Nijhout, H. F. 1994. *Insect Hormones*. Princeton University Press, Princeton

Unit 9 ❐ Significance of totipotency & pleuropotency of cells during animal development

Structure

9.1 Introduction

In higher animals, development involves the progressive restriction of cell fates. Every time a cell divides, its descendants may have to choose between alternative developmental pathways, and once that choice is made, the decision is usually irreversible. Differentiated animal cells placed in isolation therefore can not give rise to new individuals. There are examples where cells have a limited ability to dedifferentiate and recapitulate certain developmental processes, as seen in limb regeneration. However, in no case has it been possible to use a differentiated animal cell to recapitulate embryonic development.

Although differentiated animal cells cannot recapitulate the entire developmental programme, it is possible for the nuclei from those differentiated cells to do so. This type of experiment, where the nucleus of a differentiated cell is used to replace the nucleus of a fertilized egg, shows that all the information required to generate the animal is retained in the nuclei of differentiated cells.

9.2 Objectives

Through the study of this unit of the module, anybody can learn the basic impression of the potency of cells during development.

When the study will be finished, the person who reads shall be able to realize the

- Uniqueness of cell potency
- What those potency meant

- Basis of totipotency
- Nature of pluripotency
- Totipotency vs pluripotency action
- Significance of the potency

Potency - the capacity to differentiate into specialized cell types. In the strictest sense, this requires stem cells to be either totipotent or pluripotent - to be able to give rise to any mature cell type, although multipotent or unipotent progenitor cells are sometimes referred to as stem cells.

Potency specifies the differentiation potential (the potential to differentiate into different cell types) of the stem cell.[4]

- **Totipotent** (a.k.a **omnipotent**) stem cells can differentiate into embryonic and extraembryonic cell types. Such cells can construct a complete, viable, organism. These cells are produced from the fusion of an egg and sperm cell. Cells produced by the first few divisions of the fertilized egg are also totipotent
- **Pluripotent** stem cells are the descendants of totipotent cells and can differentiate into nearly all cells, i.e. cells derived from any of the three germ layers.
- **Multipotent** stem cells can differentiate into a number of cells, but only those of a closely related family of cells.
- **Oligopotent** stem cells can differentiate into only a few cells, such as lymphoid or myeloid stem cells.

Unipotent cells can produce only one cell type, their own, but have the property of self-renewal which distinguishes them from non-stem cells (e.g. muscle stem cells

Totipotency is the ability of a single cell to divide and produce all the differentiated cells in an organism, including extraembryonic tissues. Totipotent cells formed during sexual and asexual reproduction include spores and zygotes. Zygotes are the products of the fusion of two gametes (fertilization). In some organisms, cells can dedifferentiate and regain totipotency. For example, a plant cutting or callus can be used to grow an entire plant.

Human development begins when a sperm fertilizes an egg and creates a single totipotent cell (zygote). In the first hours after fertilization, this cell divides into identical totipotent cells. Approximately four days after fertilization and after several cycles of cell division, these totipotent cells begin to specialize.

Totipotent cells have total potential. They can specialize into pluripotent cells that can give rise to most, but not all, of the tissues necessary for fetal development. Pluripotent cells undergo further specialization into multipotent cells that are committed to give rise to cells that have a particular function. For example, multipotent blood stem cells give rise to the red cells, white cells and platelets in the blood.

Importantly, totipotent cells must be able to differentiate not only into any cell in the organism, but also into the extraembryonic tissue associated with that organism. For example, human stem cells are considered totipotent only if they can develop into any cell in the body, or into placental cells that do not become part of the developing fetus. This fact is an important aspect of the stem cell controversy because the human embryonic stem cells used for research purposes are pluripotent; they are collected from human embryos that have developed past the totipotent cell stage. All human embryos used in stem cell experimentation are destroyed in the process.

9.3 Basis of totipotency

The molecular mechanisms controlling totipotency are not well understood and are a subject for current research. In particular, a February 2006 report in Science suggests that in the model organism *Caenorhabditis elegans*, multiple mechanisms including RNA regulation maintain totipotency at different stages of development.

In cell biology, the definition of pluripotency has come to refer to a stem cell that has the potential to differentiate into any of the three germ layers: endoderm (interior stomach lining, gastrointestinal tract, the lungs), mesoderm (muscle, bone, blood, urogenital), or ectoderm (epidermal tissues and nervous system). Pluripotent stem cells can give rise to any fetal or adult cell type. However, alone they cannot develop into a fetal or adult animal because they lack the potential to contribute to extraembryonic tissue, such as the placenta.

In contrast to pluripotent stemcells, many progenitor cells are multipotent, i.e. they are capable of differentiating into a limited number of tissue types.

9.4 Pluripotent (biological compounds)

Pluripotent, embryonic stem cells originate as inner mass cells within a blastocyst. The stem cells can become any tissue in the body, excluding a placenta. Only the morula's cells are totipotent, able to become all tissues and a placenta.

Pluripotency can also be used (albeit less commonly) to describe the ability of certain substances to produce several distinct biological responses.

For example, in immunology many cytokines are pluripotent, in that each of these compounds can activate specific behavior in some cell types and inhibit other behavior in other cell types. Interferon gamma represents an excellent example of pluripotency. In most somatic cells it inhibits growth and upregulates expression of Major Histocompatibility Complex (MHC) antigens in a general anti-viral response. In B

lymphocytes (B cells) it stimulates antibody class switching, and in Natural Killer (NK) cells this protein hormone stimulates maturation. In macrophages it activates intracellular killing.

Pluripotent cells have the ability to phagocytize bacterial cells and lyse red blood cells. Victims with the disease Typhoid Lymphoma have a defect in the beta nucleotide in the nucleus of the pluripotent cell. This causes the cell to lyse red blood cells, eventually leading to a death by suffocation due to the lack of oxygen in the body.

9.4 Experiments to understand totipotency

The experiments of this kind were performed on *Xeonopus*. Nuclei taken from tadpole cells or from different kinds of adult cell were injected into UV-irradiated eggs. In many cases, the eggs containing tadpole nuclei developed into normal swimming tadpoles, and in few cases these produced viable adults. Adult cell nuclei from many different cell types were also able to support full development but at a much lower efficiency. However, nuclei from some adult cell types consistently failed to allow development. The results from such experiments firstly confirm the totipotency of adult cell nuclei, showing there is no irreversible change to genetic information in the nucleus during development. However, it becomes more difficult to recapitulate the entire developmental programme as development proceeds, and indeed becomes impossible in certain cell types, such as neurons. Thus, although there is no change to the genetic information in most differentiated nuclei, the DNA does undergo some change that reduce the ability of the nucleus to be reprogrammed by the intracellular environment of the egg.

9.6 Totipotency vs. pluripotency

In animal development, cell fate *and potency* are progressively restricted. For example, at the 16 –cell stage, each blastomere of a mouse embryo is totipotent; i.e. it can potentially give rise to every cell type in the adult if transferred to another embryo. Later the morula differentiates to form the inner cell mass and the trophoectoderm. The inner cell mass gives rise to the embryo, and differentiate to form three germ layers as well as other extra-embryonic structures. At this point, individual cells are still pluripotent since they can generate several different cell types, but they are no longer totipotent, since certain fates are now unavailable. Cell fates are increasingly restricted until a cell is terminally differentiated (can form only a single type). Some cells, e.g. hepatocytes, continue to divide but only produce identical daughters. Other cells, e.g. neurons, become quiescent (i.e. they exit from

the cell cycle and do not divide further). Stem cells are exceptional because they are never terminally differentiated. Instead of dividing to produce two identical daughters, stem cells produce dissimilar daughter cells, only one of which undergoes terminal differentiation.

9.7 Few more examples

Stem cells are undifferentiated cells that have the ability to become any type of body tissue or specialized cell. Scientists believe that cures for many diseases could be found from research using stem cells. There are four basic kinds of human stem cells: embryonic stem cells (hES cells), which are obtained from 5-7 day old blastocysts; fetal stem cells, which are obtained from 4-6 week old fetuses that have been aborted either spontaneously or through procured abortions; placental/cord blood stem cells, which are obtained from the umbilical cord or placenta immediately after birth; and adult stem cells, which are obtained through a biopsy of mature tissues or from bone marrow of a post-natal human being (not necessarily the tissue or bone marrow of an adult). These stem cells can also be classified according to whether they are totipotent or pluripotent. Embryonic stem cells are virtually totipotent, meaning they can become *any* type of human cell, including at their earliest stage before any differentiation has occurred those cells that make-up the trophoblast (the outer-layer of the blastocyst, which eventually becomes the placenta). Fetal stem cells, placental/cord stem cells, and adult stem cells are all pluripotent, meaning they are not totipotent, but they can become some or many types of cells found in the human body. Currently it is thought that fetal stem cells are more pluripotent, i.e., they can become more types of cells, than placental/cord stem cells, while placental/cord stem cells are more pluripotent than adult stem cells.

9.8 Significance

The current thinking in the scientific community is that embryonic stem cells may be more useful than adult stem cells in creating treatments and possible cures for diseases such as Parkinson's, Alzheimer's and spinal cord injuries. From a scientific perspective, however, the downside of totipotent embryonic stem cells is that they are more difficult to control and manipulate in the lab than pluripotent stem cells. Some researchers are exploring ways to increase the pluripotency of non-embryonic stem cells. Some studies are beginning to support the theory that adult stem cells are much more pluripotent than originally thought, and are able to turn into many more types of cells and tissues than previously suspected.

Unit 10 ❐ Roles of maternal effect gene, segment polarity gene, zygotic gene and homeotic gene in development of *Drosophila*

Structure

10.1 Introduction

Development has been an important area of research for many years. Impounding informations and research findings have revolutionarised the idea or the pattern of animal development particularly the morphogenesis in different animals. Researches have been able to identify the similarities as well as differences in the basic plan of development from a fertilized egg to an adult in organisms ranging from the sea urchin to mammals.

In this chapter we will discuss the genetic control of animal development. The central idea of this chapter is to discuss effect of different genes in the various phases of development. However, the discussion will not be focused to comment on hundreds of genes that are required for a complete development, but on the primary genes that are required for the early development an animal.

Extensive studies and researches in the field of developmental biology and the availability of mutants called as *developmental mutants* are found to affect the body plan in *Drosophila*. Undoubtedly, the most extensive and spectacular examples of genes that control development have been identified in the fruit fly, *Drosophila melanogaster*. The Drosophila genome sequence have become available in late 1999 and it has been determined that it includes about 13,600 protein-coding genes and this advantage has facilitated to identify the role of each such gene in normal development, and the available mutant specifies the actual role it plays in the developmental event. In our discussion we will pay particular attention to those that affect the segmented body plan of the organism, both in the larva and in the adult.

10.2 Maternal effect genes organize the egg cytoplasm

Early *Drosphila* development occurs in following way—

a) The structure of the egg becomes organized as it develops in the ovary of the female.

b) Store of messenger RNA (mRNA) along with yolk protein and other cytoplasmic molecules are passed into the egg from the surrounding maternal cells.

c) Immediately after the fertilization, the Zygote nucleus in the egg divides, beginning a remarkable series of 13 mitotic divisions.

d) Each of these divisions takes 5 or 10 minutes, which means that the DNA in the nucleus is replicated constantly at a very rapid rate.

During that time the nucleus do not synthesis RNA. Cytokinesis does not take place and the nucleus produced by the first seven divisions remain in the centre of the embryo until the eighth division occurs.

e) After this event, i.e. during the eighth division, most of the nuclei start to migrate out from the centre and becomes localized at the periphery of the embryo. This is known as the syncytial blastoderm stage because the nuclei are not surrounded by individual plasma membranes. Subsequently, cell membranes do form and the embryo becomes known as cellular blastoderm (Fig. 10.1).

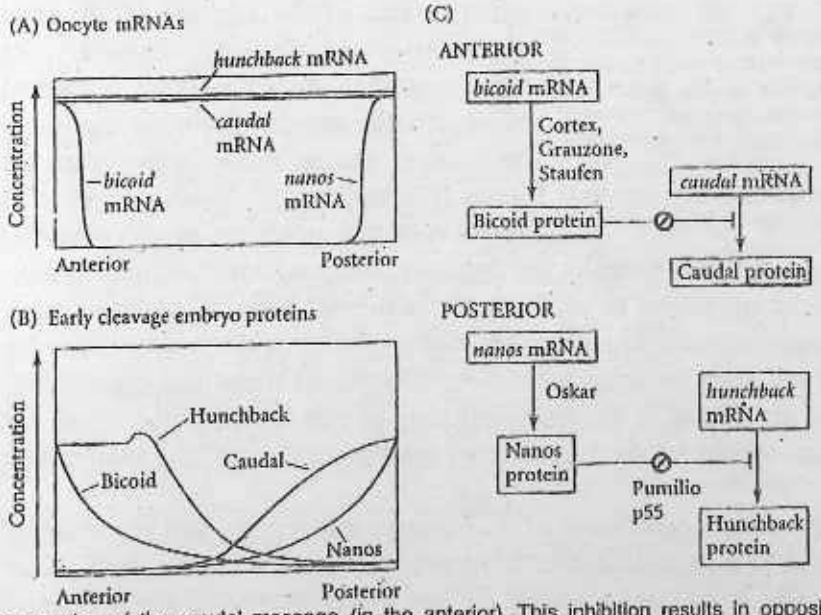


Fig. 10.1 : A model of anterior posterior pattern generation by the *Drosophila* maternal effectgenes. (A) The *bicoid, nanos, hunchback, and caudal* messanger RNA's are placed in the oocyte by the ovarian nurse cells. The *bicoid* message is sequestered anteriorly; the nanos message is sent to the posterior pole. (B) Upon translation, the Bicoid proteingradient extends from anteriorto posterior, while the Nanos protein gradient extends from posterior to anterior. Nanos inhibits the translation of the hunchback message (in the posterior), while Bicoid prevents the transaction of the caudal message (in the anterior). This inhibition results in opposing Caudal and Hunchback gradients. The Hunchback gradient is secondarily strengthened by the transcription of the hunchback gene in the anterior nuclei (since Bicoid acts as a transcription factor to activate hunchback transcription). (C) Parallel interactions whereby translational gene regulation establishes the anterior-posterior patterning of the Drosophila embryo. (C after macdonald and Smibert 1996.)

The genes that act to organize the structure of the egg cell are referred to as *maternal effect genes*. There are genes in the surrounding maternal tissues that are transcribed to produce mRNA molecules to be transported into the developing egg. It has been found that mutants defective in these genes failed to develop the polarity of the eggs. Therefore, the products of these genes (normal) are necessary in establishing the polarity of the embryo by designating which parts of the egg are dorsal or ventral and which are anterior or posterior. Thus they are known as *egg polarity genes*.

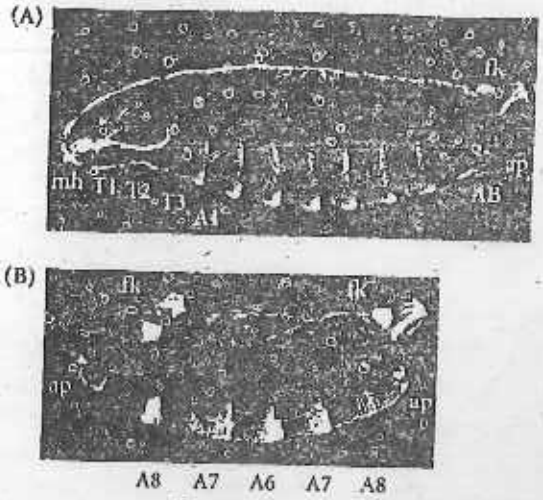


Fig. 10.2 : Phenotype of a strongly affected embryo from a female fly deficient in the *bicoid* gene. (A) Wild-type cuticle pattern. (B) *bicoid* mutant. The head and throax have been replaced by a second set of posterior telson structures. Abbreviations : fk, filzkorper neurons; ap, anal plates (both telson structures); T1-T3, thoracic segments ; A1, A8, the two terminal abdominal segments ; mh, head structures. (From Driever et al. 1990; photograph courtesy of W. Driever)

A concentration gradient of the products of the maternal effect genes is found. At about 128 nuclei stage (i.e., about 1.25 hrs after fertilization) between the seventh and eighth nuclear divisions the nuclei start to migrate to the periphery of the egg. The products of several mutant genes are localized in different regions of the egg. For example, the product of the maternal gene which defines the anterior end of the egg are localized at the anterior end of the egg. Similarly, the other maternal genes which not concerned with anterior end development are found to localize behind the anterior maternal gene (marked by *). However, prior to that event, the products of the maternal genes were found to evenly distributed in the egg and from this point a concentration gradient of the products are found to establish from the anterior to posterior axis.

At 1500 nucleic stage (about 2 hrs after fertilization) most of the nuclei reach the perimeter of the egg and start to make their own mRNA. At this stage the product of the maternal genes are found to be restricted only at the anterior end. Soon the maternal genes product would be over shadowed by the product of the own genes (Fig. 10.2).

The product (mRNA transcripts) of some of the maternal effect genes can be identified by their ability to hybridize with radioactive DNA probes obtained from cloned genes ; alternatively, their products can be identified by *antibodies that specifically bind to them*. The protein produced by translation of mRNA appear to be a part of system of determinants that organizes the early pattern of development in the embryo. A

combination of these protein gradients may provide positional information that specifies the fate of each nucleus or cell within the embryo. That information may then be

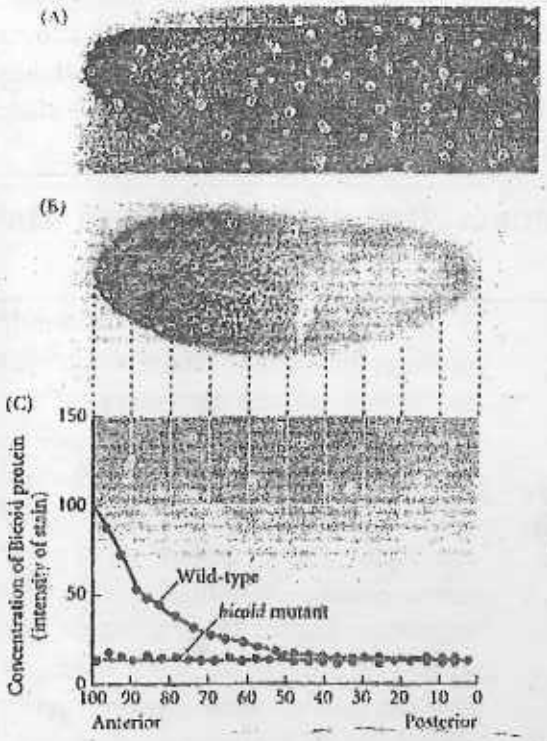


Fig. 10.3 : Gradient of Bicoid protein in the early *Drosophila* embryo. (A) Localization of *bicoid* mRNA to the anterior tip of the embryo. (B) Bicoid protein gradient shortly after fertilization. Note that the concentration is greatest anteriorly and trails off posteriorly. Notice also that Bicoid is concentrated in the nuclei. (C) Densitometric scan of the Bicoid protein gradient. The upper curve represents the Bicoid gradient in wild-type embryos. The lower curve represents Bicoid in embryo s of *bicoid* mutant mothers. (A from Kaufman et al. 1990 ; B and C from Driever and Nusslein-Volhard 1988b; photographs courtesy of the authors.)

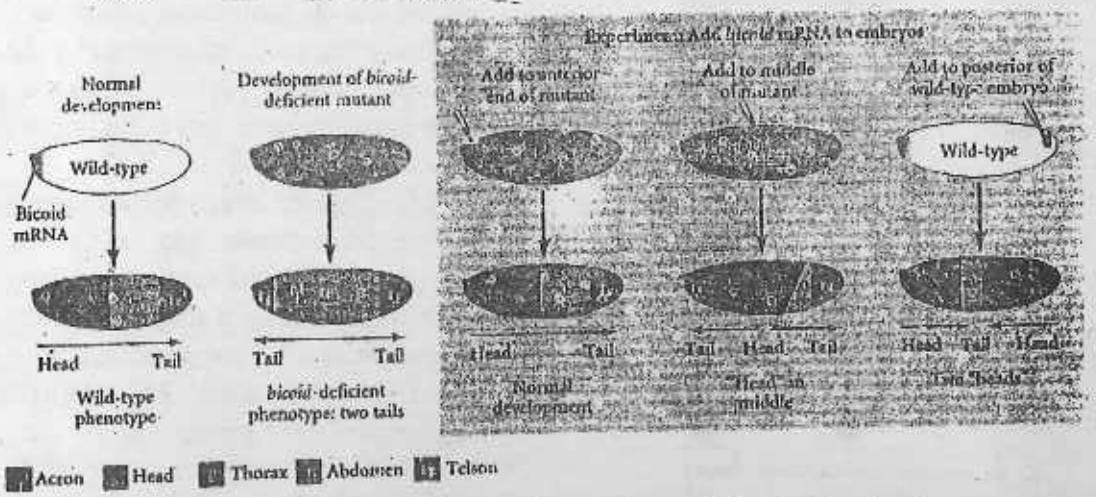


Fig. 10.4 : Schematic representation of the experiments demonstrating that the *bicoid* gene encodes the morphogen responsible for head structures in *Drosophila*. The phenotypes of *bicoid*-deficient and wild-type embryos are shown at the left. When *bicoid*-deficient embryos are injected with bicoid mRNA, the point of injection forms the head structures. When the posterior pole of an early-cleavage wild-type embryo is injected with *bicoid* mRNA, head structures form at both poles. (After Driever et al. 1990.)

interpreted by a cell as signals specifying the developmental path it should follow. For example, owing to the absence of specific signals in the egg, maternal effect mutation can produce an embryo with two heads or two posterior ends (Fig. 10.3 and 10.4).

In many cases, the phenotype associated with a maternal effect mutation can be reversed by injecting normal maternal mRNA into the mutant embryo. When this is done, the fly develop normaly, indicating that gene product is needed only for a short time at the earlier stages of development.

10.3 Zygotic segmentation genes favour and extend the developmental programme

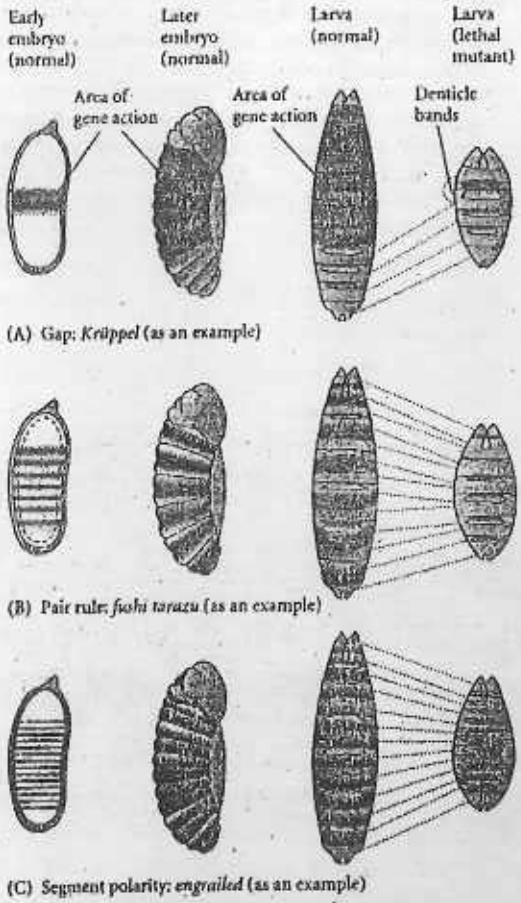


Fig. 10.5 : Three types of segmentation gene mutations. The left panel shows the early-cleavage embryo, with the region where the particular gene is normally transcribed in wild-type embryos shown in color. These are deleted as the mutants develop.

In *Drosophila*, at the eighth mitotic division the nuclei of the embryo start to migrate to the periphery of the embryo. The migration of the nucleus to the periphery under the influence of the expression of the products of the some genes called as *Zygotic genes*. Though, the stage at which the expression begins is not a zygote, but it is customary to refer them as Zygotic genes. Such Zygotic genes extend the developmenal programme beyond the pattern established by the metarnal genome include the zygotic segmentation genes and the homeotic genes.

The Zygotic segmentation genes fall into there classes 'like' gap genes, pair-rule gene, and segment polarity genes representing a rough hierarchy of gene action. So far geneticists have identified at least 24 Zygotic segmentation genes that are responsible for generating a repeating pattern of body segments within the embryo (Fig. 10.5).

(a) Gap gene : The gap gene are apparently the first set Zygotic

segmentation genes to act. These genes seem to interprete the maternal anterior-posterior information in the egg and begin organization of the body segments. A mutation in one of the gap gones usually causes the absence of one or more body segments in an embryo.

(b) Pair-rule genes and Segment polarity genes— The other two classes of segmentation genes do not act on small groups of body segments but rather affect all segments. For example mutations in pair-rule genes every other segment, whereas mutation in segment polarity genes produce segments in which one part is missing and the remain part is duplicated as a mirror image. (Table-1)

Table-1 : Classes of genes involved in pattern formation of embryonic segments in *Drosphila*

Type of gene	Site of gene activity	Effect of mutant alleles and proposed function(s) of genes
Maternal effect genes	Maternal tissues (ovary)	Many maternal effect mutation alter the polarity of the embryo, initiate pattern formation by activating zygotic genes in nuclei in certain location in embryo.
Gap genes	Embryo	Mutant cause one or more segments to be missing ; Some may influence activity of pair rule genes, segment polarity genes and homeotic genes.
Pair-rule genes	Embryo	When mutated, cause alternate segments to be missing. Some may influence activity of polarity genes and homeotic genes.
Segment polarity genes	Embryo	Mutant alleles delete part of every segment and replace it with mirror images of remaining structures and may influence homeotic genes.
Homeotic gene	Embryo	Homeotic mutations cause parts of fly to form structures normally formed in other segments. Control the identities of the segments.

10.4 Homeotic genes specify the identity of each segment

One function of the zygotic segmentation genes is to regulate the expression of a separate set of gene that actually designate the final adult structure formed by each of the *imaginal discs*. It is to note that during the very early embryogenesis in developing

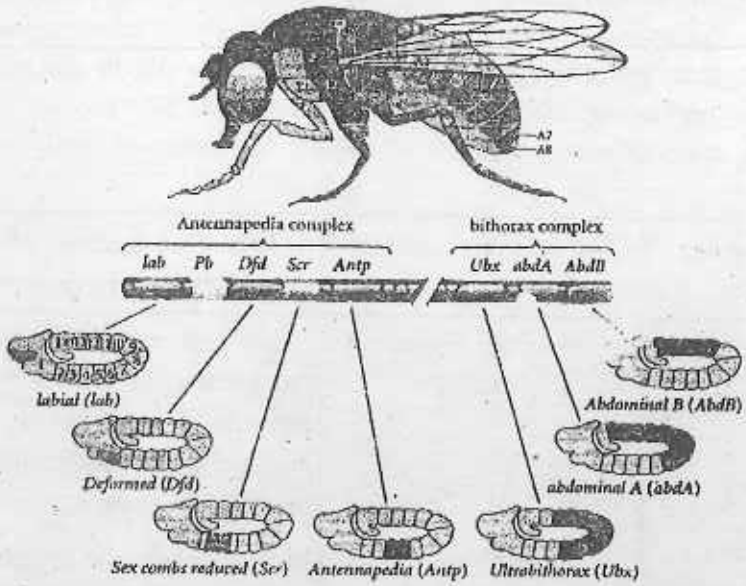


Fig. 10.6 : Homeotic gene expression in *Drosophila*. In the center are the genes of the Antennapedia and bithorax complexes and their functional domains. Below and above the gene map, the regions of homeotic gene expression (both mRNA and protein) in the blastoderm of the *Drosophila* embryo and the regions that form from them in the adult fly are shown. Darker shaded areas represent those segments or parasegments with the most product. (After Dessain et al. 1992 and Kaufman et al. 1990.)

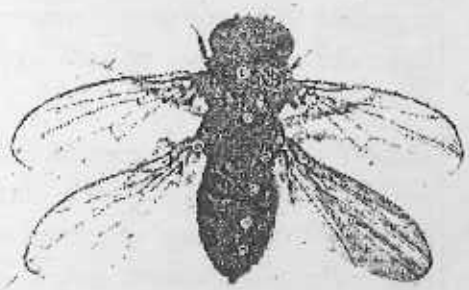


Fig. 10.7 : A four winged fruit fly constructed by putting together three mutations in *cis* regulators of the *Ultrabithorax gene*. These mutations effectively transform the third thoracic segment into another second thoracic segment (i.e., halteres into wings). (Photograph courtesy of E.B. Lewis)

larvae, precursor cells of many of the adult structures are organized as relatively undifferentiated paired structures called imaginal discs. This term comes from image, the name given to the adult form of the insect. Each imaginal disc occupies a definite position in the larva and will form a specific structure, such as a wing or a leg, in the adult body (Fig. 10.6 & 10.7).

Homeotic genes are involved to provide the segment identity and as such mutations in homeotic genes

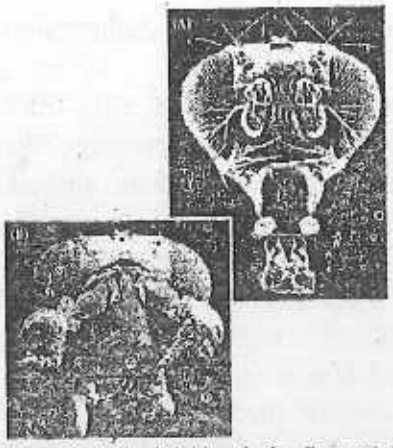


Fig. 10.8 : (A) Head of a wild-type fruit fly. (B) Head of a fly containing the *Antennapedia* mutation that converts antennae into legs. (From Kaufmann et al. 1990; photographs courtesy of T.C. Kaufmann).

cause one body part to be substituted by another and therefore produce some peculiar change in the adult. The effect of homeotic gene mutation can be best illustrated by the case of Antennapedio mutant, which have legs that grow from the head at a position where the *antennae* would normally be found (Fig 10.8).

10.5 Mechanism of action of genes which control embryonic development in *Drosophila*

Many development mutants of Drosophila are now been identified. Their effect on development in various combination have been examined and studied extensively at the molecular level. In our discussion, we will briefly comment on the moleculer mechanism of some of these genes briefly.

The earliest developmental program to operate in the egg is established by *maternal effect genes*. Such genes are active prior to fertilization and such genes are transcribed in the surrounding maternal tissues to produce mRNA molecules and such molecules are transported into the developing eggs. Using various molecular techniques, it is now possible to identify the proteins that such mRNAs are produced in the eggs. The proteins produced by translation of the mRNA appears to be part of a system of determinants that organize the early pattern of development in the embryo. A combination of these protein gradient may provide positional information that specifies the fate of each nucleus or cell in the embryo. The proof of such action can be observed when mutation in the maternal effect genes are studied. It has been observed that mutation of maternal effect genes can produce an embryo with two heads or two posterior ends. The observations strongly suggest that maternal gene products (proteins) act as signals specifying the developmental path that a cell should follow. In case of mutations in absence of such specific signals abnormal embryos are produced.

Hundreds of maternal effect genes and their products are identified of which some are named here.

The Bicoid protein, a product of bicoid gene, arise from the maternal nurse cell and injected into the anterior end of the unfertilized egg. The protein diffused through the syncytium, setting up a concentration gradient, highest at the anterior end and lowest the posterior end of the embryo. The other maternal effect gene products are also involved in setting up the anteiror posterior gradient. These are the Hunchback, Nanos and Caudal proteins. All are injected as mRNAs in to anterior region of the unfertilized eggs. The nanos mRNA is transported to the posterior part of the egg and attached to the cytoskeleton while it awaits translation. The hunchback and caudal mRNA become distributed evenly through the cytoplasm, but their proteins subsequently form gradient through the action of Bicoid and Nanos.

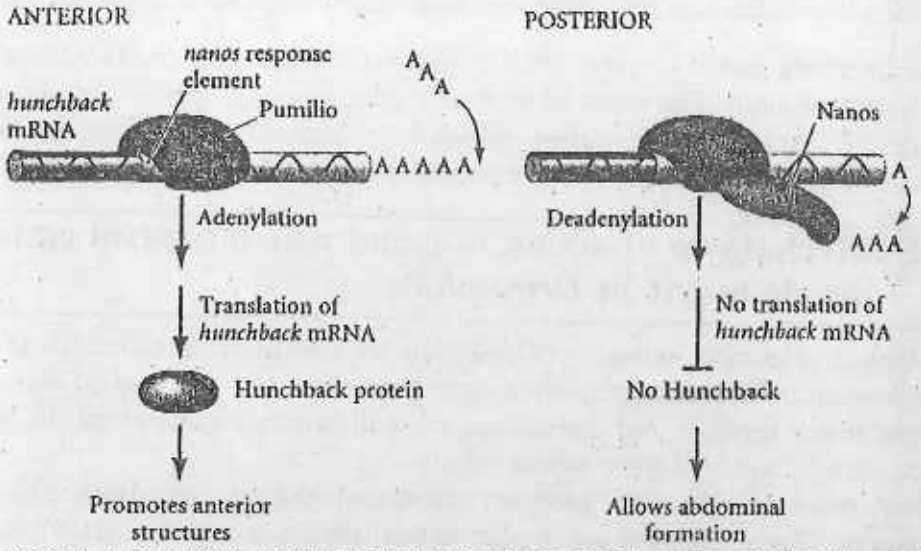


Fig. 10.9 : Control of *hunchback* mRNA translation by Nanos protein. In the anteriorof the embryo, Pumilio protein. In the anterior of the embryo, Pumilio protein binds to the Nanos response element (NRE) in the 3' UTR of the *hunchback* message, and the message is polyadenylated normally. This polyadenylated message can be translated into Hunchback protein. In the posterior of the embryo, where Nanos protein is found, Nanos binds to Pumilio to cause the dead-emylation of the *hunchback* mesage, thus preventing its translation (After Wreden et al. 1997).

Bicoid activates the hunchback gene in the embryonic nuclei, supplementing the hunchback mRNA in the anterior region and represses translation of maternal caudal mRNA. The result is an increase in the concentration of the Hunchback protein in the anterior region and a decrease in that caudal (Fig. 10.9).

Nanos represses translation of hunchback mRNA, contributing further to anterior-posterior gradient of the Hanchback protein.

The net result is a gradient of Bicoid and Hunchback in the anterior end while Nanos and Caudal operates in the posterior end. The gradient is supplimented with

Torso proteins (a maternal effect gene product) which accumulate at the extreme anterior and posterior ends. Similarly Dorsal protein gradient is formd dorsal to ventral axis.

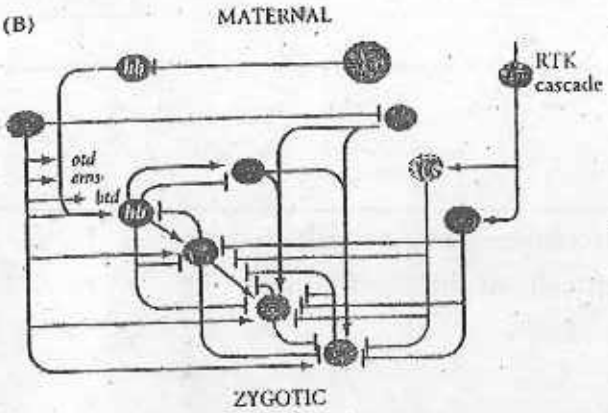


Fig.10.10 : Conversion of maternal protein gradients inot zygotic gap gene expression. (A) Gap gene expression patterns. (B) The gradients of maternal transcription factors Bicoid, Caudal, and Hunchback regulate the transcription ofthe gap genes. Hunchback and Caudal proteins come from both maternal messages and new zygotic transcription. These gap gene-encoded proteins diffuse, and the interactions between them are critical in activating the transcription of the pair rule genes. At the two tremini ofthe embryo, the interaction between Torso and Torso-like activates the *tailless* and *huckebein* gap genes. (B after Rivera-Pomar and Jackel 1996).

Zygotic segmentation genes do not become active until much later, when the embryo is no longer a zygote. They continue and extend the developmental program initiated by the maternal-effect genes. The Zygotic segmentation genes and their products interact with each other and the products of the maternal effect genes according to a hierarcheal pattern. The gap genes acting first, then the pair-rule genes and finally the segment polarity genes (Fig. 10.10).

Homeotic genes in *Drosophila* were originally identified by the altered phenotypes produced by mutant alleles. When genticits analysed the DNA sequences of several homeotic genes, they discovered a short DNA sequence of approximately 180 base pairs is the charteristic of homeotic gene, and the sequence is called the *homeobox*. Each homeobox codes for a protein functional region called *homeodomain*, Constituting of 60 amino acids that form four α-helices. Such protein acts as transcription factor and affect transcription.

Unit 11 ❐ Elementary idea of stem cell and its importance

Structure

11.1 Introduction

Stem cells are cells that has the capacity to divide indefinitely & which can give rise to more specialised cells. When they divide, stem cells produce a more specialized type of cells and more stem cells.

Some single stem cells in the early embryo are capable of generating all the structures of the embryo. These cells are known as *pleuripotent stem cells* and are capable of generating ectoderm, endoderm, mesoderm and germ cells.

These stem cells generated more pluroipotent stem cells as well as *committed stem cells*. CSCs can give rise to a smaller population of cells. For instance, one type of CSC is hemangioblast that gives rise to all the blood vessels, blood cells & lymphocytes.

CSCs can give rise to more CSCs or *progenitor cells* or *precursor*. These PCs are no longer the stem cells as they cannot produce more PCs, rather, they divide to form one or few related cell types.

Totipotency or totipotent stem cells : The very early mammalian cells that can form both the entire embryo and the fetal placenta (trophoblast) around it.

Pleuripotencey or pleuripotent stem cells : They are the Inner Cell Mass (ICM) of mammals that can form the embryo but not its surrounding tissues.

The restriction on the potency of stem cells is gradual and the potencies of these cells are determined by their surroundings. Once committed, however, they usually do not switch commitment. Once placed in a new environment, they will not change the type of cells which they can generate. Stem cells are critical for maintenance of

cell population that last for long periods of time and must be removed. Thus, in human stem cells are important for continual production of blood, hair, epidermis and intestinal epithelial cells (Fig. 11.1).

11.2 Stem cell niche

Many organs have stem cells that undergo continuous renewal. These tissues include the mammalian epidermis, hair follicle, intestinal villi, blood cells & sperm cells as well as *Drosophila* sperm and egg.

The ability of a cell to become an adult stem cell is determined by where it resides. The continuously proliferating stem cells are housed in compartments called *Stem cell niches* (regulatory microenvironment). There are particular places in the embryo that become stem cell niches.

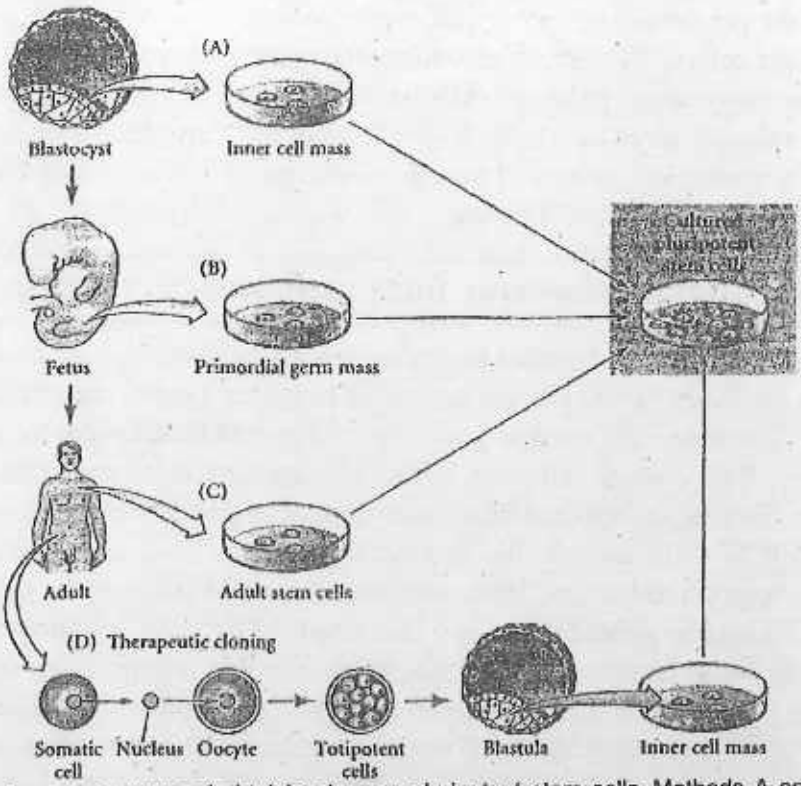


Fig. 11.1 : Four major ways of obtaining human pluripotent stem cells. Methods A and B have been documented to work; methods C and D remain experimental. (A) Cells from the inner cell mass of a blostocyst are cultured and become pluripotent embryonic stem cells. (B) The primordial germ cells a fetus are harvested and become pluripotent embryonic stem cells. (C) Adult stem cells are obtained and grown in a manner that allows them to become pluripotential. (D) "Therapeutic cloning," wherein the nucleaus of a somatic cell is transferred into an enucleated oocyte. The oocyte is activated and gives rise to a blastocytst, whose inner cel mass in harvested and clutrued to become pluripotent embryonic stem cells. (After NIH 2000.)

Stem cell niches regulate their continuous production of stem cells and their more differentiated progeny. Usually by paracrine (and sometimes by juxtacrine) factors they are produced in the niche cells. These paracrine factors retain the cells in an uncommited state. Once the cells leave the niche, the paracrine factor cannot search them and the cells begin the process differentiation.

For instance, sperms are continuously produced in the *Drosophila* testis. The stem cells for sperm resides in a regulatory microenvironment named *the hub*. The hub consists of about a dozen somatic testes cells and is surrounded by 5-9 germ stem cells. These germ stem cells that remain attached to the somatic cells remain as germ stem cell. However, their division is assymmetirc. Those remaining attached to the hub remain the stem cell population, while those daughter cells that divide in such a way that they are not touching the hub become the gonial blast cells that will divide to become the precursors of sperm cell.

The somatic cells of the hub are able to regulate stem cell proliferation by secreting the paracrine factor unpaired on the cells attached to them by activating the Jak STAT pathway in adjacent germ stem cells to specify their self renewal. Those cells that are distant form paracrine factor cannot receive this signal, so they begin their differentiation into sperm cell lineage.

11.3 Moleculer mechanism for pluripotency or totipotency

In mouse the earliest blastomeres can form both trophoblast cells and the embryonic precursor. These very early cells are said to be totipotent (Latin, capable of forming everything). The inner cell mass is said to be pluripolent (Latin, capable of forming many things). That is each cell of ICM can generate any cell type in the body but because the distinction between ICM and trophoblast has been established, it is thought that ICM cells are not able to form trophoblast.

Once the decision to become either torphoblast or ICM is done, the cells of these two regions express different genes. The trophoblast cells synthesized T box transcription factor *eomesodermin* and homeodomain containing, caudal like transcription factor Cdx2. Eomesodermin-activates those proteins characteristic of trophoblast layer [Russ et al 2000 ; Hanna. at 2002]. Cdx2 is responsible for down regulating Oct4 and Nanog, two transcription factors that along STAT 3 characterises ICM [Strumph et al 2005]. At eight cell stage cdx2, eomesodrmin and Oct4 are synthesized in all cells. But in blastocyst cdx2 and eomesodermin remains in trophoblast, whereas Oct 4 is maintained in ICM [Niwa et al 2005].

The expression of the 3 transcription factors characteristic of ICM— Oct 4, STAT 3 & Nanog— is critical for formation of the embryo and for maintenance of

pluripotency of ICM. Oct 4 is expressed first, and it is expressed in the morula as well as in the inner cell mass and early epiblast. Oct4 blocks cells to take on trophoblast fate. Later, Nanog prevents the ICM blastomeres from becoming hypoblast cells and stimulate blastomere for self renewal in epiblast.

The activated (phosphorylated) form of STAT 3 and also stimulate self renewal of ICM blastomere [Pesce and Scholar et al 2001 ; Chabers et al 2003 ; Mitri et al 2003]. If ICM blastomeres are removed in a manner-that let them retain their expression of Nanog, Oct4 and phosphorylated STAT 3 protein these cells divide and become embryonic stem cells. Pluripotency of these stem cells depend on their retaining in the expression of these 3 transcription factor.

11.3 Types of stem cells / stem cells of difrerent regions

1. Neural Stem Cells : Until recently, it was generally believed that once the nervous system is mature, no new neuron is born. However recent studies show adult mammalian brain is capable of producing new neurons and environmental stimulation can increase the number of these new neurons.

The existence of neural stem cells is now well established for the olfactory epithelium and the hippocampus [Kempermann et al. 1997a, b; Prag et al. 1999 ; Kornea & Rakie 1999 ; Kalo et al 2001]. These cells respond to sonic hedgehog and can proliferate to become multiple cell types for at least first year of a mouse's life [Alm & Joyner 2005]. It appears that the stem cells producing these neurons are located in the ependyma. Though these cells represnt only about 0.3 percent of ventricle wall cell population, they can be distinguished from other cells by their particular cell surface protein (Rietze et al 2001).

Mechanism by which neural stemeells are kept in a ready state of quiscence is not well known. Before they become neurons, neural stem cells are characterized by NRSE translational *inhibitor that prevents* neuronal differenciation by binding to a silencer region of DNA. However, when neuronal stem cells begin to differentiate, they synthesize a small, double stranded RNA that has same sequence as the silencer and which might bind NRSE and thereby permit neuronal differentiation [Kuwabara et al 2004].

2. Melanocyte stem cells : During the migration of neural crest cells, the cells that took the dorsal route become commiteed to form melanocytes. As they travel through dermis and epidermis, they eventually enter the developing hair follicles take up position at the base of the follicle bulge.

Here, they become melanocyte stem cells [Mayer-1973 ; Nishimura et al 2002]. A portion of these cells migrate outside the bulge at the beginning of each hair

development cycle to diferentiate into mature melanocyte and provide pigmentation to the hair shaft.

Nishinura et al. in (2005) have documented that the reason behind greying of hair and age in human and mice is that melanocyte stem cells become depleted from the bulge.

When the melanocytes are in stem cell niche, melanocyte stem cells are inhibited from differentiating because of the regulation of Mitf transcription factor.

Mitf activates the genes of the melanin pathway. The Mitf gene is itself activated by the Sox10 and Pax3 protein. However, on some of the genes activated by Mitf, Pax3 bind to same place on the enhancer as the Mitf, thus competing for the site [Lang et al. 2005]. So, even though Pax3 stimulates melanocyte differentiation by *activating Mitf, it also prevent* Mitf from functioning. Once outside the bulge, the Wnt signalling generated β-Catenin, which bind to a Lef/Tef transcription factor and displaces Pax3 from their sites. Thus allows Mitf to be expressed and activate the melanin producing genes.

3. Muscle progenitor cell : Adult muscles can regenerate following injury. The new myofibrils come from sets of stem cells or progenitor cells that resides along adult muscle fibre.

There may be more than one type of muscle stem cells and their function may overlap. [Polesskaya *et al.* 2003]. Lineage tracing using Chimeras indicate that these muscle progenitor cells are somatically derived myoblasts that have not fused and remain potentially available throughout adult life.

One type of putative stem cells, *the satellite cell,* is found within the basal lamina of mature myofibers. Satellite cells respond to injury or exercise by proliferating into myogenic cells that fuse and form new muscle fiber. These cells may be stem cells with the capacity to generate daughter cells for renewal or differentiation. In inactive form, these cells show Pax-7 that inhibit MYOD expression and muscle differentiation in these cells (Olguin & Olwin, 2004).

Another type of muscle stem cell [may be derived from somatic cells that migrate to form dorsal aorta] is activated by Wnt signalling from injured muscle tissue. Wnt signal appear to activate Pax 7 and it. Pax 7 protein activates the myoD family gene, thus, promoting muscle differentiation.

Comment : Most muscle progenitor cells express both Pax 7 (sattelite specific) gene and Pax 3. Recent studies also indicated that at least some satellite cells come from the central portion of the dermamyotome [Gros et al. 2005 ; Relaix et. al. 2005].

4. Development of blood cell : The bone marrow HSC is a remarkable cell in that it is the common precursor of all blood cells and lymphocytes. It is estimated that only about 1 HSC is present in every 10,000 blood cells.

The HSCs formed in the embryo are those that populate the bone marrow (in some instance, in spleen) of adult mammals. The adult HSCs riches in bone & spleen make chemo attractant proteins that attract the circulating stem cells into them (Christensen et al. 2004 ; Gotherd et al. 2005).

