Molecular Biology of Periodontium

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BASIC TISSUES

The periodontium comprises of two soft tissues and two hard tissues. The soft tissues are gingiva and periodontal ligament and the hard tissues are cementum and alveolar bone. The tooth supporting structures are the cementum, periodontal ligament and alveolar bone. Gingiva covers the alveolar bone and surrounds the tooth in a collar like manner. These structures function together as a group and thereby enable the tooth to carry out all its functions. The histological features of each tissue are designed to meet their specific functions.

A very brief overview of the gross anatomy, the histological features and their functions must be understood before the molecular processes that determine these features are discussed. A brief description of the pathological changes affecting the structures will follow, after which the molecular process that mediate these changes will be discussed.

Gingiva

Gingiva covers the alveolar process of the jaw and is anatomically divided into free gingiva, attached gingiva and interdental *gingiva*. Histologically, gingiva consists of epithelium and connective tissue, and the gingival epithelium is further subdivided into three types- oral gingival epithelium, sul-cular epithelium and junctional epithelium.

Free gingiva or marginal gingiva

Marginal gingiva extends from the crest of the gingiva to the free gingival groove which separates the marginal gingiva from the attached gingiva. The marginal gingiva is called 'free' as it is not attached to the underlying periosteum of alveolar bone. Coronally, it extends upto 1-2 mm coronal to the cementoenamel junction while apically it extends to the free gingival groove. The free gingival groove is not usually clinically visible and the apical extent of the marginal gingiva is generally determined by probing depth. The

apical extent of the probe corresponds to the level at which gingiva is attached to tooth. In health, it is at the level of the cementoenamel junction and this attachment is mediated by the junctional epithelium.

Functionally, the marginal gingiva forms the soft tissue wall of the V-shaped gingival sulcus that is formed by the tooth on one side and the gingiva on the other. The gingival sulcus can be deflected away from tooth by a blunt instrument such as the periodontal probe. In health, the gingival sulcus has a probing depth of about 2-3 mm. The sulcus is filled with the inflammatory exudate called the gingival crevicular or sulcular fluid (GCF). The gingival crevicular fluid has in addition to other constituents, factors important for the defense mechanism of gingiva. The need for defense arises from the fact that the sulcus is the primary plaque forming domain and is the portal of entry for periodontopathogenic bacteria. The gingival fibers help brace the marginal gingiva and prevent it from getting overly displaced from the tooth. Marginal gingiva is thus equipped with cellular and extracellular matrix elements that grant both esthetic and functional characteristics.

Attached gingiva

The attached gingiva is firmly attached to the periosteum of the underlying alveolar bone. It extends from the free gingival groove to the mucogingival junction apically. The attached gingiva is thus firmly entrenched between two movable structures- the marginal gingiva coronally and the alveolar mucosa apically. The dense connective tissue fibers of the attached gingiva ensure its firm attachment to the bone and grant it the required "toughness".

The dimensions of the attached gingiva vary from the anterior to the posterior teeth.

The width of the attached gingiva refers to its apico-coronal dimension and varies from 3.5-4.5 mm in the anterior teeth to 1 mm in the premolar region. The width is often least in the premolar regions due to the presence of accessory frenae in those areas. In the palatal surface, the attached gingiva blends almost imperceptibly to the palatal mucosa. Lingually, the attached gingiva gives way to the lingual mucosa and is dimensionally much lesser than on the buccal surface.

The width of attached gingiva increases with age and in supraerupted teeth. This increase in dimension occurs as a result of an increase in the height of the alveolar process which in turn is the result of passive eruption. The width of the attached gingiva is reduced either due to gingival recession or periodontal pocket formation.

The mucogingival line remains stationary throughout life as it is a genetically determined landmark that separates not *only* the attached gingiva from the alveolar mucosa, but also the alveolar process from the basal bone. Any change in dimension is thus, a direct result of changes occurring in the gingiva.

The other dimension that may play a significant role in the maintenance of the periodontal health is the thickness of the gingiva. Gingival phenotype or biotype has been classified by Eger & Muller into thick and thin or Class I, IIA and IIB. Thick gingival phenotype seems to be more conducive to periodontal health. A thin phenotype predisposes to gingival recession and increased tendency to gingival inflammation.

The functional relevance of attached gingiva relates to its firm attachment to the underlying bone. It prevents deflection of marginal gingiva due to the effects of lip musculature and frena. By preventing this deflection, it also helps avoid food entrapment

between tooth and gingival sulcus, *thus* aids plaque maintenance. The dense collagenous structure of the attached gingiva also aids in slowing down the inflammatory spread from the marginal gingiva.

Clinical features of gingiva

The clinical features of gingiva are a reflection of the underlying molecular process. The form and function of gingiva is determined by the molecular process contributing to it.

Color

Normal gingiva is coral pink in color. Factors that affect the color of the gingiva are the degree of keratinization, vascularity and pigmentation. Vascularity determines the basic color of gingiva which undergoes modification depending on the degree of keratinization and presence of pigments. Melanin pigments form the major portion of the pigmented material present in the gingiva and results in dark color. The molecular processes that are involved in keratinization and melanin pigmentation are related to the cytoskeletal elements of the cells.

Contour

Normal gingiva has an arcuate contour with a scalloped outline and a knife edge margin. The contour is dictated almost entirely by the alveolar bone which follows a positive architecture, i.e. interdental bone is at a coronal position when compared to the labial bone. The soft tissue pattern is invariably dictated by that of the hard tissue.

Consistency

In health, gingiva is firm and resilient. Firm consistency is a result of the cellular and fibrous content of the extracellular matrix. The abundant collagen fibers and the non-collagenous proteins combine to give gingiva the firm consistency that enables it to resist masticatory stresses.

Surface texture

The gingiva has a stippled appearance that has been described as that resembling an orange peel. The stippling occurs as a result of the epithelial retepegs in growth into the connective tissue. The papillary layer of connective tissue extends into the epithelium and this feature results in a stippled rather than a smooth surface. It is thought to be functional adaptation to increase resistance to mechanical stresses.

Size and shape

This corresponds to the shape of the teeth and subsequently alveolar bone. The bulk of the gingiva is composed of connective tissue comprising of cellular elements and extracellular matrix.

Position

Gingival margin is located coronal to the cementoenamel junction and rests on the enamel. The gingiva is attached through the junctional epithelium to the CEJ of the tooth.

The clinical features that have been described above are all a result of molecular processes that occur within the cell. These processes and the changes they undergo during pathology are described in subsequent chapters.

Histology

Gingival epithelium is of three types- the oral epithelium, sulcular epithelium and junctional epithelium. Each of these epithelia is present in different areas of the gingiva and

is expected to perform specific functions. The oral gingival epithelium is keratinized and has cells that are in close contact with each other through cell junctions. The primary function of the oral gingival epithelium is to provide an impermeable mechanical barrier, impervious to both fluids and bacteria.

Sulcular and junctional epithelium are both non-keratinized. Sulcular epithelium lines the gingival sulcus. Junctional epithelium has special features to allow for adhesion to tooth. The junctional epithelium along with the gingival fibers forms the dentogingival unit which provides attachment to the tooth. In addition, junctional epithelium is also expected to exhibit defense capability against the invading microorganisms. The non-keratinized epithelial cells which are not in close contact with each other allow diffusion of the sulcular fluid from the connective tissue to fill the sulcus. The crevicular fluid with its flushing effect and anti bacterial properties helps keep the pathogenic processes in check.

Plaque related gingival inflammation can be broadly of two types- edematous or fibrotic. Edematous inflammation results in bluish red color, soft consistency and a smooth surface that bleeds on probing. Fibrotic inflammation causes gingiva to remain predominantly pink in color with firm consistency, retaining its stippling and perhaps some change in contour due to enlargement.

Gingivitis is a reversible condition and the pathology may be self-limiting as long as only the gingiva is involved. The factors that trigger the change from gingivitis to periodontitis have not yet been fully elucidated but are related to the host mediated response. Periodontitis is today, known to be a result of an exaggerated response to specific bacteria present in biofilm such as Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Tanerella forsythus, etc. The distinctive changes in the periodontium occur as a result of host mediated damage. The end result of these changes is apical migration of the junctional epithelium leading to pocket formation. The molecular basis behind the process of apical migration is described in later chapters.

Cementum

Cementum is the mesenchymal tissue that covers the root surface. It provides attachment for the periodontal ligament to the root surface and is hence an integral part of the attachment apparatus. Although cementum resembles bone structurally, the avascular nature of this tissue and its subsequent low turn over makes it distinct from bone. Cementum is the least understood of all the four tissues of the periodontium.

Cementum has been classified into acellular and cellular cementum. Acellular cementum is present in the coronal 2/3rd of the root and is characterized by the lack of cells. Cellular cementum is present in the apical 1/3rd of the root surface and is characterized by the presence of the cementum forming cells called cementoblasts.