The HSC (or, CFU-M, L) appears to be dependent on the transcription factor SCL. Mice lacking SCL die from the absence of all blood & lymph lineages.

HSC is also dependent on endosteal osteoblasts that line the bone marrow and are responsible for providing the niche that attracts HSCs and keep them in a state of plasticity. These osteoblasts bind HSCs and provide several other signal.

a) one signal is provided by *jagged protein* which activate Notch protein on HSC surface.

b) A second signal comes from *Angioprotein-1* on osteoblast, which activates receptor tyrosine kinase Tie and on the surface of HSC (Arai et al. 2004).

c) A third signal is from Wnt pathway, localizing *β-catenin* into the nucleus. This pathway seems to be critical for self renewal of HSCs (Reya et. al 2003).

The HSC can give rise to blood cell precursor (common myeloid precursor cells" CMP or CFU-S) or to lymphocyte stem cells (CLP). The CMP produce *megakaryocyte /Erythroid Precursor Cells (MEP)l* which can generate either the red blood cell lineage or the platelets lineage.

CMP also produce *Granulocyte/Monocyte precursor cell (GMP)* which generate beasophil, eosinoplul, neutrophil and monocyte.

Eventually these cells produce progenitor cells that can divide but produce only one kind of cell in addition to renewing itself. eg. *Erythroid progenitor cell* [BFU-E] is a committed stem cell that can form only RBCs. Its immediete progeny is capable of responding the hormone *erythropoitin* to produce from recognizable differentiated member of erythrocyte lineage, *proerythroblast,* this cell begins to produce globin and cell is matured gradually to produce *erythroblast.* Eventually mammalian erythroblast expels the nucleus to produce *reticulocyte.* The final stage of differentiation form the erythrocyte (Fig. 11.2).

11.5 Stem cells and therapeutic cloning

Characters :

1] They are unspecialised cells.

2] They can divide until they are committed.

3] Pluripotency / totipotency.

4] They have to contain a niche.

5] They always transform into progenitor cells prior to formation of specialized cells.

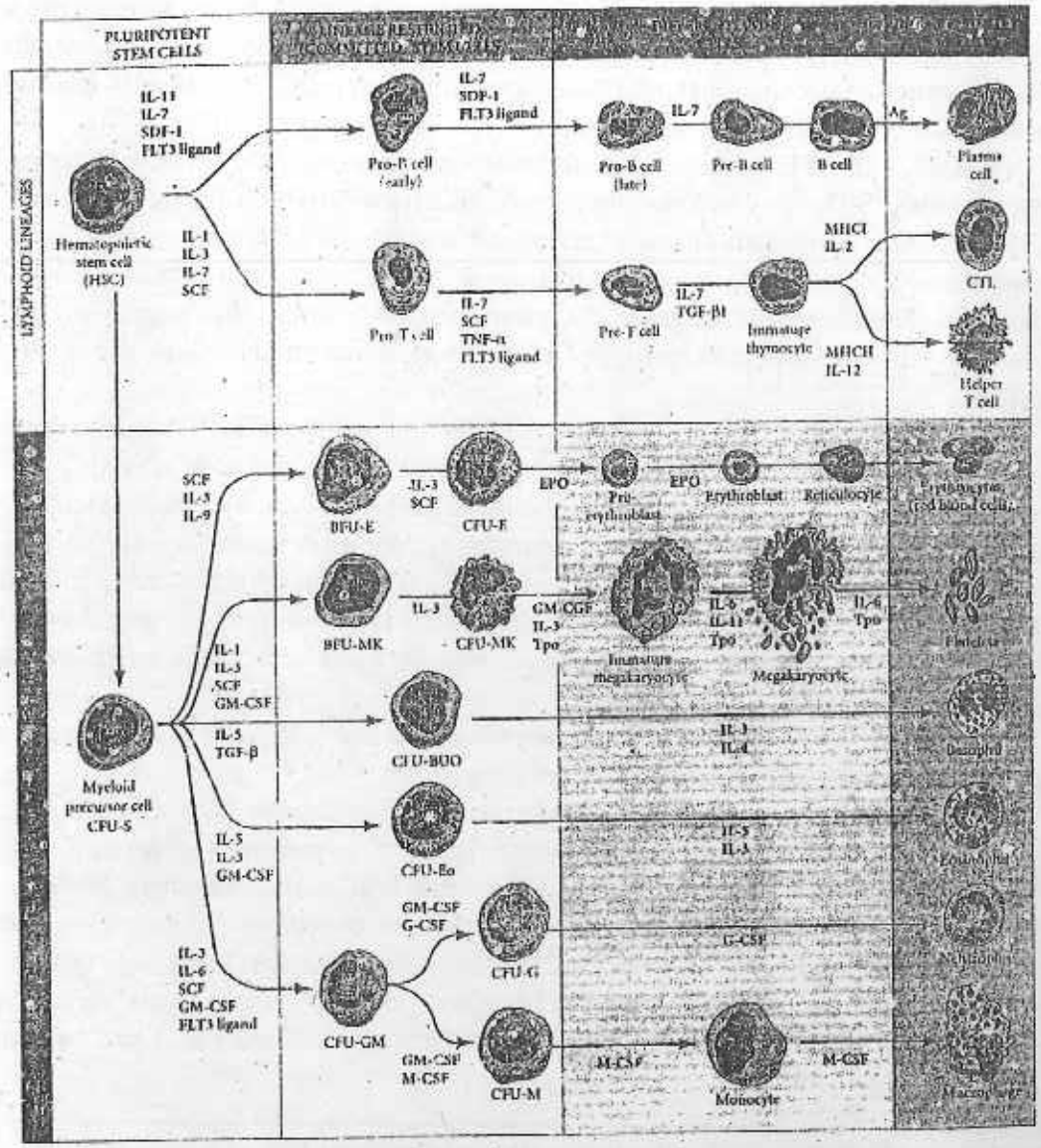


Fig. 11.2 : A model forthe origin of mammalian blood and lymphoid cells. (Other models are consistent with the data, and this one summarizes features from several models.) Factors affecting each step of differentiation are shown in red. Ag. antigen ; EPO. crythropoietin; G-CSF, granulocyte colony stimulating factor; GM-CSF, granulocyte macrophage colony-stimulating factor ; IL, interleukin ; LIF, leukemia inhibiting factor ; M-CSF, macrophage colony-stimulating factor ; MHCI, major histocampatability complex type I protein ; MHCII, major histocompatability complex type II protein ; SCF, stem cell factor ; SDF-1, stromal-derived factor-1; TNF, tumor necrosis factor ; Tpo, thrombopoietin. (After Nakauchi and Gachelin 1993 ; R&D Systems 1997.)

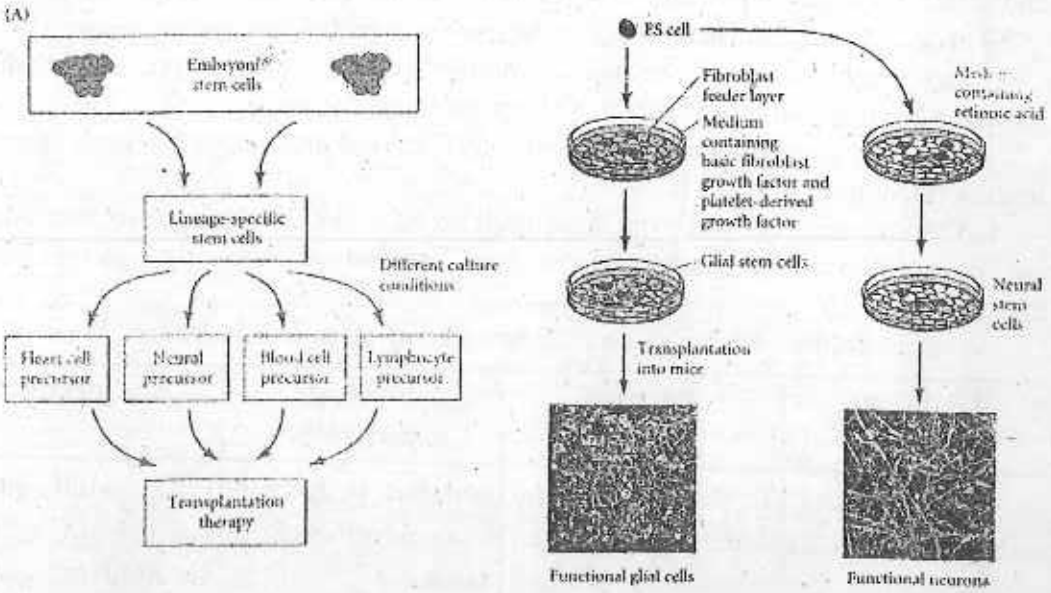
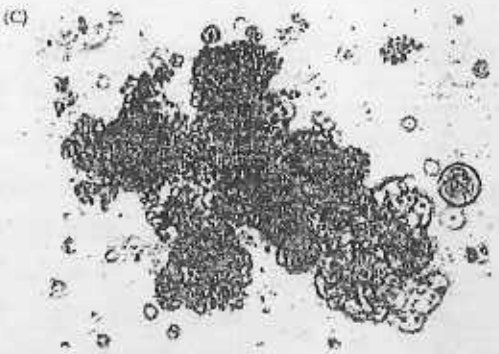


Figure 11.3 : Embryonic stem cell therapeutics, (A) Human embryonic stem cells (ES cells) can differentiate into lineage-specific stem cells, which can then be transplanted into a host. (B) The differentiation of mouse ES cells into lineage-restricted (neuronal and glial) stem cells can be accomplished by altering the media in which the ES cells grow. (C) Blood cells developing from human embryonic stem cells cultured on mouse bone marrow. (A after Gearhart 1998 ; B, photographs from Brustle et al. 1999 and Wickelgren 1999, courtsey of O. Brustle and J.W. McDonald ; C. photograph courtsey of University of Wisconsin.)



6] They are controlled by individual molecular cascade.

7] They follow contact inhibition and not form lump as in cancer.

8] There is no immuno rejection.

A) A recent review article that summarises the development of "genetic medicines".

B) Softer, D; 2006 ; from teratocarcinomas to embryonic stem cells and beyond : a history of embryonic stem cell research. Nat rev Gene 7 : 319-27.

11.6 Stem cell therapy

Embryonic Stem Cells and therapeutic cloning

ES cells are pluripotent, can be cultured indifinitely in an undifferentiated state

and retain their developmental potential after prolonged culture (Fig. 11.3).

Source : At present, human ES cells are obtained by two major sources.

a) they can be derived from ICM blastomere of human blastocyst, such as those left over from in vitro fertilization [Thomson *et al.* 1998].

b) they can be generated from germ cells derived from spontaneously aborted fetuses [Gearhart, 1998].

N.B. Some experimental evidence (Strelchenko *et. al.* 2004] suggests that it may also be possible to derive embryonic SC from late morulae before they go on to form blastocyst.

Table : Material and techniques of stem cell research

Technique	Purpose	Starting material	End product
1) Adult (or fetal) stem cell research.	To obtain undifferentiated stem cells for research and therapy.	Isolated stem cells from adult or fetal tissue.	Cells produced in culture to repair diseased or injured tissue.
2) ESC research	To obtain undifferentiated stem cells for research & therapy.	Stem cells from a blastocyst stage ambryo.	—Do—
3) Therapeutic cloning (nuclear transplantation)	To obtain undifferentiatied stem cells that are genetically matched to the recipient for therapy and tissue regeneration.	Stem cells from a blastocyst stage embryo produced from an enucleated egg supplied with nuclear material from recipient's own somatic cells.	—Do—
4) Reproductive Cloning.	To produce an embryo for implantation in womb, leading to birth of a child.	Enucleated egg supplied with material from a donor somatic cell.	Embryo (and eventual off spring) genetically identical to donor of nuclear material.

Aspects of ESC : It is thought that they can help in

— production of new neurons (in AD, PD).
— spinal cord injuries.
— produce new pancreas for diabetic.
— produce new blood cells for anaemics.
— produce new heart tissue for patients & deteriorating hearts.
— replenish new immune system.

Different experiment :

A) Kaufman & Colleagues (2001) direct human ES cells to become blood forming stem cells by placing them on mouse bone marrow or endothelial cells. These ES derived hematopoitic stem cells could further differentiate into numerous types of blood cells.

B) Human ESC. derived from germ cells were able to cure virus induced paraplegia in rats. These stem cells appear to do this by both differentiating into new neurons and by producing paracrine factors (BDNF and TGF-α) that prevent death of existing neurons [Kern et al. 2003].

C) ESC from monkey blastocyst have been able to cure a Parkinson like disease in adult monkeys whose dopaminergic neurons had been destroyed [Takagi *et al* 2005].

11.7 A potencial technique : therapeutic cloning

As human ESC develop, they express significant amount of MHC that can cause immune rejection [Drukker *et al* 2002]. To solve this problem, human ESC could be modified, or somatic cell nuclear transfer could be used to ensure that the stem cells are geneticaly identical to the person who would be receiving their progeny. To solve the problem therapeutic cloning is being introduced.

Process : In this technique, a nucleus from patient is inserted into an enucleated oocyte.

The resulting embryois grown in vitro until it has developed an ICM.

Cells from the ICM are then cultured to general stem cells that are gentically identical to the patient

Application : In "Parkinsonian" Mice

1) Barberi *et al* [2003] performed somatic cell nuclear transfer such that the nucleus of one type of mice was transferred to oocyte of another strain of mice.

2) These cells divided and became a blastocyst, and ESC were derived from blastocyst.

3) Then, the embryonic SC induced to become neural stem cells by growing on mesoderm and providing Fgf2 in medium.

4) The neural stem cells were then induced to become ventral neural stem cells by adding sonic hedge hog, to the medium.

5) Fgf2 & Fgf8 are then added to these cells, followed by exposure to brain derived neurotrophic factor (BDNF) and these produce cells that had characteristic of dopaminergic neurons.

Comment : When these cells are injected to affected mice, they restore the dopaminergic neurons and the mice become normal.

N.B. An interesting modification of this system is designed by Cowan & Collegauge (2005). They fused the somatic cell and an already existing ESC.

In many instances, the cells not only fuse, but so did their nuclei, to make a tetraploid nucleus. These hybrid cells kept the stem cell phenotype and could differentiate into 3 major germ layers.

This finding opens the possibility that transplantation of somatic nuclei into enucleated ESC may allow patient specific stem cells to be made without having to use early embryonic stages. This could circumvent many of the religious objections to using human ESC.

11.8 Multipotent adult stem cells

Numerous organs have committed multipotent stem cells, even in the adult. They are not as easy to use as Pluripotent embryonic SC, they are difficult to isolate and are often fewer than one out of every thousand cells in an organ.

Bone marrow transplantation has been used to transfer haematopioetic stem cells from one person to another in over 40,000 operations each year. This pluripotent haematopioetic stem cells are rare (1 in every 15,000 bone marrow cells) but such transplantation works well for people who are suffering from RBC. deficiencies or leukemias.

In mice, very few (perhaps even one) blood stem cells will reconstitute the muscles blood and immune systems (Asawa *et al* 1996) and a single mammary stem cell will generate an entire mammary gland in mics (Shackleton *et al* 2006).

When researchers have been able to isolate and culture such cells, they have proved to be very useful. Carvey & Colleagues (2001) have shown that when neural stem cells from midbrain of adult rats are cultured in a medium with a mixture of paracrine factors, they too, will diffentiate into dopaminergic neurons that can cure rat version of PD.

NOTE

মানুষের জ্ঞান ও ভাবকে বইয়ের মধ্যে সঞ্চিত করিবার যে একটা প্রচুর সুবিধা আছে, সে কথা কেহই অস্বীকার করিতে পারে না। কিন্তু সেই সুবিধার দ্বারা মনের স্বাভাবিক শক্তিকে একেবারে আচ্ছন্ন করিয়া ফেলিলে বুদ্ধিকে বাবু করিয়া তোলা হয়।

— রবীন্দ্রনাথ ঠাকুর

ভারতের একটা mission আছে, একটা গৌরবময় ভবিষ্যৎ আছে, সেই ভবিষ্যৎ ভারতের উত্তরাধিকারী আমরাই। নূতন ভারতের মুক্তির ইতিহাস আমরাই রচনা করছি এবং করব। এই বিশ্বাস আছে বলেই আমরা সব দুঃখ কষ্ট সহ্য করতে পারি, অন্ধকারময় বর্তমানকে অগ্রাহ্য করতে পারি, বাস্তবের নিষ্ঠুর সত্যগুলি আদর্শের কঠিন আঘাতে ধূলিসাৎ করতে পারি।

— সুভাষচন্দ্র বসু

Any system of education which ignores Indian conditions, requirements, history and sociology is too unscientific to commend itself to any rational support.

— Subhas Chandra Bose

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NETAJI SUBHAS OPEN UNIVERSITY

STUDY MATERIAL

POST GRADUATE ZOOLOGY

Paper : 7
Group : B

Endocrinology, Cell &
Tissue Structure,
Function

PREFACE

In the curricular structure introduced by this University for students of Post-Graduate degree programme, the opportunity to pursue Post-Graduate course in a subject is introduced by this University is equally available to all learners. Instead of being guided by any presumption about ability level, it would perhaps stand to reason if receptivity of a learner is judged in the course of the learning process. That would be entirely in keeping with the objectives of open education which does not believe in artificial differentiation.

Keeping this in view, study materials of the Post-Graduate level in different subjects are being prepared on the basis of a well laid-out syllabus. The course structure combines the best elements in the approved syllabi of Central and State Universities in respective subjects. It has been so designed as to be upgradable with the addition of new information as well as results of fresh thinking and analysis.

The accepted methodology of distance education has been followed in the preparation of these study materials. Co-operation in every form of experienced scholars is indispensable for a work of this kind. We, therefore, owe an enormous debt of gratitude to everyone whose tireless efforts went into the writing, editing, and devising of a proper lay-out of the materials. Practically speaking, their role amounts to an involvement in 'invisible teaching'. For, whoever makes use of these study materials would virtually derive the benefit of learning under their collective care without each being seen by the other.

The more a learner would seriously pursue these study materials the easier it will be for him or her to reach out to larger horizons of a subject. Care has also been taken to make the language lucid and presentation attractive so that they may be rated as quality self-learning materials. If anything remains still obscure or difficult to follow, arrangements are there to come to terms with them through the counselling sessions regularly available at the network of study centres set up by the University.

Needless to add, a great deal of these efforts are still experimental—in fact, pioneering in certain areas. Naturally, there is every possibility of some lapse or deficiency here and there. However, these do admit of rectification and further improvement in due course. On the whole, therefore, these study materials are expected to evoke wider appreciation the more they receive serious attention of all concerned.

Prof. (Dr.) Subha Sankar Sarkar
Vice-Chancellor

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	Writer	Editor
Units 1–2	Dr. Samiran Saha	
Unit 3	Prof. C. K. Manna	
Units 4–5	Dr. Samiran Saha	Prof. Biswaranjan Maity
Unit 6	Dr. Bibhas Guha	
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NETAJI SUBHAS
OPEN UNIVERSITY

PGZO – 7
Endocrinology, Cell and Tissue Structure, function

Group – B

Unit 1 □ Hormone as Messenger and Their Role in Metabolic Regulation

Structure

1.1 The Endocrine System

The integration of body functions in humans and other higher organisms is carried out by the nervous system, the immune system, and the endocrine system. The foundations of the **endocrine system** are the hormones and glands. The endocrine system is composed of a number of tissues that secrete their products, **endocrine hormones**, into the circulatory system. Hormone is a chemical substance, usually a peptide, amine or steroid, produced by one tissue and conveyed by the bloodstream throughout the body to affect physiological activity, such as growth or metabolism and maintaining homeostasis. (Fig. 1)

Peptides and proteins include neuropeptides, pituitary and gastrointestinal hormones.

Steroids consist of adrenal and gonadal steroids and vitamin D, which is converted to a hormone. Steroids are lipid soluble (lipophilic).

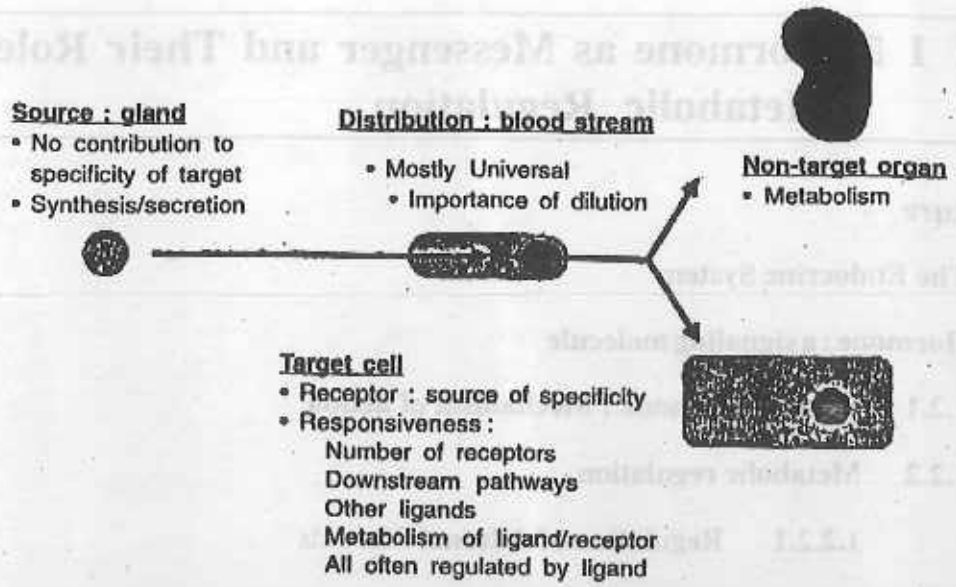


Fig. 1. Regulation of endocrine signaling

Monoamines (modified amino acids) comprise of catecholamines, histamine, serotonin, and melatonin. Catecholamines (dopamine, noradrenaline and adrenaline) are derived from tyrosine and serotonin/melatonin from tryptophan by a series of enzymatic conversions. Monoamines and amino acid hormones are water soluble just as peptides.

The term Hormone comes from the Greek *hormao*, means to urge on or excite. The essence of hormone action is that the hormone affects substances other than itself, typically by causing regulation of a metabolic pathway. At their target tissue they act by binding to a specific receptor, itself a protein. For **peptide and protein hormones** this receptor is usually an integral protein of the cell membrane, while for **steroid hormones** it is within the cell. The hormone or hormone receptor complex enters the nucleus (or is formed in the nucleus) and affects DNA transcription, and thus synthesis of specific proteins. As the body's chemical messengers, hormones transfer information and instructions from one set of cells to another. Although many different hormones circulate throughout the bloodstream, each one affects only the cells that are genetically programmed to receive and respond to its message. Hormone levels can be influenced by factors such as stress, infection, and changes in the balance of fluid and minerals in blood. Because hormones are diffused throughout the body they need to be synthesized in enormous amounts. This synthesis usually occurs in specially designed cells. Another necessity is to travel in the blood stream and diffuse in effective

concentrations into tissues. The ability of hormones to diffuse through the extracellular space relates to the local concentration of hormone at target sites, which may rapidly decrease when glandular secretion of the hormone stops. Hormones diffuse throughout extracellular fluid quickly. Hence, hormonal metabolism can occur in specialized organs such as the liver and kidney in a way that can determine their effective concentrations in other tissues.

A gland is a group of cells that produces and secretes, chemicals. A gland selects and removes materials from the blood, processes them, and secretes the finished chemical product for use somewhere in the body. Some types of glands release their secretions in specific areas. For instance, exocrine glands, such as the sweat and salivary glands, release secretions in the skin or inside the mouth. Endocrine glands, on the other hand, release more than 20 major hormones directly into the bloodstream where from they can be transported to cells in other parts of the body.

1.2 Hormone : a signaling molecule

Hormone is essentially a chemical messenger that transduce signal from one cell to another. In fact, hormonal signaling represents a special case of the more general process of signaling between cells. Even unicellular organisms like *Saccharomyces cerevisiae* secrete short peptide mating factors acting on receptors of other yeast cells. In a separate but related system, **exocrine** tissues secrete their products into ducts and then outside the body or to the intestinal tract.

The classical definition of hormone has begun to change as it is found that some secreted substances can act close to the cells that secrete them (**paracrines**), or act directly on the cell that secretes them (**autocrines**). Signals from one cell to adjacent, called paracrine signals, often trigger cellular responses that use the same molecular pathways used by hormonal signals. Because paracrine factors and hormones can share signaling mechanisms, hormones can, in some cases, act as paracrine factors. For example, Testosterone, besides being secreted into the blood stream, also acts locally to control spermatogenesis. Insulin like growth factor I (IGF-I), a hormone secreted into the blood stream from the liver and other tissues, also acts as a local paracrine factor to control cell proliferation in most tissues. Again, a single receptor can mediate the actions of a hormone (e.g. parathyroid hormone) and a paracrine factor (e.g. parathyroid hormone related protein). On the other hand, target cells respond similarly to signals that

reach them from the blood stream (hormones) or from the adjacent cell (paracrine factors); the cellular response machinery does not distinguish the sites of origin of signals. The major hormonal signaling programs are G protein-coupled receptors, tyrosine kinase receptors, serine/threonine kinase receptors, ion channels, cytokine receptors and nuclear receptors. (Fig. 2)

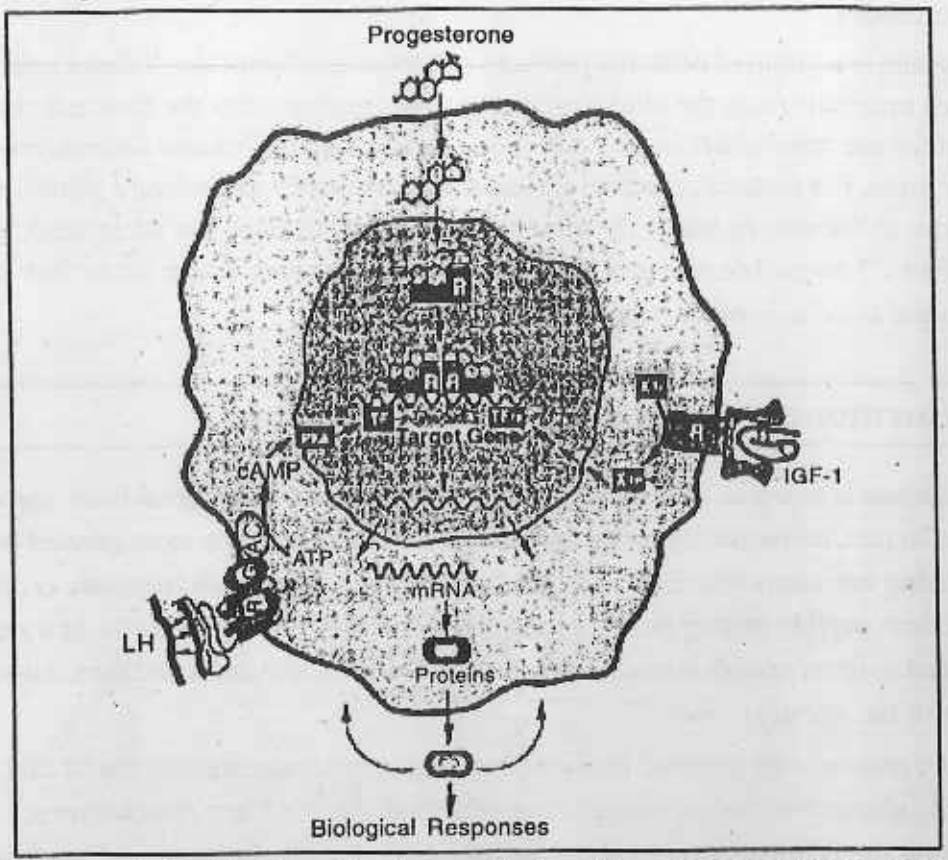


Fig. 2. Hormonal signaling by cell-surface and intracellular receptors. The receptors for the water-soluble polypeptide hormones, LH and IGF-I are integral membrane proteins located at the cell surface. They bind the hormone-utilizing extracellular sequences and transduce a signal by the generation of second messengers, cAMP for the LH receptor, and tyrosine-phosphorylated substrates for the IGF-I receptor. Although effects on gene expression are indicated, direct effects on cellular proteins, for example, ion channels, are also observed. In contrast, the receptor for the lipophilic steroid hormone progesterone resides in the cell nucleus. It binds the hormone and becomes activated and capable of direcdy modulating target gene transcription. (Tf = transcription factor; R = receptor molecule.) (Reproduced from Mayo K, In Conn PM, Melmed S (eds). Endocrinology: Basic and Clinical Principles. To-towa, NJ, Humana Press, 1997, p. 11.)

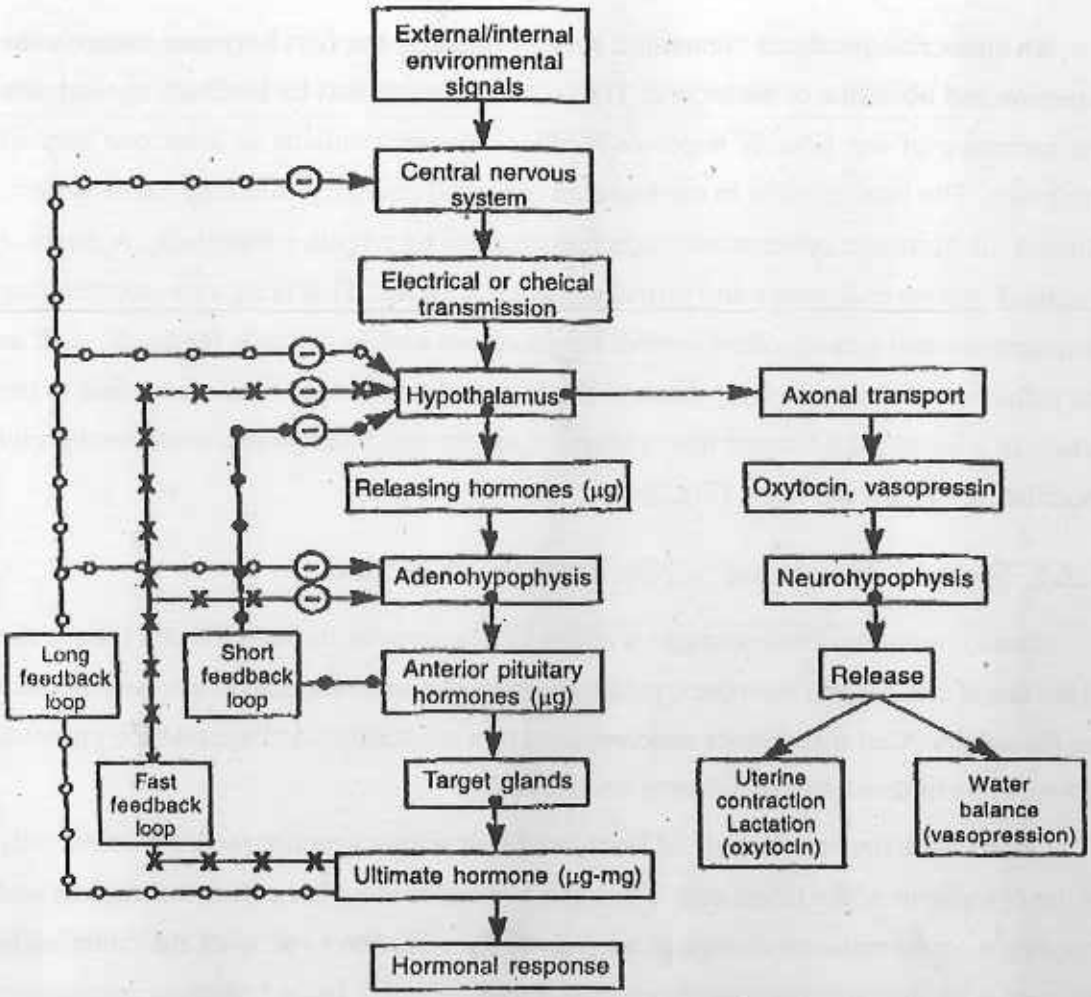


Fig. 3. Peripheral feedback mechanism and a million-fold amplifying cascade of hormonal signals. Environmental signals are transmitted to the central nervous system, which innervates the hypothalamus, which responds by secreting nanogram amounts of a specific hormone. Releasing hormones are transported down a closed portal system, pass the blood-brain barrier at either end through fenestrations, and bind to specific anterior pituitary cell membrane receptors to elicit secretion of micrograms of specific anterior pituitary hormones. These enter the venous circulation through fenestrated local capillaries, bind to specific target gland receptors, trigger release of micrograms to milligrams of daily hormone amounts, and elicit responses by binding to receptors in distal target tissues. Peripheral hormone receptors enable widespread cell signaling by a single initiating environmental signal, thus facilitating intimate homeostatic association with the external environment. Arrows with a black dot at their origin indicate a secretory process. (Reproduced from Normal AW, Litwack G. Hormones, 2nd edn. New York, Academic Press, 1997, p 14.)

An endocrine *feedback system* is a system whereby the first hormone controls the secretion and liberation of the second. The second hormone acts by feedback to modulate the secretion of the first. A *negative feedback system* contains at least one step of inhibition. The total effect is to minimise any external change introduced to the system. Almost all hormone systems maintain homeostasis by negative feedback. A *positive feedback system* exagerates any primary change initiated. This is an auto-accelerating phenomenon and a rarity. *Short feedback systems* use a short distance feedback, such as the influence of the hypophysis back to the hypothalamus. *Auto-feedback* refers to the action of a liberated hormone that is secreted on the cell from where it comes thereby modulating its own secretion. (Fig. 3)

1.2.1 Steroid Hormone : Mechanism of action

Steroid hormones cause changes within a cell by passing through the cell membrane of the target cell. Steroid hormones, unlike non-steroid hormones, can do this because they are fat-soluble. Cell membranes are composed of a phospholipid bilayer which prevents fat-insoluble molecules from diffusing into the cell.

Once inside the cell the steroid hormone binds with a specific receptor found only in the cytoplasm of the target cell. The steroid receptor complex enters the nucleus and initiates a conformational change that involves dimerization to activate the complex to interact with specific regions on cellular DNA referred to as hormone responsive elements (HRE). Once bound to the chromatin, this steroid hormone-receptor complex calls for the production of messenger RNA (mRNA) molecules through a process called transcription. The mRNA molecules are then modified and transported to the cytoplasm. The mRNA molecules code for the production of proteins through a process called translation. These proteins regulate cell function, growth differentiation, etc. So it is the process of expression of proteins that these hormones regulate.

The steroid hormone mechanism of action can be summarized as follows :

1. Steroid hormones pass through the cell membrane of the target cell.
2. The steroid hormone binds with a specific receptor in the cytoplasm.

3. The receptor bound steroid hormone travels into the nucleus and binds to another specific receptor on the chromatin.
4. The steroid hormone-receptor complex calls for the production of messenger RNA (mRNA) molecules, which code for the production of proteins.

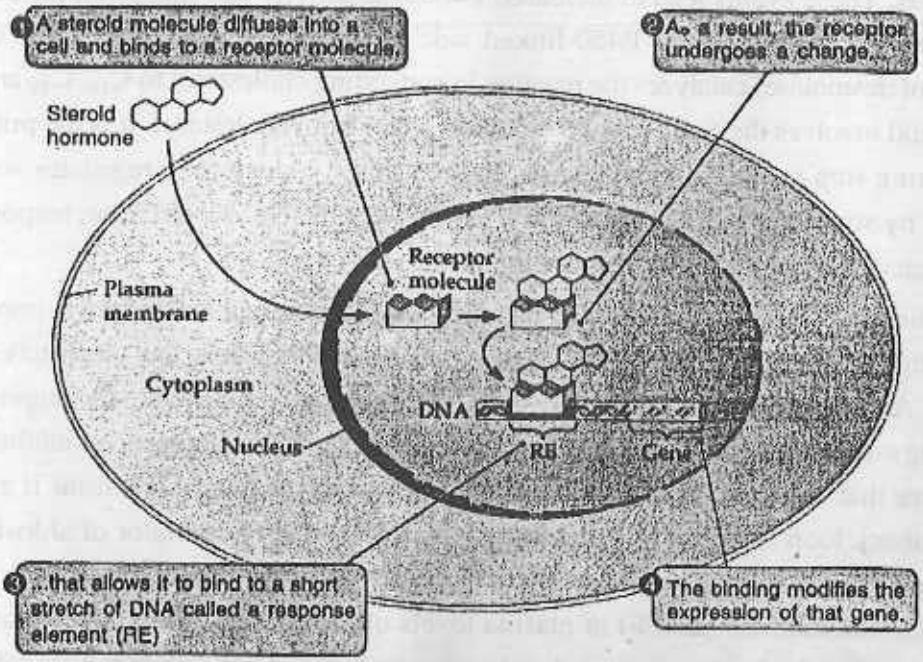


Fig. 4. Steroid hormone : mechanism of action.

1.2.2 Metabolic regulation

1.2.2.1 Regulation of Adrenal Steroids

Adrenocorticotropic hormone (ACTH) of the anterior pituitary regulates the hormone production of the zona fasciculata and zona reticularis. ACTH receptors in the plasma membrane of the cells of these tissues activate adenylate cyclase with production of the second messenger, cAMP. The effect of ACTH on the production of cortisol is particularly important, because a classic feedback loop is prominent in regulating the circulating levels of corticotropin releasing hormone (CRH), ACTH, and cortisol.

Mineralocorticoid secretion from the zona glomerulosa is stimulated by an entirely different mechanism. Angiotensins II and III, derived from the action of the kidney protease, renin on liver-derived angiotensinogen, stimulate zona glomerulosa cells by binding a plasma membrane receptor coupled to phospholipase C. Thus, angiotensin II and III binding to their receptor leads to the activation of PKC and elevates intracellular Ca^{2+} levels. These events lead to increased P450SCE activity and increased production of aldosterone. P450SCE or P450-linked side chain cleaving enzyme (also called cholesterol desmolase) catalyzes the reaction in converting cholesterol to C_{18}, C_{19} and C_{21} steroids and involves the cleavage of a 6-carbon group from cholesterol. It is the principal rate-limiting step in steroid biosynthesis. In the kidney, aldosterone regulates sodium retention by stimulating gene expression of mRNA for the Na^+/K^+-ATPase, responsible for the reaccumulation of sodium from the urine.

The interplay between renin from the kidney and plasma angiotensinogen is important in regulating plasma aldosterone levels, sodium and potassium levels, and ultimately blood pressure. Among the drugs most widely used to lower blood pressure are the angiotensin converting enzyme (ACE) inhibitors. These compounds are potent competitive inhibitors of the enzyme that converts angiotensin I to the physiologically active angiotensins II and III. This feedback loop is closed by potassium, which is a potent stimulator of aldosterone secretion. Changes in plasma potassium of as little as 0.1 millimolar concentration can cause wide fluctuations (±50%) in plasma levels of aldosterone. Potassium increases aldosterone secretion by depolarizing the plasma membrane of zona glomerulosa cells and opening a voltage-gated calcium channel, with a resultant increase in cytoplasmic calcium and the stimulation of calcium-dependent processes.

Although fasciculata and reticularis cells have the capability of synthesizing androgens and glucocorticoids respectively, but normally the fasciculate glucocorticoids production. However, when genetic defects occur in the enzyme complexes leading to glucocorticoid production, or in case of tumour, large amount of androgen, dehydroepiandrosterone (DHEA) is produced and lead to hirsutism and other masculinizing changes in secondary sex characteristics.

1.2.2.2 Regulation of Sex steroids

Although many steroids are produced by the testes and the ovaries, the two most important sex hormones are testosterone and estradiol-17β. These compounds are under

tight biosynthetic control, with short and long negative feedback loops that regulate the secretion of follicle stimulating hormone (FSH) and luteinizing hormone (LH) by the pituitary and gonadotropin releasing hormone (GnRH) by the hypothalamus. Low levels of circulating sex hormone reduce feedback inhibition on GnRH synthesis (the long loop), leading to elevated FSH and LH. The latter peptide hormones bind to gonadal tissue and stimulate P450SCE activity, resulting in sex hormone production via cAMP and PKA mediated pathways. The roles of cAMP and PKA in gonadal tissue are the same as that described for glucocorticoid production in the adrenals, but in this case adenylate cyclase activation is coupled to the binding of LH to plasma membrane receptors.

The biosynthetic pathway to sex hormones in male and female gonadal tissue includes the production of androgens, androstenedione and dehydroepiandrosterone. Testes and ovaries contain an additional enzyme, a 17β-hydroxysteroid dehydrogenase that enables androgens to be converted to testosterone.

In males, LH binds to Leydig cells, stimulating production of the principal Leydig cell hormone, testosterone. Testosterone is secreted to the plasma and also carried to Sertoli cells by androgen binding protein (ABP). In Sertoli cells the Δ4 double bond of testosterone is reduced, producing dihydrotestosterone. Testosterone and dihydrotestosterone are carried in the plasma, and delivered to target tissue, by a specific gonadal-steroid binding globulin (GBG). In a number of target tissues, testosterone can be converted to dihydrotestosterone (DHT). DHT is the most potent of the male steroid hormones, with an activity that is 10 times that of testosterone. Because of its relatively lower potency, testosterone is sometimes considered to be a prohormone. Testosterone is also produced by Sertoli cells but in these cells it is regulated by FSH, again acting through a cAMP- and PKA-regulatory pathway. In addition, FSH stimulates Sertoli cells to secrete androgen-binding protein (ABP), which transports testosterone and DHT from Leydig cells to sites of spermatogenesis. There, testosterone acts to stimulate protein synthesis and sperm development.

Aromatase activity is also found in granulosa cells, but in these cells the activity is stimulated by FSH. Normally, thecal cell androgens produced in response to LH diffuse to granulosa cells, where granulosa cell aromatase converts these androgens to estrogens. As granulosa cells mature they develop competent large numbers of LH receptors in the plasma membrane and become increasingly responsive to LH, increasing the quantity of estrogen produced from these cells. Granulosa cell estrogens are largely, if

not all, secreted into follicular fluid. Thecal cell estrogens are secreted largely into the circulation, where they are delivered to target tissue by the same globulin (GBG) used to transport testosterone.

1.2.3 Protein/peptide and Biogenic Amine Hormones: Mechanism of action

Such water-soluble hormones (first messengers) bind to hormone receptors on the lipid-rich plasma membrane. Peptide hormone and catecholamine receptors are membrane receptors with a binding domain located extracellularly and an effector domain intracellularly.

The second messengers involved are cyclic adenosine monophosphate (cAMP), cyclic guanosine monophosphate (cGMP), inositol trisphosphate (IP_3), Ca^{2+}, diacylglycerol (DAG) etc. The Ca^{2+}-ion is an important second messenger. The Ca^{2+}-influx to the cytosol

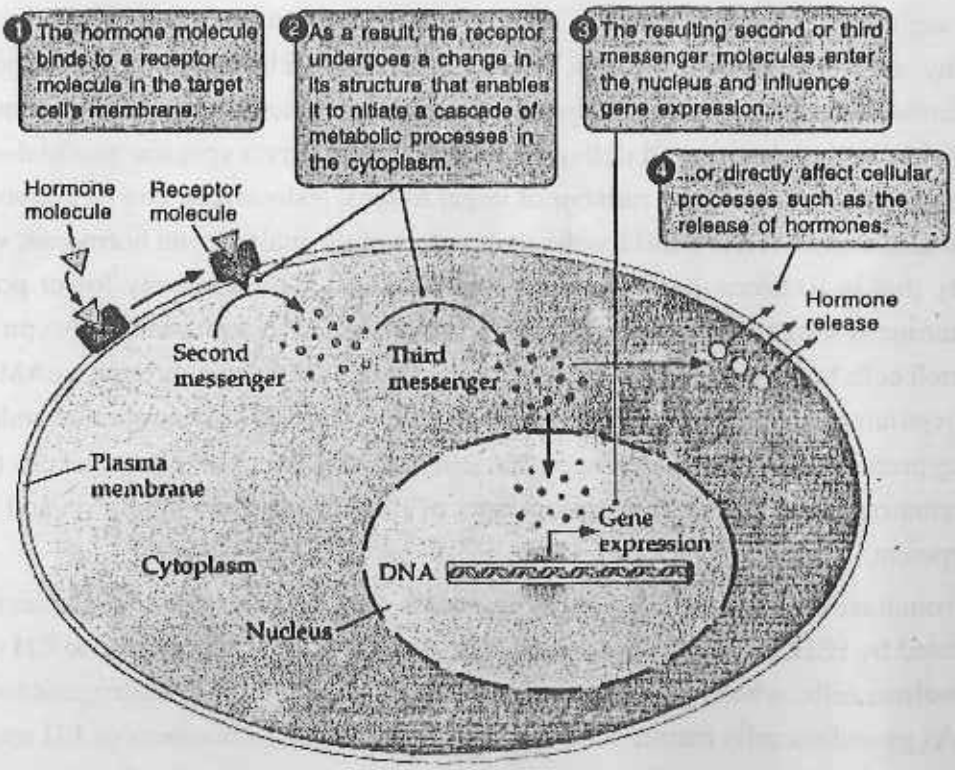


Fig 5. Peptide/Amine hormone : mechanism of action.

is controlled by hormone receptor binding, neural stimuli or modified by other second messengers. Sutherland received Nobel Prize in 1971 for discovering cAMP and demonstration of its role as a second messenger in mediating body functions.

1.2.4 Metabolic regulation of Peptide/Amine hormone

Increased activity of the sympathetic nervous system including release of adrenaline triggers fight-or-flight reactions. In the heart, adrenaline molecules diffuse to the myocardial cells, where they bind to membrane b-receptors. A stimulatory signal is hereby transmitted to an associated enzyme called adenylcyclase. This enzyme catalyses the conversion of ATP to cAMP. The importance of cAMP is that it activates protein kinase A, which, among many other functions, phosphorylates the Ca^{2+}-channel protein. This activation is correlated with an increase in the magnitude of the Ca^{2+}-influx, the force of contraction, and the heart rate.

The parasympathetic system counteracts the sympathetic by slowing the heart rate and decreasing the force of contraction. Acetylcholine is bound to another set of specific membrane receptors located on the heart cell membrane. Acetylcholine reduces the Ca^{2+}-influx that was increased by adrenaline.

Most hormones have a blood concentration of approximately 10^{-10} mol per l. One molecule bound to a cell receptor releases 10 000 times more cAMP in the cell. Hence, cAMP works as an amplifier of the hormone signal. Phosphodiesterase (PDE) destroys cAMP. PDE enhances hydrolysis of cAMP to the inactive 5'- AMP by a highly exergonic process. Inhibitors of the PDE (theophylline and caffeine) act synergistically with hormones that use cAMP as a second messenger. cAMP stimulates catabolic processes such as lipolysis, glycogenolysis (glucagon), gluconeogenesis, and ketogenesis. The cAMP also stimulates amylase liberation in the saliva by the parotid gland, the HCl secretion by the parietal cells, the insulin release by the b-cells in pancreas, and the increased ion permeability of many cell membranes. When the glucose concentration increases in the arterial blood and close to the b-cells of the pancreatic islets of Langerhans, it triggers an increase in Ca^{2+} -influx to the cell. The initial surge in insulin secretion is caused by calmodulin-dependent protein kinases.

The high cytosolic [Ca^{2+}] activates the membrane phospholipase A_2 and C. Phospholipase A_2 releases arachidonic acid (AA) which stimulates insulin secretion. Phospholipase C catalyses the formation of IP_3 and DAG. The IP_3 releases more Ca^{2+} from the

endoplasmic reticulum, and DAG activates protein kinase C. The decrease in insulin secretion after the initial surge and its subsequent increase can be explained by the action of protein kinase C.

Initially, the active protein kinase C stimulates the Ca^{2+}-pump in the plasma membrane, reduces cytosolic [Ca^{2+}] and thus reduces the initial calmodulin-dependent insulin secretion. Later, protein kinase C stimulates the formation of cAMP and amplifies the induction of calmodulin-dependent protein kinase thereby causing a gradual increase in insulin secretion. Prolonged glucose stimulation probably leads to down-regulation of protein kinase C. An abnormally prolonged glucose stimulation may render b-cells glucose blind and thus spoil their function.

Insulin secretion is not only stimulated by glucose, but also potentated by acetylcholine via phospholipase C and by glucagon via activation of adenylcyclase. b-Agonists stimulate b-receptors on the glucagon producing a-cells, whereas a-agonists inhibit insulin secretion via a_2-receptors on the b-cells. Acetylcholine and glucagon react by activating protein kinase C and cAMP dependent protein kinase A, respectively. Both mechanisms potentate the Ca^{2+}-triggered insulin secretion.