Cementum is also classified based on the fibers present in its matrix into intrinsic and extrinsic cementum. Extrinsic cementum consists of fibers that arise from the periodontal ligament and are inserted perpendicularly to the root surface. These fibers are mineralized and are called Sharpey's fibers. Sharpey's fibers are arranged perpendicular to the root surface and in regular bundles corresponding to the principal fibers of the periodontal ligament. Intrinsic fibers are secreted by the

cementoblasts and are present within the matrix. These fibers are randomly arranged and are not present in bundles like the extrinsic fibers.

Cementum is thus classified as–

Acellular afibrillar cementum

This cementum is present just apical to the cementoenamel junction. It is devoid of both cellular and fibrillar elements. Instead, there is a cementoid matrix that is poorly differentiated and unorganized. This cementum forms a weak link which may have important clinical implications in that iatrogenic or inflammatory damage to this structure may result in rapid spread of inflammation to the pulp.

Acellular extrinsic fiber cementum

This form of cementum is present in the coronal portion of the root and is the most mineralized. This cementum provides the mechanical rigidity that is important for the attachment apparatus. Loss of this cementum as a result of periodontal disease results in loss of the most important attachment structure of the tooth.

Cellular mixed stratified cementum

This is a combination of both extrinsic and intrinsic fiber cementum with regularly arranged extrinsic fibers interspersed with the random intrinsic fibers.

Cellular intrinsic fiber cementum

Intrinsic fiber cementum is present in only the apical areas of the tooth. It is this cementum that undergoes continuous deposition throughout life. This deposition is important to compensate for the physiological attrition that necessitates continuous passive eruption.

Intermediate cementum

The presence of this type of cementum is somewhat controversial. Some investigators have claimed that this cementum is infact an experimental artefact comprising of a proteoglycan-glycoprotein layer. This cementum is formed as a result of failure of the hertwigs's epithelial root sheath to retract as a result of which the cementoblasts are unable to gain entry to this area.

Cementum was for long considered to be an inert mineralized tissue due of its avascular nature and lack of turn over. It is now recognized that, cementum is associated with several biological proteins that are involved in the attachment as well as homeostatic mechanisms.

Cementum attachment protein

Cementum attachment protein (CAP) is present on the outer surface of the cementum and mediates two important functions. CAP is known to play a role in chemotaxis and differentiation of cementoblasts prior to laying down of the cementoid matrix. In addition, CAP mediates the attachment of the periodontal ligament fibers to the root surface.

Cementum growth factor

It bears a structural homology to insulin like growth factor. It is a tyrosine phosphatase that is involved in the cell signaling processes that are thought to be involved in the differentiation of cementoblasts and hence play a role in organization and formation of cementum.

The biological characteristics of the acellular and cellular cementum are yet to be clearly delineated and offers considerable scope for further research. Similarly the origin and factors that affect the differentiation of

cementoblasts are yet to be fully elucidated. Currently available information about these factors has been described in later chapters.

Periodontal ligament

Periodontal ligament is the mesenchymal tissue that is present between the cementum and alveolar bone. Periodontal ligament attaches the tooth to alveolar bone and absorbs the mechanical load that is applied during mastication. It consists of collagen bundles that are arranged in specific groups termed as the principal fibers of the periodontal ligament. The principal fibers are divided into groups as follows.

Transseptal fibers

These fibers start apical to the cementoenamel junction of one tooth, traverse the alveolar bone and are inserted into the cementum of the adjacent tooth. Transssseptal fibers are important for maintenance of spatial arrangement of teeth. They reorganize rapidly and once they form back, try to get teeth back to their original position.

Alveolar crest group

This group of fibers run from the cementum to the crest of the alveolar bone. These fibers resist any forces that tend to lift the teeth away from the socket, i.e. extrusive forces.

Oblique fibers

They are the most numerous of the principal fibers. They arise from the root and run in an oblique direction to the alveolar bone. These fibers bear the brunt of the masticatory forces subjected to the tooth.

Horizontal group

These fibers run from the root to the alveolar bone in a perpendicular direction to the long axis of the tooth. These fibers resist torsional forces and prevent any laterotrusive movement.

Apical group

Apical fibers run from the root apex to the alveolar bone. They are the most resistant among the principle fibers. These fibers are meant to resist intrusive forces.

Inter-radicular fibers

These fibers are present in multirooted teeth between two or three roots as the case may be. These fibers reinforce anchorage to bone.

The principal fibers are made of type I collagen. In addition to type I, the periodontal fibers have other collagens like type III, XVIII etc. In addition to collagen, periodontal ligament also has oxytalan fibers which belong to the elastin network (elastin, elaunin and oxytalan). Periodontal ligament also has a characteristic distribution of glycoproteins and proteoglycans present in its extracellular matrix.