Transcription in the cell nucleus produces a precursor messenger RNA molecule complementary to part of a DNA. The precursor is processed into messenger RNA and transported through the nuclear membrane into the cytoplasm. Translation produces big precursor molecules (pre-pro-hormones). Precursors have a signal peptide that contains processing information to ensure that the protein enters the rough endoplasmic reticulum. Here enzymes split the precursor into a signal molecule and a prohormone. Finally, peptide hormones undergo post-translational processing (for eg, thyroid stimulating hormone, TSH, and gonadotropins are glycosylated; insulin forms a zinc-complex). The hormones reach the Golgi complex, where they are packed into secretory granules that migrate to the cell surface.

Roger Guillemin synthesized brain peptides that regulate the pituitary secretion in vitro. He received the Nobel Prize in 1977.

Unit 2 □ Thyroid Biosynthesis and Function

Structure

2.1 Thyroid Hormone Synthesis

Thyroid hormone synthesis requires the uptake of iodide by active transport, thyroglobulin biosynthesis, oxidation and binding of iodide to thyroglobulin, and within the matrix of this protein, oxidative coupling of two iodotyrosines into iodothyronines. All these steps are regulated by the cascades of enzymes.

The thyroid contains two hormones, L-thyroxine (tetraiodothyronine, T_4) and L-triiodothyronine (T_3) (Fig. 1). Iodine is an indispensable component of the thyroid hormones, comprising 65% of T_4's weight, and 58% of T_3's. The thyroid hormones are the only iodine-containing compounds with established physiologic significance in vertebrates.

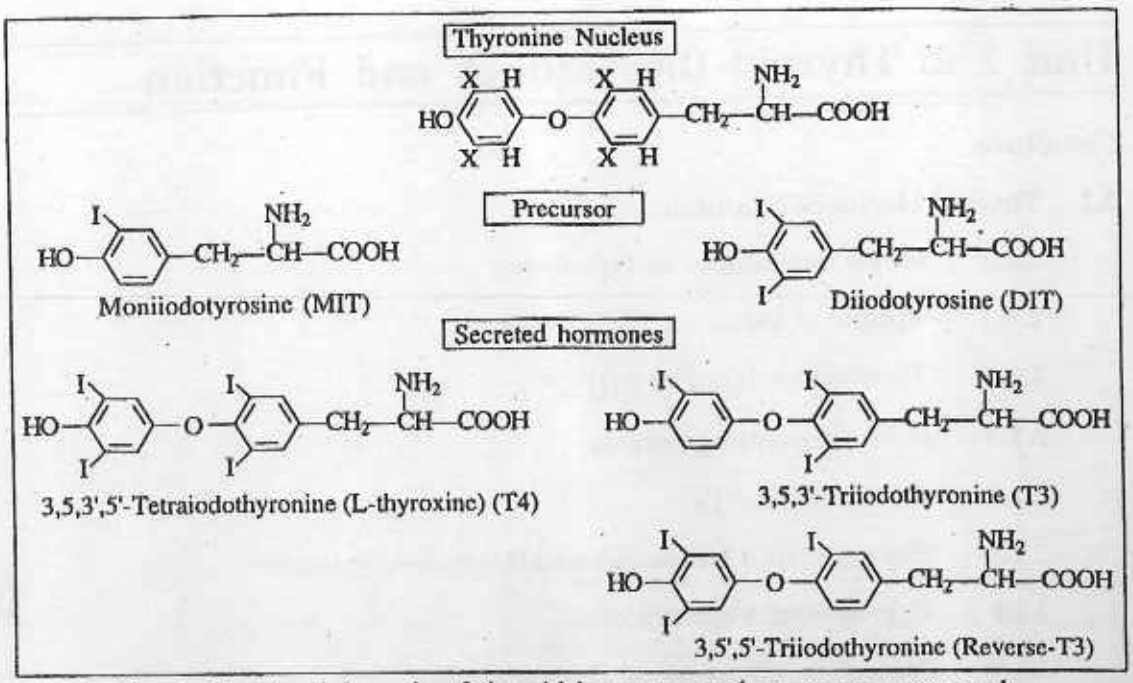


Fig. 1. Structural formula of thyroid hormones and precursor compounds.

The term "iodine" occasionally causes confusion because it may refer to the iodine atom itself but also to molecular iodine (I_2). In this chapter "iodine" refers to the element in general, and "molecular iodine" refers to I_2. "Iodide" refers specifically to the ion I^-.

Ingested iodine is absorbed through the small intestine and transported in the plasma to the thyroid, where it is concentrated, oxidized, and then incorporated into thyroglobulin (Tg) to form MIT and DIT and later T_4 and T_3 (Fig. 2). After a variable period of storage in thyroid follicles, Tg is subjected to proteolysis and the released hormones are secreted into the circulation, where specific binding proteins carry them to target tissues.

This chapter discusses these broad steps as :

(a) iodine availability and absorption;
(b) uptake of iodide by the thyroid;
(c) oxidation of iodide, which involves the thyroperoxidase (TPO), H_2O_2, and H_2O_2 generation;
(d) Tg, whose iodination leads to hormone formation;
(e) storage of thyroid hormones in a Tg-bound form;
(f) Tg breakdown and hormone release;
(g) control of synthesis and secretion by iodine supply and TSH; and
(h) effects of drugs and other external agents on synthesis and secretion of thyroid hormones.

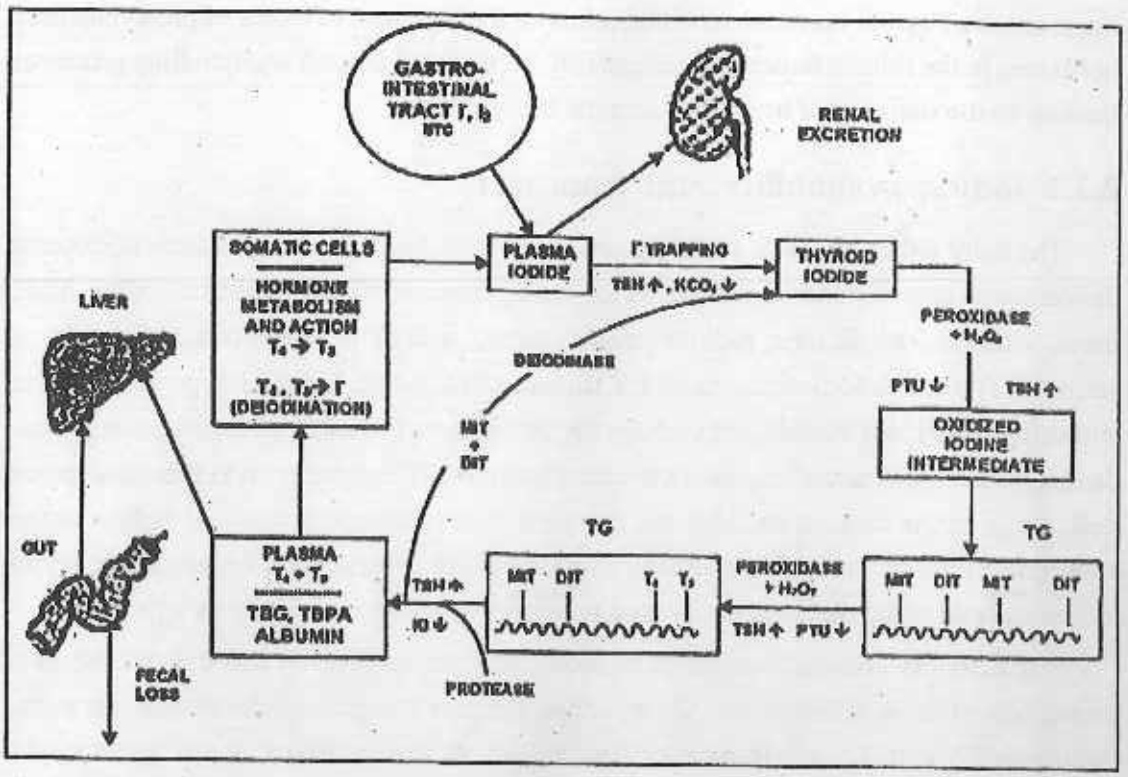


Fig. 2. The iodide cycle. Ingested iodide is trapped in the thyroid, oxidized, and bound to tyrosine to form iodotyrosines in thyroglobulin (TG); coupling of iodotyrosyl residues forms T_4 and T_3. Hormone secreted by the gland is transported in serum. Some T_4 is deiodinated to T_3. The hormone exerts its metabolic effect on the cell and is ultimately deiodinated; the iodide is reused or excreted by the kidney. A second cycle goes on inside the thyroid gland, with deiodination of iodotyrosines generating iodide, some of which is reused without leaving the thyroid.

The production of thyroid hormones is based on the organization of thyroid epithelial cells in functional units, the thyroid follicles. A single layer of polarized cells (Fig. 2-4A.) forms the enveloppe of a spherical structure with an internal compartment, the follicle lumen. Thyroid hormone synthesis is dependent on the cell polarity that conditions the targeting of specific membrane protein, either on the external side of the follicle (facing the blood capillaries) or on the internal side (at the cell-lumen boundary) and on the tightness of the follicle lumen that allows the gathering of substrates and the storage of products of

the reactions.Thyroid hormone secretion relies on the existence of stores of pre-synthetized hormones in the follicle lumen and cell polarity-dependent transport and handling processes leading to the delivery of hormones into the blood stream.

2.1.2 Iodine availability and transport

The daily iodine intake of adult humans varies from less than 10 μg in areas of extreme deficiency to several hundred milligrams for some persons receiving medicinal iodine. Milk, meat, vitamins, medicines, radiocontrast material, and skin antiseptics are important sources. Too much iodine increases the incidence of iodine-induced hyperthyroidism, autoimmune thyroid disease and perhaps thyroid cancer. Deficiency causes mental retardation, goiter, hypothyroidism, and other manifestations. The global push to eliminate iodine deficiency in the current decades has put both excess and deficiency of iodine in the spotlight. Some countries have already moved rapidly from severe iodine deficiency to iodine excess, while others are only now recognizing iodine deficiency as a problem.

Most dietary iodine is reduced to iodide before absorption throughout the gut, principally in the small intestine. Absorption is virtually complete. Iodinated amino acids, including T_4 and T_3, are transported intact across the intestinal wall. Short-chain iodopeptides may also be absorbed without cleavage of peptide bonds. Absorbed iodide has a volume of distribution numerically equal to about 38% of body weight (in kilograms), mostly extracellular, but small amounts are found in red blood cells and bones. Milk is the principal source of virtually all the newborn's iodine, so milk substitutes need to provide adequate amounts.

2.1.3 Uptake of iodine by the thyroid

Thyroid cells extract and concentrate iodide from plasma. The normal thyroid maintains a concentration of free iodide 20 to 50 times higher than that of plasma, depending on the amount of available iodine and the activity of the gland. This concentration gradient may be more than 100:1 in the hyperactive thyroid of patients with **Graves' disease**. The thyroid can also concentrate other ions, including bromide, astatide, pertechnetate, rhenate, and chlorate, but not fluoride. Iodide transport is energy-dependent and requires O_2. Ouabain, digitoxin, and other cardiac glycosides block iodide transport in vitro. Iodide uptake by thyroid cells is dependent on membrane ATPase.

The protein responsible for iodide transport, called sodium iodide symporter or NIS, is located at the basolateral plasma membrane of thyrocytes (Fig. 3.). NIS-mediated I^-

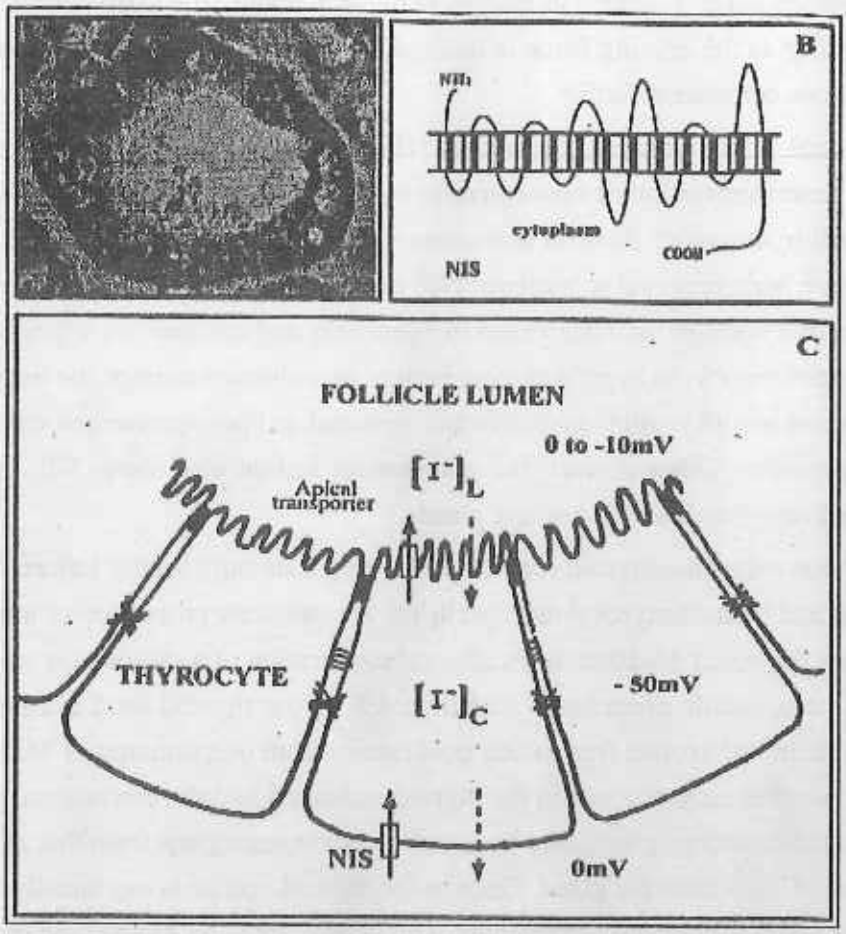


Fig. 3. NIS-mediated transport of iodide. A, immunolocalization of the human NIS protein at the basolateral plasma membrane of thyrocytes in their typical follicle organization. B, schematic representation of the membrane topology of the NIS polypeptide chain deduced from secondary structure prediction analyses. C, transport of iodide from the extracellular fluid (or plasma) to the thyroid follicle lumen. The uptake of iodide at the basolateral plasma membrane of thyrocytes must be active; it operates against an electrical gradient (0 to-50mV) and a concentration gradient, $[I^-]_c$ being higher than extracellular $[I^-]$. The transport of iodide from the cytoplasm to the follicle lumen should be a passive process, the electrical and concentration gradients being favorable.

accumulation is a Na^+-dependent active transport process that couples the energy released by the inward translocation of Na^+ down to its electrochemical gradient to the simultaneous inward translocation of I^- against its electrochemical gradient. The maintenance of the Na^+ gradient acting as the driving force is insured by Na^+-K^+-ATPase. NIS belongs to the sodium/glucose cotransport family.

Functional studies clearly show that NIS is responsible for most of the events previously described for iodide concentration by the thyroid. TSH stimulates NIS expression and iodide transport. Several mutations in the NIS gene causing defective iodide transport have been reported in humans. NIS expression is increased in Grave's disease and hyperactive nodules, and decreased in adenomas and carcinomas appearing as cold nodules at scintigraphy. In hypofuctioning benign or malignant tumors, the impairment of iodide transport would result from both transcriptional and post-transcriptional alterations of NIS expression. Other tissues that concentrate iodide also show NIS expression, including salivary glands and mammary glands.

Iodide that enters the thyroid remains in the free state only briefly before it is further metabolized and bound to tyrosyl residues in Tg. A significant proportion of intrathyroidal iodide is free for about 10-20 minutes after administration of a radioactive tracer, but in the steady state, iodide contributes less than 1% of the thyroid total iodine. A major fraction of the intrathyroidal free iodide pool comes from deiodination of MIT and DIT; this iodide is either recycled within the thyroid or leaked into the circulation. Some data suggest that iodide entering the gland by active transport segregates from that generated by deiodination of Tg within the gland. Once in the thyroid, iodide is organically bound at a rate of 50 to 100% of the pool each minute. The proportion of an iodide load that is bound varies little, despite wide shifts in daily intake. In contrast, NIS activity is sensitive to both iodine availability and TSH stimulation, and transport rather than intrathyroidal binding is the controlling factor in making iodide available for hormonogenesis.

2.1.4 Thyroperoxidase (TPO)

After concentrating iodide, the thyroid rapidly oxidizes it and binds it to tyrosyl residues in Tg, followed by coupling of iodotyrosines to form T_4 and T_3. The process requires the presence of iodide, a peroxidase (TPO), a supply of H_2O_2, and an iodine acceptor protein (Tg).

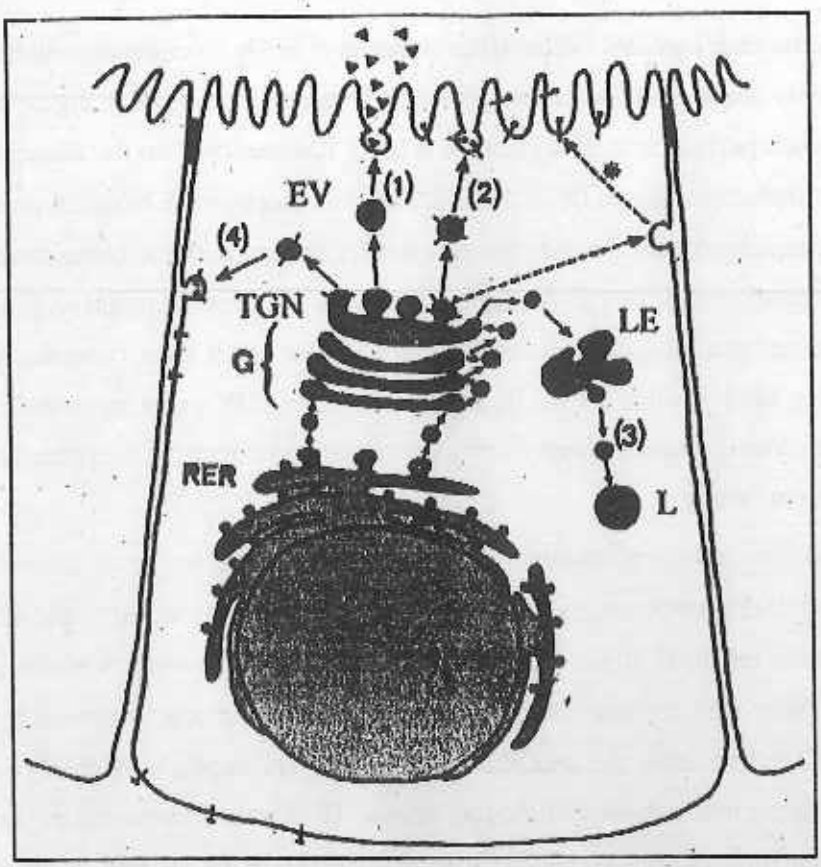


Fig. 4. A polarized thyroid epithelial cell synthesizing soluble proteins, Tg (▲) and lysosomal enzymes (X) and membrane proteins, NIS (⊥) and TPO (°). The polypeptide chain(s) generated by RER membrane-bound polysomes, enter the lumen of RER for the former and remain inserted into the RER membrane for the latter. Inside the lumen of RER, newly-synthesized proteins undergo core glycosylation and by interacting with chaperones acquire their conformation. Proteins are then transported to the Golgi apparatus (G), where terminal glycosylation and other post-translational reactions take place. In the Trans-Golgi network (TGN), mature proteins undergo sorting processes and are packed into transport vesicles. The vesicles carrying soluble proteins (inside the vesicle) and membrane proteins (as integral vesicle membrane protein) deliver them at the appropriate plasma membrane domain: the apical domain (1) and (2) or the basolateral domain (4). Vesicles carrying lysosomal enzymes (3) conveyed their content to prelysosomes or late endosomes (LE) and lysosomes (L). Apical plasma membrane proteins may reach their final destination by an alternative route involving a transient transfer to and then a retrieval and transport (,) from the basolateral membrane domain to the apical domain.

Thyroperoxidase oxidizes iodide in the presence of H_2O_2. In crude thyroid homogenates, enzyme activity is associated to cell membranes. It can be solubilized using detergents such as deoxycholate or digitonin. The enzyme activity is dependent on the association with a heme, the ferriprotoporphyrin IX or a closely related porphyrin. Chemical removal of the prosthetic group inactivates the enzyme, and recombination with the heme protein restores activity. The apoprotein from human thyroid is not always fully saturated with its prosthetic group. Some congenitally goitrous children have poor peroxidase function because the apoprotein has weak binding for the heme group. Human TPO, which has 46% nucleotide and 44% amino acid sequence homology with human myeloperoxidase, clearly belongs to the same protein family.

TPO synthesized on polysomes is inserted in the membrane of the endoplasmic reticulum and undergoes core glycosylation. TPO is then transported to the Golgi where it is subjected to terminal glycosylation and packaged into transport vesicles along with Tg (Fig. 4). These vesicles fuse with the apical plasma membrane in a process stimulated by TSH. TPO delivered at the apical pole of thyrocytes exposes its catalytic site with the attached heme in the thyroid follicular lumen. TPO activity is restricted to the apical membrane, but most of the thyroid TPO is intracellular, being located in the perinuclear part of the endoplasmic reticulum. Most of this intracellular protein is incompletely or improperly folded; it contains only high mannose-type carbohydrate units, while the membrane TPO has complex carbohydrate units. Glycosylation is essential for enzymatic activity. Chronic TSH stimulation increases the amount of TPO and its targetting at the apical membrane.

2.1.5 H_2O_2 Generating system

By definition, a peroxidase requires H_2O_2 for its oxidative function. H_2O_2 is produced at the apical plasma membrane by an enzyme that requires both calcium and NADPH. There are two members of the NADPH oxidase family, viz. ThOX1 and ThOX2. The current model assigns seven transmembrane domains to ThOX1 and ThOX2 (Fig. 5B).

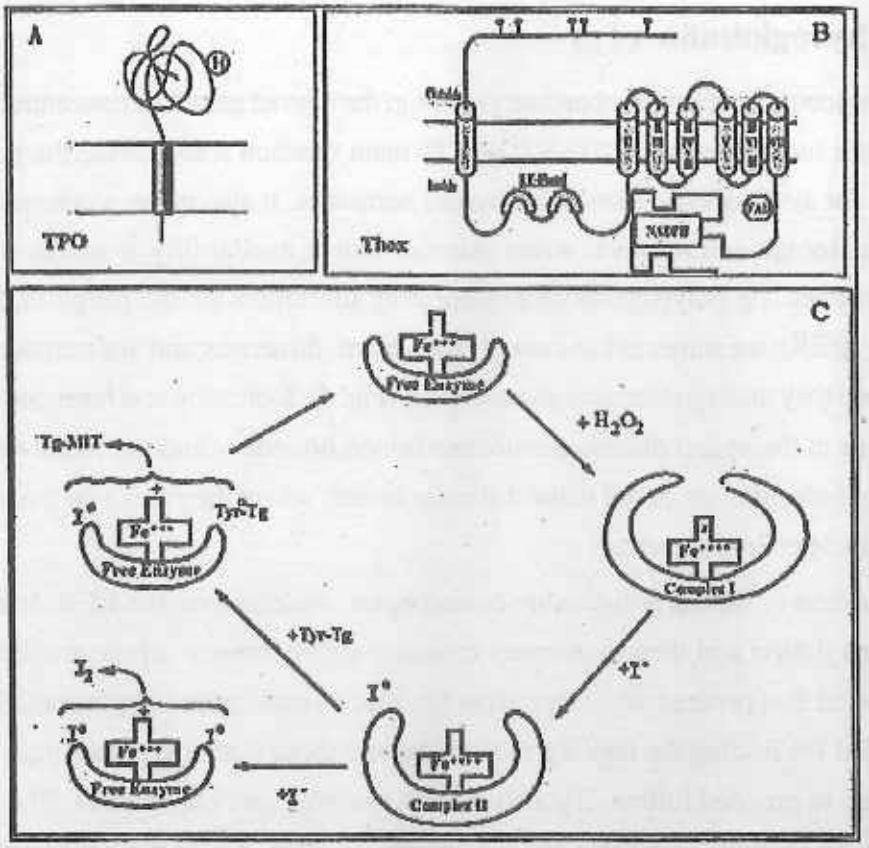


Fig. 5. Schematic representation of the membrane topology of Thyroperoxidase, TPO (A) and NADPH thyroid oxidase, ThOX (B) at the apical plasma membrane of thyrocytes. C, hypothetical reaction scheme for TPO. H_2O_2 is presumed to oxidize the free enzyme with a loss of two electrons leading to the formation of complex I. Iodide binds to complex I, is oxidized and form complex II, which then reacts with a tyrosyl residue of Tg, Tyr-Tg.The newly-formed I^0 and Tyr^0-Tg free radicals interact to form MIT-Tg and the enzyme returns to its free state. I_2 may be generated from two oxidized iodine atoms.

The two proteins are glycoproteins, their apparent molecular mass ranges from 170-180 kDa. Immunolabeling experiments revealed the presence of ThOX inside the cells and at the apical plasma membrane. ThOX proteins are components of the H_2O_2 generating system, but additional polypeptide chains are required to get the complete thyroid H_2O_2 generating system.

2.1.6 Thyroglobulin (Tg)

Thyroglobulin is the most abundant protein in the thyroid gland; its concentration within the follicular lumen can reach 200-300 g/L. Its main function is to provide the polypeptide backbone for synthesis and storage of thyroid hormones. It also offers a convenient depot for iodine storage and retrieval when external iodine availability is scarce or uneven. Neosynthesised Tg polypeptide chains entering the lumen of the rough endoplasmic reticulum (RER) are subjected to core glycosylation, dimerises and are transferred to the Golgi where they undergo terminal glycosylation (Fig. 4). Iodination and hormone formation of Tg occur at the apical plasma membrane-lumen boundary and the mature hormone-containing molecules are stored in the follicular lumen, where they make up the bulk of the thyroid follicle colloid content.

Maturation of the Tg polypeptide chain begins while still on the RER. It undergoes core glycosylation and then monomers fold into stable dimers. Arvan and co-workers have mapped this process and emphasize the role of molecular chaperones. The latter are essential for folding the new Tg molecules, and those that are folded improperly are not allowed to proceed further. Tg also contains sulphur and phosphorus. The former is present in the chondroitin sulfate and the complex carbohydrate units, although its form and role are not known. Several studies have reported presence of phosphate in Tg, up to 12 mol. per mol Tg. Of this, about half is in the complex carbohydrate units, the remainder is present as phosphoserine and phosphotyrosine. This may relate to protein kinase A activity.

2.1.7. Thyroglobulin Iodination and Hormone Synthesis

The step preliminary to thyroid hormone formation is the attachment of iodine to tyrosyl residues in Tg to produce MIT and DIT. This process occurs at the apical plasma membrane-follicle lumen boundary and involves H_2O_2, iodide, TPO, and glycosylated Tg. All rendezvous occur at the apical membrane to achieve Tg iodination (Fig. 6.).

First, iodide must be oxidized to an iodinating form (iodine). One scheme proposes that oxidation produces free radicals of iodine and tyrosine, while both are bound to TPO to form MIT which then separates from the enzyme (Fig. 5C). Further reaction between

free radicals of iodine and MIT gives DIT. Experimental studies by Taurog and others suggest that the TPO reduction occurs directly in a two electron reaction.

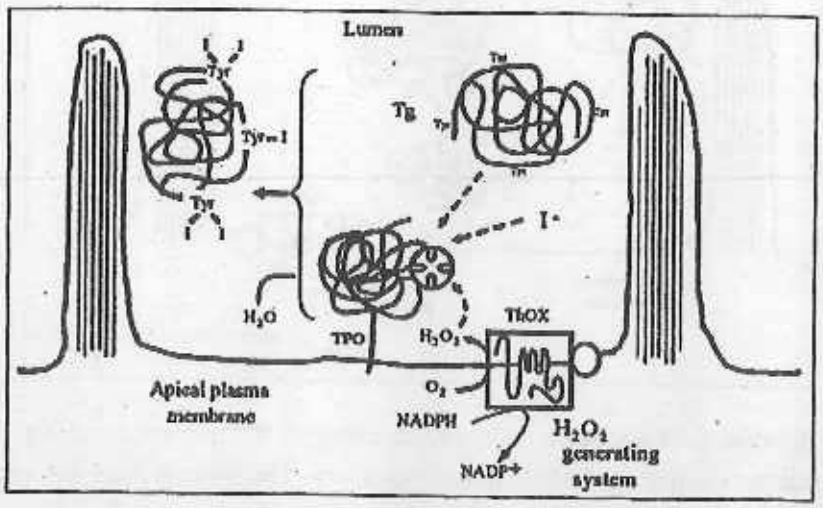


Fig. 6. Iodination of Tg at the apical plasma membrane-follicle lumen boundary. The scheme does not account for the relative size of the intervening molecules.

A second proposal, based on studies of rapid spectral absorption changes is that TPO-I^+ is the iodination intermediate and that the preferred route is oxidation of TPO by H_2O_2 followed by two electron oxidation of I^- to I^+, which then reacts within a tyrosine.

As a third possibility, Taurog proposed a reaction between oxidized TPO and I^- to produce hypoiodite (OI^-), which also involves a two electron reaction. Whatever the precise nature of the iodinating species, it is clear that iodide is oxidized by H_2O_2 and TPO, and transferred to the tyrosyl groups of Tg. All tyrosine residues of Tg are not equally accessible to iodination. The molecule has about 132 tyrosyl residues among its two identical chains; at most, only about 1/3 of the tyrosyls are iodinated. As isolated from the thyroid, Tg rarely contains more than 1% iodine or about 52 iodine atoms.

The final step in hormone synthesis is the coupling of two consenting iodotyrosyl residues to form iodothyronine (Fig. 7). Two DIT form T_4; one DIT and one MIT form T_3. Coupling takes place while both acceptor and donor iodotyrosyl are in peptide linkage within the Tg molecule.The reaction is catalyzed by TPO, required H_2O_2 and is stringently

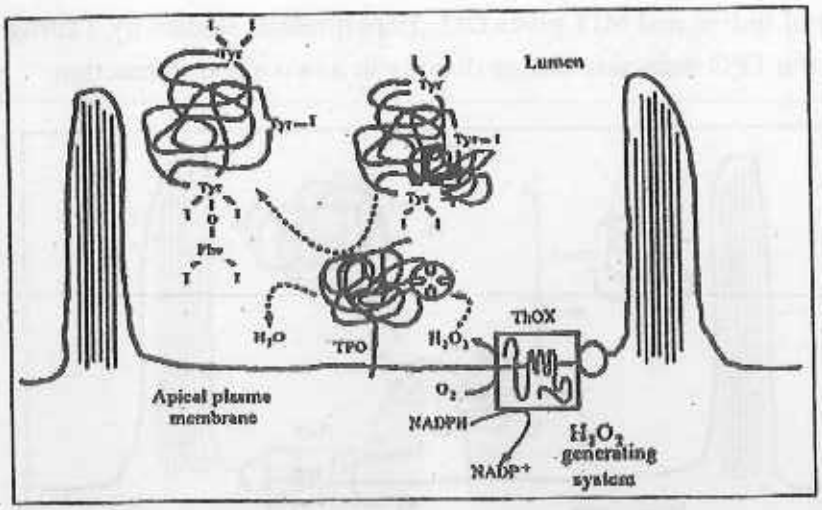


Fig. 7. Synthesis of hormone residues (coupling of iodotyrosines) in Tg at the apical plasma membrane-follicle lumen boundary. The scheme does not account for the relative size of the intervening molecules

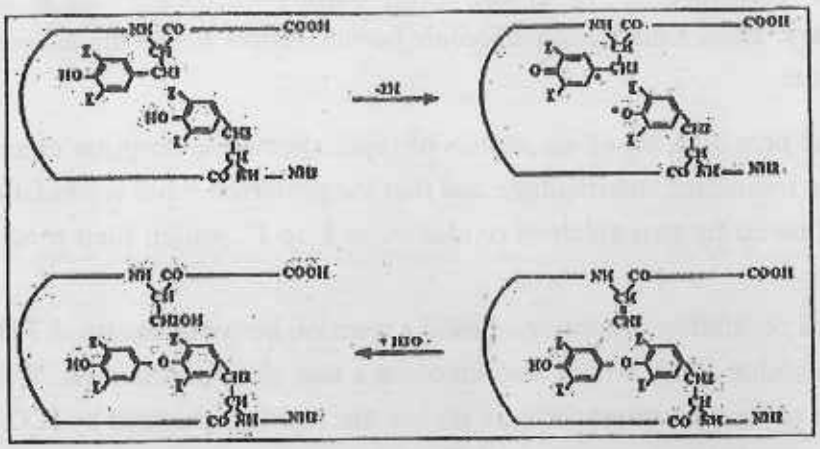


Fig. 8. Possible coupling reaction sequence. Oxidation of iodotyrosines may produce iodotyrosyl radicals. The free radicals could combine to generate the iodothyronine residue (at the tyrosine acceptor site) and a dehydroalanine residue (at the tyrosine donor site), which in the presence of H_2O_2 converts into a serine.

dependent on Tg structure.The generation of the iodothyronine residue involves the formation of an ether bond between the iodophenol part of a donor tyrosyl and the hydroxyl group of the acceptor tyrosyl (Fig 8). After the cleavage reaction that gives the

iodophenol, the alanine side chain of the donor tyrosyl remains in the Tg polypeptide chain as dehydroalanine. Observations both in vivo and in vitro show an appreciable delay in coupling after initial formation of iodotyrosines.

In addition to its role as component of the iodoamino acids, iodine is associated with cleavage of peptide bonds of Tg, at least in vitro. This has been attributed to generation of free radicals during oxidation. Exposure of Tg to reducing agents yields an N-terminal peptide of about 20-26kDa, depending on the animal species, that contains the major hormonogenic site of Tg. This peptide appears in parallel with iodination or may slightly precede it. Further addition of iodine cleaves the 26kDa further, to produce an 18kDa (in human Tg), an event that also occurs with TSH stimulation. Thus, iodination-associated cleavage appears to be part of the maturation of the Tg molecule.

The amount of iodine has important effects on thyroid hormone production. The initial reaction between TPO and H_2O_2 produces the so-called "compound I," which oxidizes iodide and iodinates Tg. Next, the two reactants form compound II, which is necessary for the coupling reaction to make thyroid hormones. However, if excessive iodine is present, conversion to compound II does not take place, and hormone synthesis is impaired.

2.1.8 Thyroglobulin Endocytosis

To be useful, thyroid hormones must be released from Tg and delivered to the circulation for action at their distant target tissues. Depending on numerous factors including - the supply of iodide as substrate, the activity of enzymes catalyzing hormone formation, the concentration and physico-chemical state of Tg - the hormone content of lumenal Tg molecules varies to a rather large extent. Tg molecules newly arrived in the follicle lumen with negligible hormone content would co-exist with "older" Tg exhibiting up to 6-8 hormone residues. The downstream processes responsible for the production of free thyroid hormones from these prohormonal molecules must therefore adequately manage the use of these lumenal heterogeneous Tg stores to provide appropriate amounts of hormones for peripheral utilization.

The way the thyroid follicle proceeds to generate free hormones from stored hormone containing Tg molecules has been known for a long time. Tg molecules are first taken up by polarized thyrocytes and then conveyed to lysosomal compartments for proteolytic cleavage that release T_4 and T_3 from their peptide linkages. The first step represents the limiting point in the thyroid hormone secretory pathway. Over the last decade, there has

been substantial improvement in the knowledge of the cellular and molecular mechanisms governing the internalization or endocytosis and intracellular transport of the prohormone ,Tg. The evolution has first been to consider that it could proceed via a mechanism different from phagocytosis, also named macropinocytosis, evidenced in rats under acute TSH stimulation. Results obtained in rats have been known for a long time extrapolated to the different animal species including human. There is now a number of experimental data indicating that in the thyroid of different species under physiological circumstances, internalization of Tg, mainly if not exclusively, occurs via vesicle-mediated endocytosis or micropinocytosis (Fig. 9), an ubiquitous cellular process accounting for macromolecule internalization by all cell types.

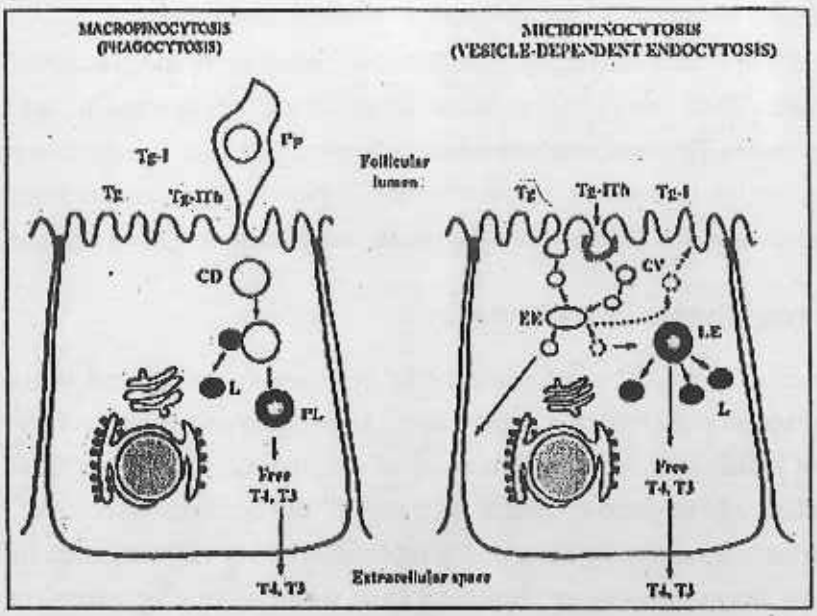


Fig 9. Schematic representation of the two modes of internalization of Tg; Micropinocytosis (on the right) and Macropinocytosis or phagocytosis (on the left). Intraluminal Tg stores potentially subjected to endocytosis are composed of recently secreted non-iodinated Tg, iodinated Tg (Tg-I) and iodinated Tg containing iodothyronine residues (Tg-Ith).Abbreviations are: CV, Coated Vesicle; EE, Early Endosome; LE, Late Endosome; L, Lysosome; Pp, Pseudopod; CD, Colloid Droplet; PL, Phagolysosome. The scheme on the right indicates the three possible routes of transport of internalized Tg molecules reaching the EE: transport to LE, recycling towards the follicle lumen and transcytosis i.e.transport towards the basolateral plasma membrane.

Under TSH stimulation, macropinocytosis would be triggered and would become operative in Tg internalization. Pseudopods representing extensions of the apical plasma membrane project into the follicle lumen and pinch off to form a resorption vacuole known as colloid droplet. The colloid droplets then deliver their content to lysosomes. Pseudopod formation is one of the earliest effects of TSH on the gland, evident within several minutes after administration. In species other than rat, TSH stimulates macropinocytosis through the activation of the cyclic AMP cascade.

2.1.9 Proteolytic Cleavage of Thyroglobulin

Internalized Tg molecules that are conveyed to lysosome compartments, are subjected to diverse hydrolytic reactions leading to the generation of free thyroid hormones and to complete degradation of the protein. Given its composition, Tg is likely the substrate for the different lysosomal enzymes. Efforts have been made to identify proteases involved in the release of hormonal residues from their peptide linkage in Tg. Endopeptidases such as cathepsin D, H and L are capable of cleaving Tg.

Initial cleavage occurs by endopeptidases and resulting products are further processed by exopeptidases. Dunn et al showed that cathepsin B has exopeptidase activity as well as an endopeptidase action. Starting from highly purified preparations of thyroid lysosomes, Rousset et al have identified intralysosomal Tg molecules with very limited structural alterations without hormone residue. After Tg digestion, T_4 and T_3 must be tree from the lysosomal compartments to the cytoplasm and are transported out of the cell for circulation. It has been postulated for decades that thyroid hormones are released from thyrocytes by simple diffusion.

Among other products which are released or leaked out from the thyroid, is Tg. The secretion of Tg is clinically important. Its presence in serum can be detected by a routine assay and provides a sensitive (although not always specific) marker for increased thyroid activity. Attempts have been made to determine the biochemical characteristics of circulating Tg molecules in terms of iodine content, structural integrity and hormone content. Serum levels are elevated in patients with hyperplastic thyroid

or thyroid nodules including differentiated thyroid cancer. Tg measurement can identify congenital hyperplastic goiter, endemic goiter, and many benign multinodular goiters, but its greatest application is in the follow-up of differentiated thyroid cancer. Most papillary and follicular cancers retain some of the metabolic functions of the normal thyrocyte, including the ability to synthesize and secrete Tg. Subjects who have

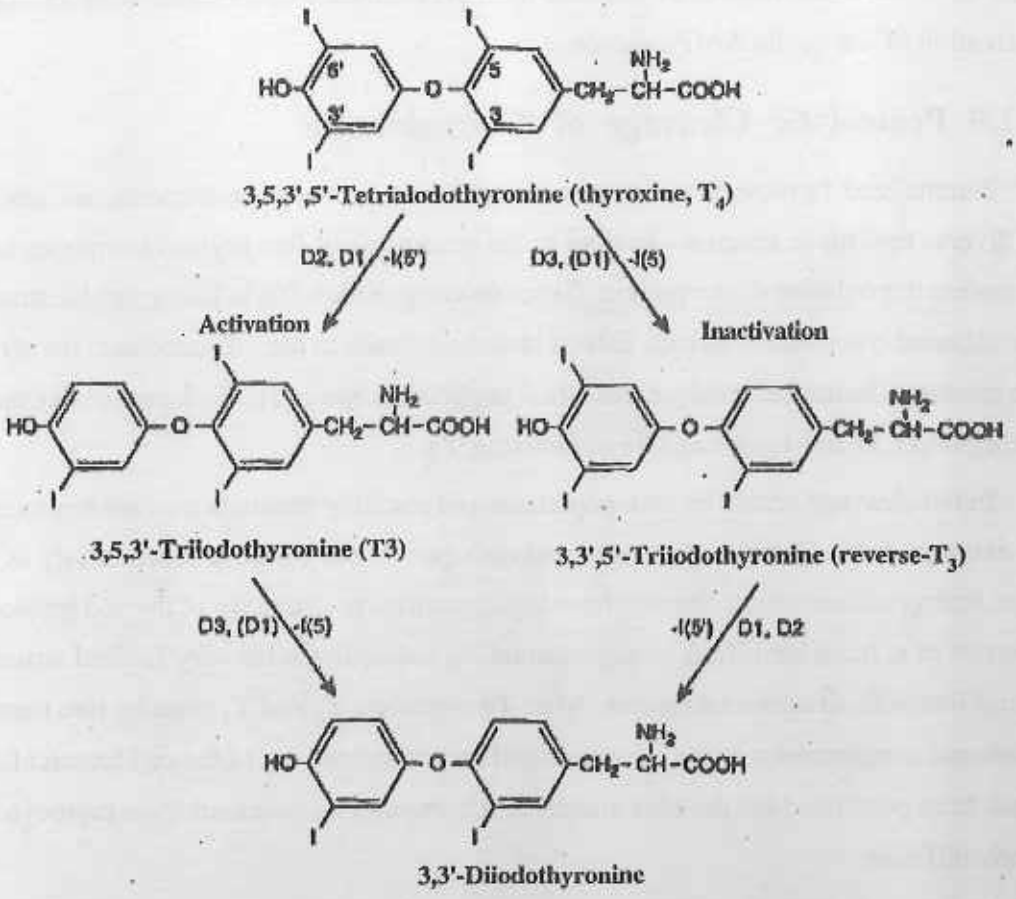


Fig. 10. Pathways for thyroid hormone activation and inactivation catalyzed by human iodothyronine selenodeiodinases. Numbers refer to the iodine positions in the iodothyronine nucleus. The iodothyronine deiodinases are abbreviated Dl, D2, and D3 for types 1, 2, and 3 deiodinases, respectively. *Arrows* refer to mono-deiodination of the outer or inner ring of the iodothyronine nucleus, termed 5' or 5 by convention. The parentheses around Dl emphasize that D3, not Dl, is probably the major enzyme catalyzing inner ring deiodination of T_4 and T_3.

differentiated thyroid cancer treated by surgery and radioiodine should not have normal thyroid tissue left, and therefore, should not secrete Tg. If Tg is found in their serum, it reflects the presence of normal thyroid tissue, unlikely after its ablation, or of thyroid cancer. Tracking serum Tg levels is probably the most sensitive and practical means for the follow-up of such patients. It is more sensitive when the subject is stimulated by TSH. Until recently, this could only be done by withdrawal of thyroid hormone and consequent symptomatic hypothyroidism, but now recombinant human TSH can be administered to enhance the sensitivity of the serum Tg and thyroid scan.

2.1.10 Iodide Metabolism by the Thyroid Cell

Because the concentration of iodide in plasma is extremely low, a mechanism is required for the thyroid cell to concentrate the required amounts of this element. This process, called *iodide trapping*, is accomplished by a membrane protein, the *sodium-iodide symporter* (NIS). Human NIS is a 643 amino acid protein with 13 membrane-spanning domains.

The transport of iodide is an active process, depending on the presence of sodium gradient across the basal membrane of the thyroid cell such that downhill transport of 2 Na^+ ions results in the entry of one iodide atom against an electrochemical gradient. In addition to being expressed in the basolateral membrane of the thyroid cell, NIS has also been identified in other iodide concentrating cells, including salivary and mammary glands, choroid plexus, gastric mucosa, and in the cytotrophoblast and syncytiotrophoblast. The iodide transport system generates an iodide gradient of 20 to 40 over the cell membrane and NIS also transports TcO_4^-, ClO_4^-, and SCN^-, accounting for the utility of radioactive TcO_4^- as a thyroid scanning tool and the capacity of potassium perchlorate ($KClO_4^-$) to block iodide uptake. In fact, these anions have a higher affinity for NIS than does iodide itself. On the other hand, the affinity of NIS for iodide is much higher than it is for the other inorganic anions, such as bromide and chloride, accounting for the selectivity of the thyroid transport mechanism.

It has been known for decades that the iodide-concentrating mechanism is required for normal thyroid function, as its absence is associated with congenital hypothyroidism and goiter unless large quantities of inorganic iodide are provided. A number of families have

now been identified in which various mutations in the NIS gene are associated with congenital hypothyroidism and an iodide transport defect. Transcription of the NTS gene is increased by TSH. The mechanism for this has not been completely elucidated, but studies of the rat NIS promoter suggest that there is an NTS *upstream enhancer,* which confers a cyclic adenosine monophosphate (cAMP) response but also contains binding sites for the thyroid specific transcription factors PAX-8 and TTF-1, as well as a degenerate cAMP response element sequence. Importantly, several studies have documented decreases in NIS expression in human thyroid adenomas and carcinomas that contribute to the loss of iodine uptake in neoplastic thyroid cells, which thus present as "cold" nodules on radioisotopic imaging.

A second thyroid cell protein involved in iodide metabolism, *pendrin,* the product of die *PDS gene,* has now been identified by positional cloning using genornic DNA from families with I the autosomal recessive disorder, *Pendred's syndrome.* This is a long-recognized inherited condition in which sensorineural hearing loss is combined with varying degrees of impaired thyroid hormone synthesis, leading to goiter. Pendrin is a transmembrane protein, a member of the sulfate transport protein family. Initially thought to be a sulfate transporter, it is now recognized to transport chloride, iodide, and bicarbonate (HCO_3^-). Pendrin is expressed in the apical border of the thyroid cell, die inner ear, and the kidney (see Fig. 10-2). Mutations in pendrin cause an inner ear malformation, although not all patients have goiter. It is postulated that pendrin is required for iodide transport across the apical membrane of die thyrocyte into die follicular lumen, where it is then oxidized and coupled to tyrosine in Tg.

The presence of thyroid dysfunction in Pendred's syndrome can be ascertained by the *perchlorate discharge test,* which illustrates die physiologic role of pendrin in thyroidal iodine metabolism. In normal individuals, more than 90% of thyroidal radioiodine is present as iodotyrosine and iodothyronine within minutes of its entry into the thyroid. It is then no longer in the intracellular iodide pool. In patients with Pendred's syndrome, or with other disorders inhibiting the iodination of tyrosine (see later topics, such as Hashimoto's thyroiditis), this process is delayed, as shown by the exit (discharge) of more than 10% of the thyroidal radioiodine within 2 hours of administration of 500 mg of $KC1O_4$. Perchlorate inhibits NIS function by an as yet unidentified mechanism eliminating the iodide gradient,

which is required for maintaining the ra-dioiodide in the gland. This illustrates that both iodide transport by NIS at the basal pole of the diyrocyte and its efflux across die apical membrane by pendrin are required for thyroid hormone synthesis. Deafness in patients with Pendred's syndrome is due to formation of a common cavity in the upper coils of the cochiea with dilatation of the vestibular aqueducts, not to the hypothyroidism per se.

In addition to being brought into die thyroid gland by active transport from the extracellular fluid, thyroidal iodide is generated by the deiodination of iodotyrosines liberated during the hydrolysis of Tg. A portion of this iodide is oxidized and used to iodinate tyrosine, and the remainder is lost from the gland as the *iodide leak* (see Fig. 10-2). This conservation process is interrupted when antithyroid drugs—which inhibit iodide oxidation, such as methimazole (MMI), carbimazole (CB) or propylthiouracil (PTU)—are given, thus further enhancing the effectiveness of these thyroid peroxidase inhibitors in blocking thyroid hormone synthesis.

2.1.11 Control of Hormone Synthesis

The most important controlling factors are iodine availability and TSH. Inadequate amount of iodine leads to inadequate thyroid hormone production, increased TSH secretion and thyroid stimulation, and goiter. Excess iodine acutely inhibits thyroid hormone synthesis, known as the Wolff-Chaikoff effect, that occurs apparently by inhibiting H_2O_2 generation resulting blocking Tg iodination. A proposed mechanism is that the excess iodide leads to the formation of 2-iodohexadecanal, which is endowed with an inhibitory action on H_2O_2 generation. TSH influences virtually every step in thyroid hormone synthesis and release. All these effects appear to be mediated through the cAMP cascade.

In summary, TSH stimulates the expression of NIS, TPO, Tg and generation of H_2O_2, which in turn increase the formation of T_3 and T_4, that alters the priority of iodination and hormonogenesis among tyrosyls and promotes the rapid internalization of Tg by thyrocytes. These several steps are interrelated and have the net effects of increasing the amount of iodine available to the cells for synthesis and releasing of a larger amount and a more effective type of thyroid hormone (T_3).

Anti-thyroid drugs are external compounds influencing thyroid hormone synthesis. The major inhibitory drugs are the thionamides: propylthiouracil and methimazole. In the thyroid,

they appear to act by competing with tyrosyl residues of Tg for oxidized iodine, at least in the rat. Iodotyrosyl coupling is also inhibited by these drugs and appears more sensitive than tyrosyl iodination.

2.2 Thyroid : Function

2.2.1 Thyroid Hormone Secretion

Secretion of thyroid hormone requires endocytosis of human thyroglobulin, its hydrolysis, and the release of thyroid hormones from the cell. Thyroglobulin can be ingested by the thyrocyte by three mechanisms.

In *macropinocytosis*, at first, pseudopods engulf clumps of thyroglobulin. In all species this process is triggered by acute activation of the cAMP/PKA cascade induced by TSH. Stimulation of macropinocytosis is preceded and accompanied by an enhancement of thyroglobulin exocytosis. In dog thyroid slices and even primary cultures, TSH and PKA activation acutely induces phagocytosis and macropinocytosis of thyroglobulin involved in stimulated thyroid hormone secretion. This process might be mediated by inactivation of the Rho family small G proteins, resulting in microfilament depolymerization and stress fiber disruption accompanied by dephosphorylation of cofilin and myosin light chains.

Subsequently in *micropinocytosis* a small amount of colloid fluid is ingested. This process does not appear to be greatly influenced by acute modulation of the regulatory cascades. It is enhanced in chronically stimulated thyroids and thyroid cells by early mobilization in the membrane and later by induction of vesicle transport proteins Rab 5 and 7. It probably accounts for most of basal secretion. Eventually receptor-mediated endocytosis is enhanced in chronically stimulated thyroid cells. The protein involved is megalin and asyaloglycoprotein. This process probably accounts for the transcytosis of low hormone containing thyroglobulin. Macropinocytosis is inhibited by microfilament and microtubule poisons and by lowering of the temperature (below 23°C). Whatever is the mechanism, endocytosis is followed by lysosomal digestion with complete hydrolysis of thyroglobulin. The main iodothyronine in thyroglobulin is thyroxine. However, during its secretion a small fraction is deiodinated by type I 5 and in main type II 5 -deiodinase to triiodothyronine (T_3), resulting in increasing T_3 (the active hormone) secretion.

The free thyroid hormones are released by an unknown mechanism, which may be diffusion or transport. The iodotyrosines are deiodinated by specific deiodinases and their iodides are recirculated in the thyroid iodide compartments. Under acute stimulation, a release (spillover) of amino acids and iodide from the thyroid is observed. A mechanism for lysosome retention of poorly iodinated thyroglobulin on N-acetylglucosamine receptors and recirculation to the lumen has been proposed. Under normal physiologic conditions, endocytosis is the limiting step of secretion, but after acute stimulation, hydrolysis may be the limiting step of thyroid secretion. Secretion by macropinocytosis is triggered by activation of the cAMP cascade and inhibited by Ca^{2+}. It is also inhibited in some thyroids by protein kinase C downstream from cAMP. Thus the PIP_2 cascade negatively controls macropinocytosis.

2.2.2 Cellular Action of Thyroid Hormone

The thyroid hormones (THs, thyroxine (T_4) and triiodothyronine (T_3)) have important effects on development, growth, and metabolism. Some of the most prominent effects of TH occur during fetal development and early childhood. In humans, the early developmental role of TH is illustrated by the distinctive clinical features of cretinism observed in iodine-deficient areas. In childhood, lack of TH can cause delayed growth. However, in the latter case, many of the effects of TH may be metabolic rather than developmental, as growth is restored rapidly after TH treatment. In adults, the primary effects of THs are manifested by alterations in metabolism. These effects include changes in oxygen consumption, protein, carbohydrate, lipid, and vitamin metabolism. The clinical features of hypothyroidism and hyperthyroidism emphasize the pleiotropic effects of these hormones on many different pathways and target organs.

Since the initial description of TH effects on metabolic rate more than 100 years ago, many theories have been proposed to explain its mechanism of hormone action. The proposed models include: uncoupling oxidative phosphorylation, stimulation of energy expenditure by the activation of Na^+-K^+ ATPase activity, and direct modulation of TH transporters and enzymes in the plasma membrane and mitochondria. Recently, there has been increasing evidence for non-genomic actions; however, the major effects of TH occur via nuclear receptors that mediate changes in gene expression.

In many respects, T_4 can be regarded as a prohormone for the more potent hormone, T_3. Most of the TH bound to receptors is in the form of T_3, either secreted into the

circulation by the thyroid gland or derived from T_4 to T_3 conversion by 5' monodeiodinases. There are three distinct deiodinases- type I, type II, and type III. The distribution and regulation of these enzymes can have important effects on TH action. For example, Type II deiodinase has high affinity for T_4 and is found primarily in the pituitary gland, brain, and brown fat where conversion of T_4 to T_3 modulates the intracellular concentration of T_3. Thus, tissues that contain type II deiodinase can respond differently to a given circulating concentration of T_4 (by intracellular conversion to T_3) compared to the organs that only can respond to T_3. T_3 binds to its receptors with approximately 10-15 fold higher affinity than T_4. Nuclear receptors are approximately 75% saturated with TH in brain and pituitary and 50% saturated with TH in liver and kidney. It is notable that the extent of TH receptor occupancy varies in different tissues, providing a mechanism for alterations in circulating TH levels to alter receptor activity. In contrast to the related steroid hormone receptors, TRs are mostly nuclear both in the absence and presence of TH. In fact, TH receptors are tightly associated with chromatin, consistent with their proposed role as DNA-binding proteins that regulate gene expression.

Unit 3 □ Anterior Pituitary Structure, Hormone and Function

Structure

3.1 The Normal Pituitary Gland

The pituitary is a bean-shaped gland located at the base of the brain in the midline. It measures 0.6 cm SI × 0.9 cm AP × 1.3 cm and an average gland weighs 0.6. Females tend to have larger glands, especially during or after pregnancy, with the weight up to 1 g. The gland lies within the bony sella turcica that surrounds it inferiorly and laterally. Superiorly it is covered by the diaphragm sella, a reflection of the dura matter. Lateral to the sella are the cavernous sinuses; anteroinferior is the sphenoid sinus; anterosuperior is the optic chiasma; superior to it is the hypothalamus. The pituitary is composed of two anatomically and functionally distinct parts: the neurohypophysis and the adenohypophysis.

3.1.1 Development

The pituitary gland is formed as a result of two separate developmental processes giving the anterior and posterior lobes.

- The posterior pituitary develops as an extension of the hypothalamus itself. The infundibulum is formed from the neuroectoderm of the floor of the third ventricle and develops to form the posterior pituitary. The median eminance is also formed from neuroectoderm.
- The anterior pituitary is derived from oral epithelium from the roof of the mouth cavity, which migrates upwards towards the neural tube. This outgrowth is known

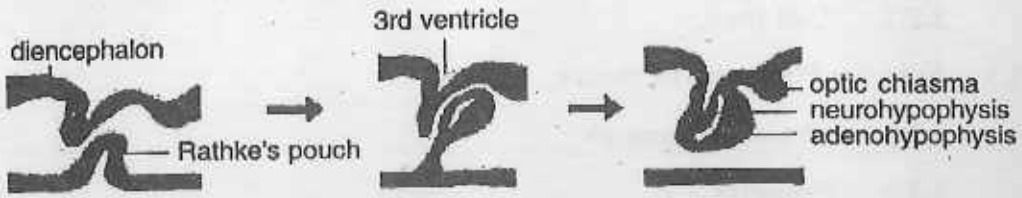


Fig. 3.1 : Development of hypophysial-portal system

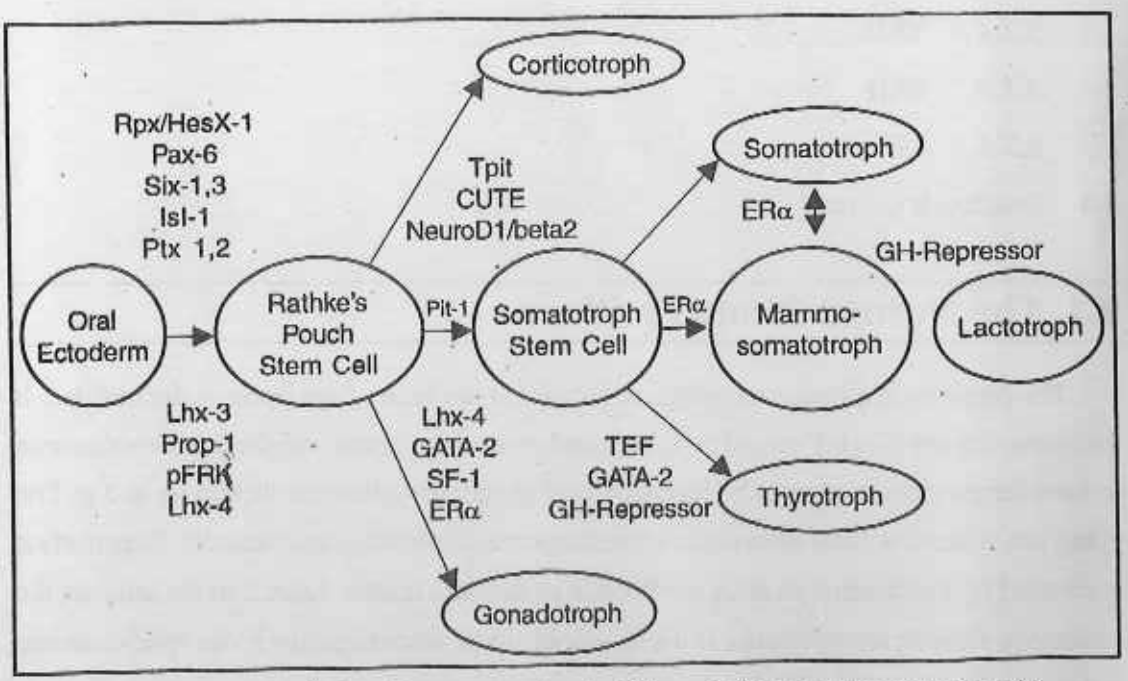


Fig. 3.2 : Pituitary development and cytodifferentiation of adenohypophysis. Transcription factors implicated in each step are identified.

as **Rathke's pouch.** It detaches itself by the 6th week of development although detachment is not always complete and may cause problems such as craniopharyngioma.

- The hypothalamo-pituitary axis is a functional unit by mid gestation.

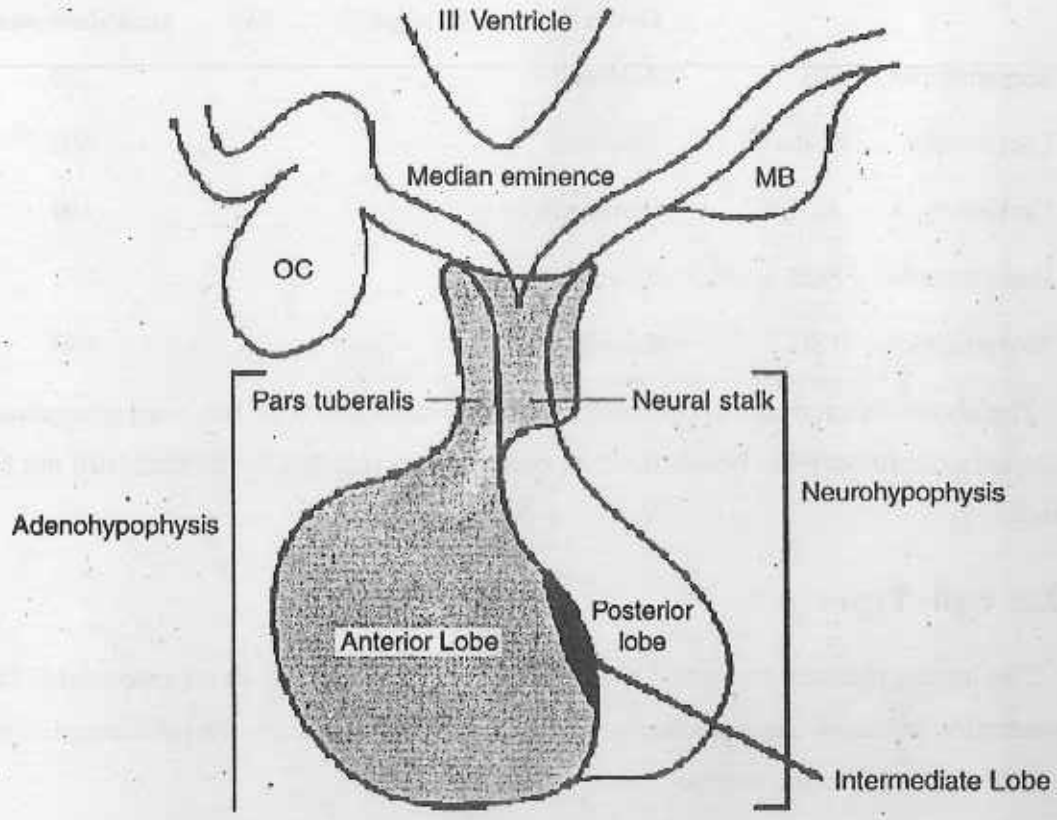


Fig. 3.3 : Anatomical organisation showing hypothalamic and hypophyseal neurovascular link for the control of hormonal secretion from hypothysis cerebri. (OC = optic chiasmea; MB = mammillary body)

3.2 Anterior Pituitary

3.2.1 Histology

The cells of the anterior pituitary were originally classified using techniques for staining intracellular granules as acidophils, basophils and chromophobe cells. Immunocytochemical

and electron microscopic techniques now permit classification of cells by their specific secretary products—

CELL TYPES	HORMONE SECRETED	STAINING REACTION			GRANULAR SIZE
		General	Orange-G	PAS	(nm/diameter)
1. Somertotophs	GH	Acidophil	+	–	350
2. Lactotrophs	Prolactin	Acidophil	+	–	600
3. Corticotrophs	ACTH	Chromophobe	–	–	100
4. Gonadotrophs	FSH + LH	Basophil	–	+	200
5. Thyrotrophs	TSH	Basophil	–	+	140

The above different cell types which secrete specific hormone has been recognised in the antaior pituitary but beside that few cells also present but their nature still not be found.

3.2.2 Cell Types :

The human pituitary contains 5 or more distinct cell types, which are responsible for the secretion of atleast 6 independent hormon. It is logical to classify the pituitary cells on the basic of the hormone secreted.

1. **Somatotrophic cell :** It is rounded or ovoid, the cytoplasm is packed with dense and round granules 350-400 nm in size. These cells account for about 4-10% of the net weight of the anterior pituitary.

2. **Lactotrophic cell :** A second but distinct staining cell randomly distributed in the anterior pituitary has been associated with prolactin secretion. Granule are frequently ovoid or elleypsoidal and approx. 600 nm on plectron microscopy. *These cells proliferate during pregnancy* as a result of elevated estrogen levels and account for the increase in gland size.

3. **Thyrotrophic cell :** These TSH—secreting cells, because of their glycoprotein product, are basophilic and also show a positive reaction with PAS stain. The thyrotroph granules are small (50-140 nm) polygonal (found in rat) with small nucleus. These cells are usually located in the anteromedial and anterolateral portions of the gland.

4. **Corticotrophic cell :** Characterisation of these cell have been difficult on morphological ground. Immanofluorescent studies with antibodies that react with corticotrophin have definitely localised the hormone within basophilic cells, but some cells also may fluorescene with antibodies against thyrotrophin. Considering the similarities in the amino acid sequences in the structure of melanocyte stimulating hormone (MSH) and ACTH, it is reported that these two hormones are secreted from the same cells.

5. **Gonadotrophic cell :** The gonadotrophins are secreted by basophilic cells which are PAS+ve. These cells are located in the lateral portion of the gland. They become hypertrophied and cause the gland to enlarge during states of primary gonadal failure. Such as Klinefelter's Syndrome and Turner's Syndrome. A provisional separation of two types of gonadotrophin cells has been made by *Barnes* :

(a) FSH gonadotrophic cells are larger, rounded whose secretory granules are spherical between 150 and 300 nm in diameter. Certain other differences in the E.R and mitochondria have been noted.

(b) LH or ICSH gonadotrophic cells are comparatively small with scant cytoplasm. The cells are rounded or polygonal and found in proximity of the sinusoidal capillaries. These cells also contain spherical secretory granules having diameter 100-300 nm which are of uniform electron dense appearence.

6. **Other cell types :** Despite immunocytochemical staining with antibodies directed against all of the known anterior pituitory hormones, some cells remain unstained.

These are chromophobes by conventional staining methods, but electron microscopy has identified secretory granules in many of them. It is not certain whether they represent undifferentiated primitive secretary cells of whether they produce an as yet unidentified hormone, such as adrenal androgen—stimulating hormone or ovarian growth factor.

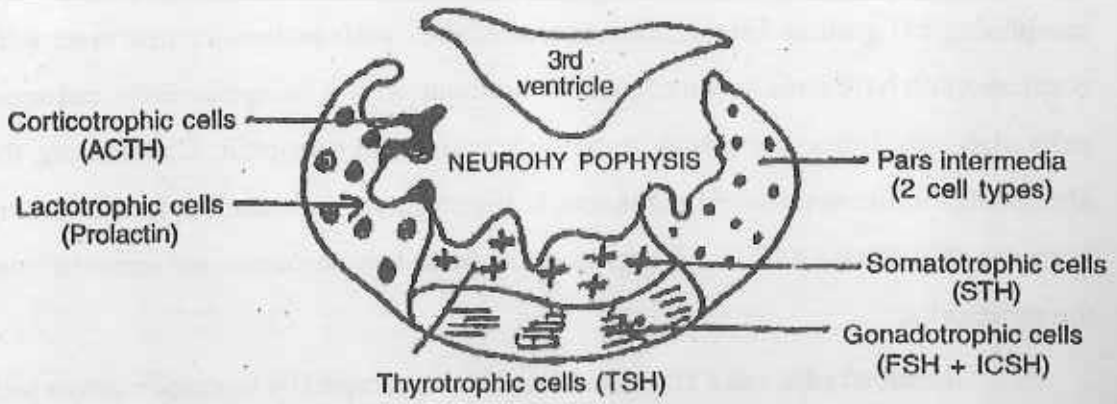


Fig. 3.4 : Diagram of a mid sagital section of the pituitary gland of a bony fish (*Poecilia*) showing Localisation of cell types in adenohypophysis.

3.3 Anterior Pituitary Hormones

There are 6 major anterior pituitary hormones whose biosynthesis, structure, function and secretary control have been well characterized and which can be accurately measured in tissue of body fluids. These hormone—ACTH, GH, PRL, TSH, LH and FSH may be classified into 3 goups : corticotropin related peptides (ACTH, LPH, MSH and endorphins), the somato mammotropins (GH and PRL), which are also peptides; and the glycoproteins (LH, FSH and TSH).

3.3.1 Growth hormones or Somatotrophic hormone (GH or STH) :

Chemistry : The human growth hormone (HGH) is the smallest of the growth hormone that have been examined with molecular weight 21,500. It is composed of a single

chain of 188 amino acids *without carbohydrate substituents.* In man the amino acid chain probably exists with a large and small loop formed from intramolecular disulphide bonds

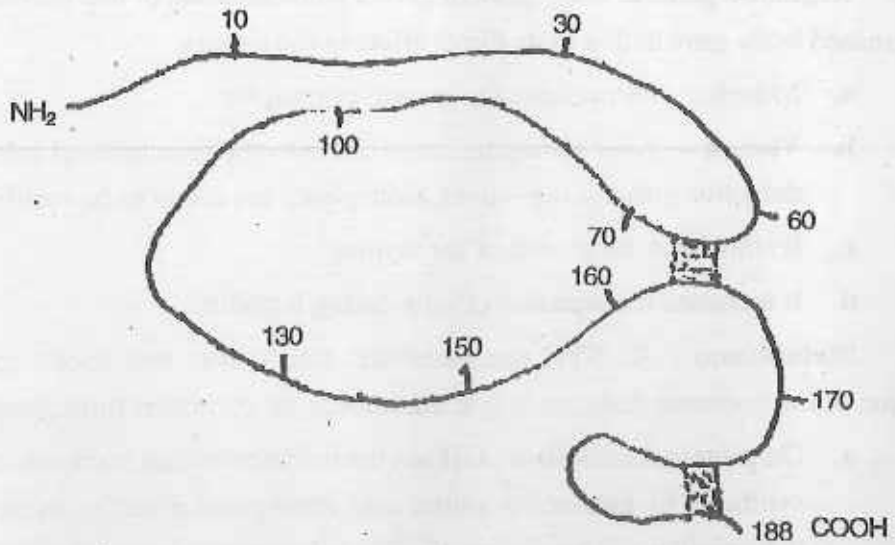


Fig. : Schematic representation of HGH.

unlike the disulphide bonds of insulin and the neurohypophysial hormones, the disulfide bonds of HGH are not essential for biologic activity.

Growth hormone from pig, whale, sheep and cow pituitary is believed to have a significantly larger molecular weight than HGH; Bovine (cattle) GH weights about 46,000 with 400 amino acids. End-group analysis of the bovine hormone has revealed one amino end-group and two carboxyl end groups which suggest that this hormone exists as a branched chain.

It has been noted that human growth hormone (HGH) exerts lactogenic effect along with the growth stimulating one.

Biological action : As its name implies, the primary function of growth hormone (somatotropin) is promotion of linear growth. Its basic metabolic effects serve to achieve this result, but most of the growth promoting effects are mediated by the somatomedins, a family of small peptides produced in the liver.

1. **Skeletal growth :** Stimulates the multiplication of the epiphyseal cartilage and this

increases the length of the cartilage bones. In adult animals with closed epiphysis growth hormone causes thickening of bone.

2. **Regulates general body growth :** After administration of this hormone there is an increased body growth due to its direct effect in the tissues.

   a. **Muscles** — Stimulates the growth of muscles.

   b. **Viscera** — After administration of GH in hypophysectomised animal various defective growths (eg—liver, kidney etc.) are found to be rectified.

   c. It stimulates the growth of the thymus.

   d. It increases the secretion of milk during lactation.

3. **Metabolism :** As STH has metabolic effect over and above growth, the secretion of the hormone does not stop at adulthood, but continues throughout the life.

   a. On protein metabolism : GH via the somatomedians increases the protein synthesis by enhancing amino acid uptake and directly accelerating the transcription and translation of mRNA. It decreeses nitrogen excretion in the urine and the nitrogen thus retained helps in the synthesis of tissue protein. GH is a protein-anaebolic hormone and prevents the catabolism of amino acids.

   b. On fat metabolism : GH tends to decrease protein catabolism by mobilizing fat as a more efficient fuel source. It directly causes the release of fatty acids from adipose tissue and enhances their conversion to acetyl CoA, from which energy is derived. This protein sparing effect may be the most imp. mechanism by which GH promotes growth and development.

   c. On carbohydrate metabolism : GH also affects carbohydrate metabolism. In excess, it decreases carbohydrate utilization and impairs glucose uptake into cells. The high blood glucose level then leads to overproduction of insulin by β-cells and finally to its exhaustion and atrophyse the GH is diabetogenic specially in man.

   d. Ion or mineral metabolism : GH (STH) increases intestinal absorption of calcium as well as its excretion. In addition to calcium, sodium, potassium, magnesium phosphate and chloride are also retained.

4. STH stimulates proliferations of thymic lymphocytes both in *vivo* and in *vitro*.
5. The GH also has lactogenic effect.

3.3.2 Lactogenic hormone/Prolactin :

It is secreted during preguancy and lactation in women by acidophil pregnancy cells.

Chemistry : Prolactin (PRL) is a 198(180–205) amino acid polypeptide hormone (MW-22,000) synthesized and secreted from the lactotrophs of the anterior pituitary. Despite evolution from an ancestral hormone common to GH and human placental lactogen (hpL), PRL share only 16% of its residue with the former and 13% with hpL.) It has one free amino acid group, but on free carboxyl end–group. For this reason, Li has suggested that the peptide chain has an intrachain disultphide bridge forming a ring similar to that present in rasopressin and oxytocin. No bound carbohydrate has been found in prolactin. The nature of human prolactin remains a mystery of current pituitary endocrinology.

Biological Action :

1. Prolactin (LTH) is responsible for lactation in the post partum women, the breast having been prepared by oestrogen and progesterone. It helps initiating (lactogenesis) rather than maintaing milk secretion. Growth or somatotrophic and thyroid hormones help in the maintenance of the secretion of milk (golactopoiesis). The level of prolactin increases during the night. Oestradiol stimulates prolactin release where as L-dopa inhibits it by promoting the discharge of PIF.
2. It stimulates slightly the proliteration of the glandular elements of the mammary glands during pregnancy and thus helps to complete the development of brests.
3. It helps in maintenance of secretary activity of corpus luteum and secretion of the hormone, Progesterone, due to combined action of LH and Prolactin.
4. Prolactin induces changes in maternal behaviour which are imp. for the helpless young. In some birds of both sexes it promotes the resting behaviour. The actual pathways by which prolactin induces these changes have not been established.

5. Under suitable experimental conditions prolactin has been shown to be calorigenic, to be diabetogenic, to promote *protein synthesis,* and to inercase the rate of chondroitin sulphate formation in cartilage. An increase in the weight of the liver and several other organs has been observed in prolactin-treated pigeons.

The possible significance of these various actions of prolactin under physiologic conditions remains to be determined. As yet there is no known role of the hormone in the male sex.

3.3.3 Adrenocorticotrophic hormone (ACTH) :

Chemistry : ACTH is a 39-amino acid peptide hormone. Moleculer weight about 4,500. The amino acids are numbered from the end with the free NH_2 group (the N-terminal end). The first 24 are common to ACTH from man and other mammals and biological activity is provided by the first 20. The arangement of amino acids 25 to 33 varies in difference species (found in man is shown). It has been isolated in α and β forms. Both these forms contain a large number of amino acids and have got straight chain linkage. Unlike other pituitary hormones, can withstand heating to 100°C.

Biological action :

1. The primary effect of ACTH is to stimulate the secretion of glucocorticoids, mineralocorticoids and androgenic steroids from the adrenal cortex is responsible for this biologic activity. ACTH binds to receptors on the adrenal cortex and provokes steroidogenesis through the mediation of cyclic 3', 5'-adenosine monophosphate (cyclic AMP, CAMP).

2. In addition to this, some of the effects of ACTH on the adrenal cortex are the following :

   a. Angmented oxidative phosphorylation.
   b. Increased protein synthesis.
   c. Accelerated glycolysis.
   d. Altered lipid metabolism.
   e. Ascorbic acid depletion.

3. Corticotropic also induces changes in carbohydrate metabolism in the adrenalectomized animal.

Administration of ACTH to normal human being produces the following effect :

a. Increased excretion of N_2, K and P.
b. Retention of sodium chloride and secondary retention of water.
c. Elevation of fasting blood sugar and a diabetic glucose tolerance curoc.
d. Increase in circulating free fatty acids.
e. Increased excretion of uric acid.
f. Decline in circulating cosinophils and lymphocytes, and increase in neutrophils.
g. It has a slight melanocyte-stimulating effect.

3.3.4 Thyrotropin/Thyroid Stimulating Hormone (TSH) :

Chemistry : Chemically, TSH is a basic glycoprotein with a molecular weight of about 28,000. It is composed of 2 non covalently linked subunits termd α and β. α-chain consists of 89 amino acids residues and αβ-chain with 112 amino acid residues. The structure of the α-subunit of TSH resembles—FSH, LH and human chorionic gonadotropin (hcG)—but the G subunit differs in these glycoproteins and is responsible for their biologic and immunologic specificity.

Biological action : Controls the growth and activity of the thyroid gland. Primary physiologic action of TSH is probably to stimulate the release of thyroid hormone from the intrafollicular thyroglobulin. TSH is necessory for ocoupling of di-iodotyrosine to form thyroxine (T_4).

1. Thyrotropin can be shown to increase iodine uptake, iodine clearance from the plasma, iodotyrosine and iodothyronine formation. Thyroglobulin Proteolysis and thyroxine release from thyroid gland.

This occurs through activation of adenylate cyclase and the generation of cAMP.

2. There is an increase in respiration, nucleic acid synthesis, glucose utilization and fatty acid release.
3. TSH accelerates lipolysis by isolated rat adipose tissue in vitro.
4. TSH may exert a direct effect upon extra-ocular retrobuller structures (lipid, muscle, connective tissue), with consequent proteusion of the eye ball (exoph thalmos).

3.3.5 Follicle-Stimulating Hormone (FSH) :

FSH is a heterodimeric glycoprotein consisting of

- the same alpha chain found in TSH (and LH)
- a beta chain of 115 amino acids, which gives it its unique properties.

Synthesis and release of FSH is triggered by the ***gonadotropin-releasing hormone*** (**GnRH**) from the hypothalamus. The effect of FSH depends on one's sex.

FSH in females

In sexually-mature females, FSH (assisted by LH) acts on the follicle to stimulate it to release ***estrogens***. FSH produced by recombinant DNA technology is available to promote ovulation in women planning to undergo *in vitro fertilization* (IVF) and other forms of assisted reproductive technology.

FSH in males

In sexually-mature males, FSH acts on spermatogonia to stimulate (with the aid of testosterone) the production of sperm.

3.3.6 Luteinizing Hormone (LH) :

LH is synthesized within the same pituitary cells as FSH and under the same stimulus (**GnRH**). It is also a heterodimeric glycoprotein consisting of

- the same 89-amino acid alpha subunit found in FSH and TSH (as well as in chorionic gonadotropin);
- a **beta** chain of 115 amino acids is responsible for its charecteristic properties.

The effects of LH also depend on sex.

LH in females

In sexually-mature females,

- a surge of LH triggers the completion of *meiosis I* of the egg and its release (**ovulation**) in the middle of the cycle;
- LH also stimulates the empty follicle cells to develop into the **corpus luteum**, which secretes **progesterone** during the latter half of the menstrual cycle.

Women with a severe LH deficiency can now be treated with human LH produced by recombinant DNA technology.

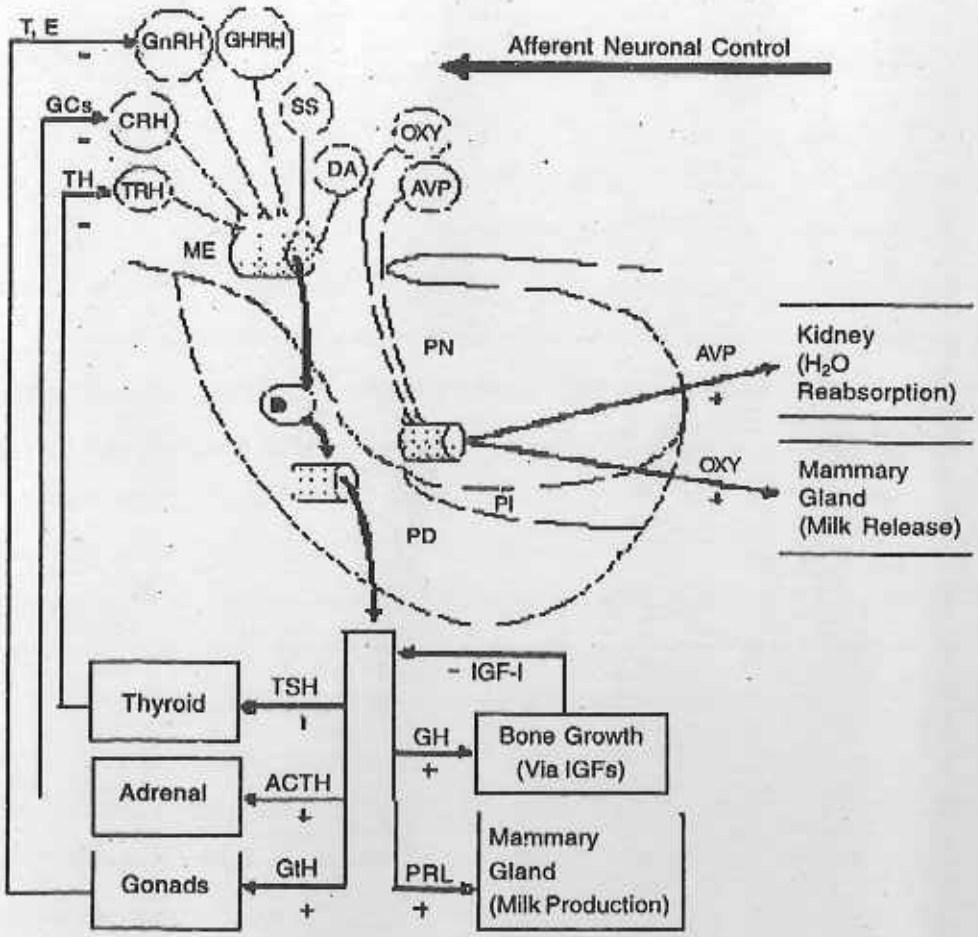
LH in males

LH acts on the interstitial cells (also known as Leydig cells) of the **testes** stimulating them to synthesize and secrete the male sex hormone, **testosterone**. LH in males is also known as **interstitial cell stimulating hormone (ICSH).**

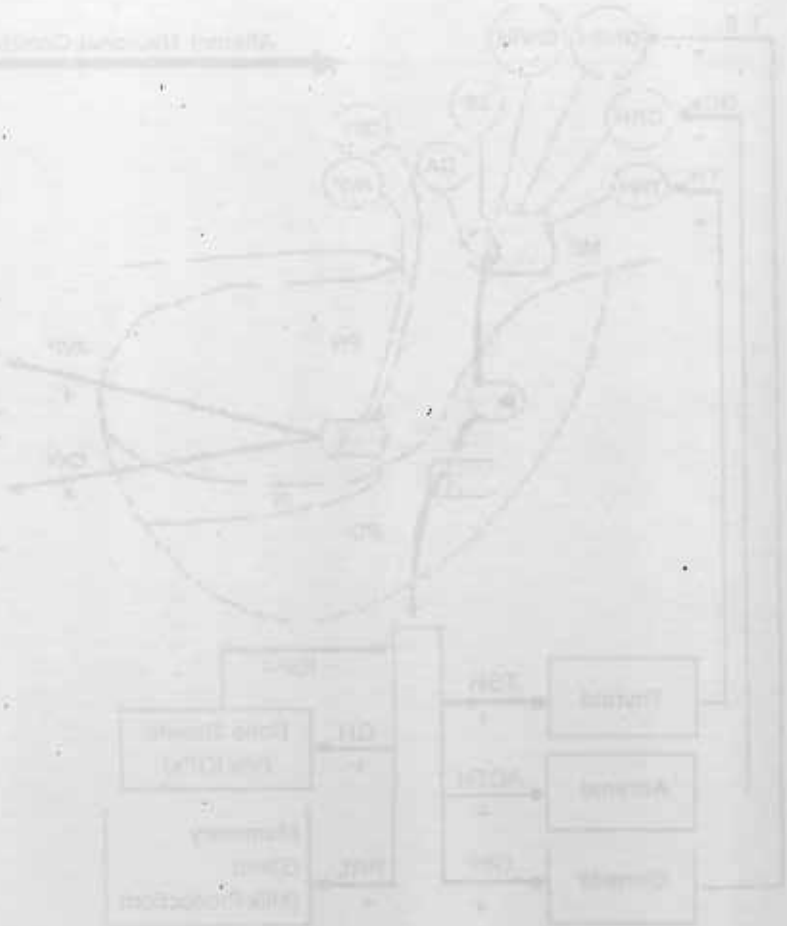
3.4 Feedback Control

Negative feedback is an important factor in controlling the hypothalamic-pituitary-target organ axis function. Once hypothalamic hormones stimulate the release or inhibition

Regulation of the Pituitary gland by the Hypothalamus



of the pituitary hormone, this may then act on a target gland, such as the thyroid, causing release of further hormones or causing metabolic effects. The action of hypothalamic hormones may be inhibited by long feedback loops from the target gland hormone or by short feedback loops from the pituitary hormone. There may also be direct feedback from the target gland hormone to the pituitary gland. Input is also received at the hypothalamus from higher brain centres, which can be due to internal or external influences. Positive feedback action also plays a partial in certain systems. For example, in the situation where high levels of oestradiol in the blood causes a surge in LH levels during the menstrual cycle.



Unit 4 □ Adrenal Cortical Hormone, Biosynthesis and Function

Structure

4.1 Introduction

The adrenal glands are small paired structures situated above each kidney. Both in anatomy and in function, they consist of two distinct regions :

an outer layer, the **adrenal cortex**, which surrounds the **adrenal medulla**.

4.2 The Adrenal Cortex

Using cholesterol as the starting material, the cells of the adrenal cortex secrete a variety of **steroid** hormones. **Glucocorticoids** (e.g., cortisol), **Mineralocorticoids** (e.g., aldosterone) and **Androgens** (e.g., testosterone)

Production of all these hormones are triggered by the secretion of ACTH from the anterior lobe of the pituitary.

These hormones achieve their effects by:

- travelling through the body in the blood. Because they are so hydrophobic, they must be carried bound to a serum globulin.
- entering from the blood into all cells
- binding to their **receptor** — a protein present in the cytoplasm and/or nucleus of "target" cells
- The hormone-receptor complex binds to a second to form a **dimer.**
- The dimer migrates into the nucleus (if it did not form there).
- The hormone-receptor dimer binds to specific **hormone response elements** in DNA.
- These are specific DNA sequences in the promoter of genes that will be turned on (sometimes off) by the interaction.
- Other **transcription factors** are recruited to the promoter and gene transcription begins.

4.2.1 Glucocorticoids

Glucocorticoids (GC) are a class of steroid hormones that bind to the glucocorticoid receptor (GR), which is present in almost every vertebrate animal cell. The glucocorticoids get their name from their effect of raising the level of blood sugar (glucose). One way they do this is by stimulating **gluconeogenesis** in the liver: the conversion of fat and protein into intermediate metabolites that are ultimately converted into glucose. (Fig. 1)

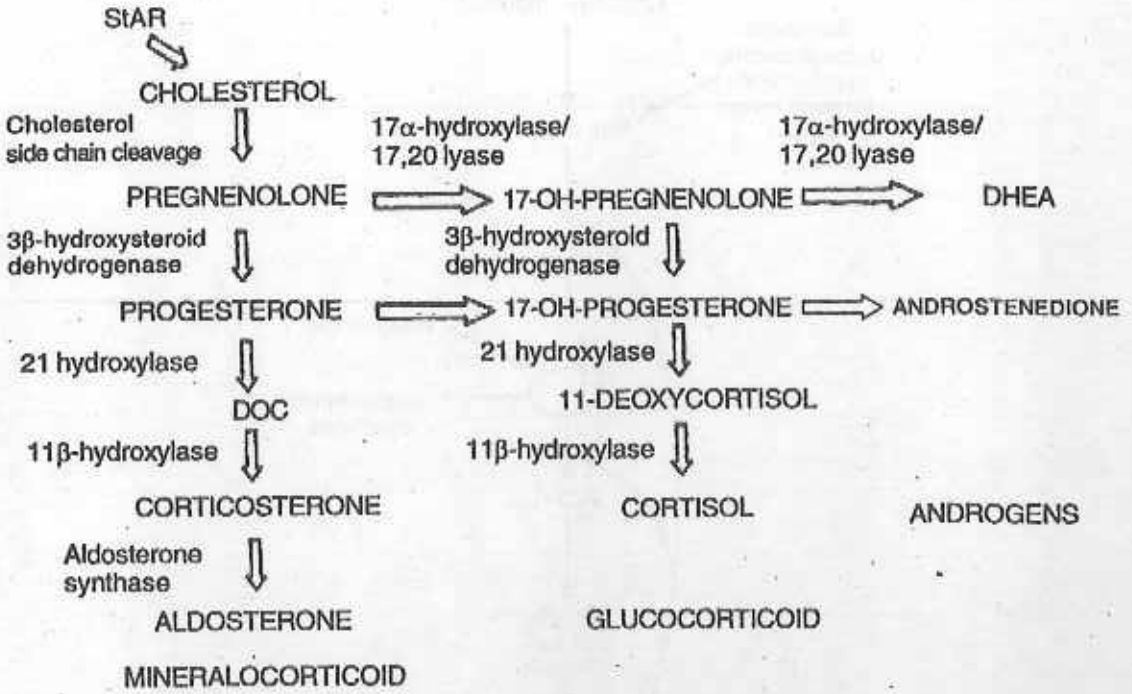


Fig. 1. Adrenal steroidogenesis. After the steroidogenic acute regulatory (StAR) protein-mediated uptake of cholesterol into mitochondria within adrenocortical cells, aldosterone, cortisol, and adrenal androgens are synthesized through the coordinated action of a series of steroidogenic enzymes in a zone-specific fashion. Androstenedione; DHEA, dehydroepiandrosterone; DOC, deoxycorticosterone.

GCs are part of the feedback mechanism in the immune system that turns immune activity (inflammation) down. They are therefore used in medicine to treat diseases that are caused by an overactive immune system, such as allergies, asthma, autoimmune diseases and sepsis. GCs have many diverse (pleiotropic) effects, including potentially harmful side effects. They also interfere with some of the abnormal mechanisms in cancer cells, so they are used in high doses to treat cancer.

GCs cause their effects by binding to the glucocorticoid receptor (GR). The activated GR complex in turn up-regulates the expression of anti-inflammatory proteins in the nucleus (a process known as transactivation) and represses the expression of pro-inflammatory proteins in the cytosol by preventing the translocation of other transcription factors from the cytosol into the nucleus (transrepression).

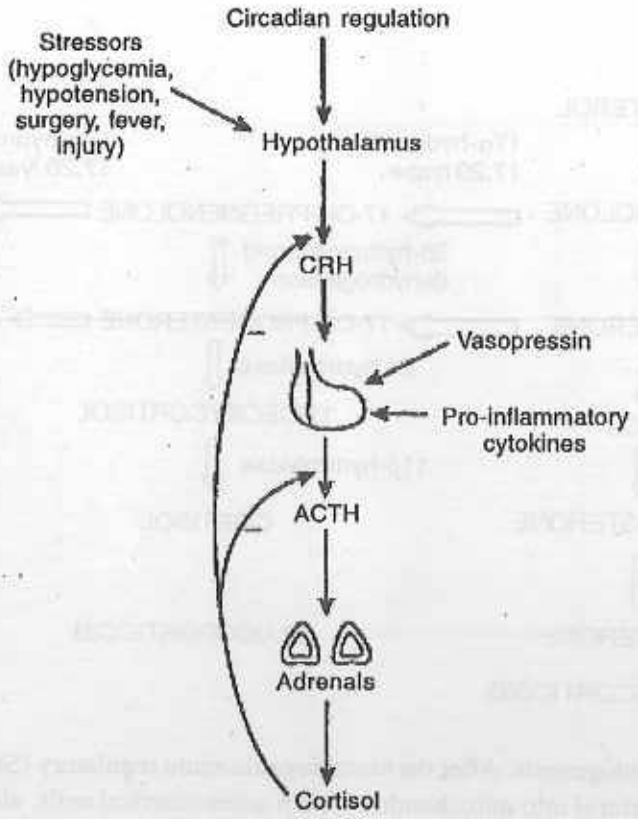


Fig. 2. Normal regulation of adrenal glucocorticoid secretion Adrenocorticotropic hormone (ACTH) is secreted from the interior pituitary under the influence of two principal secretagogues, cor tropin-relcasing hormone (CRH) and arginine vasopressin; other factors including cytokines also play a role. CRH secretion is regulated by an inbuilt circadian rhythm and additional stressors oper through the hypothalamus. Secretion of both CRH and ACTH inhibited by cortisol, highlighting the importance of negative feed control.

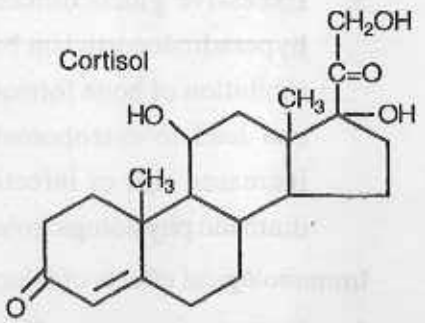
GCs are distinguished from mineralocorticoids and sex steroids by their specific receptors, target cells, and effects. Corticosteroid refers to both glucocorticoids and mineralocorticoids (as both are mimics of hormones produced by the adrenal cortex).

Cortisol (or hydrocortisone) is the most important and abundant human glucocorticoid. It is essential for life, and it regulates or supports a variety of important cardiovascular, metabolic, immunologic, and homeostatic functions. Glucocorticoid receptors are found in

the cells of almost all vertebrate tissues. Various synthetic glucocorticoids are available; these are used either as replacement therapy in glucocorticoid deficiency or to suppress the immune system.

Cortisol and the other glucocorticoids also have a potent anti-inflammatory effect on the body. They depress the immune response, especially cell-mediated immune responses.

For this reason glucocorticoids are widely used in therapy to reduce the inflammatory destruction of rheumatoid arthritis and other autoimmune diseases, to prevent the rejection of transplanted organs and to control asthma.



4.2.1.1 Functions

Glucocorticoid effects may be broadly classified into two major categories: metabolic and immunological. In addition, glucocorticoids play important roles in fetal development.

As discussed in more detail below, glucocorticoids through interaction with the glucocorticoid receptor up-regulate the expression of anti-inflammatory proteins and down-regulate the expression of pro-inflammatory proteins.

The name "glucocorticoid" derives from early observations that these hormones were involved in glucose metabolism. In the fasted state, cortisol stimulates several processes that collectively serve to increase and maintain normal concentrations of glucose in blood.

Metabolic effects of Glucocorticoids :

1. Stimulation of gluconeogenesis, particularly in the liver : This pathway results in the synthesis of glucose from non-hexose substrates such as amino acids and glycerol from triglyceride breakdown, and is particularly important in carnivores and certain herbivores. Enhancing the expression of enzymes involved in gluconeogenesis is probably the best-known metabolic function of glucocorticoids.
2. Mobilization of amino acids from extrahepatic tissues : These serve as substrates for gluconeogenesis.

3. Inhibition of glucose uptake in muscle and adipose tissue : A mechanism to conserve glucose.
4. Stimulation of fat breakdown in adipose tissue : The fatty acids released by lipolysis are used for production of energy in tissues like muscle, and the released glycerol provide another substrate for gluconeogenesis.
5. Excessive glucocorticoid levels resulting from administration as a drug or hyperadrenocorticism have effects on many systems. Some examples include inhibition of bone formation, suppression of calcium absorption (both of which can lead to osteoporosis), delayed wound healing, muscle weakness, and increased risk of infection. These observations suggest a multitude of less-dramatic physiologic roles for glucocorticoids.