Cellular elements present in the periodontal ligament are fibroblasts, osteoblasts, undifferentiated mesenchymal cells and the ECRM. The role of each of these cells, their unique features and the molecular process each of them undergo are described later in this chapter.

Functions

The functions of the periodontal ligament are physical, formative, sensory and nutritive.

This book will deal with only the molecular aspects of all these functions. The mechanosensory signals are transmitted to specific receptors. The pathway of communication required for the receptors to function is described in the chapter "cell communication". Similarly, the growth factors and their function in response to forces are also described in this chapter. A remarkable feature of the periodontal ligament is the maintenance of the periodontal ligament. Maintenance of this space is made possible through a number of factors like ECRM, nitric oxide, osteocalcin and other genetic factors, all of which have been described in later sections.

Alveolar bone

Alveolar bone is the "raison d'tere" for the existence of tooth in a way. Alveolar bone has been divided into alveolar bone proper and supporting alveolar bone. The alveolar bone proper refers to that part of the alveolar bone that bears the root of the teeth, while the supporting alveolar bone is the basal bone of the maxilla and mandible. Alveolar bone proper has also been divided into bundle bone and lamellated bone. Bundle bone is called so because of the insertion of numerous collagen bundles of the principle fibers of the periodontal ligament being inserted into it. Lamellated bone is that part of alveolar bone adjacent to bundle bone. Histologically, the appearance of osteoblasts arranged in concentric circles called lamella, led to the usage of this term. Radiographically, a thick radioopaque line is present around the root of the teeth. This is referred to as the lamina dura and is the radiographic reperesentation of alveolar bone proper.

The alveolar process is in most parts dictated by teeth. The shape of the root,

alignment of teeth and their position in the arch determine the shape and size of alveolar bone. The bone cells are osteoblasts, osteoclasts *and* osteocytes, and together they regulate bone formation. These cells and their regulating functions are described later in this chapter. Bone loss is perhaps one of the most definitive features of periodontitis.

The characteristic features of each of these tissues have been described briefly. The intracellular process that goes towards formation and maintenance of these tissues in health, the changes they undergo during pathology and their potential use as markers of periodontal disease forms the rest of the book.

CELL ORGANELLES

Each tissue of the periodontium thus has its own distinct functional and biochemical characteristics that are a result of their matrix components. The synthesis and turn over of these matrix components are controlled by
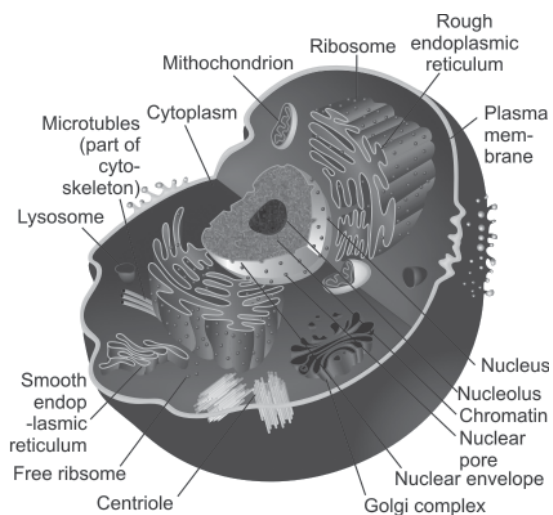

Fig. 1.1: Structure of cell *(For color version, see Plate 1)*

intracellular events in organelles that are specifically designed for this purpose. The structural organization of these intracellular organelles is basically similar in most eukaryotic cells (Fig. 1.1).

All cells are enclosed by a plasma membrane within which is the cytoplasm or the cytosol of the cell. Each cell has several structures called organelles that are required for synthesis, transport of substances (esp. proteins) and to perform important functions that are required for basic cell survival. The important organelles of the cell are- cytosol, nucleus, Golgi complex, endoplasmic reticulum, ribosomes and lysosomes. These organelles have been briefly discussed so as to offer an understanding of the common processes that are involved in production of structural molecules that are relevant to the periodontium.

Plasma membrane

The plasma membrane forms the outer boundary of the cell, thereby separating the cytosol from the external environment. As all cells are bathed in an aqueous environment, it is important to prevent ingress of water from the external environment into the cytosol. At the same time, the intracellular components (fluid) must be prevented form leaking out of the cell. The plasma membrane provides a barrier that prevents both the above mentioned events. The plasma membrane is however, not an inert rigid barrier.