Immunological effects of Glucocorticoids :

1. Immunosuppression: Glucocorticoids suppress the cell-mediated immunity. They act by inhibiting genes that code for the cytokines IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-8 and IFN-γ, the most important of which is IL-2. Smaller cytokine production reduces the T cell proliferation. Glucocorticoids do however not only reduce T cell proliferation, but also lead to another well known effect called glucocorticoid induced apoptosis. The effect is more prominent in immature T cells that still reside in the thymus, but also affect peripheral T cells. The exact mechanism underlying this glucocorticoid sensitivity still remains to be elucidated. Glucocorticoids also suppress the humoral immunity, causing B cells to express smaller amounts of IL-2 and of IL-2 receptors. This diminishes both B cell clone expansion and antibody synthesis. The diminished amounts of IL-2 also causes fewer T lymphocyte cells to be activated. Since glucocorticoid is a steroid, it regulates transcription factors.
2. Anti-inflammatory: Glucocorticoids are potent anti-inflammatories, regardless of the inflammation's cause. Glucocorticoids' primary anti-inflammatory mechanism is lipocortin-1 (annexin-1) synthesis. Lipocortin-1 both suppresses phospholipase A2, thereby blocking eicosanoid production, and inhibits various leukocyte inflammatory events (epithelial adhesion, imigration, chemotaxis, phagocytosis, respiratory burst, etc.). In other words, Glucocorticoids not only suppress immune response, but also inhibit the two main products of inflammation,

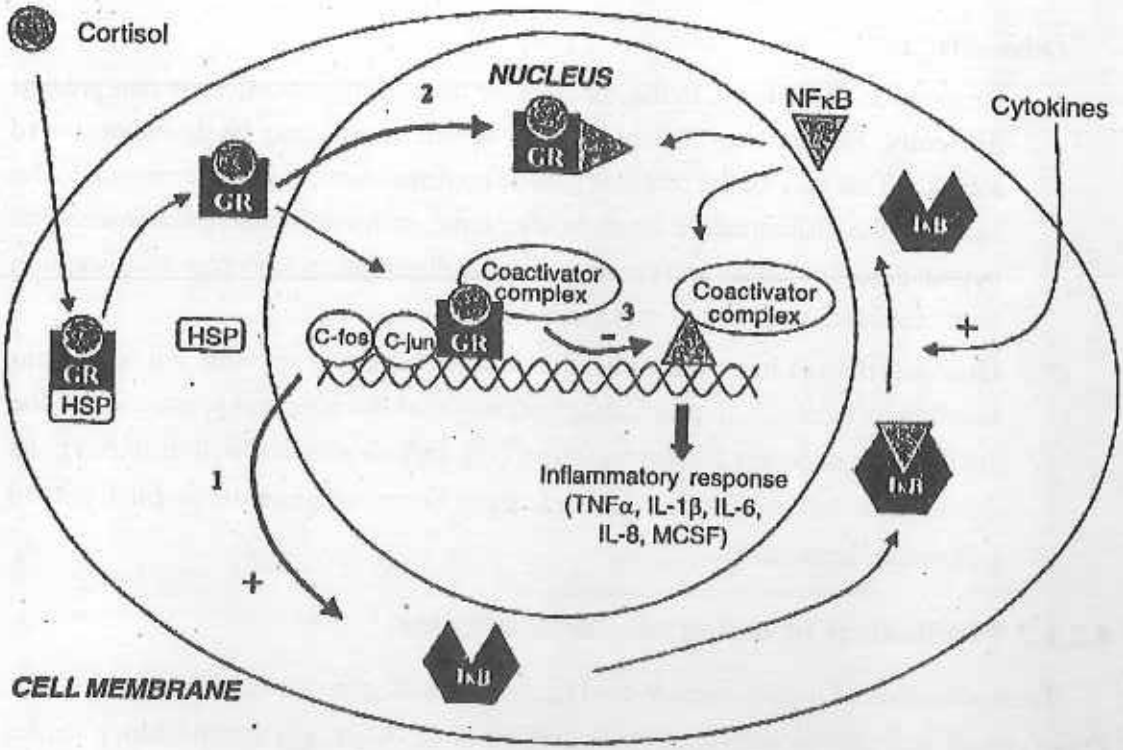


Fig. 3. The anti-inflammatory action of glucocorticoids. Cortisol binds to the cytoplasmic glucocorticoid receptor (GR). Conformational changes in the receptor-ligand complex result in dissociation from heat shock proteins (HSPs) 70 and 90 and migration to the nucleus. Binding occurs to specific DNA motifs—glucocorticoid response elements in association with the activator protein-1 (AP-1) comprising c-fos and c-jun. Glucocorticoids mediate their anti-inflammatory effects through several mechanisms: (1) The inhibitory protein I/cB, which binds and inactivates nuclear factor «B (NFxB), is induced. (2) The GR-cortisol complex is able to bind NFicB and thus prevent initiation of an inflammatory process. (3) Both GR and NF/cB compete for the limited availability of coactivators that include cyclic adenosine monophosphate response element binding protein (CREB) and steroid receptor coactivator-1.

prostaglandins and leukotrienes. In addition, glucocorticoids also suppress cyclooxygenase (both COX-1 and COX-2) expression much like NSAIDs, potentiating the anti-inflammatory effect. Glucocorticoids marketed as anti-inflammatories are often topical formulations, such as nasal sprays for rhinitis or inhalers for asthma. These preparations have the advantage of only affecting the targeted area, thereby reducing side effects or potential interactions.

Other effects :

1. Resistance: Resistance to the therapeutic uses of glucocorticoids can present difficulty; for instance, 25% of cases of severe asthma may be unresponsive to steroids. This may be the result of genetic predisposition, ongoing exposure to the cause of the inflammation (such as allergens), immunological phenomena that bypass glucocorticoids, and pharmacokinetic disturbances (incomplete absorption or accelerated excretion or metabolism).
2. Glucocorticoids have multiple effects on fetal development. An important example is their role in promoting maturation of the lung and production of the surfactant necessary for extrauterine lung function. Mice with homozygous disruptions in the corticotropin-releasing hormone gene die at birth due to pulmonary immaturity.

4.2.1.2 Mechanism of action of glucocorticoids

Transactivation : Glucocorticoids bind to the cytosolic glucocorticoid receptor (GR). This type of receptor is activated by ligand binding. After a hormone binds to the corresponding receptor, the newly-formed receptor-ligand complex translocates itself into the cell nucleus, where it binds to glucocorticoid response elements (GRE) in the promoter region of the target genes resulting in the regulation of gene expression. This process is commonly referred to as transactivation.

Dissociation : The ordinary glucocorticoids do not distinguish among transactivation and transrepression and influence both the "wanted" immune and "unwanted" genes regulating the metabolic and cardiovascular functions. Intensive research is aimed at discovering selectively acting glucocorticoids that will be able to repress only the immune system.

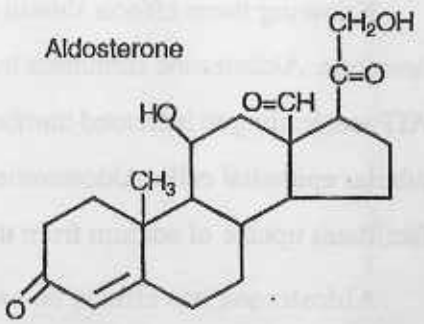
Therapeutic use : Glucocorticoids may be used in low doses in adrenal insufficiency. In much higher doses, glucocorticoids are used to suppress various allergic, inflammatory, and autoimmune disorders. They are also administered as posttransplantory immunosuppressants to prevent the acute transplant rejection and the graft-versus-host disease. Nevertheless, they do not prevent an infection and also inhibit later reparative processes.

Physiological replacement : Any glucocorticoid can be given in a dose that provides approximately the same glucocorticoid effects as normal cortisol production. This is approximately 6-12 mg/m²/day (m² refers to body surface area (BSA), and is a measure of body size; an average man is 1.7 m²).

4.2.2 Mineralocorticoids

Mineralocorticoids are acutely critical for maintenance of life. The mineralocorticoids get their name from their effect on mineral metabolism. Removal of the adrenal glands leads to death within just a few days reflecting a direct result of loss of mineralocorticoid activity Observation of such a unfortunate subject would reveal several key derangements :

- the concentration of potassium in extracelluar fluid becomes dramatically elevated
- urinary excretion of sodium is high and the concentration of sodium in extracellular fluid decreases significantly
- volume of extracellular fluid and blood decrease
- the heart begins to function poorly, cardiac output declines and shock ensues



4.2.2.1 Aldosterone and Mineralocorticoid Receptors

The most important mineralocorticoid is **aldosterone.**

Aldosterone acts on the kidney promoting the reabsorption of sodium ions (Na^+) into the blood. Water follows the salt and this helps maintain normal blood pressure. Aldosterone also acts on sweat glands to reduce the loss of sodium in perspiration and taste cells to increase the sensitivity of the taste buds to sources of sodium. The secretion of aldosterone is stimulated by a drop in the level of sodium ions in the blood, a rise in the level of potassium ions in the blood and angiotensin II.

4.2.2.2 Functions of Mineralocorticoids

Mineralocorticoids play a critical role in regulating concentrations of minerals - particularly sodium and potassium - in extracellular fluids. As described above, loss of these hormones leads rapidly to life-threatening abnormalities in electrolyte and fluid balance. The major target of aldosterone is the distal tubule of the kidney, where it stimulates exchange of sodium and potassium. Three primary physiologic effects of aldosterone are known. Increased resorption of sodiumic sodium loss in urine is decreased under aldosterone stimulation. Increased resorption of water, with consequent expansion of extracellular fluid volume. This is an osmotic effect directly related to increased resorption of sodium. Increased renal excretion of potassium.

Knowing these effects should quickly suggest the cellular mechanism of action this hormone. Aldosterone stimulates transcription of the gene encoding the sodium-potassium ATPase, leading to increased numbers of "sodium pumps" in the basolateral membranes of tubular epithelial cells. Aldosterone also stimulates expression of a sodium channel which facilitates uptake of sodium from the tubular lumen.

Aldosterone has effects on sweat glands, salivary glands and the colon which are essentially identical to those seen in the distal tubule of the kidney. The major net effect is again to conserve body sodium by stimulating its resorption or, in the case of the colon, absorption from the intestinal lumen. Conservation of water follows conservation of sodium.

4.2.2.3 Physiology

The name mineralocorticoid derives from early observations that these hormones are involved in the retention of sodium–a mineral. The primary endogenous mineralocorticoid is aldosterone, although a number of other endogenous hormones (including progesterone and deoxycorticosterone) have mineralocorticoid function.

Aldosterone acts on the kidneys to provide active reabsorption of sodium and an associated passive reabsorption of water, as well as the active secretion of potassium in the principal cells of the cortical collecting tubule and active secretion of protons via proton ATPases in the luminal membrane of the intercalated cells of the collecting tubule. This in turn results in an increase of blood pressure and blood volume.

Control over aldosterone secretion is truly multifactorial and tied into a spider web of other factors which regulate fluid and electrolyte composition and blood pressure. If the major effects of aldosterone are considered, it is rather easy to predict factors which stimulate or suppress aldosterone secretion. (Fig. 4)

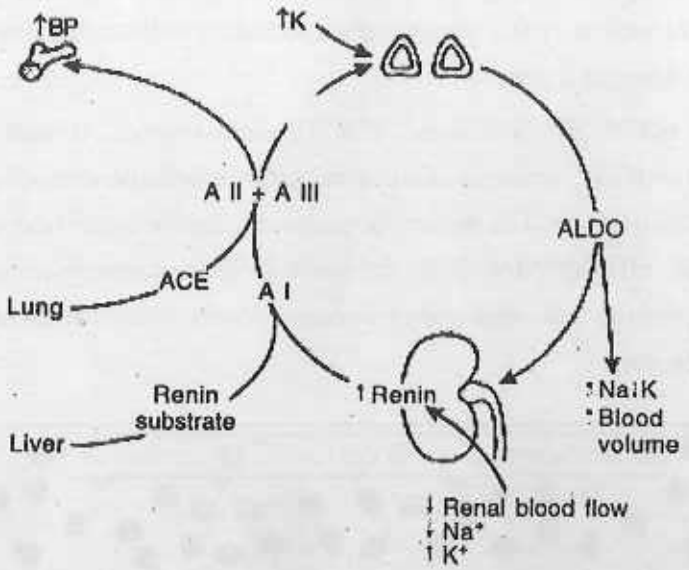


Fig. 4. The normal renin-angiotensin-aldosterone regulatory system. Renin, secreted by die kidney, cleaves angiotensin I (A I) from renin substrate (angiotensinogen), an α_2-globulin produced by the liver. Angiotensin I is converted into biologically active angiotensin II by angiotensin-converting enzyme (ACE), mainly in the lung. Angiotensin II increases peripheral vascular resistance, and, together with angiotensin III, stimulates aldosterone (ALDO); secretion, which results in sodium retention and increased plasma volume.

The two most significant regulators of aldosterone secretion :

- Concentration of potassium ions in extracellular fluid: Small increases in blood levels of potassium strongly stimulate aldosterone secretion.

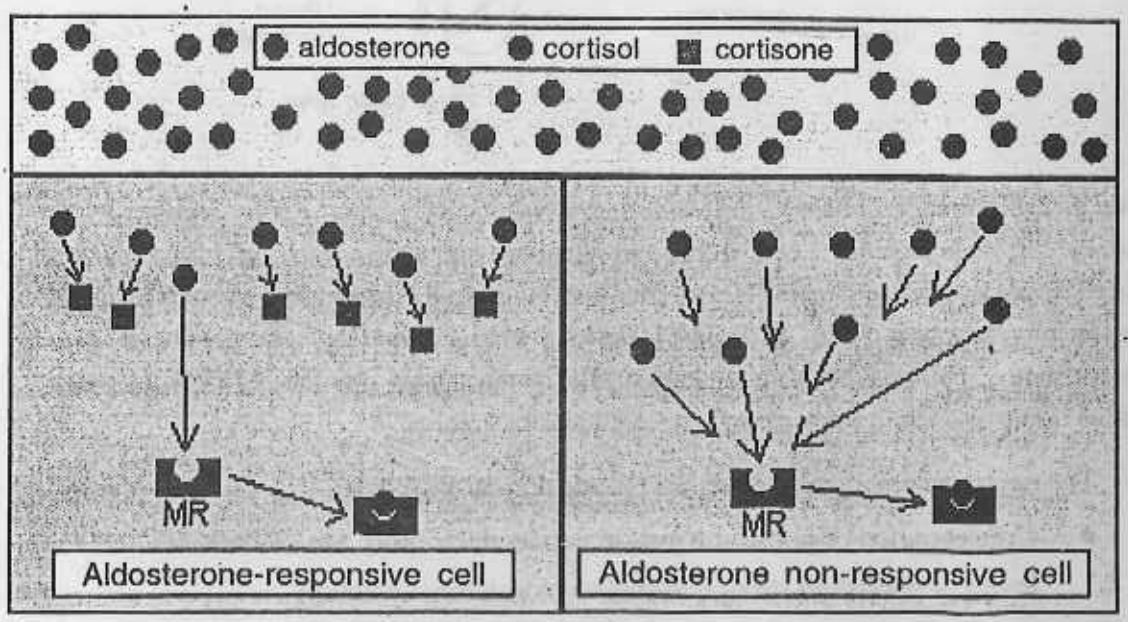
- Angiotensin II: Activation of the renin-angiotensin system as a result of decreased renal blood flow (usually due to decreased vascular volume) results in release of angiotensin II, which stimulates aldosterone secretion.

Other factors which stimulate aldosterone secretion include adrenocorticotropic hormone (short-term stimulation only) and sodium deficiency. Factors which suppress aldosterone secretion include atrial naturetic hormone, high sodium concentration and potassium deficiency.

4.2.2.4 Mechanism of action of mineralocorticoids

The effects of mineralocorticoids are mediated by slow genomic mechanisms through nuclear receptors as well as by fast nongenomic mechanisms through membrane-associated receptors and signaling cascades.

Cortisol, the major glucocorticoid in non-rodent species, is said to have "weak mineralocorticoid activity", which is of some importance because cortisol is secreted more abundantly than aldosterone. The mineralocorticoid receptor binds both aldosterone and cortisol with equal affinity. Moreover, the same DNA sequence serves as a hormone response element for the activated (steroid-bound) forms of both mineralocorticoid and glucocorticoid receptors.



In aldosterone-responsive cells, cortisol is effectively destroyed, allowing aldosterone to bind its receptor without competition. Target cells for aldosterone express the enzyme 11-beta-hydroxysteroid dehydrogenase, which has no effect on aldosterone, but converts cortisol to cortisone, which has only a very weak affinity for the mineralocorticoid receptor. In essence, this enzyme "protects" the cell from cortisol and allows aldosterone to act appropriately. Some tissues (e.g. hippocampus) express abundant mineralocorticoid receptors but not 11-beta HSD - they therefore do not show responses to aldosterone because aldosterone is not present in quantities sufficient to compete with cortisol. 11-beta hydroxysteroid dehydrogenase type II catalyzes the deactivation of glucocorticoids to 11-dehydro metabolites.

18 hydroxy 11 deoxycorticosterone (also designated 18OH-DOC) is a steroid hormone probably used to conserve sodium and stimulate hydrogen ion (or acid) excretion. 18OH-DOC lowers urine pH but has no affect on potassium excretion. This would seem to indicate that 18OH-DOC's primary purpose is to stimulate hydrogen ion or ammonium excretion. Under low sodium intake 18 OH DOC is increased in serum. There is a marked increase in serum 18OH DOC after injection of insulin and this may be due to the hypokalemic (low serum potassium) tendency after a rise in insulin which in turn would make the serum more acidic. Since 18OH-DOC lowers urine pH (increases acidity) but has no affect on potassium excretion. This would seem to indicate that 18OH-DOC's primary purpose is to stimulate hydrogen ion or ammonium excretion. Its use by the body to conserve potassium would be indirect by virtue of hydrogen ion's interference with potassium excretion. This interference is further indicated because injecting sodium bicarbonate or even hyperventilating (breathing rapidly beyond need) can triple potassium excretion. The daily rhythm for potassium and hydrogen ion excretion show a rather close inverse relationship, which gives additional circumstantial support to the supposition that they compete at a common site. 18OH-DOC is strongly dependent on the potassium cell or plasma content, because in potassium deficient rats markedly less 18OH-DOC is converted to 18OH-corticosterone and less yet if sodium is deficient.

ACTH (a peptide hormone) has a large affect on 18OH DOC, causing 18OH DOC to go down to zero when ACTH does. This could be for the primary purpose of keeping

serum immune enzymes and cell fluids at a high pH (alkaline) during internal infection, but not doing so during the intestinal infection of diarrhea, during which disease the resulting dehydration forces ACTH to decline. It probably is important normally to keep the vacuoles where pathogens are digested at a high pH because if the pH or alkalinity is not high enough, the pathogens inside the immune cells are not digested and thus released intact. So when an intestinal disease is not calling for ACTH to decline, the indirect potassium conserving attribute of 18OH-DOC by virtue of stimulating acid excretion would be valuable, as would also increased acid excretion during internal disease be valuable.

18OH DOC may act primarily by blocking aldosterone's effect on potassium, and must have aldosterone to assist it with sodium. Nichols, et al., have been able to show that injection of 18OH-DOC, which raised blood levels of this hormone ten times, were more retentive of sodium than a similar amount of aldosterone. So there must be a synergism involved. At the same time, the ratio of sodium to potassium excretion declined very little for 18OH-DOC, while for aldosterone, the ratio fell to as little as 1/3 that of control men. This implies a considerable sparing of potassium by 18OH-DOC. Urine potassium excretion is not altered by 18OH-DOC injection.

Angiotensin II has very little effect on 18OH-DOC and is ambiguous nor does serum potassium above 4.8 mEq/litter (187 mg). This last is not surprising since 18OH-DOC should not be used by the body at high serum potassium. Under low sodium intake, 18OH-DOC rises in the serum. ACTH causes a marked increase in 18OH-DOC, probably by a generalized affect on the zona fasciculata of the adrenal cortex where 18OH-DOC is synthesized. So when it is necessary for sodium to be unloaded during the dehydration induced decline of ACTH during diarrhea in order to preserve osmotic pressure, the resulting 18OH-DOC decline would assist in this.

18OH-DOC is deeply involved in one of the three forms (at least) of hypertension (high blood pressure).

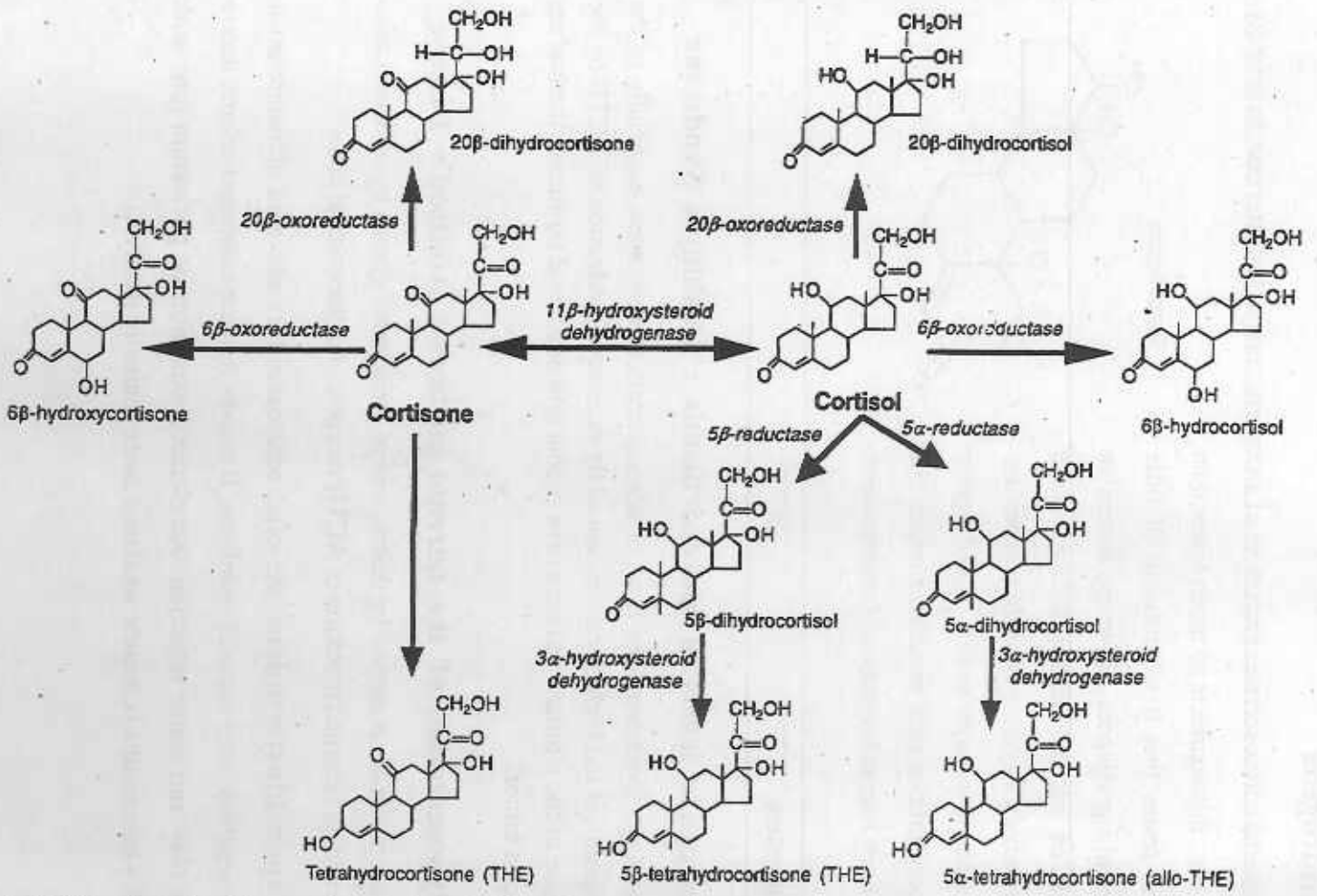
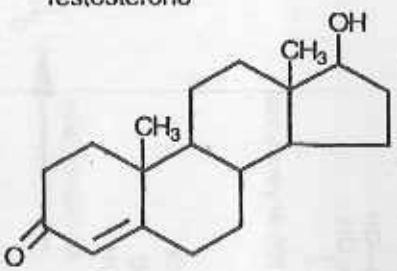


Fig. 5. The principal pathways of cortisol metabolism. Interconversion of hormonally active cortisol to inactive cortisone is catalyzed by two isozymes of 11β-hydroxysteroid dehydrogenase (11β-HSD), 11β-HSD1 principally converting cortisone to cortisol and 11β-HSD2 the reverse. Cortisol can be hydroxylated at the C6 and C20 positions. A ring reduction is undertaken by 5α-reductase or 5β-reductase and 3α-hydroxysteroid dehydrogenase.

4.2.3 Androgens

The adrenal cortex secretes precursors of androgens such as **testosterone**. In sexually-mature males, this source is so much lower than that of the testes that it is probably of little physiological significance. However, excessive production of adrenal androgens can cause premature puberty in young boys. In females, the adrenal cortex is a major source of androgens. Their hypersecretion may produce a masculine pattern of body hair and cessation of menstruation.

Testosterone



4.3 Diseases

4.3.1 Excessive levels of glucocorticoids : Cushing's Syndrome

In Cushing's syndrome, the level of adrenal cortical hormones, especially of the glucocorticoids, is too high. It can be caused by excessive production of ACTH by the anterior lobe of the pituitary and excessive production of adrenal hormones themselves (because of a tumor).

4.3.2 Hyposecretion of the adrenal cortices : Addison's Disease

Addison's disease is caused by destruction of the adrenal glands by infection, auto immunity and an inherited mutation of ACTH receptor on adinocortical cells.

4.3.3 Hyperaldosteronism (the syndrome caused by elevated aldosterone) is generally resulted from adrenal neoplasm. It causes hypertension and edema due to excessive Na+ and water retention. Accelerated excretion of potassium ions with extreme K+ loss results is muscle weakness and eventually paralysis.

Unit 5 □ Norepinephrine and Epinephrine : Hormones of the Adrenal Medulla

Structure

5.1 Introduction

The adrenal medulla consists of masses of neurons that are part of the sympathetic branch of the autonomic nervous system. Instead of releasing at a synapse these neurons release their neurotransmitters called **Catecholamines** into the blood. Catecholamines are released in response to stress. They are called catecholamines because they contain a catechol group, and are derived from the amino acid tyrosine.

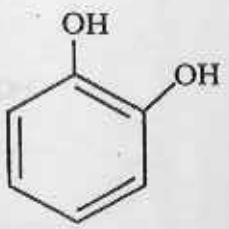


Fig. 1. Catechol group

Thus, although part of the nervous system, the adrenal medulla functions as an endocrine gland. Basically, Norepinephrine, Epinephrine and Dopamine are the principal catecholamines found in the body. Both Norepinephrine and Epinephrine are derived from the amino acid tyrosine. The hormones bind to adrenergic receptors — transmembrane proteins in the plasma membrane of many cell types. The term epinephrine is derived from the Greek roots epi- and nephros, and literally means above the kidney, in reference to the gland's anatomic location. The Latin roots ad- and renes have similar meanings, and give rise to adrenaline. The term "norepinephrine" is derived from the chemical prefix nor-, which indicates that norepinephrine is the next lower homolog of epinephrine. In particular, the two structures are identical except that epinephrine has a methyl group attached to its nitrogen, while the methyl group is replaced by a hydrogen atom in norepinephrine.

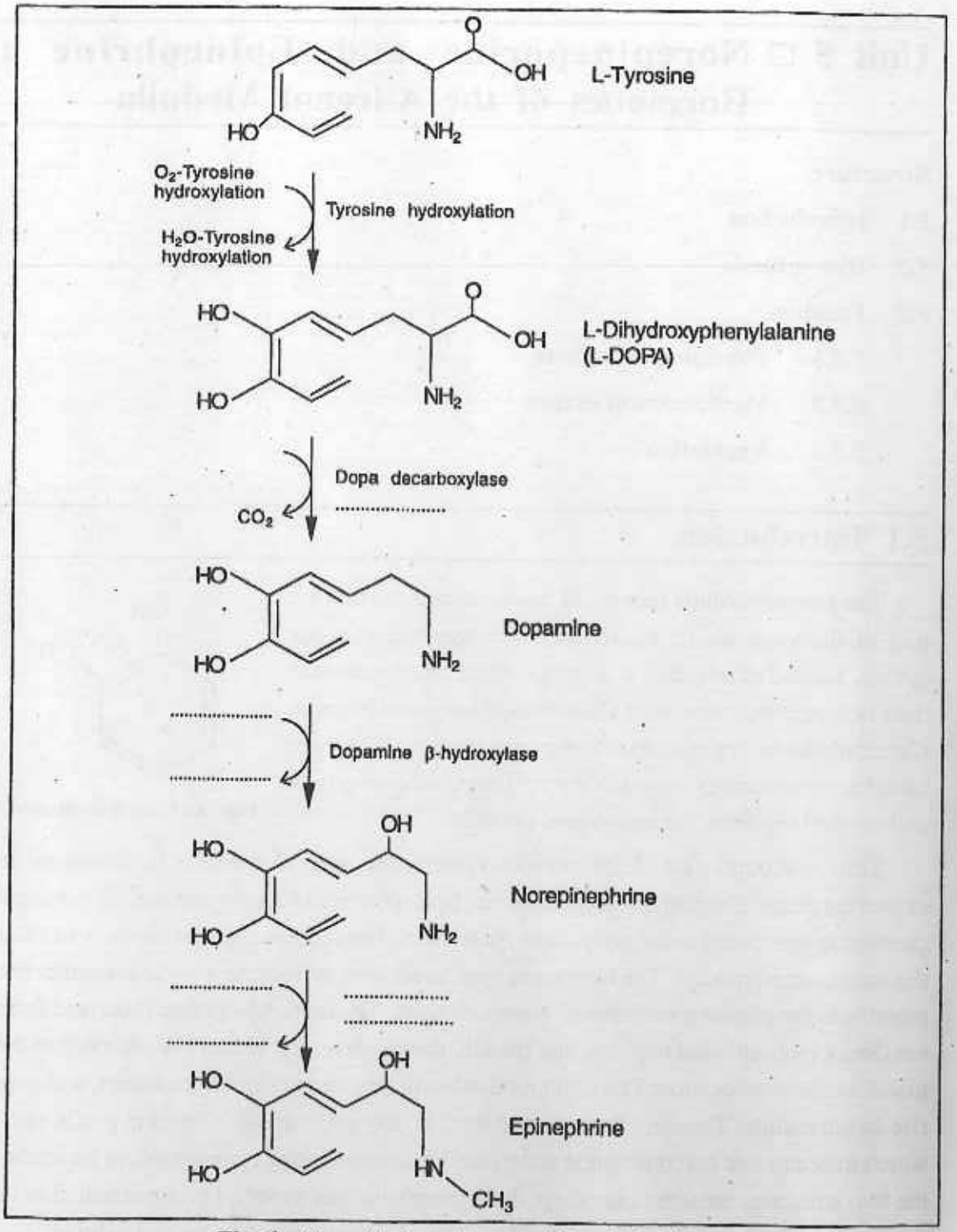


Fig. 2. Biosynthetic pathway of Catecholamines.

5.2 Biosynthesis

Norepinephrine is formed by hydroxylation and decarboxylation of tyrosine, and **Epinephrine** is synthesized by the methylation of norepinephrine. The synthetic pathway is shared by all catecholamines. Some of the Tyrosine is formed from Phenylalanine, but mostly is of dietary origin. Phenylalanine hydroxylase is found primarily in the liver. Tyrosine is transported into catecholamine secreting adrenal medullary cells by a concentrating mechanism. It is converted to DOPA and then to Dopamine in the cytoplasm of the cells by tyrosine hydroxylase and Dopa decarboxylase. The Dopamine then enters the granulated vesicles and is converted to Norepinephrine by Dopamine β-hydroxylase. L-Dopa is the isomer involved, but the norepinephrine is formed in the D configuration. The rate-limiting step in synthesis is the conversion of tyrosine to Dopa. Tyrosine hydroxylase, catalyzing this step is subject to feedback inhibition by dopamine and norepinephrine. The cofactor for tyrosine hydroxylase is tetrahydrobiopterin. This is converted to dihydrobiopterin, when tyrosine is converted to Dopa.

Some neurons and adrenal medullary cells contain the cytoplasmic enzyme phenylethanolamine-N-Methyltransferase (PNMT). This enzyme catalyzes the conversion of norepinephrine to epinephrine. In these cells, norepinephrine apparently leaves the vesicles, is converted to epinephrine, and then enters the storage vesicles. The catecholamines are held in the granulated vesicles by an active transport system, and the action of this transport system is inhibited by the drug Reserpine.

5.3 Function

5.3.1 Physiological effects

Both of them mimic the effects of noradrenergic nervous discharge. Along with epinephrine, norepinephrine mediates the **fight-or-flight response**, directly increasing heart rate, triggering the release of glucose, and increasing blood flow to skeletal muscle. The fight-or-flight response, also called the 'fright, fight or flight response', 'hyperarousal' or 'the acute stress response', was first described by Walter Cannon in 1915. Animals react to threats with a general discharge of the sympathetic nervous system, priming the

animal for fighting or fleeing. This response was later recognized as the first stage of a general adaptation syndrome (GAS) that regulates stress responses.

However, when norepinephrine acts as a drug it increases blood pressure by its prominent increasing effects on the vascular tone from α-adrenergic receptor activation. The resulting increase in vascular resistance triggers a compensatory reflex that overcomes its direct stimulatory effects on the heart, called the baroreceptor reflex, which results in a drop in heart rate called reflex bradycardia. Epinephrine, when in the bloodstream, it rapidly prepares the body for action in emergency situations. The hormone boosts the supply of oxygen and glucose to the brain and muscles, while suppressing other non-emergency bodily processes (digestion in particular). It increases heart rate and stroke volume, dilates the pupils, and constricts arterioles in the skin and gastrointestinal tract while dilating arterioles in skeletal muscles. It elevates the blood sugar level by increasing catabolism of glycogen to glucose in the liver, and at the same time begins the breakdown of lipids in fat cells. Like some other stress hormones, epinephrine has a suppressive effect on the immune system. All of these effects prepare the body to take immediate and vigorous action. The type of action in various cell types depends on their expression of adrenergic receptors.

Norepinephrine and epinephrine also produce a prompt rise in the metabolic rate that is independent of the liver. This calorigenic action does not occur in the absence of the thyroid and the adrenal cortex. The cause of the initial rise in metabolic rate may be due to cutaneous vasoconstriction, that decreases heat loss leading to an increase in body temperature or mascular activity, or both.

5.3.2 Mechanism of action

The effects of these two hormones are brought about by actions on α- and β-adrenergic receptors. There are two types of α receptors, α_1 and α_2 receptors, while β receptors are are subdivided into β_1, β_2 and β_3 receptors. Both these hormones incearse heart rate mediated by β_1 receptors. Norepinephrine produces vasoconstriction in most organs via α_1 receptors, while Epinephrine dialates the blood vessels in skeletal muscle and liver via β_2 receptors. This usually overbalances the vasoconstriction produced by epinephrine elsewhere, and the total peripheral resistance drops. Epinephrine causes a widening of the pulse pressure, but because baroreceptor stimulation is insufficient to obscure the direct effect of the hormone on the heart, cardiac rate and output increase.

Both epinephrine and norepinephrine cause glycogenolysis either via β receptors by increasing cyclic AMP, with activation of phosphorylase, or via α receptors, by increasing intracellular Ca^{2+}. Additionally, both the catecholamines increase secretions of insulin and glucagon via β adrenergic reception mechanism and inhibit their secretion via α adrenergic mechanisms.

5.3.3 Regulation

Neural control :

The physiologic stimuli affect medullary secretion through the nervous system at basal states, the secretion of these hormones is low, and their secretion is further reduced during sleep. Increased adrenal medullary secretion is part of the diffuse sympathetic discharge provoked in emergency. The small granulated vesicles in post ganglionic noradrenergic neurons contain ATP and norepinephrine, and the large granulated vesicles contain neuropeptide Y. There is evidence that low frequency stimulation promotes release of ATP and high frequency stimulation casuses release of neuropeptide Y.

The sympathetic nervous system, which acts via splanchnic nerves to the adrenal medulla, stimulates the release of epinephrine. Acetylcholine released by preganglionic sympathetic fibers of these nerves acts on nicotinic acetylcholine receptors and causes cell depolarization and an influx of calcium. Calcium triggers the exocytosis of chromaffin granules and thus the release of epinephrine (and norepinephrine) into the bloodstream. Epinephrine (as with norepinephrine) does exert negative feedback to down-regulate its own synthesis at the presynaptic α_2 adrenergic receptor.

The calorigenic action of catecholamines in animals exposed to cold is important. With such exposure, animals with experimental denervation of the adrenal glands shiver sooner and more vigorously than normal controls. Again, hypoglycemia is a potent stimulus to catecholamine secretion, which enhances the glycogenolysis.

Unit 6 □ Biosynthesis of Sex Steroids

Structure

6.1 Hormones of the Ovary

The mature ovary actively synthesizes and secretes a variety of hormones. Among these are the steroids, which include estrogens, progesterone, androgens and their precursors. In addition to these substances the ovary also produces relaxin, prostaglandins and other substances that act locally to regulate its function.

The Steroid Hormones :

The ovary is normally the major source of estrogen. The ovary also produces and secretes large amounts of progesterone during the luteal phase of the cycle. It is also the source of small amounts of testosterone and other androgens that serve not only as precursors to estrogen synthesis but also are released into the circulation to act on peripheral tissues.

Biosynthesis of Steroid Hormones :

The steroid hormones are synthesized from cholesterol, which is present in the gland both free and esterified to fatly acids (cholesterol esters). Cholesterol derived either from

circulating lipoproteins or from cholesterol esters in the gland is converted to pregnenolone by removal of a 6-carbon fragment, isocaproic acid. The reaction of or group of reactions is the rate-limiting step in the biosynthetic process and is controlled by luteinzing hormone (LH) from the anterior pituitary.

Pregnenelone formed by this reaction may be converted either to progesterone or to 17α-hydroxy-pregnenolone. The conversion to progesterone requires the action of 3β-hydroxysteroid dehydrogenase and $\Delta^{5,4}$-ketosteroid isomerase, which shifts from the Δ^5 to the Δ^4 position. Progesterone is secreted by the corpus luteum in large amounts following ovulation. However, it also serves as a precursor for androgen and estrogen, which converts it to 17α-hydroxyprogesterone in the ER. Following 17α-hydroxylation, the 2-carbon (20-21) side chain may be cleaved by the C17, 20-lyase enzyme to form androgens.

17α-Hydroxypregnenelone is converted by the lyase enzyme to dehydroepiandrosterone (DHEA). This compound can then be converted to androstenedione by the 2 successive action of enzymes Δ^5, 3β-hydroxysteroid dehydrogenase and Δ^5, 3-ketosteroid isomerase respectively. Androstenedione is the major androgen secreted by the ovary, but small amounts of DHEA and testosterone are also released.

Androstenedione is converted to oestrogen by aromatisation. Aromatisation of androgens to oestrogens occur greatly in the microsome of the cell by a group of enzymes known as the aromatase complex or system and also requires NADAH + molecular O_2. Estradiol, the major oestrogen produced by the ovary, is synthesized by 3 steps hydroxylation or the methyl group of carbone 19, oxidation of this group and hydroxylation at the 2α position. A 17-hydrogenes enzyme can lead to an interconversion of androstenedione and testosterone, and the interconversion of oestrone and oestradiol.

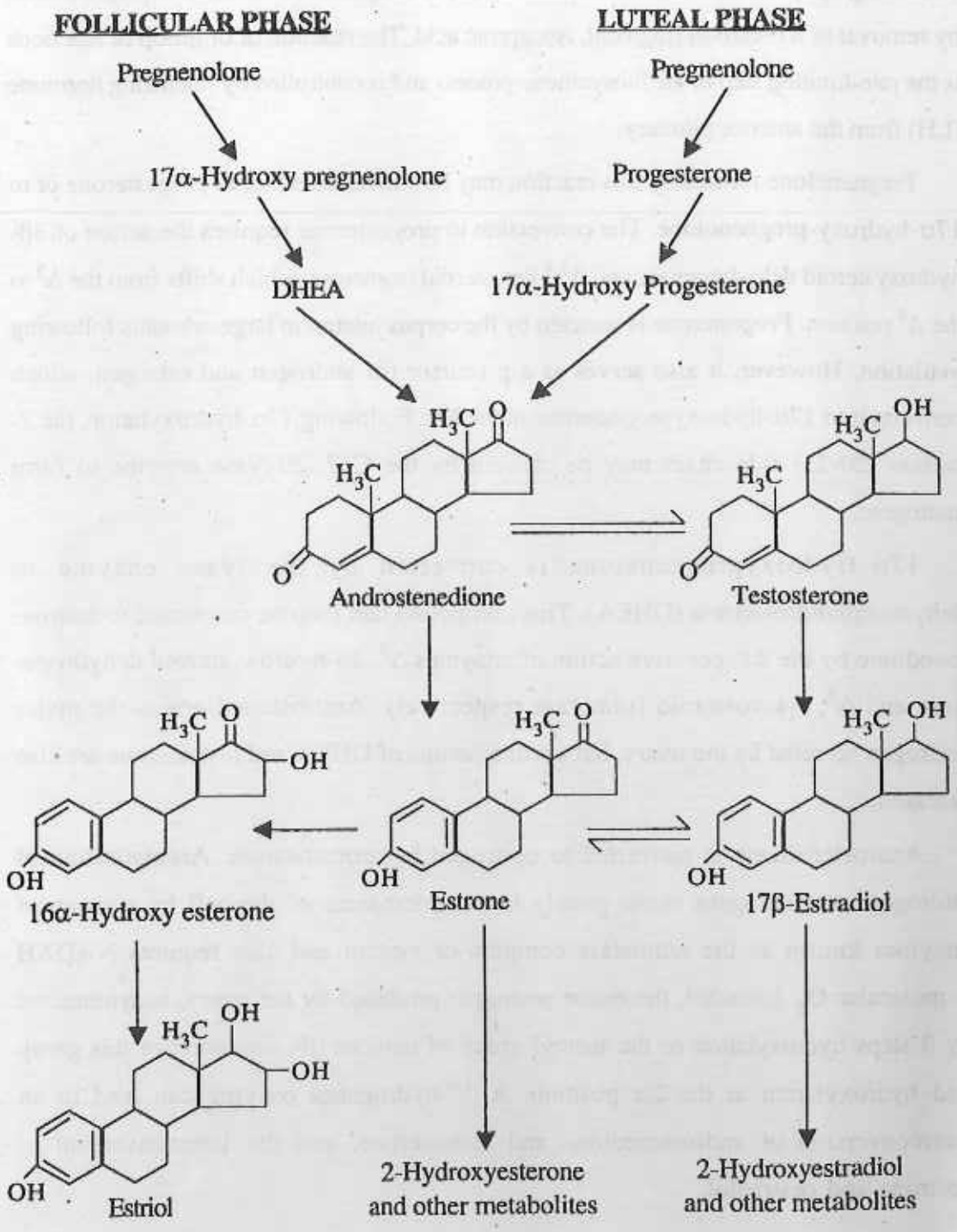


Fig. Biosynthesis and metabolism of estrogens (Basic & clinical pharmacology, lange 1982).

6.2 Metabolism of Sex Steroid Hormones

(1) Estrogens : Circulating estradiol is rapidly converted in the liver to estrone by 17β-hydroxy steroid dehydrogenase. Some of the estrone re-enters the circulation,

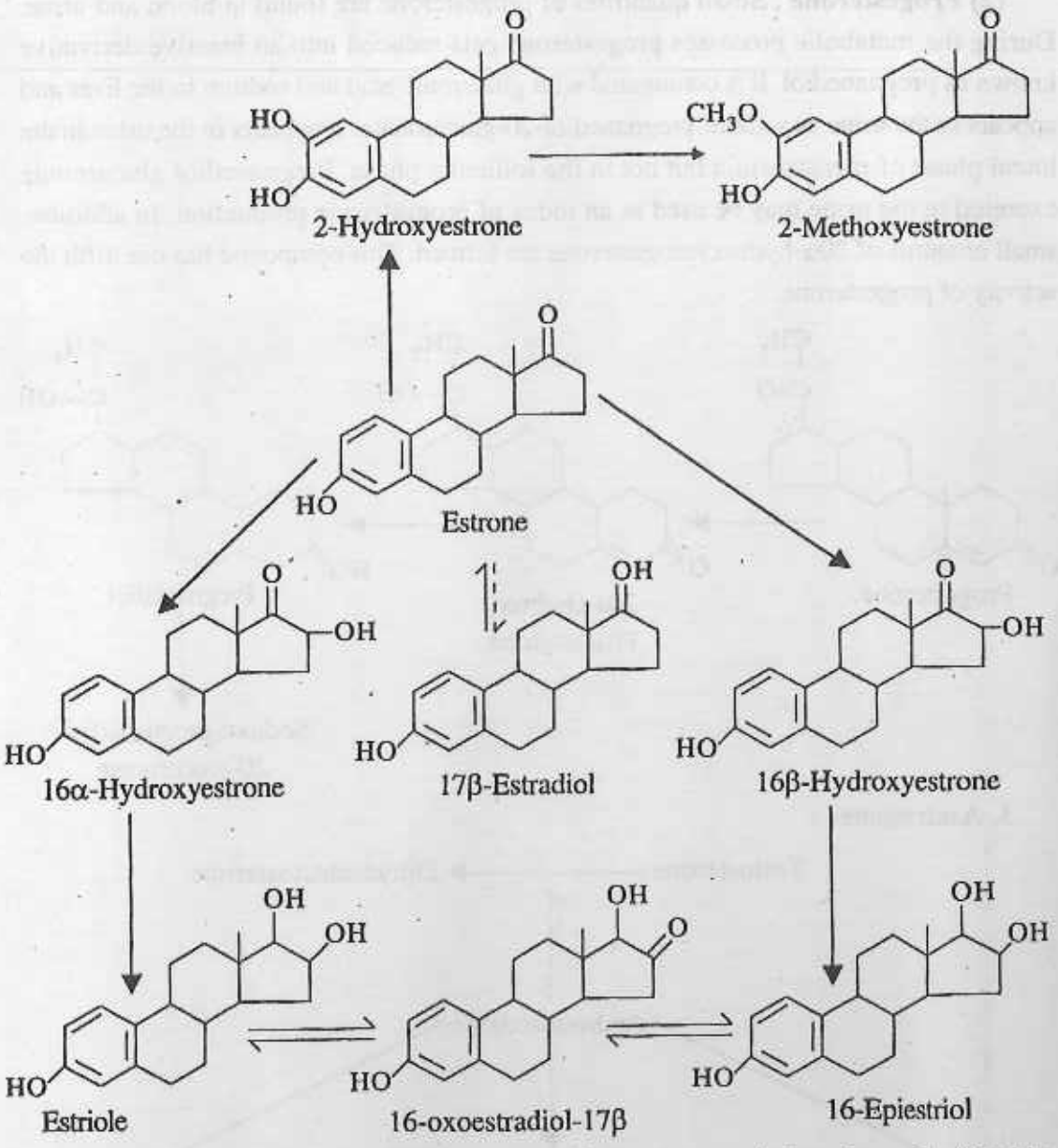
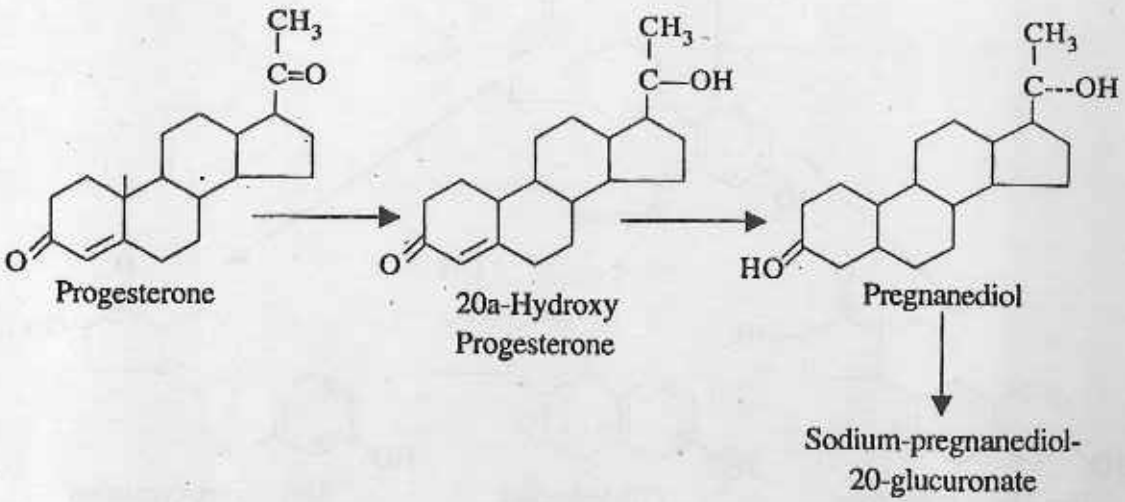


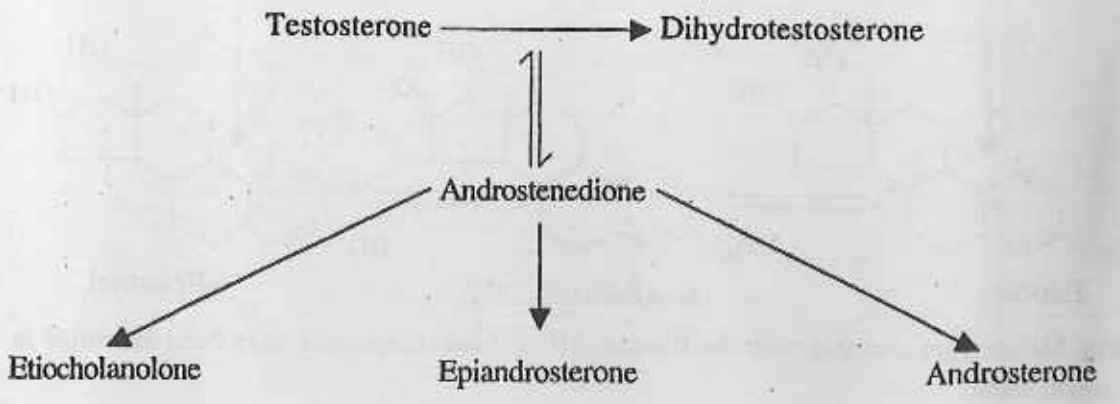
Fig. Metabolism of estrogen in the Human. All of these compounds have been identified in human urine.

however, most of it is further metabolized to 16α-hydroxyestrone (which is then converted to estriol) or to 2-hydroxy-estrone. Much of the estrone is conjugated to form estrone sulfate. Estriol is converted largely to form estriol 3-sulfate-16-glucuronide before excretion by the kidney.

(2) Progesterone : Small quantities of progesterone are found in blood and urine. During the metabolic processes progesterone gets reduced into an inactive derivative known as pregnanediol. It is conjugated with glucuronic acid and sodium in the liver and appears in the urine as sodium-pregnanediol-20-glucuronate. It appears in the urine in the luteal phase of menstruation but not in the follicular phase. Pregnanediol glucuronide excreted in the urine may be used as an index of progesterone production. In addition, small amounts of 20α-hydroxyprogesterone are formed. This compound has one-fifth the activity of progesterone.

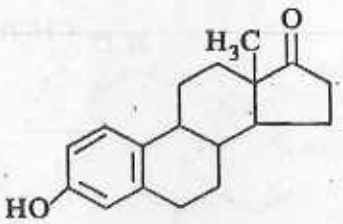


3. Androgenes :

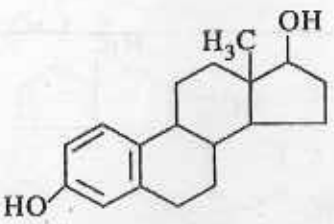


6.3 Oestrogen

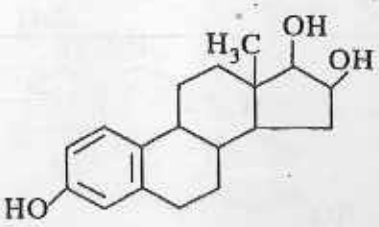
Oestrogen are compounds which can produce oestrus in ovariectomised animals. They are all sterol derivatives. They are less effective by mouth. Their structures are as follows :



Oestrone



17β-oestradiol



Oestriol

Oestradiol (with—OH at the 17C position)—it is the hormone secreted by the ovary.

Oestrone (with =O at the 17C position) is the possible circulating hormone.

Oestriol (with—OH at the 17C position and an additional ---OH at the 16C position) — It is found in adult female urine and increased during pregenecy, is also to liberated from the placenta.

There is close relationship between oestradiol, oestrone and oestriol in the body such as—

Oestradiol $\rightleftharpoons$ Oestrone $\longrightarrow$ Oestriol.

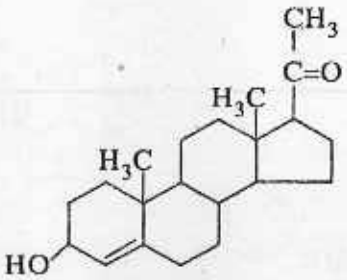
- **Sources :** Ovary is the chief source of oestrogen.

1. From the Graafian follicles—The liquor folliculi and the follicular epithelium are rich in oestrogens.
2. From the ovarian interstitial cells.
3. The oestrogen secreted during the luteal phase of the cycle is formed by the theca lutein cells of the corpus luteum.

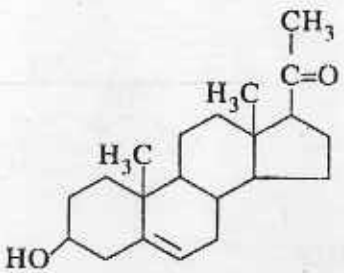
In addition, the oestrogen also is secreted from adrenal cortex, testes and placenta.

6.4 Progesterone

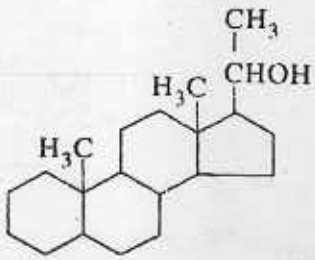
Progesterone is the active principle of corpus luteum. It is a sterol derivative with a side chain at the 17C position. It is found in two crystaline forms, eg-α and β.



Progesterone



Pregnenolone



Pregnanediol

- **Sources :** (i) Corpus between
  (ii) Placenta
  (iii) Adrenal cortex.

6.5 Control of Ovarian Functions

1. The Hypothalamic—Hypophysial—Ovarian axis

After hypophysectomy the ovaries are atrophed and follicles do not develop beyond the antrum stage. Regulation of gamete release and of hormone secretion by the ovary is mediated by the pituitary gonadotropins. These protein hormones are synthesized and released by the pituitary under hypothalamic regulation.

(a) Effect of pituitary gonadotrophins :

FSH controls (a) maturation of the Graafian follicles and (b) Secretion of oestrogens. luteinizing hormone (LH) causes ovulaation of the follicle that has been ripened by FSH, formation, growth and maintenance of corpus luteum and secretion of progesterone.

It seems probable that pure FSH does not cause estrogen secretion but that small amounts of LH are necessary for oestrogen production.

The structural and functional maintenance of the corpus luteum by a luteotropin (probably prolactin) is clearly established only in the rat. In larger mammals including the human, the corpus luteum probably has a predestined life and an intrinsic secretory activity that are initiated by LH at the time of ovulation.

(b) Effect of Hypothalamic centre :

The synthesis and release of gonadotropins by the pituitary is regulated by centers in the hypothalamus that mediated their effects by neurohumoral substances which are transported to the anterior lobe through the portal vessels. These vessels originate in the median eminence of the hypothalamus and terminate in the anterior pituitary. Direct nervous control of the anterior pituitary appears to be of negligible importance, because lesion of the hypothalamus causes abolition of gonadotrophic hormone secretion and atrophy of the ovary along with changes in the reproductive organs and stoppage of sexual cycle.

In the lower species that have been extensirely investigated, different are as of the hypothalamus appears to regulate release of different gonadotropins, but other centers inhibit the release of gonadotropins. The human child becomes sexually nature precociously because of the presence of lesions in the posterior hypothalamus that normally inhibits untimely release of gonadotropins.

(c) Effect of ovarian steroids :

Ovarian steroids exert a regulating influence on gonadotropin secretion, mediated probably through hypothalamic centers.

1. Negative feed-back : Under normal conditions, ovarian steroids limit or reduce the secretions of pituitary gonadotropins. Serum concentrations of both FSH + LH are increased markedly following ovariectomy or in postmenopausal women, whereas administration of estrogen (or estrogen and progesterone) lowers serum gonadotropins levels. Throughout the normal menstrual cycle, the negative feed back effects of ovarian steroids predominate.

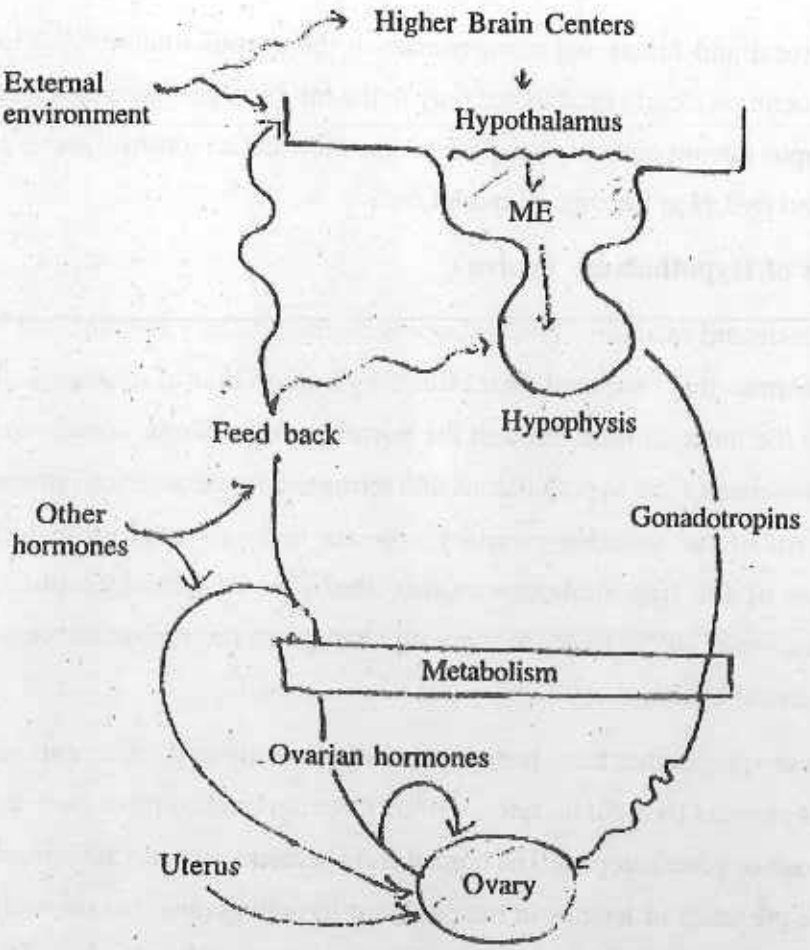


Fig. Hypothalamic-hypophysial-ovarian circuit. Schematic representation with some of the variables that may modify function. The undulations in the arrows indicate rhythmicity.

2. Positive feed-back : Estradiol and progesterone under certain conditions, can induce the release of LH and FSH. During the menstrual cycle, rising concentration of estradiol in the latter part of the follicular phase initiate the preovulatory surge of LH via this mechanism. This increase in LH secretion in turn stimulates a small but significant increase in the secretion of progesterone which in turn with estradiol initiates the mid cycle surge of FSH.

It is probable that the negative and positive feedback actions of ovarian sex steroids result from both (1) a direct effect of the steroids on the pituitary gonadotropes that alters their sensitivity to GnRH end (2) Modulation of the frequency and magnitude of the pulses of hypothalamic GnRH.

Uterus : It is possible that uterine endometrium synthesizes a speific luteolytic factor named as luteolysin, which acting on the ovary, causes involution of corpus luteum. The uterine effect is not mediated through pituitary, it is a direct one.

3. Pineal gland : It is postulated that melatonin or some other active principle present in the pineal gland has antigonadal activity. The action may occur directly or through anterior pituitary and control nervous system.

4. In addition, there are some other endocrine glands such as adrenal cortex, thyroid, and thymus, and physical factors such as diet, vitamins and temperature may influence ovarial function.

6.6 Androgen

Androgens are substances having masculinising properties. Both in the testis and adrenal of acids, the androgens can be synthesized either from cholesterol or directly from acetyl CoA.

Chemistry and varieties :

Androgens are C-19 sterol compounds. They have 2 varieties (a) natural (b) synthetic. The chief natural androgens is called Testosterone. Methyl testosterone and testosterone propionate are important synthesis androgens, which are effective by mouth and erereely absorbed and unaffected by liver. Natural androgens are mostly inactivated by liver. Hence not much effective by mouth. *Activity increases if combined with fatty acids, such as propionic acid.* The chief degradation products of androgens are the 17-ketosteroids (17-Ks), androsterone and dehydroepiandrosterone (DHEA).

Testosterone, Δ4-androstenedione and dehydroepiandrosterone are the main circulatory androgens of testicular origin. Testosterone is more potent than the others. In contrast the adrenal cortex secretes DHEA and Δ4-androsteredione as its major androgens.

In 1965, it was shown that androgen stimulation of accessory sexual glands is not due to testosterone but *due to the reduction of testosterone dehydrotestosterone (DHT)* 5γ 5α rathotase. DHT is the principal intracellular androgen that occurs mainly in the liver. DHT is bound by specific nuclear and cytoplasmic protein. The accessory sexual glands are sites of specific retention and accumulation of DHT complexes. The DHT receptor complex binds to DNA giving rise to stimulation of RNA synthesis.

Biosynthesis of androgens :

Interstitial cells of Leydig are the target cells that are stimulated by *gonadotrophins* for an increased synthesis of androgens. The chief products which are secreted into the spermatic venous blood of adult testes are testosterone and smaller quantities of Δ^4-androsteredione and DHEA. Tissues other than Leydig cells also have the ability to transform steroid precursors to testosterone. These include the seminiferous tubules, liver, adrenal cortex, prostate gland and skeletal muscle.

The immediate precursor of the gonadal steroid as with the adrenal steroid is cholesterol. The initial alternation of the cholesterol molecule involves clearage at the side chain to yield Δ^5 pregnenolone. The conversion of pregnenolone to estrone requires the action of 5 enzymes (i) 3β-hydroxysteroid dehydrogenase (ii) Δ^5, Δ^4-isomerase (iii) 17-hydroxylase (iv) 17, 20-desmolase (v) 17-ketoreductase.

Pregenenolone seems to be the common substrate for all of the normonal steroids. Conversion of pregnenolone requires 3β-hydroxysteroid dehydrogenase which oxidises the hydroxyl group at position 3 and an isomerase which changes the Δ^5 double bond to Δ^4, to yielding progesterone. The progesterone can sense as a precursores for androgens in both the ovaries and testis.

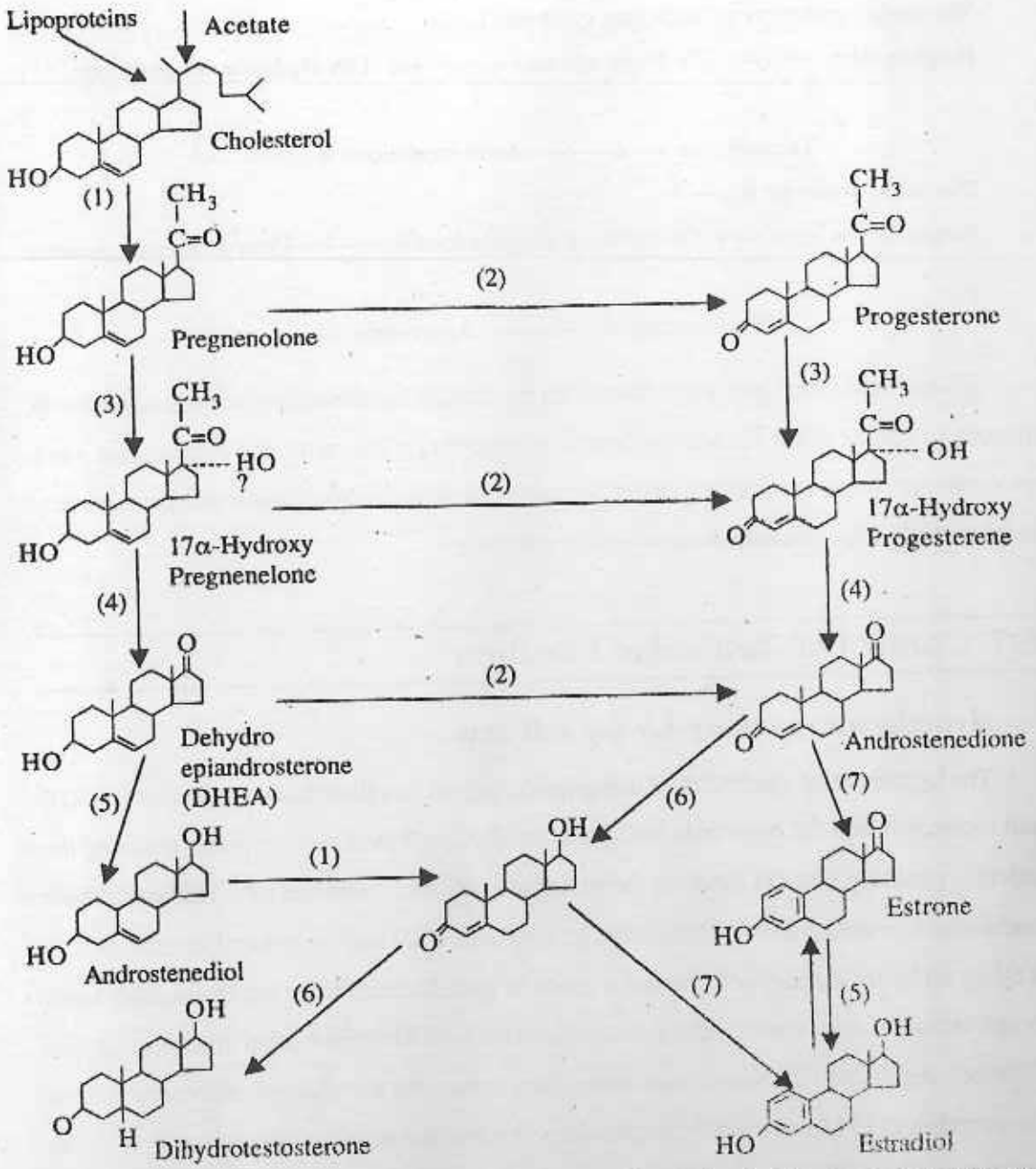
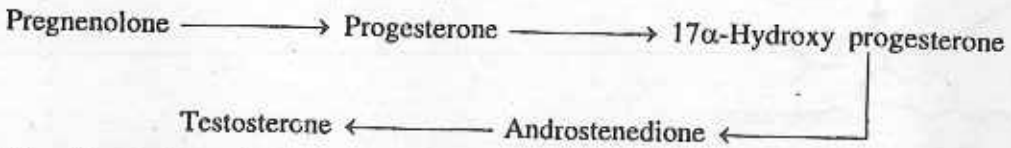
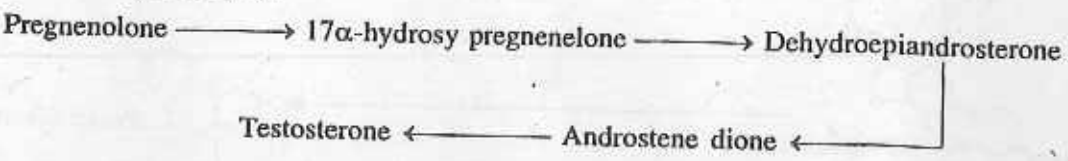


Fig. Pathways for testicular androgen and estrogen biosynthesis. Heavy arrows indicate major pathways circled number represent enzymes as follows : (1) = 20,22—desmolase (2) = 3β-hydroxysteroid dehydrogenase and Δ5, Δ4-ketosteroid isomerase (3) = 17-hydroxylase (4) = 17, 20-demolase (5) = 17-ketoreduclase (6) = 5α-reductase (7) = aromatase

The major pathways of androgen synthesis is :—



The other pathway is :—



Several additional pathways have been postulated but the ability of human endocrin tissues to utilize them for steroid hormone production has not been proved one such possible pathways involves the direct formation of dehydroepiandrosterone from cholesterol without a C_{21} intermediate.

6.7 Control of Testicular Function

Hypothalmic-pituitary-Leydig cell axis :

The hypothalmus synthesizes a decapeptide, gonadotropin-releasing hormone (GnRH) and secretes it into the hypothalamo hypothyseal portal blood system. After reaching the anterior pituitary, GnRH binds to the gonadotropes and stimulates the release of both luteinizing hormone (LH/ICSH) and, to a lesser extent, FSH into the general circulation. LH is taken up by the Leydig cells, where it binds to specific membrane receptors. This leads to activation of adenylate cyclase and generation of cAMP and other messengers that ultimately results into the secretion of androgens. In turn, the elevation of androgens inhibits the secretion of LH from the anterior pipuitary through the negative few lack action on the pituitary and an inhibitory effect at the hypothalamic level. The inhibitory effect of androgens on the hypothalamus is mediated principally by estradiol, which is formed locally in the hypothalamus from androgens.

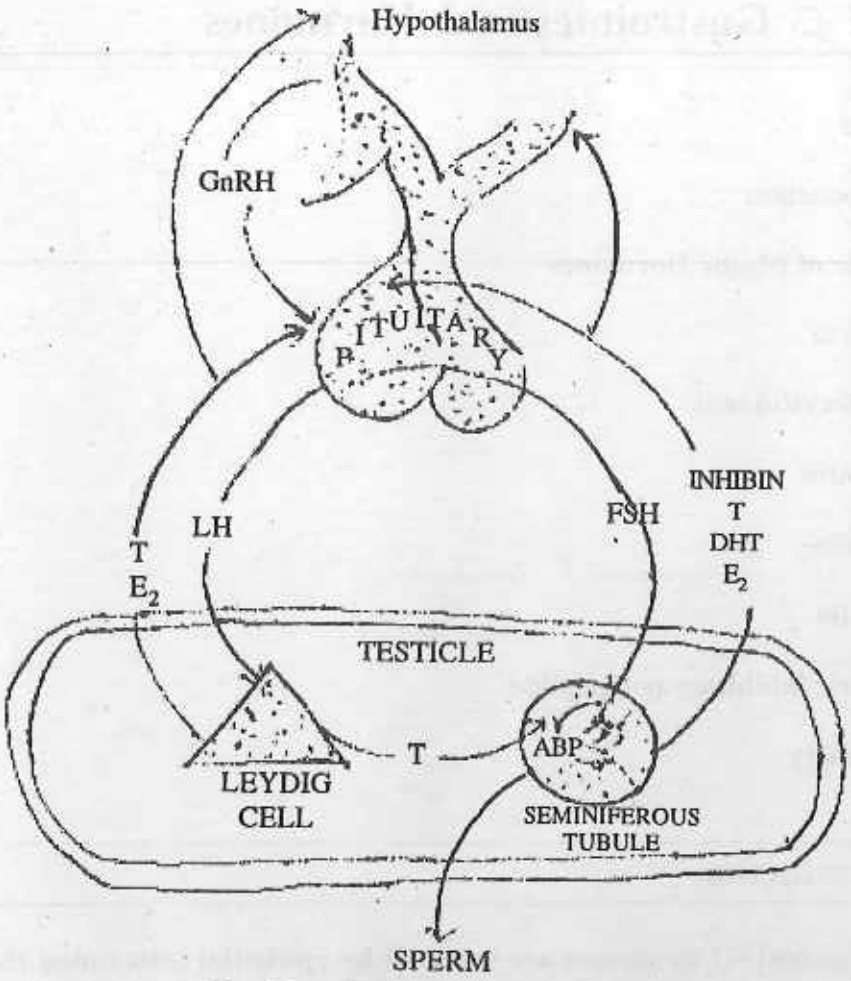


Fig. Hypothalmic-pituitary-testicular axis.
GnRH gonadotropin-releasing hormone
LH luteinizing hormone
FSH Follicle-stimulating hormone
T Testosterone
DHT dihydrotestosterone
ABP androgen binding protein
E_2 Estradiol
+ +ve influence
– –ve influence

Unit 7 □ Gastrointestinal Hormones

Structure

7.1 Introduction

The classical GI hormones are secreted by epithelial cells lining the lumen of the stomach and small intestine. These hormone-secreting cells - endocrinocytes - are interspersed among a much larger number of epithelial cells that secrete their products (acid, mucus, etc.) into the lumen or take up nutrients from the lumen. GI hormones are secreted into blood, and hence circulate systemically, where they affect function of other parts of the digestive tube, liver, pancreas, brain and a variety of other targets.

The following table summarizes the effects and stimuli for release of the major gastrointestinal hormones, each of which is discussed in more detail in subsequent pages :

7.2 Table of Major Hormones

Hormone	Major Activities	Stimuli for Release
Gastrin	Stimulates gastric acid secretion and proliferation of gastric epithelium	Presence of peptides and amino acids in gastric lumen
Cholecystokinin	Stimulates secretion of pancreatic enzymes, and contraction and emptying of the gall bladder	Presence of fatty acids and amino acids in the small intestine
Secretin	Stimulates secretion of water and bicarbonate from the pancreas and bile ducts	Acidic pH in the lumen of the small intestine
Ghrelin	Appears to be a strong stimulant for appetite and feeding; also a potent stimulator of growth hormone secretion	Not clear, but secretion peaks prior to feeding and diminishes with gastric filling
Motilin	Apparently involved in stimulating housekeeping patterns of motility in the stomach and small intestine	Not clear, but secretion is associated with fasting
Gastric inhibitory polypeptide	Inhibits gastric secretion and motility, and potentiates release of insulin from beta cells in response to elevated blood glucose concentration	Presence of fat and glucose in the small intestine

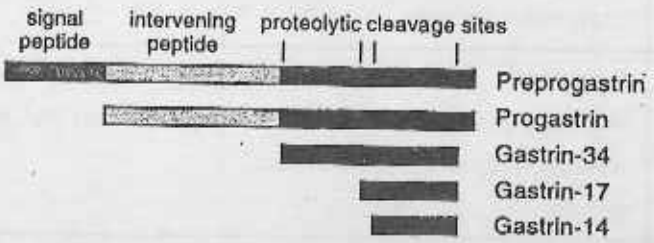
There are *a bunch* of hormones, neuropeptides and neurotransmitters that affect gastrointestinal function. Interestingly, a number of the classical GI hormones are also synthesized in the brain, and sometimes referred to as "brain-gut peptides". The significance of this pattern of expression is not clear.

7.3 Gastrin

Gastrin is a major physiological regulator of gastric acid secretion. It also has an important trophic or growth-promoting influence on the gastric mucosa. Gastrin is synthesized in G cells, which are located in gastric pits, primarily in the antrum region of the stomach and binds with the receptors, found predominantly on parietal and enterochromaffin-like cells.

Structure of Gastrin and the Gastrin Receptor

Gastrin is a linear peptide that is synthesized as a preprohormone and is post-translationally cleaved to form a family of peptides with identical carboxytermini. The predominant circulating form is gastrin-34 ("big gastrin"), but full biologic activity is present in the smallest peptide (gastrin-14 or minigastrin). Further, full bioactivity is preserved in the five C-terminal amino acids of gastrin, which is known as pentagastrin. The five C-terminal amino acids of gastrin and cholecystokinin are identical, which explains their overlapping biological effects.



The gastrin receptor is also one of the receptors that bind cholecystokinin, and is known as the CCK-B receptor. It is a member of the G protein-coupled receptor family. Binding of gastrin stimulates an increase in intracellular Ca^{++}, activation of protein kinase C, and production of inositol phosphate.

Control and Physiologic Effects of Gastrin

The primary stimulus for secretion of gastrin is the presence of certain foodstuffs, especially peptides, certain amino acids and calcium, in the gastric lumen. Also, as yet unidentified compounds in coffee, wine and beer are potent stimulants for gastrin secretion. Secretion of this hormone is inhibited when the luminal pH of the stomach becomes very low (less than 3 approximately).

Gastrin appears to have at least two major effects on gastrointestinal function :

- **Stimulation of gastric acid secretion:** Gastrin receptors are found on parietal cells, and binding of gastrin, along with histamine and acetylcholine, leads to fully-stimulated acid secretion by those cells. Canine parietal cells have roughly 44,000 gastrin receptors each, and in that species, it has been demonstrated that immunoneutralization of gastrin blocks secretion of acid in response to intragastric administration of peptides. Enterochromaffin-like (ECL) cells also bear gastrin receptors, and recent evidence indicates that this cell may be the most important target of gastrin with regard to regulating acid secretion. Stimulation of ECL cells by gastrin leads to histamine release, and histamine binding to H2 receptors on parietal cells is necessary for full-blown acid secretion.
- **Promotion of gastric mucosal growth:** Gastrin clearly has the ability to stimulate many aspects of mucosal development and growth in the stomach. Treatment with gastrin stimulates DNA, RNA and protein synthesis in gastric mucosa and increases the number of parietal cells. Another observation supporting this function is that humans with hypergastrinemia (abnormally high blood levels of gastrin) consistently show gastric mucosal hypertrophy.

In addition to parietal and ECL cell targets, gastrin also stimulates pancreatic acinar cells via binding to cholecystokinin receptors, and gastrin receptors have been demonstrated on certain populations of gastric smooth muscle cells, supporting pharmacologic studies that demonstrate a role for gastrin in regulating gastric motility.

Disease States

Excessive secretion of gastrin, or **hypergastrinemia**, is a well-recognized cause of a severe disease known as Zollinger-Ellison syndrome, which is seen at low frequency in man and dogs. The hallmark of this disease is gastric and duodenal ulceration due to excessive and unregulated secretion of gastric acid. Most commonly, hypergastrinemia is the result of gastrin-secreting tumors (gastrinomas), which develop in the pancreas or duodenum.

7.4 Cholecystokinin

Cholecystokinin plays a key role in facilitating digestion within the small intestine. It is secreted from mucosal epithelial cells in the first segment of the small

intestine (duodenum), and stimulates delivery into the small intestine of digestive enzymes from the pancreas and bile from the gallbladder. Cholecystokinin is also produced by neurons in the enteric nervous system, and is widely and abundantly distributed in the brain.

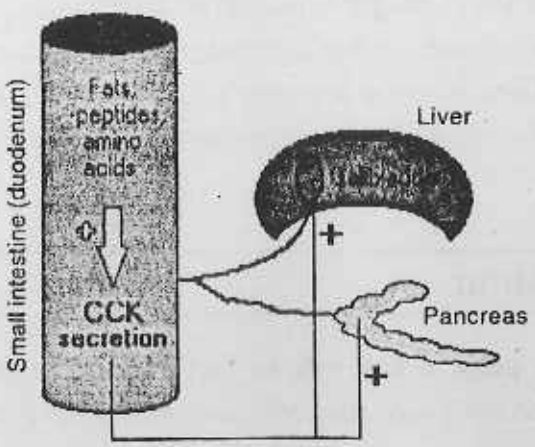
Structure of Cholecystokinin and Its Receptors

As mentioned previously, cholecystokinin and gastrin are highly similar peptides. Like gastrin, cholecystokinin is a linear peptide that is synthesized as a preprohormone, then proteolytically cleaved to generate a family of peptides having the same carboxy ends. Full biologic activity is retained in CCK-8 (8 amino acids), but peptides of 33, 38 and 59 amino acids are also produced. In all of these CCK peptides, the tyrosine seven residues from the end is sulfated, which is necessary for activity.

Two receptors that bind cholecystokinin have been identified. The CCK_A receptor is found abundantly on pancreatic acinar cells. The CCK_B receptor, which also functions as the gastrin receptor, is the predominant form in brain and stomach. Both receptors are having seven transmembrane domains typical of G protein-coupled receptors.

Control and Physiologic Effects of Cholecystokinin

Foodstuffs flowing into the small intestine consist mostly of large macromolecules (proteins, polysaccharides and triglyceride) that must be digested into small molecules (amino acids, monosaccharides, fatty acids) in order to be absorbed. Digestive enzymes from the pancreas and bile salts from the liver (which are stored in the gallbladder) are critical for such digestion. Cholecystokinin is the principle stimulus for delivery of pancreatic enzymes and bile into the small intestine.



The most potent stimuli for secretion of cholecystokinin are the presence of partially-digested fats and proteins in the lumen of the duodenum (a particularly potent stimulus is pictured above). An elevation in blood concentration of cholecystokinin has two major effects that facilitate digestion :

- **Release of digestive enzymes from the pancreas** into the duodenum. Older literature refers to cholecystokinin as *pancreozymin*, a term coined to describe this effect.
- **Contraction of the gallbladder to deliver bile** into the duodenum. The name cholecystokinin (to "move the gallbladder") was given to describe this effect. Cholecystokinin is also known to stimulate secretion of bile salts into the biliary system.

Pancreatic enzymes and bile flow through ducts into the duodenum, leading to digestion and absorption of the molecules that stimulate cholecystokinin secretion. Thus, when absorption is completed, cholecystokinin secretion ceases.

Injection of cholecystokinin into the ventricles of the brain induces satiety (lack of hunger) in laboratory animals. In view of its pattern of secretion relative to feeding, it would make physiologic sense that this hormone might participate in control of food intake. However, recent experiments suggest that cholecystokinin is at best a minor player in regulation of food intake.

In addition to its synthesis in small intestinal epithelial cells, cholecystokinin has been clearly demonstrated in neurons within the wall of the intestine and in many areas of the brain. It seems, in fact, to be the most abundant neuropeptide in the central nervous system. Secretion of cholecystokinin from neurons appears to modulate the activity of other hormones and neuropeptides, but it seems safe to say the understanding its role in function of the brain is rudimentary at best.

Disease States

Diseases resulting from excessive or deficient secretion of cholecystokinin are rare. Cholecystokinin deficiency has been described in humans as part of autoimmune polyglandular syndrome, and manifests as a malabsorption syndrome clinically similar to pancreatic exocrine insufficiency. Additionally, there is mounting evidence that aberrations in expression of cholesystokinin or its receptor within the human brain may cause certain types of anxiety

and schizophrenia. Clearly, a much better understanding of the role of cholecystokinin in brain function is required.

7.5 Secretin

The small intestine is periodically assaulted by a flood of acid from the stomach, and it is important to put out that fire in a hurry to avoid acid burns. *Secretin functions as a type of fireman: it is released in response to acid in the small intestine, and stimulates the pancreas and bile ducts to release a flood of bicarbonate base, which neutralizes the acid.* Secretin also has some historical interest, as it was discovered as the first hormone.

Structure of Secretin and Its Receptors

Secretin is synthesized as a preprohormone, then proteolytically processed to yield a single 27-amino acid peptide by removal of the signal peptide plus amino and carboxy-terminal extensions. The sequence of the mature peptide is related to that of glucagon, vasoactive intestinal peptide and gastric inhibitory peptide.

The secretin receptor has seven membrane-spanning domains, characteristics of a G protein-coupled receptor.

Control and Physiologic Effects of Secretin

Secretin is secreted in response to one known stimulus : acidification of the duodenum, which occurs most commonly when liquified ingested food from the stomach are released into the small intestine.

The principal target for secretin is the pancreas, which responds by secreting a bicarbonate-rich fluid, which flows into the first part of the intestine through the pancreatic duct. Bicarbonate ion is a base and serves to neutralize the acid, thus preventing acid burns and establishing a pH conducive to the action of other digestive enzymes. A similar, but quantitatively less important response to secretin is elicited by bile duct cells, resulting in additional bicarbonate being dumped into the small gut.

As acid is neutralized by bicarbonate, the intestinal pH rises toward neutrality, and secretion of secretin is turned off.

Disease States

Diseases associated with excessive or deficient secretion of secretin are not recognized.

7.6 Ghrelin

Structure of Ghrelin and Its Receptor

Ghrelin is synthesized as a preprohormone, then proteolytically processed to yield a 28-amino acid peptide. An interesting and unique modification is imposed on the hormone during synthesis in the form of an n-octanoic acid bound to one of its amino acids; this modification is necessary for biologic activity.

Synthesis of ghrelin occurs predominantly in epithelial cells lining the fundus of the stomach, with smaller amounts produced in the placenta, kidney, pituitary and hypothalamus.

The ghrelin receptor was known well before ghrelin was discovered. Cells within the anterior pituitary bear a receptor that, when activated, potently stimulates secretion of growth hormone and that receptor was named as the **growth hormone secretagoue receptor** (GHS-R). The natural ligand for the GHS-R was announced in 1999 as ghrelin, and ghrelin was named for its ability to provoke growth hormone secretion (the suffix ghre means "grow").

Ghrelin receptors are present on the cells in the pituitary that secrete growth hormone, and also have been identified in the hypothalamus, heart and adipose tissue.

Control and Physiologic Effects of Ghrelin

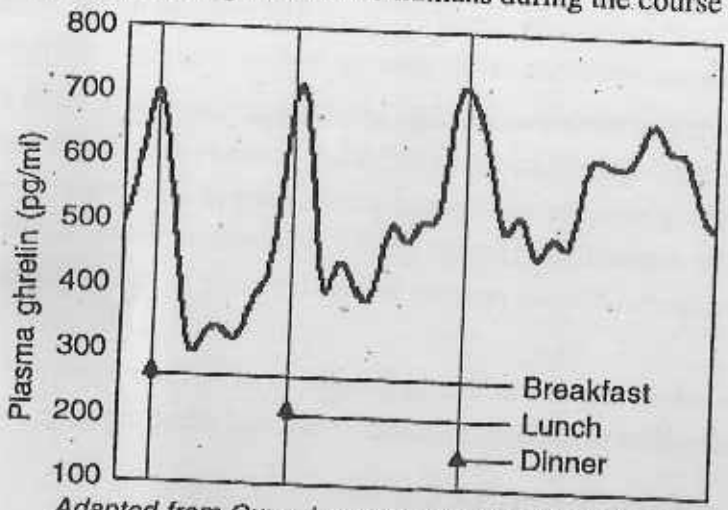
At least two major biologic activites have been ascribed to ghrelin :

- **Stimulation of growth hormone secretion :** Ghrelin, as the ligand for the growth hormone secretagogue receptor, potently stimulates secretion of growth hormone. The ghrelin signal is integrated with that of growth hormone releasing hormone and somatostatin to control the timing and magnitude of growth hormone secretion.
- **Regulation of energy balance :** In both rodents and humans, **ghrelin functions to increase hunger** though its action on hypothalamic feeding centers. This makes sense relative to increasing plasma ghrelin concentrations observed during fasting (see below). Additionally, humans injected with ghrelin reported sensations

of intense hunger. Ghrelin also appears to **suppress fat utilization in adipose tissue**, which is somewhat paradoxical considering that growth hormone has the opposite effect. Ghrelin seems to be one of several hormonal signals that communicates the state of energy balance in the body to the brain.

Other effects of ghrelin include stimulating gastric emptying activity having a variety of positive effects on cardiovascular function (e.g. increased cardiac output). It is not totally clear whether the cardiovascular effects are a direct effect of ghrelin or represent an indirect effect of ghrelin's ability to stimulate growth hormone secretion.

Blood concentrations of ghrelin are lowest shortly after consumption of a meal, then rise during the fast just prior to the next meal. The figure to the right shows this pattern based on assays of plasma ghrelin in 10 humans during the course of a day.



Adapted from Cumming et al. Diabetes 50:1714, 2001.

Given the effects of ghrelin on energy metabolism and hunger, it is a prominent target for development of anti-obesity treatments. It has been reported that immunization of rats against ghrelin resulted in decreased weight gain and adiposity relative to control rats, even though both the groups consumed equivalent amount of food. This intriguing experiment suggests the possibility of a vaccine against obesity.

Disease States

Ghrelin concentrations in blood are reduced in obese humans compared to lean control subjects, but whether this is the cause or effect is not defined. Patients with anorexia nervosa have higher than normal plasma ghrelin levels, which is decreased if weight gain occurs.

Prader-Willi syndrome is another disorder relevant to ghrelin science. Affected patients develop extreme obesity associated with uncontrolled appetite. The plasma ghrelin levels are exceptionally high in comparison to obese patients due to other causes. Prader-Willi syndrome is clearly a complex disease with many defects; it may be that excessive ghrelin production contributes to the appetite and obesity components.

7.7 Motilin

Motilin is a 22 amino acid peptide secreted by endocrinocytes in the mucosa of the proximal small intestine. Based on amino acid sequence, motilin is unrelated to other hormones.

Motilin participates in controlling the pattern of smooth muscle contractions in the upper gastrointestinal tract. There are two basic states of motility of the stomach and small intestine: the fed state, when foodstuffs are present, and the interdigestive state between meals. Motilin is secreted into the circulation during the fasted state at intervals of roughly 100 minutes. These bursts of motilin secretion are temporily related to the onset of "housekeeping contractions", which sweep the stomach and small intestine clear of undigested material (also called the migrating motor complex).

Control of motilin secretion is largely unknown, although some studies suggest that alkaline pH in the duodenum stimulates its release.

An interesting aspect of the motilin activity is that erythromycin and related antibiotics act as nonpeptide motilin agonists, and are sometimes used for their ability to stimulate gastrointestinal motility. Administration of low dose of erythromycin induces migrating motor complex, which provides additional support for the conclusion that motilin secretion triggers this pattern of GI motility, rather than results from it.

7.9 Summary

A large number of peptides are synthesized and secreted by endocrine cells of the pancreas and gastrointestinal tract. Many of these peptides circulate as hormones, but

they also function as paracrine modulators or neurotransmitters not only in the gut but in the central and peripheral nervous systems. Although some biologic actions for many of these peptides have been delineated, it seems likely that new peptides, receptors, and novel biologic functions will continue to be discovered, which may provide new opportunities for understanding the pathophysiology, diagnosis, and treatment of endocrine disease.

Unit 8 □ Biomembrane

Structure

8.1 Introduction

Membranes are crucial to the life of the cell. The cell has internal milieu which is different from its external environment .This difference is maintained throughout the life of the cell by thin surface membrane, cell or plasma membrane, which controls the entrance and exit of molecules and ions. Inside the eukaryotic cells the membranes of the endoplasmic reticulum (ER), Golgi bodies, mitochondria and other membrane bound organelles maintain the characteristic differences between the content of each organelle and the cytosol. The function of the membrane in regulating this exchange between cell and external medium as well as between the organelles and cytoplasm is called **permeability.** Although all biomembranes have the same basic phospholipid bilayer structure and certain common function, each type of cellular membrane also has certain distinctive activities determined largely by the unique set of protein associated with that membrane. There are two basic categories of proteins: integral proteins, all or part of which penetrate or span the phospholipid bilayer and peripheral proteins, which do not interact with the hydrophobic core of the bilayer. In this section basic principal that governs the organization of the phospholipids and integral proteins in all biological membranes has been discussed.

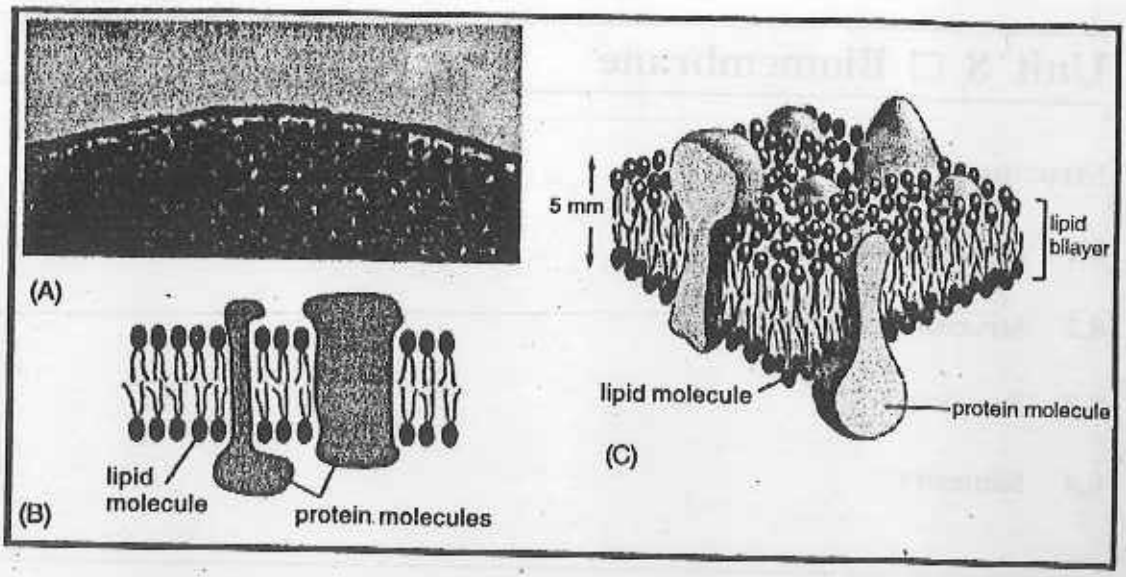


Fig. 1.

All biomembranes have a common general structure: each is a very thin film of lipid and protein molecules, held together mainly by noncovalent interaction. Cell membranes are dynamic, fluid structure and most of their molecules are able to move about in the plane of the membrane. Lipid molecules are arranged as a continuous sheet of double layer above 5nm thick. This lipid bilayer provides the basic fluid structure of the membrane and serves as relatively impermeable barrier to the passage of most water soluble molecules. Protein molecules that span the lipid bilayer mediate nearly all other functions of the membrane, transporting specific molecules across it.

8.2 Structural organization

In plasma membrane of human RBC, protein represents approximately 52% of its mass, lipids 40% and carbohydrate 8%. Oligosccharides are bound to lipids and proteins as glycolipids and glycoproteins respectively.

Lipid bilayer :

All lipid molecules in cell membrane are amphipathic (or amphiphilic), because they have a hydrophilic (water-loving) or polar end and a hydrophobic (water-fearing) or non-polar end. The most abundant lipid components are phospholipids. These have a polar

head group and two hydrophobic hydrocarbon tails. Tails are usually fatty acids and they can differ in length. In phosphoglycerides, a major class of phospholipids, fatty acyl side chains are esterified to two of the three hydroxyl group is glycerol, and the third hydroxyl group is esterified to phosphate. The phosphate group is also esterified to a hydroxyl group of another hydrophilic compound, such as choline in phosphatidyl-choline. Instead of choline, alcohol such as ethanolamine, serine and the sugar derivatives inositol are linked to the phosphate group in other phosphoglycerides.

It is the shape and amphipathic nature of the lipid molecules that cause them to form bilayers spontaneously in aquous environments. Hydrophilic molecules dissolve readily in water because they contain charged or uncharged groups that can form either favorable electrostatic interactions or hydrogen bonds with water molecules. Lipid molecules spontaneously aggregate to bury their hydrophobic tails in the interior and expose their hydrophilic heads to water. They can do this in either of the two ways : they can form spherical micelles with the tail inwards or can form bimolecular sheets or bilayers with hydrophobic tails sandwiched between the hydrophilic head groups (Fig. 10.4). A lipid bilayer has other characteristics besides its self sealing property that make it an ideal structure for cell membrane. Most important is its fluidity, which is crucial to many membrane functions.

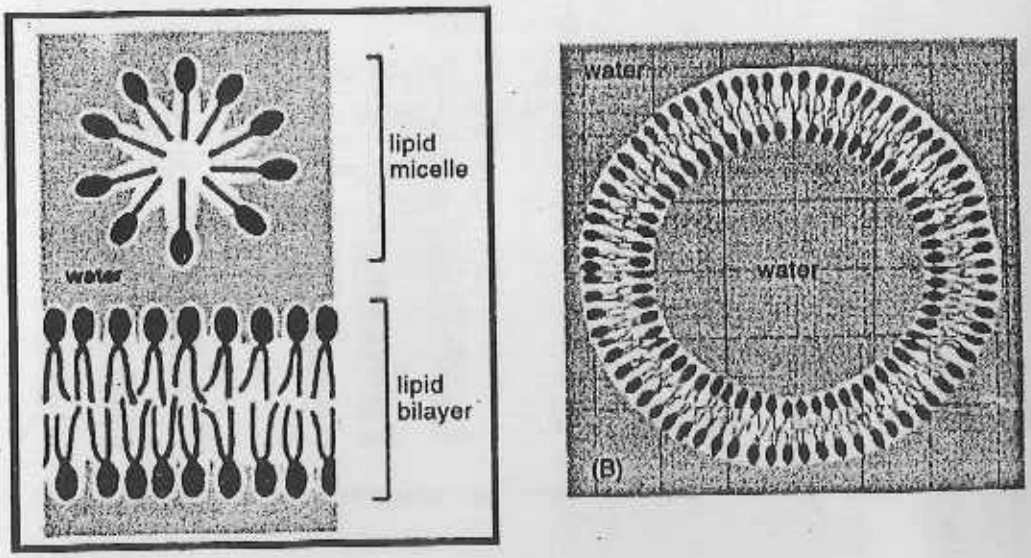


Fig. 2.

Sphingomylin and phospholipids that lack glycerol backbone, is found mainly in plasma membrane. It contains sphingosine, an amino alcohol with long unsaturated hydrocarbon chains. Cholesterol and its derivatives constitute another important class of membrane lipid. Although cholesterol is almost entirely hydrocarbon in composition, it is amphipathic because its hydroxyl group can interact with water. Cholesterol is specially abundant in plasma membrane of mammalian cells, but is absent in most prokaryotic cells. Carbohydrate is found in many membranes, covalently bound either to proteins as constituents of glycoprotein or to lipids as glycolipids. (fig. 3). Bound carbohydrate increases the hydrophilic character of lipids and proteins and help to stabilize the conformation of many proteins. The simple glycolipid and glycosylcrebrosides contain a single glucose unit attached to serimides.

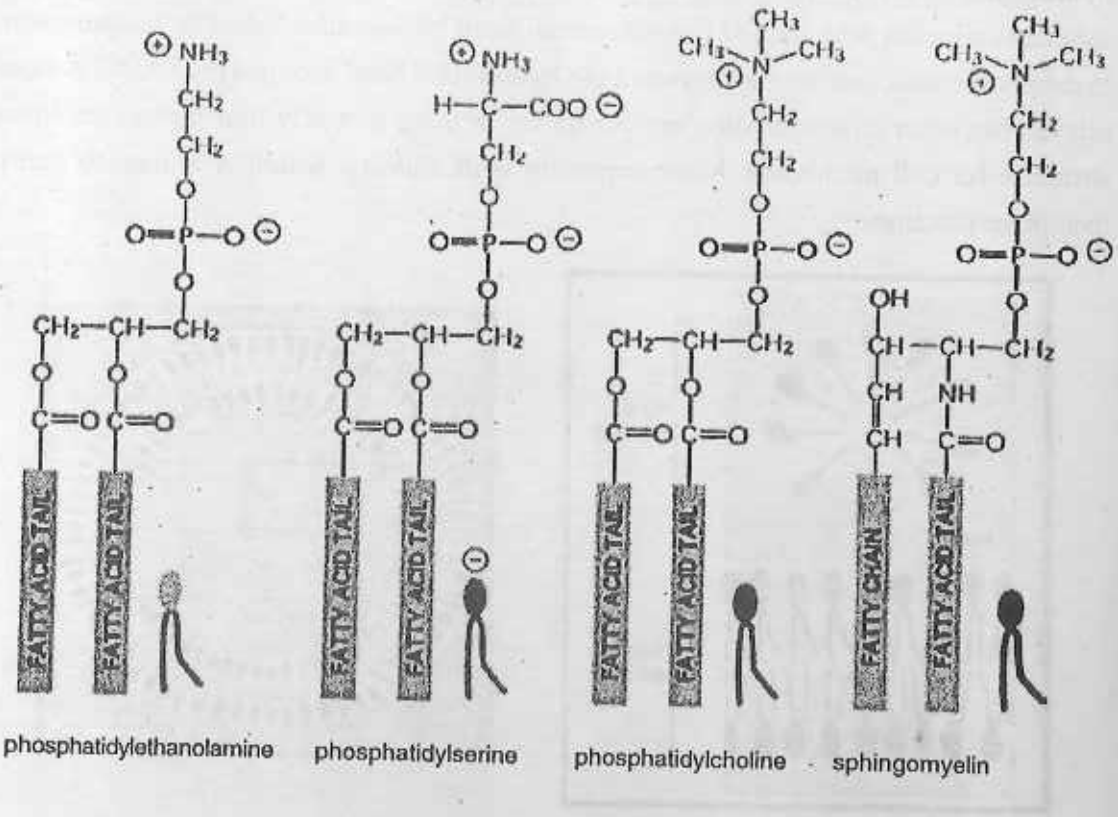


Fig. 3.

Membrane proteins

While lipids are fundamental structural elements of membrane, proteins are responsible for carrying out specific membrane functions. Most of the plasma membrane consists of approximately 50% lipid and 50% protein by weight, with carbohydrate as glycolipids or glycoprotein, constituting 5 to 10% of the membrane mass. Since proteins are much larger than lipids, there is always many more lipid molecules than protein molecules present in the membrane. About 50 lipid molecules are present for each protein molecule. In 1972 Jonathan Singer and Gorth Nicolson proposed the fluid mosaic model of membrane structure which is now accepted as basic paradigm for organization of all the biological cell membrane.

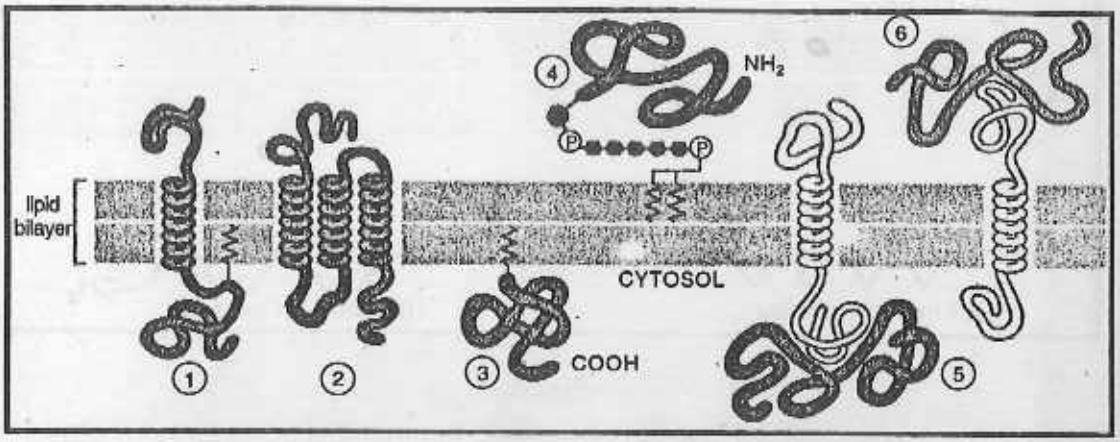


Fig. 4.

In this model, membranes are viewed as a two-dimensional fluid structures in which proteins are inserted into lipid layers. Singer and Nicolson distinguished two classes membrane proteins, peripherial and integral. Peripherial membrane proteins are defined as proteins that dissociate from the membrane following treatment with polar reagents such as solution of extreme pH or high salt concentrations. These proteins are not inserted into hydrophobic interior of the lipid bilayer. Instead they are indirectly associated with membrane through protein-protein interactions. These interactions frequently involve ionic bond. Integral membrane proteins can be released only by treatment in the phospholipids

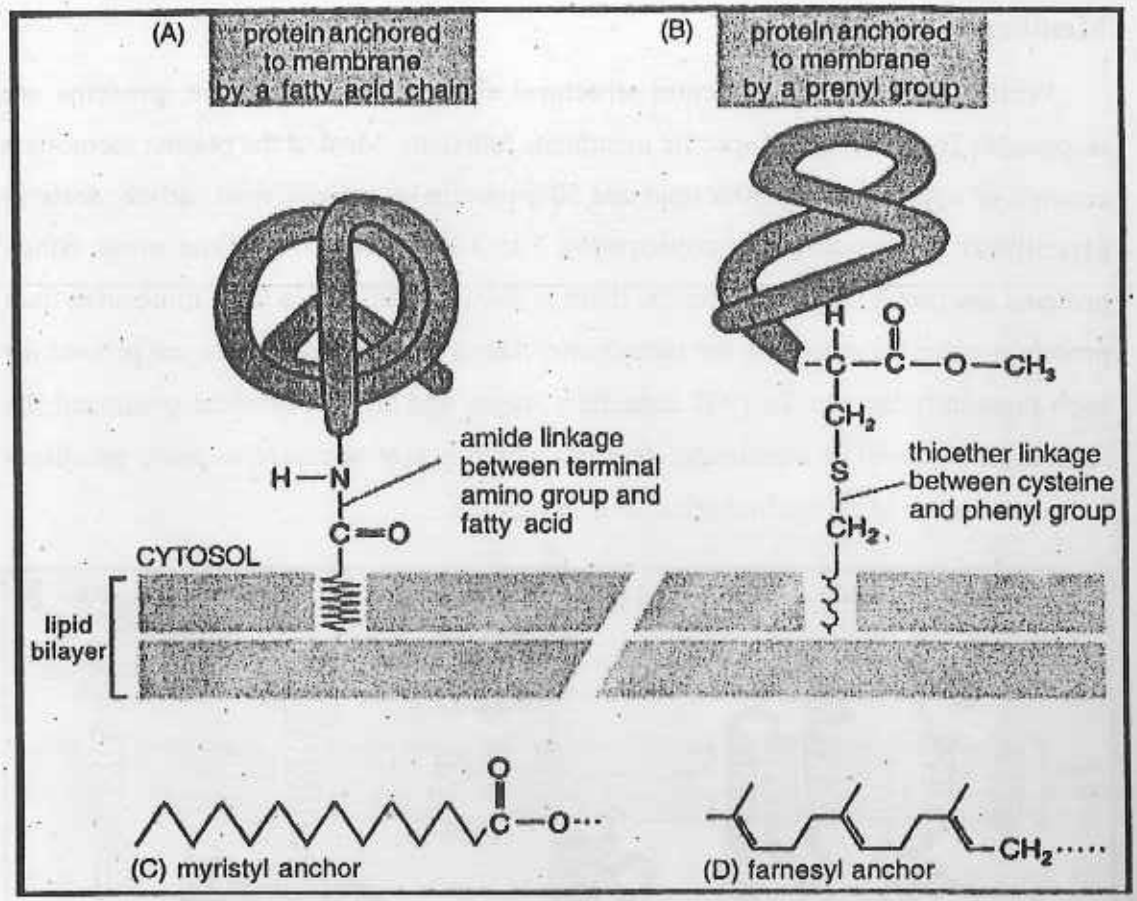


Fig. 5.

bilayer. Portions of these membrane proteins are inserted into the lipid bilayers, Most commonly used reagents for solubilization of integral membrane proteins are detergents, which are small amphipathic molecules containing both hydrophobic and hydrophilic groups. Many integral proteins are transmembrane proteins, which span the lipid bilayers with portions exposed on both sides of the membrane. They are also amphipathic and their hydrophilic regions pass through the membrane and interact with hydrophobic tails of the lipid molecules in the interior of the bilayer lipid. Their hydrophobic regions are exposed to water on either side of the membrane. Other membrane proteins are located entirely in the cytosolic monolayer of the lipid bilayer either by an amphipathic helix exposed to either surface of the proteins or by one or more covalently attached lipid chain, which can be fatty

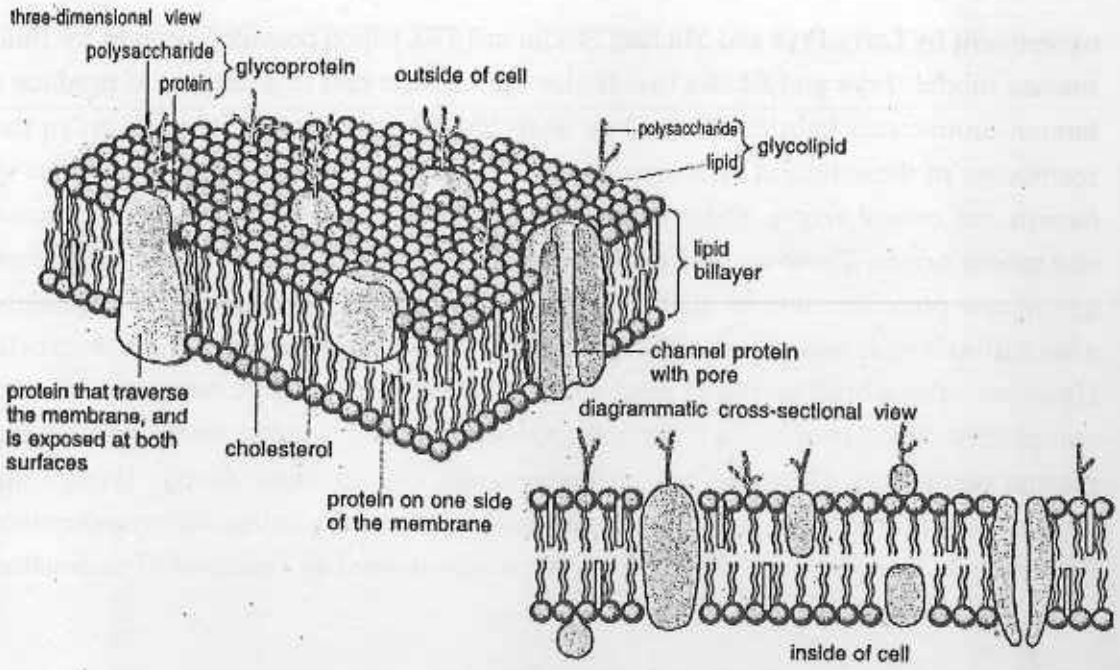


Fig. 6.

acid chains or phenyl groups. Other membrane proteins are entirely exposed at the external cell surface, being attached to the lipid bilayer only by covalent linkage to phosphatidylinositol in outer lipid monolayer.

The lipid-linked proteins are made as soluble proteins in the cytosol and are subsequently directed to the membrane by the covalent attachment of the liquid. Some proteins are made as single transmembrane proteins in the endoplasmic reticulum, while in the ER the transmembrane segments of the protein are cleaved off and a glycophosphatidylinositol anchor is added, leaving the protein bound to the noncytosolic surface of the membrane solely by their anchor.

Mobility of membrane proteins :

Membrane proteins and phospholipids are unable to move back and forth between the inner and outer leaflets of the membrane at an appreciable rate. However, because they are inserted to a fluid lipid bilayer, both proteins and lipid are able to diffuse laterally through the membrane. This lateral movement was first shown directly in an

experiment by Larry Frye and Michael Edidin in 1970, which provided support for fluid mosaic model. Frye and Edidin fuse human and mouse cell in a culture to produce a human-mouse cell hybrids. Then they analyzed the distribution of proteins in the membrane of these hybrid *cells using antibodies sufficiently to recognize proteins of human and mouse origin*. These antibodies specifically recognize proteins of human and mouse origin. These antibodies were labeled with fluorescent dyes. So the human and mouse proteins could be distinguished by fluorescence microscope. Immediately after fusion human and mouse cells are localized to different halves of the hybrid cell. However, after a brief period of incubation at 37°C, human and mouse proteins were completely mixed over the cell surface, indicating that they move freely through the plasma membrane. However, not all proteins are able to move freely through the membrane. In some cases, mobility are restricted by their association with cytoskeleton. For example, function band 3 RBC membrane is immobilized as a result of its association with ankylon spectrin.

Many membrane proteins are glycosylated

The great majority of transmembrane proteins in animal cells are glycosylated. As in glycolipids, the sugar residues are added in the lumen of ER and Golgi apparatus. For this reason, the disaccharide chains are always present in noncytosolic side of the plasma membrane.

8.3 Function

In all cells plasma membrane has several essential functions. These include transporting nutrients and metabolic waste out of the cells, preventing unwanted materials extracellular milieu into the from entering the cell into the thusly preventing loss of needed metabolites and maintaining the proper ionic composition, pH (7.2), and osmotic pressure of the cytosol. To carry out these functions, the plasma membrane contains specific transporter proteins that permit the passage for entry of certain small molecules. Several of these proteins use the energy released by ATP hydrolysis to pump ions and other molecules out of the cells against their concentration gradient. Small charged molecules such as ATP and amino acids can diffuse freely within the cytosol but are restricted in their ability to leave or enter across the plasma membrane.

In addition to their universal function, the plasma membrane has other crucial roles in multicellular organism. Few of the cells in multicellular plants and animals exist as isolated entities, rather groups of cells with related specialization combine to form tissues. Specilized area of the plasma membrane contains proteins and glycolipids that form specific contact and junction between the cells to strengthen tissues and allow the exchange of metabolites between the cells. Other proteins in the plasma membrane act as anchoring proteins for many of the cytoskeletal fibers that present in the cytosol, imparting shape and strength of the cell. In addition, enzymes bound on the plasma membrane catalyzes reactions that would occur with difficulty in environment. Plasma membrane of many types of cell also contains receptor proteins that bind specific signaling molecules, leading to various cellular responses.

8.4 Summary

The phospholipid bilayer forms the basic structure of all biomembranes, which also contain proteins, glycoproteins, cholesterol, and other sterols, and glycolipids. The presence of specific sets of membrane proteins permit each type of membrane to carry out distinctive functions. In a phospholipid bilayer, the long fatty acyl side chains in each leaflet are oriented towards one another, forming a hydrophobic core; the polar head groups line both surface. All the cellular membranes have closed compartments, and have a cytosolic and an exoplasmic components. The asymmetry of biological membranes is reflected in the specific orientation of each type of integral and peripheral membrane protein with respect to the cytosolic and exoplasmic faces. The presence of glycolipids exclusively in the exoplasmic leaflets also contribute to membrane asymmetry. Most integral proteins and lipids are laterally mobile in biomembranes. According to the fluid mosaic model, the membrane is viewed as a two-dimensional mosaic of phospholipids and protein molecules.

Unit 9 □ Basic Mechanism of Cell Signaling Pathway

Structure

9.1 Basic mechanism of cell signaling

No cell lives in isolation. In all multicellular organisms, survival depends on elaborate intracellular communication network that coordinate growth, differentiation and metabolism of the multitude of cells in diverse tissues and organs. These communication mechanisms depend heavily on extracellular signaling molecules, which are produced by the cells to signal to their neighboring cells or further away from those cells. They also depend on elaborate system of proteins that each cell contains to enable to respond to a particular subset of these signals in a cell-specific pathway. These proteins include cell surface receptor proteins, which bind the signaling molecules and a variety of intracellular signaling proteins that distribute the signal to appropriate part of the cell. Among the intracellular signaling proteins are kinases, phosphatases, GTP-binding proteins etc. At the end of each intracellular signaling pathway are target proteins, which are altered when the pathway is active and change the behavior of the cell. These target proteins can be gene regulated proteins, ion channels, components of a metabolic pathway, part of cytoskeleton etc.

Mechanism enabling one cell to influence the behavior of the cells certainly exists in unicellular organism, long before multicellular organism appears in the earth. Yeast cells normally live independently. They can communicate and influence one another's behavior in preparation on mating. In such organism when a haploid individual is ready to mate it secretes a peptide mating factor that sends signals to the cells of the opposite sex to mate.

Communication by extracellular signaling usually involves six steps : i) synthesis and ii) release of signaling molecules by the cell, iii) transport of the signaling molecules to the

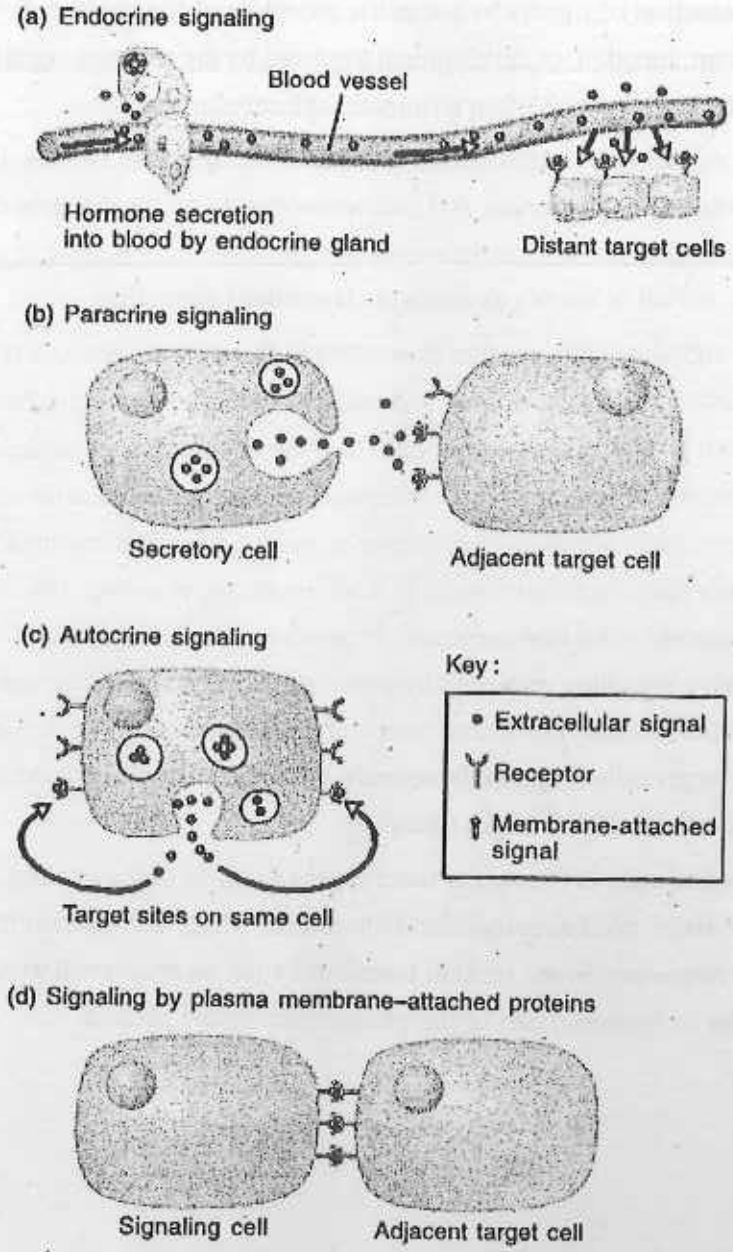


Fig. 1. General schemes of intercellular signaling in animals. (a–c) Cell-to-cell signaling by extracellular chemicals occurs over distances from a few micrometers in autocrine and paracrine signaling to several meters in endocrine signaling. (d) Proteins attached to the plasma membrane of one cell can interact directly with receptors on an adjacent cell.

target cell, iv) detection of signals by a specific receptors of the target cell, v) a change in cellular metabolism, function, or development triggered by the receptor-signal complex, and vi) removal of the signal which often terminates the cellular response.

In animals, signaling by extracellular secreted molecules can be classified into three types namely, **autocrine**, **paracrine**, and **endocrine**, based on the distance over which the signals acts. In addition certain membrane-bound proteins on one cell can directly signal an adjacent cell, which is known as **contact-dependent signaling**.

In autocrine signaling cells respond to substances that they themselves release (Fig.1c). Many growth factors act in this fashion, and cultured cell often secrete growth factors that stimulate their own growth and proliferation. This type of signaling is particularly common in tumor cells, many of which overproduce and release growth factors that stimulate inappropriate uncontrolled growth. In paracrine signaling, the signaling molecules secreted by a cell affect only the target cells present in close proximity to it (Fig. 1b). The conduction of an electric impulsre from one nerve cell to another, or from nerve cell to muscle cell occurs via paracrine signaling mediated by neurotransmitters. In endocrine signaling, the signaling molecules, called hormones, which are synthesised at one site and act on the distantly located target cells (Fig. 1a). In animals, a hormone usually is carried by the blood from its site of synthesis to the target tissue.

Some compounds can act in two or three types of cell-to-cell signaling. Certain small amino acid derivatives, such as epinephrine functions l th as neurotransmitter (paracrine) and as systemic hormone. Some protein hormones such as epidermal growth factor are synthesized as the exoplasmic part of the plasma membrane protein.

Unit 10 □ Cell Surface Receptors, Second Messenger System, MAP kinase pathway

Structure

10.1 Introduction

All water soluble signal molecules including neurotransmitters and all signal proteins bind to specific receptor protein of the plasma membrane of the target cells and influence the target cell activity. They convert an extracellular ligand-binding events into intracellular signals that alter the behavior of the target cell. Binding of ligand to some of these receptors induces second-messenger formation, whereas ligand binding to others do not. Most cell surface receptor belongs to one of the four classes, defined by the transduction mechanism which are described below.

10.2 Types of receptors

a) **G-protein-coupled receptors :** Ligand binding activates a GTP-binding protein (G-protein), which in turn activates or inhibits an enzyme that generates a second messenger or modulate an ion channel, causing a change in membrane potential. All G-protein-coupled receptors belong to a large family of homologous seven-transmembrane domain.

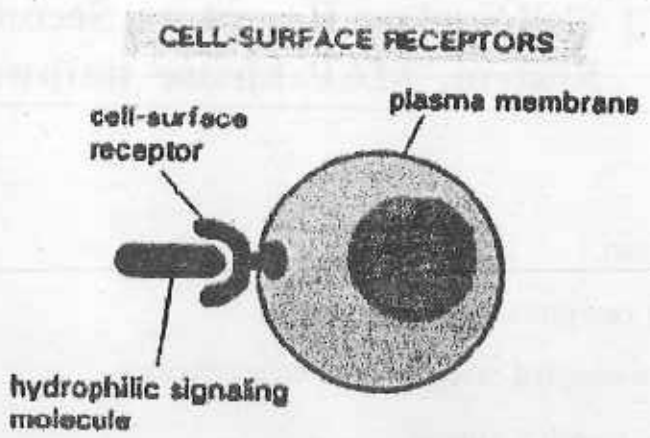


Fig. 1.

b) **Ion-channel receptors :** They are also known as transmitter-gated ion channel or ionotropic receptors. Ligand binding changes the conformation of the receptors so that specific ions flow through it. The resultant ion movement alters the electric potential across the membrane. This type of receptors are involved in rapid synaptic signaling between electrically excitable cells and is mediated by a small number of neurotransmitters that transiently open or close an ion channel. The ion-channel-linked receptors belong to large family of homologous, multipass transmembrane protein.

c) **Tyrosine kinase-linked receptor :** These receptors lack intrinsic catalytic activity, but ligand binding stimulates formation of a dimeric receptor, which then interacts with and activates one or more cytosolic protein-tyrosine kinases. The receptors for many cytokines, the interferons, and human growth factors are of these types. These tyrosine-kinase receptors are sometimes referred to as cytokine-receptor superfamily.

d) **Receptor with intrinsic tyrosine-kinase activity :** When activated these receptors can function directly as enzymes. They can be formed by a single-pass transmembrane proteins that have their ligand binding site out side the cell and their catalytic or enzyme-binding site inside the cell. Some activated receptors catalyze conversion of GTP or GMP, other act as protein phosphatases, removing phosphate group from phosphotyrosine residue in the substrate proteins, thereby

modifying their activity. The receptors for insulin and many growth factors are ligand-triggered protein kinases. In most cases the ligand binds as a dimer, leading to dimerization of the receptors and activation of its kinase activity. These receptors often referred to as receptor serine/threonine kinase or receptor tyrosine kinases.

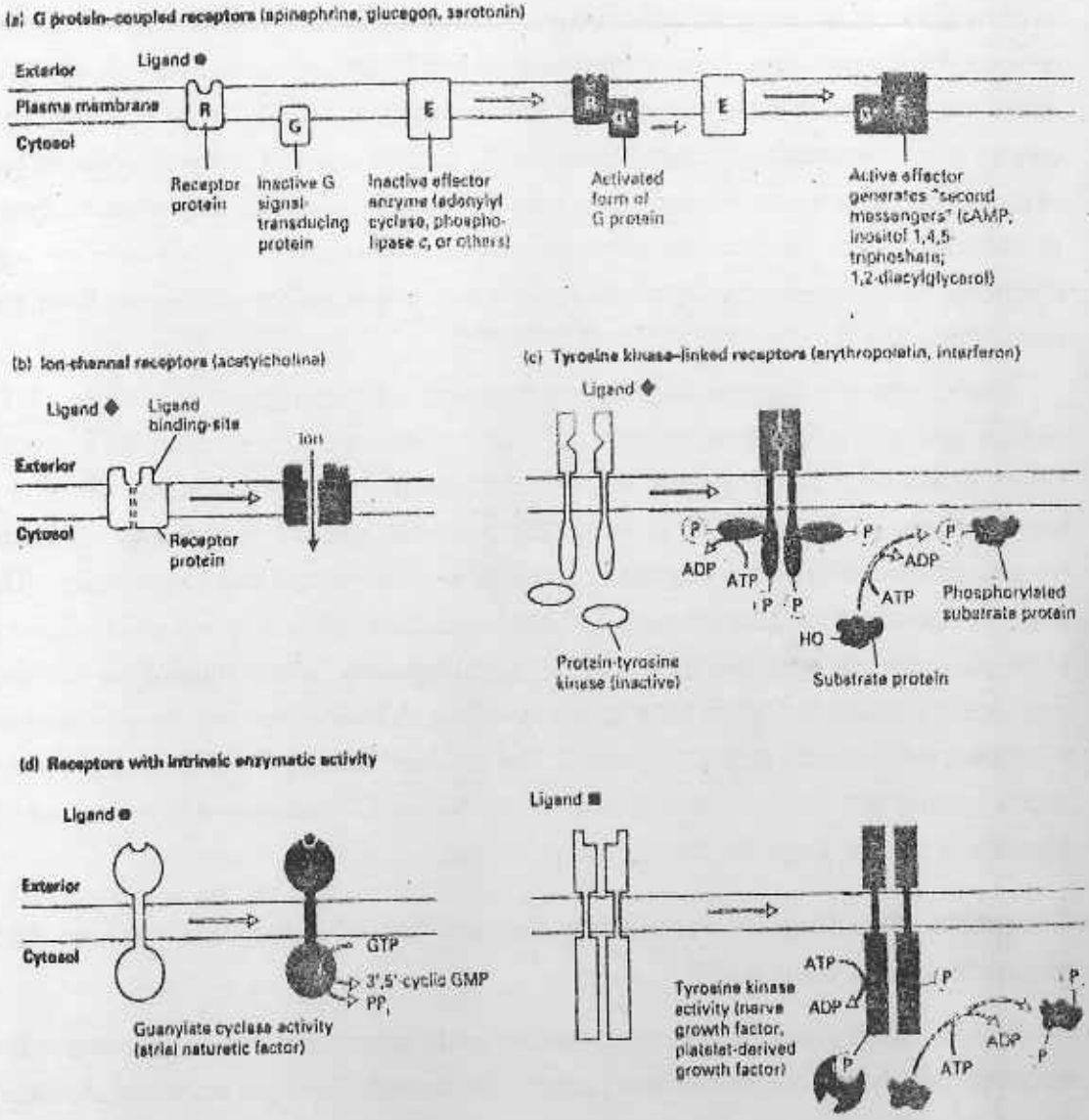


Fig. 2.

10.3 G-protein-coupled receptors and their effectors

G-protein coupled receptors (GPCRs) are the largest family of cell-surface receptors and are found in all eukaryotes. G-protein-coupled receptors mediate the response to an enormous diversity of signal molecules. These signaling molecules that activate them are as varied in structure as they are in function and include protein and small peptides, as well as derivatives of amino acids and fatty acids. Same ligand can activate many different receptor family members. Despite the chemical and functional diversity of the signaling molecules that bind to them, all G-protein-coupled receptors have a similar structure. They contain seven membrane-spanning regions with C-terminal segment on the cytoplasmic face of the plasma membrane. These receptors are also sometimes called serpentine receptors. In addition to their characteristics orientation in the plasma membrane, they have the same functional relationship to the G proteins which they use to signal the cell interior having an extracellular ligand.

The G proteins that transduce signals from extracellular ligands is a trimeric GTP binding proteins having three subunits- α, β and γ. In the unstimulated state, the α subunit has GDP bound and the G protein is inactive. When stimulated by activated receptor, α subunit releases its bound GDP, allowing GTP to bind its place. This exchange causes the trimer to dissociate into two activated components- an α subunit and a $\beta\gamma$ complex. The targets of the dissociated components of the G proteins are either enzymes or ion channels in the plasma membrane, and they relay the signal onwards. The α subunit is a GTPase, and once it is bound to GTP to GDP, it reassociates with $\beta\gamma$ complex to reform an inactive G-protein, reversing the activation process. The activated state of G-protein is short-lived. Alpha subunit of G-proteins may G.stimulatory (G_s) or G-inhibitory (G_i) or may be G_q depending upon its targets in the plasma membrane.

G-protein signaling by regulating the activity of adenylate cyclase and production of cyclic AMP

In many types of cell, for example, binding of different hormones to their respective receptors induces the activation of adenylate cyclase through G-protein activation. Acivated adenylate cyclase then converts membrane bound ATP to cyclic AMP (cAMP). Cyclic AMP was first identified as small intracellular mediator in the 1950s. The normal

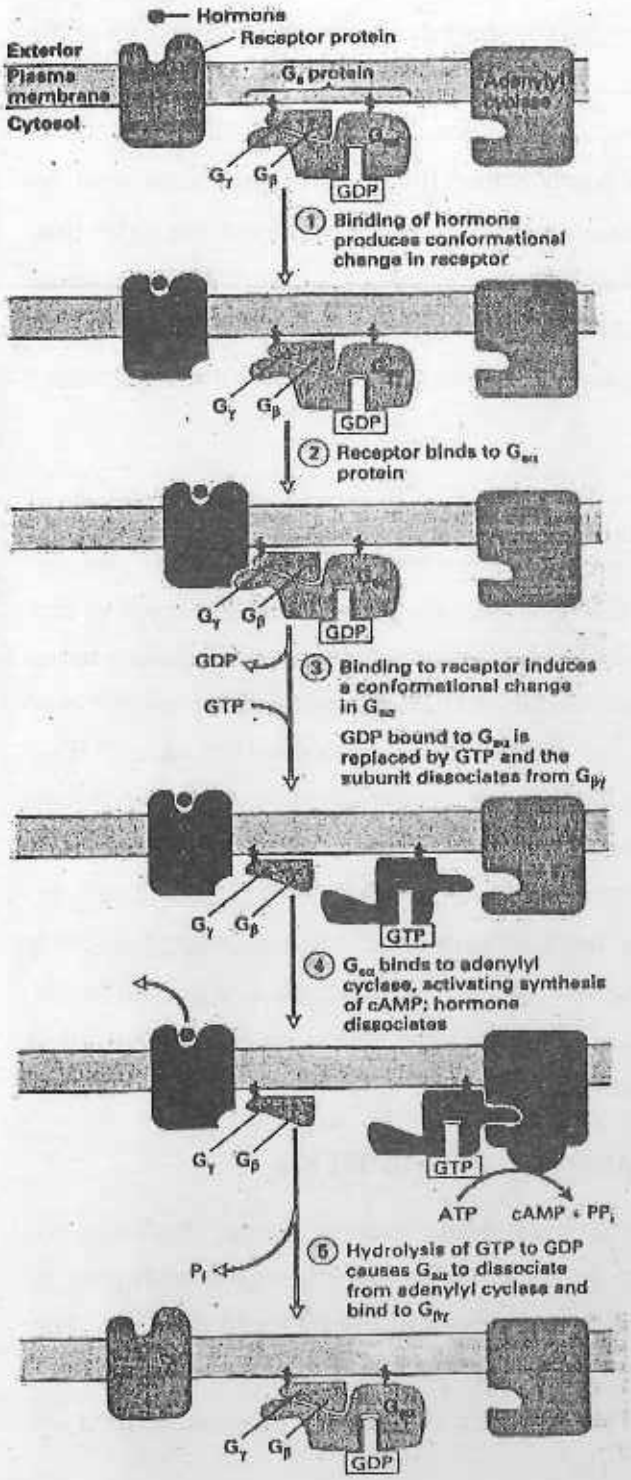


Fig. 3. Activation of adenylyl cyclase following binding of an appropriate hormone (e.g., epinephrine, glucagon) to a G_s protein–coupled receptor. Following ligand binding to the receptor, the G_s protein relays the hormone signal to the effector protein, in this case adenylyl cyclase. G_s cycles between an inactive form with bound GDP and an active form with bound GTP. Dissociation of the active form yields the $G_{s\alpha}$·GTP complex, which directly activates adenylyl cyclase. Activation is short-lived because GTP is rapidly hydrolyzed (step 5). This terminates the hormone signal and leads to reassembly of the inactive G_s·GDP form, returning the system to the resting state. Binding of another hormone molecule causes repetition of the cycle. Both the G_γ and $G_{s\alpha}$ subunits are linked to the membrane by covalent attachment to lipids. Binding of the activated receptor to $G_{s\alpha}$ promotes dissociation of GDP and its replacement with GTP.

concentration of cAMP inside the cell is about 10^{-7} M, but an extracellular signal can cause cAMP levels to change more than twenty fold in seconds. Such a rapid synthesis of the molecule is balanced by its rapid breakdown or removal. In fact, cAMP is continuously destroyed by one or more cAMP phosphodiesterase that hydrolyzes cAMP to adenosine 5'-monophosphate (5'-monophosphate). Many extracellular signal molecules work by increasing cAMP and they do so by increasing the activity of adenylate cyclase rather than decreasing the activity of phosphodiesterase. All receptors that act via cAMP are coupled to stimulatory G protein. Another G protein, called inhibitory G protein inhibits adenylate cyclase but it mainly acts by directly regulating the ion channels rather than decreasng cAMP content.

10.4 Receptor tyrosine kinases

Receptor tyrosine kinae (RTKs) are second major types of cell surface receptors and ligand for RTKs are soluble or membrane-bound peptide/protein hormones including nerve growth factor (NGF), platelet-derived growth factor (PDGF) , fibroblast growth factor (FGF), epidermal growth factor (EGF), insulin and insulin-like growth factor (IGFs). Binding of ligand to this type of receptor stimulates receptor's intrinsic protein-tyrosine kinase activity, which subsequently stimulates a signal transduction cascade leading to changes in cellular physiology and/or pattern of gene expression. Some RTKs have been identified in studies in human cancers associated with mutant forms of growth factor receptors, which send a proliferative signal to cells even in the absence of growth factor. One such mutant receptor, encoded at new locus, contributes to the uncontrolled proliferation of certain human breast cancers.

Ligand binding leads to autophosphorylation of of RTKs

All RTKs comprises an extracellular ligand binding domain, a single hydrophobic transmembrane domain and a cytosolic domain that includes a region with protein tyrosine kinase activity. Binding of ligand causes most of the RTKs to dimerise. The protein kinase of each receptor monomer then phosphorylates a distinct set of tyrosine residues in the cytosolic domain of its dimmer partner, a process termed as

autophosphorylation (Fig. 4). Autophosphorylation occurs in two stages. First, tyrosine residues in the phosphorylation tip near the catalytic site are phosphorylated. This leads to a conformational change that facilitates binding of ATP in some receptors (e.g., insulin receptors) and binding of protein substrates in other receptors (e.g., FGF receptors). The receptor kinase activity then phosphorylates other sites in the cytosolic domain; the resulting phosphotyrosines serve as docking sites for other proteins involved in RTK-mediated signal transduction. Phosphotyrosine residues in several RTKs interact with adapter proteins containing SH2 PTB domains. These proteins couple the activated receptors with the other component of the signal transduction pathway but have no intrinsic signaling properties.

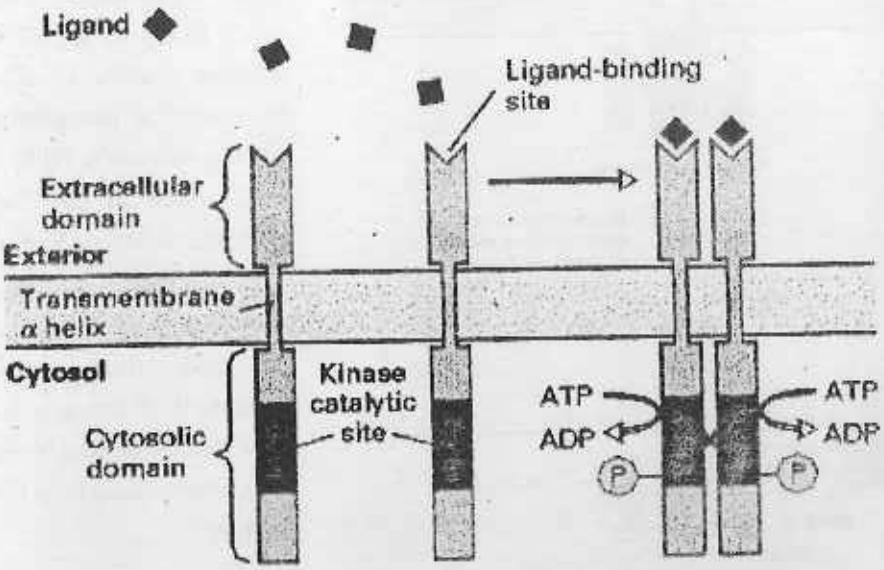


Fig. 4. General structure and activation of receptor tyrosine kinases (RTKs). The ligands for some RTKs, such as the receptor for EGF depicted here, are monomeric; ligand binding induces a conformational change in receptor monomers that promotes their dimerization. The ligands for other RTKs are dimeric; their binding brings two receptor monomers together directly. In either case, the kinase activity of each subunit of the dimeric receptor initially phosphorylates tyrosine residues near the catalytic site in the other subunit. Subsequently, tyrosine residues in other parts of the cytosolic domain are autophosphorylated.

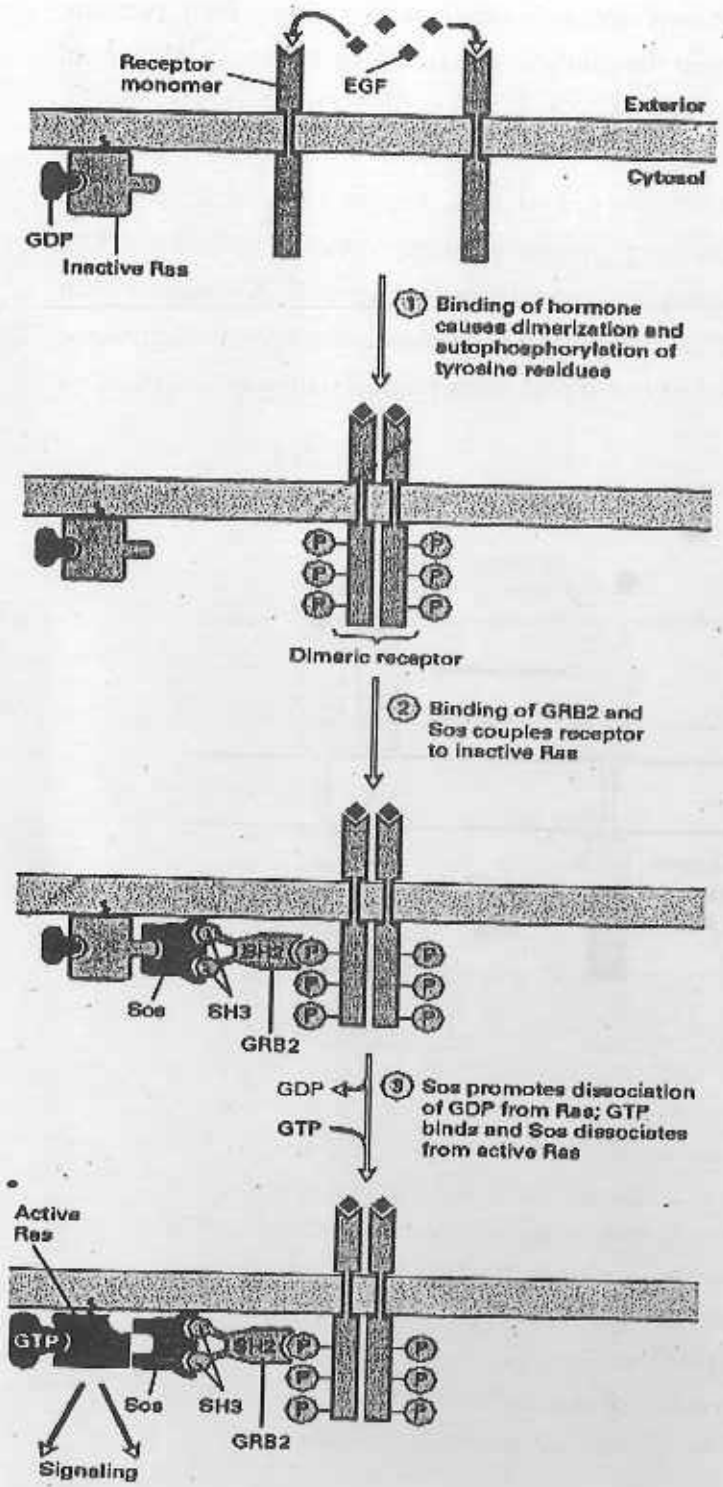


Fig. 5. Activation of Ras following binding of a hormone (e.g., EGF) to an RTK. The adapter protein GRB2 binds to a specific phosphotyrosine on the activated RTK and to Sos, which in turn interacts with the inactive Ras · GDP. The guanine nucleotide–exchange factor (GEF) activity of Sos then promotes formation of the active Ras · GTP. Note that Ras is tethered to the membrane by a farnesyl anchor.

Activation of Ras

Ras is a GTP-binding switch protein that alternates between an active on state with bound GTP and an inactive off state with bound GDP. Activation of Ras is triggered by ligand binding to RTKs. An adaptor protein and a guanosine neucleotide exchange factor (GEF) link most activated RTKs to Ras. GEF which binds to the Ras-GDP complex, causing dissociation of bound GDP yielding Ras-GTP form. Because GTP is present in cells at a higher concentration than GDP, GTP binds spontenously to "empty" Ras molecules, with release of GEF-GTPase-activating proteins (GAPs) increase the rate of hydrolysis of bound GTP by Ras, thereby inactivating Ras (Fig. 5).

Ras belong to large Ras superfamily of monomeric GTPases. The family also contains two other subfamilies: the Rho family, involved in relaying signals from cell-surface receptors to the actin cytoskeleton and elsewhere, and the rab family is involved in regulating the traffic of intracellular transport vesicles.

An adaptor protein and GEF link most activated RTKs to Ras

The first indication that Ras functions downstream a RTKs in a common signaling pathway came from experiments in which cultured fibroblast cells were induced to proliferate by treatment with a mixture of platelet-derived growth factor (PDGF) and epidermal growth factor (EGF). Microinjection of anti-Ras antibodies into these cells blocked cell proliferation. Conversely, injection of a constitutively active mutant Ras protein (i.e., Ras^{D}), which hydrolyzes GTP very inefficiently and thus persists in the active state, caused the cells to proliferate in the absence of the growth factors. These findings are consistent with studies showing that addition of fibroblast growth factor (FGF) to fibroblasts leads to a rapid increase in the proportion of Ras present in the GTP-bound active form.

But how does binding of a growth factor (e.g., EGF) to the RTK (e.g., the EGF receptor) leads to activation of Ras? Two cytosolic proteins—GRB2 and Sos—provide the key links. An *SH2 domain* in GRB2 binds to a specific phosphotyrosine residue in the activated receptor. GRB2 also contains two *SH3 domains,* which bind and activate Sos. GRB2 thus functions as an adapter protein for the EGF receptor. Sos functions as a

guanine nucleotide–exchange protein (GEF), which helps to convert inactive GDP-bound Ras to the active GTP-bound form.

Genetic analysis of mutants blocked at particular stages of differentiation have provided considerable insight into RTK signaling pathways. Most of these genetic studies were done in the worm *C. elegans* and in the fly *Drosophila*. Mutants in these species in which development of specific cells is blocked is particularly useful in elucidating the pathway from an RTK to Ras.

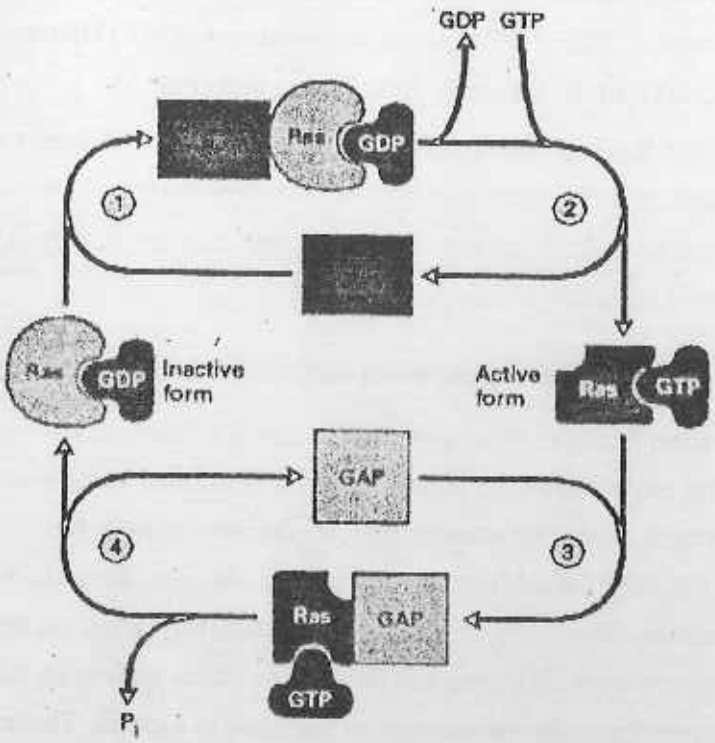


Fig. 6. Cycling of the Ras protein between the inactive form with bound GDP and the active form with bound GTP occurs in four steps. By mechanisms discussed later, binding of certain growth factors to their receptors induces formation of the active Ras · GTP complex. Step **1** : Guanine nucleotide–exchange factor (GEF) facilitates dissociation of GDP from Ras. Step **2** : GTP then binds spontaneously, and GEF dissociates yielding the active Ras · GTP form. Steps **3** and **4** : Hydrolysis of the bound GTP to regenerate the inactive Ras · GDP form is accelerated hundredfold by GTPase-activating protein (GAP). Unlike G_α, cycling of Ras thus requires two proteins, GEF and GAP; otherwise, G_α and Ras exhibit many common features.

SH2 Domain in GRB2 Adapter Protein Binds to a Specific Phosphotyrosine in an Activated RTK

To identify proteins that associate with phosphotyrosine residues in the cytosolic domain of activated EGF receptors, scientists used an expression cloning strategy. cDNAs synthesized from mRNAs isolated from human brain-stem tissue were inserted into a ygt11 expression vector, which then was plated on a lawn of *E. coli* cells. When the resulting cDNA library was screened using a fragment of phosphorylated human EGF receptor as the probe, two cDNA clones were identified. One encoded a subunit of PI-3 kinase that contains an SH2 domain and the other encoded GRB2, a homolog of the SH2-containing adapter protein identified in the *Drosophila* Sev pathway. Thus GRB2 and its *Drosophila* homolog are adapter proteins that function downstream from RTKs but upstream of Ras in both the flies and mammalian cells.

GRB2 and similar adapter proteins bind to different phosphotyrosine residues on RTKs via the conserved SH2 domain. This domain derived its full name, the *Src homology 2 domain*, from its homology with a region in the prototypical cytosolic tyrosine kinase encoded by *src*. The three-dimensional structures of SH2 domains in different proteins are very similar. Each binds to a distinct sequence of amino acids surrounding a phosphotyrosine residue. The unique amino acid sequence of each SH2 domain determines the specific phosphotyrosine residues it binds. The SH2 domain of the Src tyrosine kinase, for example, binds strongly to any peptide containing the critical core sequence of phosphotyrosine–glutamic acid–glutamic acid–isoleucine. These four amino acids make intimate contact with the peptide-binding site in the Src SH2 domain. Binding resembles the insertion of a two-pronged "plug"—the phosphotyrosine and isoleucine residues of the peptide—into a two-pronged "socket" in the SH2 domain. The two glutamic acids fit singly into the surface of the SH2 domain between the phosphotyrosine socket and the hydrophobic socket that accepts the isoleucine residue. Variations in the nature of the hydrophobic socket in different SH2 domains allow them to bind to phosphotyrosines adjacent to different sequences, accounting for differences in their binding specificity.

Activated RTKs also can recruit signaling molecules through a different domain called the phosphotyrosine binding (PTB) domain. While SH2-binding specificity is largely

determined by residues C-terminal to the phosphotyrosine, PTB-binding specificity is determined by specific residues five to eight residues N-terminal to the phosphotyrosine residue.

Sos, a Guanine Nucleotide–Exchange Factor, Binds to the SH3 Domains in GRB2

In addition to one SH2 domain, which binds to phosphotyrosine residues in RTKs, GRB2 contains two SH3 domains, which bind to Sos, a guanine nucleotide–exchange factor. SH3 domains, which contain ≈55–70 residues, are present in a large number of proteins involved in intracellular signaling. Although the three-dimensional structures of various SH3 domains are similar, their specific amino acid sequences differ. SH3 domains selectively bind to proline-rich sequences in Sos and other proteins; different SH3 domains bind to different proline-rich sequences.

Proline residues play two roles in the interaction between an SH3 domain in an adapter protein (e.g., GRB2) and a proline-rich sequence in another protein (e.g., Sos). First, the proline-rich sequence assumes an extended conformation that permits extensive contacts with the SH3 domain, thereby facilitating interaction. Second, a subset of these prolines fit into binding "pockets" on the surface of the SH3 domain. Several nonproline residues also interact with the SH3 domain and are responsible for determining the binding specificity. Hence the binding of peptides to SH2 and SH3 domains follows a similar strategy: certain residues provide the overall structural motif necessary for binding, and neighboring residues confer specificity to the binding.

Following hormone-induced activation of an RTK (e.g., the EGF receptor), a complex containing the activated RTK, GRB2, and Sos is formed on the cytosolic face of the plasma membrane. Formation of this complex depends on the dual binding ability of GRB2. Receptor activation thus leads to relocalization of Sos from the cytosol to the membrane, bringing Sos near to its substrate, membrane-bound Ras-GDP. Biochemical and genetic studies indicate that the C-terminus of Sos inhibits its nucleotide exchange activity and that GRB2 binding relieves this inhibition. Binding of Sos to Ras-GDP leads to changes in the conformation of two regions of Ras, switch I and switch II, thereby

opening the binding pocket for GDP so it can diffuse out. Because GTP is present in cells at a concentration 10 times higher than GDP, GTP binding occurs preferentially, leading to activation of Ras. The activation of Ras and $G_{s\alpha}$ thus occurs by similar mechanisms: a conformational change induced by binding of a protein—Sos and an activated GPCR, respectively—that opens the protein structure. So bound GDP is released to be replaced by GTP. As we discuss in the next section, binding of GTP to Ras, in turn, induces a specific conformation of switch I and II that allow Ras-GTP to activate downstream effector molecules.

Several other proteins, including GAP, bind to specific phosphotyrosines in activated RTKs. This binding localizes GAP close to Ras-GTP, so it can promote the cycling of Ras; exactly how GAP interacts with Ras and perhaps other components of the RTK-Ras pathway is unclear.

10.5 MAP Kinase Pathways

All Ras-linked RTKs in mammalian cells appear to utilize a highly conserved signal-transduction pathway in which the signal induced by ligand binding is carried via GRB2 and Sos to Ras, leading to its activation. Activated Ras then induces a kinase cascade that culminates in activation of MAP kinase. This serine/threonine kinase, which can translocate into the nucleus, phosphorylates many different proteins including transcription factors that regulate expression of important cell-cycle and differentiation of specific proteins. In this section, we first examine the components of the kinase cascade downstream from Ras in RTK-Ras signaling pathways in mammalian cells. Then we discuss the linkage of other signaling pathways to similar kinase cascades and recent studies indicating that both yeasts and cells of higher eukaryotes contain multiple MAP kinases.

Activation of MAP kinase in two different cells can lead to similar or different cellular responses, and activation in the same cell occurs by stimulation of different RTKs. The mechanisms controlling the response specificity of MAP kinases are poorly understood and are not considered in this chapter.

Signal Transduction from Activated Ras to a Cascade of Protein Kinases

A remarkable convergence of biochemical and genetic studies in yeast, *C. elegans*, *Drosophila*, and mammals has revealed a highly conserved cascade of protein kinases that operate in sequential fashion downstream from activated Ras as follows :

1. Activated Ras binds to the N-terminal domain of Raf, a serine/threonine kinase.
2. Raf binds to and phosphorylates MEK, a dual-specificity protein kinase that phosphorylates both tyrosine and serine residues.
3. MEK phosphorylates and activates MAP kinase, another serine/threonine kinase.
4. MAP kinase phosphorylates many different proteins, including nuclear transcription factors, that mediate cellular responses.

Several types of experiments have demonstrated that Raf, MEK, and MAP kinase lie downstream of Ras and their sequential order in the pathway. For example, constitutively active mutant Raf proteins induce quiescent cultured cells to proliferate in the absence of hormone stimulation. These mutant Raf proteins, which initially were identified in tumor cells, are encoded by oncogenes and stimulate uncontrolled cell proliferation. Conversely, cultured mammalian cells that express a mutant, defective Raf protein cannot be stimulated to proliferate uncontrollably by a mutant, constitutively active Ras^D protein. This finding establishes a link between the Raf and Ras proteins. In vitro binding studies have shown that purified Ras-GTP protein binds directly to Raf. An interaction between the mammalian Ras and Raf proteins also has been demonstrated in the yeast two-hybrid system, a genetic system in yeast used to select cDNAs encoding proteins that bind to target, or "bait" proteins. The binding of Ras and Raf to each other does *not* induce the Raf kinase activity.

The location of MAP kinase downstream of Ras was evidenced by the finding that in quiescent cultured cells expressing a constitutively active Ras^D, activated MAP kinase is generated in the absence of hormone stimulation. More importantly, in *Drosophila* mutants that lack a functional Ras or Raf but express a constitutively active MAP kinase specifically

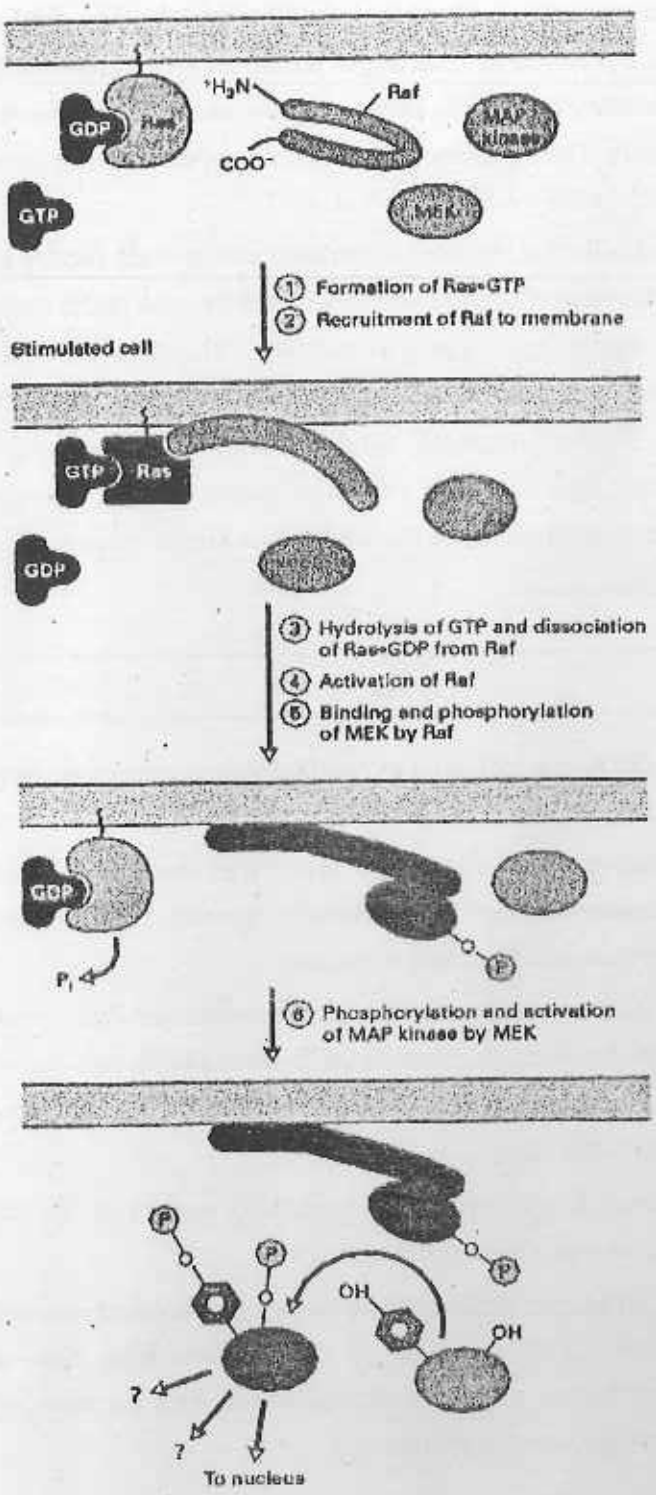


Fig. 7. Figure 20-28. Kinase cascade that transmits signals downstream from activated Ras protein. In unstimulated cells, most Ras is in the inactive form with bound GDP *(top);* binding of a growth factor to its RTK leads to formation of the active Ras · GTP. A signaling complex then is assembled downstream of Ras, leading to activation of MAP · kinase by phosphorylation of threonine and tyrosine residues separated by a single amino acid. Phosphorylation at both sites is necessary for activation of MAP kinase.

in the developing eye, R7 photoreceptors were found to develop normally. This finding indicates that activation of MAP kinase is sufficient to transmit a proliferation or differentiation signal normally initiated by ligand binding to an RTK. Biochemical studies showed that Raf does not activate MAP kinase directly. The signaling pathway thus appears to be a linear one: activated RTK → Ras → Raf → (?) → MAP kinase.

Finally, fractionation of cultured cells that had been stimulated with growth factors led to identification of MEK, a kinase that specifically phosphorylates threonine and tyrosine residues on MAP kinase, thereby activating its catalytic activity. (The acronym MEK comes from MAP and ERK kinase, where ERK is another acronym for MAP.) Later studies showed that MEK binds to the C-terminal catalytic domain of Raf and is phosphorylated by the Raf serine/ threonine kinase activity; this phosphorylation activates the catalytic activity of MEK. Hence, activation of Ras induces a kinase cascade that includes Raf, MEK, and MAP kinase.

10.6 Summary

- Receptor tyrosine kinases (RTKs), which bind to peptide/protein hormones, may exist as dimers or dimerize during binding to ligands.
- Ligand binding leads to activation of the kinase activity of the receptor and autophosphorylation of tyrosine residues in its cytosolic domain. The activated receptor also can phosphorylate other protein substrates.
- Ras is an intracellular GTPase switch protein that acts downstream from most RTKs. Like $G_{s\alpha}$, Ras cycles between an inactive GDP-bound form and active GTP-bound form. Ras cycling requires the assistance of two proteins, GEF and GAP, , whereas $G_{s\alpha}$ cycling does not.
- Unlike GPCRs, which interact directly with an associated G protein, RTKs are linked indirectly to Ras via two proteins, GRB2 and Sos.
- The SH2 domain in GRB2, an adapter protein, binds to specific phosphotyrosines in activated RTKs. The two SH3 domains in GRB2 then bind Sos, a guaninenucleotide exchange factor, thereby bringing Sos close to membrane-bound Ras-GDP and activating its exchange function.

- Binding of Sos to inactive Ras causes a large conformational change that permits release of GDP and binding of GTP.
- Normally, Ras activation and the subsequent cellular response is induced by ligand binding to an RTK. However, in cells that contain a constitutively active Ras, the cellular response occurs in the absence of ligand binding.
- Activated Ras promotes formation of signaling complexes at the membrane containing three sequentially acting protein kinases and a scaffold protein Ksr. Raf is recruited to the membrane by binding to Ras · GTP and then activated. It then phosphorylates MEK, a dual specificity kinase that phosphorylates MAP kinase. Phosphorylated MAP kinase dimerizes and translocates to the nucleus where it regulates gene expression.
- RTKs, GPCRs, and other receptor classes can activate MAP kinase pathways. Single-cell eukaryotes, such as yeast, and multicellular organisms contain multiple M/ P kinase pathways that regulate diverse cellular processes.
- Although different MAP kinase pathways share some upstream components, activation of one pathway by extracellular signals does not lead to activation of others containing shared components.
- In MAP kinase pathways containing common components, the activity of shared components is restricted to only a subset of MAP kinases by their assembly into large pathway-specific signaling complexes.
- Some MAP kinases have kinase-independent functions that can restrict signals to only a subset of MAP kinases.

Unit 11 □ Apoptosis

Structure

11.1 Introduction

The cells of multicellular organism are members of highly organized community. The number of these cells in this community is tightly regulated - not simply by controlling the rate of cell division, but also by controlling the cell death. If cells are no longer needed, they commit suicide by activating an intracellular death programme. This process is **Programmed Cell Death (PCD)** or **Apoptosis** (from a Greek word, "falling off" as leaves from a tree). Apoptosis when not regulated, can contribute to the various diseases, mainly cancer, autoimmune or neurodegenerative diseases. The biological hallmark of apoptosis includes activations of endonucleases, DNA fragmentation into oligosomal fragments and activation of a family of cysteine protease called caspases.

11.2 How does apoptosis differ from necrosis

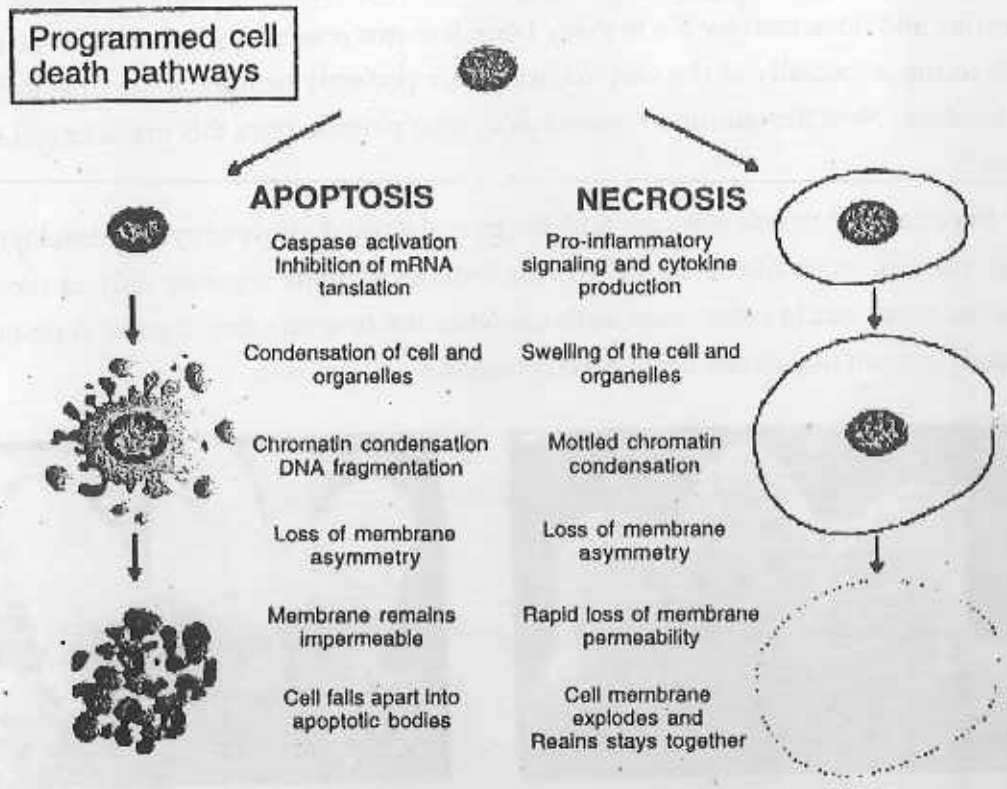


Fig. 1 : Basic differences between apoptosis and necrosis

Cells that die as a result of injury typically, swell and burst. They spill over their contents over their neighbours - a process called **cell necrosis** causing a potentially damaging inflammatory response. By contrast, a cell undergoes apoptosis dies neatly, without damaging its neighbour. The cell shrinks and condenses, the cytoskeleton collapses, the nuclear envelop disassembles and the nuclear DNA breaks up into fragments. Most importantly, the cell surface is altered, displaying property that causes the dying cell to be rapidly phagocytosed, either by neighbouring cells or by macrophages before any leakage of its content occurs. These events not only avoid the damaging consequence of the cell necrosis, but also allow the organic components of the dead cells to be recycled by the cells that ingest them.

The amount of apoptosis in developing and adult animal tissues can be astonishing. For example, in the developing vertebrate nervous system about half of the nerve cells normally die soon after they are formed. In healthy adult humans, billions of cells in the intestine and bone marrow die in every hour. It seems reasonably wasteful for so many cells to die, especially as the vast majorities are perfectly healthy at the time they kill themselves. Now the question is vasted as to what purpose does this massive cell death serve?

For example, mouse paws are sculpted by cell death during embryonic development. They start as spade like structure and the individual digits separate only as the cells between them die. In other cases, cells die when the structure they formed is no longer needed. In adult tissues cell death exactly balances cell division.

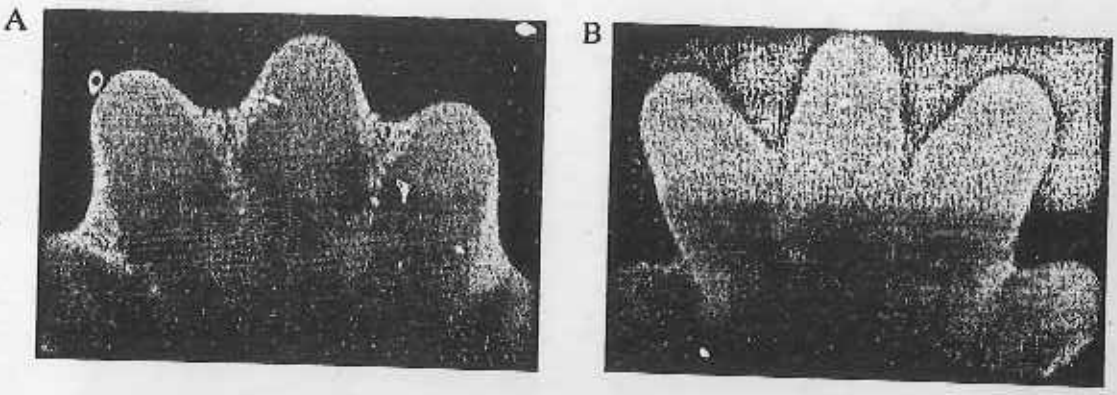


Fig 2. : Sculpting the digits in the developing mouse paw by apoptosis. (A) The paw in this mouse embryo has been stained with a dye that specifically labels cells that have undergone apoptosis. The apoptotic cells appear as *bright green* dots between the developing digits. (B) This inter-digital cell death eliminates the tissue between the developing digits, as seen one day later, when few, if any, apoptotic cells can be seen.

11.3 Apoptotic process

The process of apoptosis can be divided into three distinct phases namely **i) initiation, ii) effectors, and iii) degradation.** During initiation cell receives death inducing signals. The cells at this stage lack the obligatory survival factors and the cells have shortage of

metabolic supply which is followed by appearance of death-signal transducing receptors. During the effector phase the signals are translated into metabolic reaction and the decision to die is taken. Beyond this stage i.e., during degradation phase an increase in the overall entrophy, including activation of catabolic enzymes, DNA fragmentation and massive protein degradation become apparent. Fragmented DNA encapsulated to form apoptotic bodies that are quietly consumed by the adjacent cells. Thus pathway of apoptotic cell death is demarcated when the cells sense or receive the death signals depending on the type of stimulus of the particular cell type.

Biochemically, apoptotic cells are characterized by reduction in the mitochondrial transmembrane potential, intracellular acidification, production of reactive oxygen species (ROS), externalization of phosphatidyl serine residues in the membrane bilayer, selective proteolysis of subset of cellular proteins and degradation of DNA into internucleosomal fragments.

11.4 When cells die?

The cell dies by apoptosis in the developing embryo during morphogenesis and in the adult animal during tissue turn over, immune regulation or at the end of immune response. So aberration of this process is detrimental. Thus unscheduled apoptosis of certain brain neurons contribute to cause disorder such as Alzheimer's and Parkinson's disease, whereas failure of the dividing cells to initiate apoptosis after sustaining DNA damage contribute to the cancer. PCD has also been reported in the pathological dysfunction, such as T-cell depletion in HIV infection and mononuclear cell loss in *Plasmodium falciparum* infection.

11.5 Basic apoptotic machinery

Genetic studies of nematode *C. elegans* contribute a conceptual frame work involving three genes. Two of these genes are cell death defective genes (*ced*) – *ced3* and *ced4* are both required for the apoptosis in worm whereas another gene *ced9*,

inhibits action of *ced3* and *ced4* by helping cell survival. Gene product of *ced3* is a caspase that cleaves certain proteins after specific aspartic amino acid residue. This is activated through self cleavage. Normally *ced4* binds *ced3* and promotes *ced3* activation, whereas *ced9* binds to *ced4*, and prevents it from activating *ced3*. Normally *ced9* is combines with *ced3* keeping *ced3* inactive. Apoptotic stimuli cause *ced9* dissociation, allowing *ced4* to activate *ced3* and thereby causing cell death by apoptosis. Vertebrate animals have evolved the entire gene families that resemble the cell death genes of *C. elegans*. Mammalian caspases are similar to ced3. Apoptotic activation factor (Apaf-1) is the only mammalian *ced4* homologue known so far. The products of the mammalian *Bcl2* gene family are related to *ced9*, but include two subgroups of proteins that either inhibit or promote apoptosis.

11.6 Mechanism of apoptosis

All nucleated animal cells contain the seed of their own destruction in the form of various inactive procaspases that lie waiting for a signal to destroy cells. The basic mechanism is similar in animal cells. This machinery depends on family of proteases that have a cystine at their active site and cleaves their target protein at specific aspartic acids. These enzymes are called caspases.

Caspases are synthesized in the cell as inactive "procaspases", which are usually activated by cleavage at aspartic acids by other caspase. Once activated caspases cleave and thereby activate other procaspases, resulting in an amplifying proteolytic cascade. Some activated caspases then cleave other key proteins in the cells. Some cleave the nuclear lamina, for example causing irreversible breakdown of the nuclear lamina, and other cleaves a protein that normally holds a DNA degrading enzyme (DNAase) in an active form freeing the DNAase to cut out the DNA in the cell nucleus. In this way the cell dismantles itself quickly and neatly, and its corpse is rapidly taken up and digested by another cell. The protease cascade is not only destructive and self amplifying but also irreversible, so that once a cell reaches a critical point it can not be turned back.

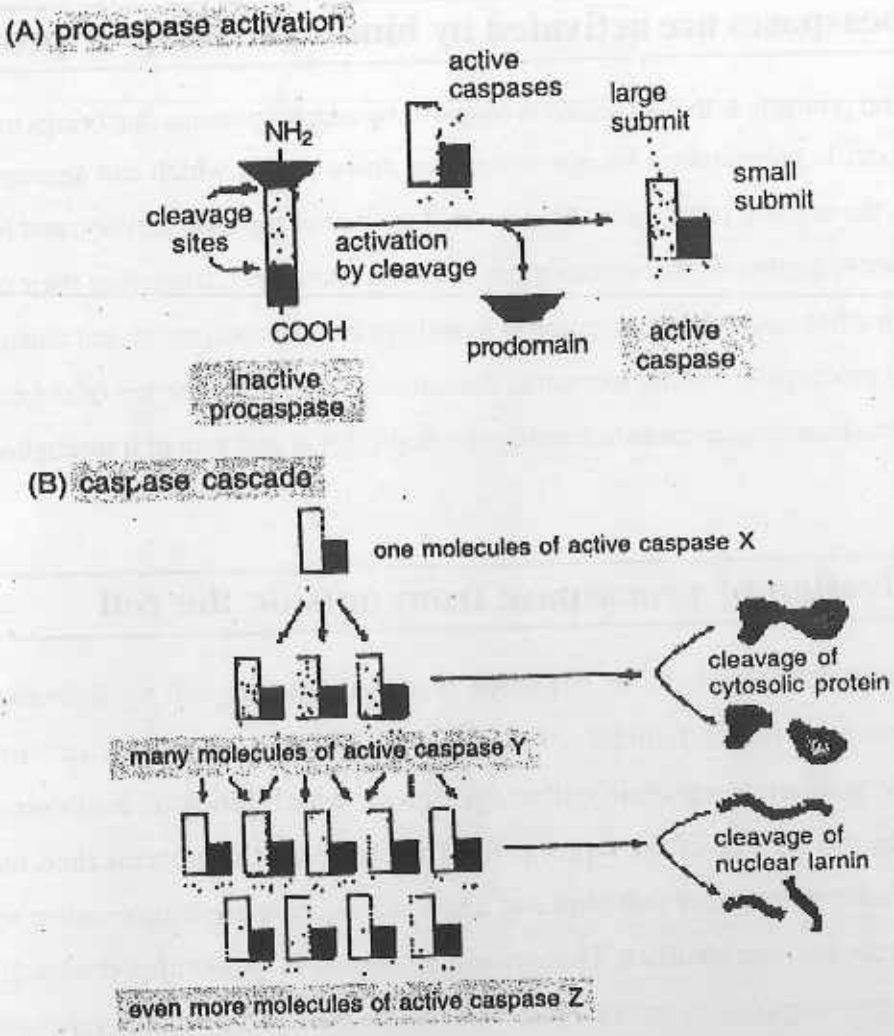


Fig 3. The caspase cascade involved in apoptosis. (A) Each suicide protease is made as an inactive proenzyme (procaspase), which is usually activated by proteolytic cleavage by another member of the caspase family. As indicated, two of the cleaved fragments associate to form the active site of the caspase. The active enzyme is thought to be a tetramer of two of these units (not shown). (B) Each activated caspase molecule can cleave many procaspase molecules, thereby activating them, and these can then activate even more procaspase molecules. In this way, an initial activation of a small number of procaspase molecules (called initiator caspases) can lead, via an amplifying chain reaction (a cascade), to the explosive activation of a large number of procaspase molecules. Some of the activated caspases (called effector caspases) then cleave a number of key proteins in the cell, including specific cytosolic proteins and nuclear lamins, leading to the controlled death of the cell.

11.7 Procaspases are activated by binding to adaptor proteins

A general principle is that activation is triggered by adaptor proteins that brings multiple copies of specific procaspases, known as *initiator procaspases*, which can aggregate. In some cases, the initiator procaspases have a small amount of protease activity, and forcing them together to aggregate that causes them to cleave each other, triggering their mutual activation. In other cases, the aggregation is thought to cause a conformational change that activates the procaspase. Within moments, the activated caspase at the top of the cascade cleaves downstream procaspases to amplify the death signal and spread it throughout the cell.

11.8 Activation of procaspase from outside the cell

Procaspase activation can be triggered from out side the cell by activation of death receptors on the cell surface. For example, killer lymphocytes can induce apoptosis by producing a protein called fas ligand, which binds to death receptor protein fas on the surface of the target cells. The clustered fas proteins then recruit intracellular adaptor proteins that bind and aggregate procaspase-8 molecules, which cleave and activate one another. The activated caspase-8 molecules then activate downstream procaspases to induce apoptosis. Some stressed or damaged cells kill themselves by producing both fas ligand and fas protein, thereby triggering an intracellular caspase cascade.

When cells are damaged or stressed, they can also kill themselves by triggering procaspase aggression and activation from within the cell. In a best understood pathway mitochondria are involved and induce to release the electron carrier protein cytochrome C into cytosol, where it binds and activate an adaptor protein called Apaf-1. This mitochondrial pathway of procaspase activation is recruited in most of the apoptosis to initiate or to accelerate and amplify caspase cascade. This response

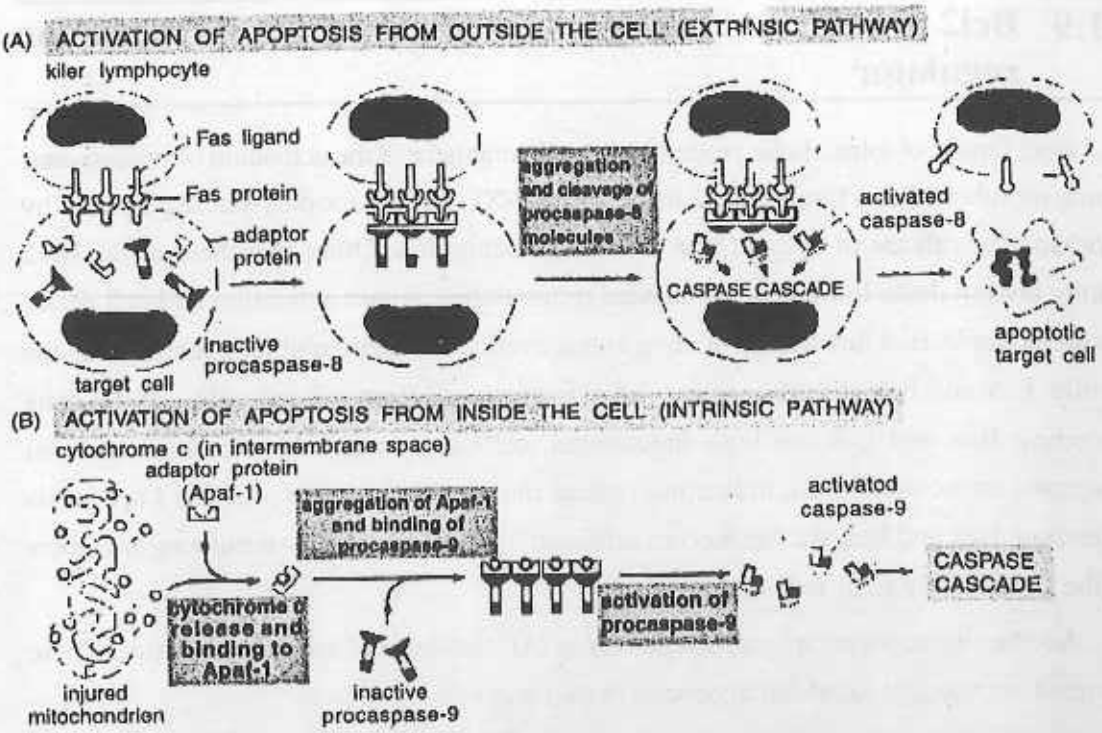


Fig 4. Induction of apoptosis by either extracellular or intracellular stimuli. (A) Extracellular activation. A killer lymphocyte carrying the Fas ligand binds and activates Fas proteins on the surface of the target cell. Adaptor proteins bind to the intracellular region of aggregated Fas proteins, causing the aggregation of procaspase-8 molecules. These then cleave one another to initiate the caspase cascade. (B) Intracellular activation. Mitochondria release cytochrome *C*, which binds and causes the aggregation of the adaptor protein Apaf-1. Apaf-1 binds and aggregates procaspase-9 molecules, which leads to the cleavage of these molecules and the triggering of a caspase cascade. Other proteins that contribute to apoptosis are also released from the mitochondrial intermembrane space (not shown).

usually requires p53 which can activate the transcription of gene that encodes the protein and promote the release of cytochrome C from mitochondria. This protein belongs to Bcl2 family.

11.9 Bcl2 protein and IAP proteins are the main intracellular regulator

Bcl2 family of intracellular protein is the main regulator of the activation of procaspases. Some member of this family, Bcl2 itself and Bcl-XL inhibit apoptosis at least partly by blocking the release of cytochrome C from mitochondria. Other members of the Bcl2 family are not death inhibitors, but instead promote procaspase activation and cell death. As for example, Bad function by binding to inactivating the death inhibiting members of the family. Bax and Bak stimulate release of cytochrome C from mitochondria. If the gene encoding Bax and Bak are both inactivated, cells are remarkably resistant to most apoptosis inducing stimuli, indicating crucial importance of these proteins I apoptosis induction. Bax and Bak are themselves activated by other apoptosis stimulating members of the Bcl2 family such as Bid.

Another intracellular apoptosis regulator is IAP (inhibitor of apioptosis) family. These proteins are thought to inhibit apoptosis in two ways :

1) they bind to some procaspases to prevent their activation and
2) they bind to caspases to inhibit their activities.

IAP protein was originally discovered as protein induced by certain insect virus, which use them to prevent the infected cells from killing itself before the virus have had time to replicate. When mitochondria release cytochrome C to activate Apaf-1, they also release a protein to block IAPs, thereby greatly increasing the efficiency of death activating process. Signals, which can either activate apoptosis or inhibit it. These signal molecules mainly act by regulating the levels or activity of members of Bcl2 and IAP families.

11.7 Summary

In multicellular organisms, cells that are no longer needed or are a threat to the organism are destroyed by a tightly regulated cell suicide process known as programmed cell death, or apoptosis. Apoptosis is mediated by proteolytic enzymes called caspases, which trigger cell death by cleaving specific proteins in the cytoplasm and nucleus.

Caspases exist in all cells as inactive precursors, or procaspases, which are usually activated by cleavage by other caspases, producing a proteolytic caspase cascade. The activation process is initiated by either extracellular or intracellular death signals, which cause intracellular adaptor molecules to aggregate and activate procaspases. Caspase activation is regulated by members of the Bcl-2 and IAP protein families.

Procaspase activation can be triggered from outside the cell by the activation of death receptors on the cell surface. Killer lymphocytes, for example, can induce apoptosis by producing a protein called **Fas ligand**, which binds to the death receptor protein **Fas** on the surface of the target cell. The clustered Fas proteins then recruit intracellular adaptor proteins that bind and aggregate procaspase-8 molecules, which cleave and activate one another. The activated caspase-8 molecules then activate downstream procaspases to induce apoptosis. Some stressed or damaged cells kill themselves by producing both the Fas ligand and the Fas protein, thereby triggering an intracellular caspase cascade.

Unit 12 □ Synthesis, Sorting, Trafficking of Protein hormone

Structure

12.1 Introduction

The polypeptide hormones constitute a critically important and diverse set of regulatory molecules encoded by the genome whose functions are to convey specific information among cells and organs. This type of molecular communication appear early in the development of life and evolves a complex system for the control of growth, development and reproduction, and for the maintenance of metabolic homeostasis. These hormones consist of approximately 400 or more small proteins ranging from as few as three amino acids (thyrotropin-releasing hormone, TRH) to 192 amino acids (growth hormone). In a broader sense, these polypeptides function as hormones, whose actions on distant organs are mediated by way of their transport through the blood stream, and act locally as cell-to-cell communicators. The latter function of the polypeptide hormones is exemplified by their elaboration and secretion within neurons of the central, autonomic, and peripheral nervous systems, where they act as neurotransmitters. These multiple modes of expression of the polypeptide hormone genes have aroused great interest in the specific functions of these peptides and the mechanisms of their synthesis and release.

12.2 Steps in Expression of a Protein-encoding Gene

The steps involved in transfer of information encoded in the polynucleotide language of DNA to the poly-amino acid language of biologically active proteins involve gene transcription, post-transcriptional processing of ribonucleic acids (RNAs), translation, and post-translational processing of the proteins. The expression of genes and protein synthesis can be considered in terms of several major processes, any one or more of which may serve as specific control points in the regulation of gene expression :

1. *Rearrangements and transpositions of DNA segments.* These processes occur over many years in evolution, with the exception of uncommon mechanisms of somatic gene rearrangements such as the rearrangements in the immunoglobulin genes during the lifetime of an individual.
2. *Transcription.* Synthesis of RNA results in the formation of RNA copies of the two gene alleles and is catalyzed by the basal RNA polymerase II-associated transcription factors.
3. *Post-transcriptional processing.* Specific modifications of the RNA include the formation of messenger RNA (mRNA) from the precursor RNA by way of excision and rejoining of RNA segments (introns and exons) and modifications of the 3' end of the RNA by polyadenylation and of the 5' end by addition of 7-methylguanine "caps."
4. *Translation.* Amino acids are assembled by base pairing of the nucleotide triplets (anticodons) of the specific "carrier" aminoacylated transfer RNAs to the corresponding codons of the mRNA bound to polyribosomes and are polymerized into the polypeptide chains.
5. *Post-translational processing and modification.* Final steps in protein synthesis may involve one or more cleavages of peptide bonds, which result in the

conversion of biosynthetic precursors (prohormones), to intermediate or final forms of the protein; derivation of amino acids (e.g., glycosylation, phosphorylation, acetylation, myristoylation); and the folding of the processed polypeptide chain into its native conformation. Each of the specific steps of gene expression requires the integration of precise enzymatic and other biochemical reactions. These processes have developed to provide high fidelity in the reproduction of the encoded information and to provide control points for the expression of the specific phenotype of cells. The post-translational processing of proteins creates diversity in gene expression through modifications of the protein. Although the functional information contained in a protein is ultimately encoded in the primary amino acid sequence, the specific biologic activities are a consequence of the higher orders of the secondary, tertiary, and quaternary structures of the polypeptide. Given the wide range of possible specific modifications of the amino acids, such as glycosylation, phosphorylation, acetylation, and sulfation, any one of which may affect the conformation or function of the protein, a single gene may ultimately encode a wide variety of specific proteins as a result of post-translational processes. Polypeptide hormones are synthesized in the form of larger precursors that appear to fulfill several functions in biologic systems, including (1) intracellular trafficking, by which the cell distinguishes among specific classes of proteins and directs them to act their sites of action, and (2) the generation of multiple biologic activities from a common genetically encoded protein by regulated or cell-specific variations in the post-translational modifications.

All the peptide hormones and regulatory peptides studied thus contain signal or leader sequences at the amino termini; these hydrophobic sequences recognize specific sites on the membranes of the rough endoplasmic reticulum, which results in the transport

of nascent polypeptides into the secretory pathway of the cell. The consequence of the specialized signal sequences of the precursor proteins is that proteins destined for secretion are selected from a great many other cellular proteins for sequestration and subsequent packaging into secretory granules and export from the cell. In addition, most, if not all, of the smaller hormones and regulatory peptides are produced as a consequence of post-translational cleavages of the precursors within the Golgi complex of secretory cells.

12.3 Subcellular Structure of Cells that Secrete Protein Hormones

Cells whose principal functions are the synthesis and export of proteins contain highly developed, specialized subcellular organelles for the translocation of secreted proteins and their packaging into secretory granules. The subcellular pathways utilized in protein secretion have been elucidated largely through the early efforts of Palade and colleagues. Secretory cells contain an abundance of endoplasmic reticulum, Golgi complexes, and secretory granules. The proteins that are to be secreted from the cells are transferred during their synthesis into these subcellular organelles, which transport the proteins to the plasma membrane. Protein secretion begins with translation of the mRNA encoding the precursor of the protein on the rough endoplasmic reticulum, which consists of polyribosomes attached to elaborate membranous saccules that contain cavities (cisternae). The newly synthesized, nascent proteins are discharged into the cisternae by transport across the lipid bilayer of the membrane. Within the cisternae of the endoplasmic reticulum, proteins are carried to the Golgi complex by mechanisms that are incompletely understood. The proteins gain access to the Golgi complex either by direct transfer from the cisternae, which are in continuity with the membranous channels of the Golgi

complex, or by way of shuttling vesicles known as transition elements. Within the Golgi complex, the proteins are packaged into secretory vesicles or secretory granules by their budding from the Golgi stacks in the form of immature granules. Immature granules undergo maturation through condensation of the proteinaceous material and application of a specific coat around the initial Golgi membrane. On receiving the appropriate extracellular stimuli (regulated pathway of secretion), the granules migrate to the cell surface and fuse to become continuous with the plasma membrane, which results in the release of proteins into the extracellular space, a process known as exocytosis. The second pathway of intracellular transport and secretion involves the transport of proteins contained within secretory vesicles and immature secretory granules. Although the use of this alternative vesicle-mediated transport pathway remains to be demonstrated conclusively (it is generally considered to be a constitutive, or unregulated, pathway), different extracellular stimuli may modulate hormone secretion differently, depending on the pathway of secretion. For example, in the parathyroid gland and in the pituitary cell line derived from corticotropic cells (AtT-20), newly synthesized hormone is released more rapidly than hormone synthesized earlier. These findings suggest that the newly synthesized hormone may be transported by way of a vesicle-mediated pathway without incorporation into mature storage granules.

12.4 Intracellular Segregation and Transport of Polypeptide Hormones

Specific amino acid sequences encoded in the proteins serve as directional signals in the sorting of proteins within subcellular organelles. A typical eukaryotic cell synthesizes an estimated 5000 different proteins during its life span. These different proteins are synthesized by a common pool of polyribosomes. However, each of the different proteins is directed

to a specific location within the cell, where its biologic function is expressed. For example, specific groups of proteins are transported into mitochondria, membranes, the nucleus, or into the other subcellular organelles, where they serve as regulatory proteins, enzymes, or structural proteins. A subset of proteins is specifically designed for export from the cell (e.g., immunoglobulins, serum albumin, blood coagulation factors, and protein and polypeptide hormones). This process of directional transport of proteins involves sophisticated informational signals. Because the information for these translocation processes must reside either wholly or in part w ..n the primary structure or in the conformational properties of the protein, sequential post-translational modifications may be crucial for determining the specificity of protein function.

12.4.1 Signal Sequences in Peptide Prohormone Processing and Secretion

The early processes of protein secretion that result in the specific transport of exported proteins into the secretory pathway are now becoming better understood. Initial clues to this process came from determinations of the amino acid sequences of the proteins programmed by the cell-free translation of mRNAs encoding secreted polypeptides. Secreted proteins are synthesized as precursors that are extended at their NH 2 termini by sequences of 15 to 30 amino acids, called *signal* or *leader sequences*. Signal sequence extensions, or their functional equivalents, are required for targeting the ribosomal or nascent protein to specific membranes and for the vectorial transport of the protein across the membrane of the endoplasmic reticulum. On emergence of the signal sequence from the large ribosomal subunit, the ribosomal complex specifically makes contact with the membrane, which results in translocation of the nascent polypeptide across the endoplasmic reticulum membrane into the cisterna as the first step in the transport of the polypeptide within the secretory pathway. These observations initially left unanswered the question of

how specific polyribosomes that translate mRNAs encoding secretory proteins recognize and attach to the endoplasmic reticulum. Because microsomal membranes in vitro reproduce the processing activity of intact cells, it was possible to identify macromolecules responsible for processing of the precursor and for translocation activities. The endoplasmic reticulum and the cytoplasm contain an aggregate of molecules, called a *signal recognition particle complex*, that consists of at least 16 different proteins, including three guanosine triphosphatases to generate energy and a 7S RNA. This complex, or particle, binds to the polyribosomes involved in the translation of mRNAs encoding secretory polypeptides when the NH 2 -terminal signal sequence first emerges from the large subunit of the ribosome. The specific interaction of the signal recognition particle with the nascent signal sequence and the polyribosome arrests further translation of mRNA. The nascent protein remains in a state of arrested translation until it finds a high-affinity binding protein on the endoplasmic reticulum, the signal recognition particle receptor, or docking protein. On interaction with the specific docking protein, the translational block is released and protein synthesis resumes. The protein is then transferred across the membrane of the endoplasmic reticulum through a proteinaceous tunnel. At some point, near the termination of synthesis of the polypeptide chain, the NH 2-terminal signal sequence is cleaved from the polypeptide by a specific signal peptidase located on the cisternal surface of the endoplasmic reticulum membrane. The removal of the hydrophobic signal sequence frees the protein (prohormone or hormone) so that it may assume its characteristic secondary structure during transport through the endoplasmic reticulum and the Golgi apparatus. Interestingly, after its cleavage from the protein by signal peptidase, the signal peptide may sometimes be further cleaved in the endoplasmic reticulum membrane to produce a biologically active peptide. The signal sequence of preprolactin of 30 amino acids, for example, is cleaved by a signal peptide peptidase to give a charged peptide of 20 amino acids that is released into the cytosol, where it binds to calmodulin and inhibits Ca^{2+}-calmodulin-dependent phosphodiesterase.

This sequence in the directional transport of specific polypeptides ensures optimal cotranslational processing of secretory proteins, even when synthesis commences on free ribosomes. The presence of a cytoplasmic form of the signal recognition particle complex that blocks translation guarantees that the synthesis of the presecretory proteins is not completed in the cytoplasm; the efficient transfer of proteins occurs only after contact has been made with the specific receptor or docking protein on the membrane. Although the identification of the signal recognition particle and the docking protein explains the specificity of the binding of ribosomes containing mRNAs encoding the secretory proteins, it does not explain the mode of translocation of the nascent polypeptide chain across the membrane bilayer. Further dissection and analysis of the membrane have identified other macromolecules that are responsible for the transport process.

NOTES

মানুষের জ্ঞান ও ভাবকে বইয়ের মধ্যে সঞ্চিত করিবার যে একটা প্রচুর সুবিধা আছে, সে কথা কেহই অস্বীকার করিতে পারে না। কিন্তু সেই সুবিধার দ্বারা মনের স্বাভাবিক শক্তিকে একেবারে আচ্ছন্ন করিয়া ফেলিলে বুদ্ধিকে বাবু করিয়া তোলা হয়।

— রবীন্দ্রনাথ ঠাকুর

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— Subhas Chandra Bose

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