The ability of cell to expand and adapt to their external stimuli is dependent on transfer of ions and solutes in and out of the cell. A rigid impermeable barrier would not allow exchange of material between the cell and the external environment. For this purpose, the plasma membrane needs to be semipermeable

that would retain essential intracellular substances at the same time allow transfer of solutes that are required to maintain homeostasis.

Structure

Structurally, the plasma membrane consists of a lipid bilayer and a protein coat which help carry out its functions. The lipid bilayer provides the impermeability, while the proteins help in transfer of ions and other molecules in and out of the cell.

Unlike other membranes within the cell, plasma membrane needs to maintain its rigidity to prevent any distortion due to external pressure. This rigidity is conferred by the cytoskeleton.

All biological membranes that surround the cell and *other* intracellular organelles have a common general structure - an outer membrane and an inner membrane (Fig. 1.2). The lipid bilayer confers impermeability *to the* plasma membrane. This lipid bilayer has a hydrophilic end that is exposed to the external environment and a hydrophobic end that faces the interior of the cell. Apart from the lipids, the chief constituents of the plasma
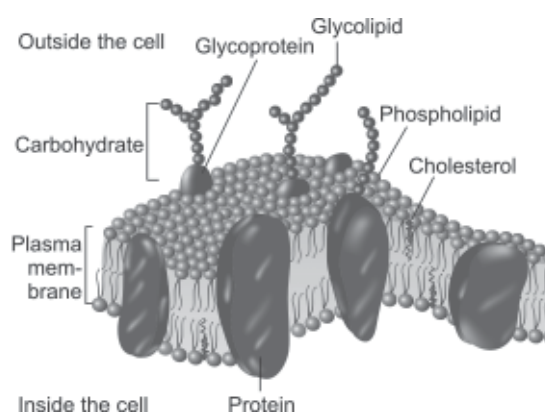

Fig. 1.2: Cell membrane
(For color version, see Plate 1)

membrane are proteins. These proteins link the lipid bilayer to the cytoskeleton as well as to adjacent cells. *Further,* these proteins are involved in all the functions of the cell membrane such as ion transport, ATP synthesis and receptor functions.

Membrane lipids

The lipids found in the plasma membrane are *of three types–*
- Phospholipids,
- Cholesterol, and
- glycolipids.

Phospholipids

The phospholipids are the most abundant among all the lipids present in the plasma membrane. They have a hydrophilic protein head and two hydrophobic tails of variable length. The tail portion of these molecules contains largely nonpolar fatty acids. While the hydrophilic polar end readily dissolves in water because of its charged surface; the non-polar tails do not interact with water molecules because of lack of any charge. The important phospholipids present in the cell membrane are phosphotidyl choline, phosphotidyl ethanolamine, phosphotidyl serine and sphingomyelin. Other phospholipids such as inositol phospholipids are present in much lesser proportions. Although present in small quantities, these phospholipids are functionally important as they play an important role in the cell signaling process.

Cholesterol

The cholesterol molecules present in the plasma membrane are aligned along the phospholipids with their hydroxyl groups joined to the polar ends of the phospholipids.

The presence of cholesterol enhances the water impermeability of the plasma membrane.

Glycolipids

Glycolipids are sugar containing lipid molecules that exhibit extreme asymmetry in their distribution. They are found only in the non-cytosolic monolayer of the membrane, i.e., the outer membrane. In epithelial cells, these glycolipids are found only in the apical surface. Other important glycolipids are those that bind the surfaces of adjacent cells together.

The presence of the glyco lipids on the apical surface of the epithelial cells grants these cells their polarity. Cell polarity is important for the motility of the keratinocytes as we shall see in following chapters.

The lipid bilayer is not similar to each other and there is considerable asymmetry in their protein binding capability. This asymmetry has biological effects that are functionally relevant to the cell. There are specific proteins that bind to phospholipids that are asymmetrically distributed on each of the lipid layer, e.g., protein kinase C (PKC) binds to the hydrophobic tail of the lipid monolayer, specifically to phosphotidyl serine. The negative charge produced by the phosphotidyl serine is required for this binding to take place. On the other hand, phosphotidyl inositol is not present in the hydrophobic portion of the lipid bilayer. Following signal activation, this molecule transforms its binding to the hydrophilic portion of the membrane.

Membrane proteins

Proteins are an integral part of membrane complex and regulate their activities in various ways. Like their lipid counterparts,

the proteins too have a hydrophobic and a hydrophilic end. The hydrophobic ends are embedded within the lipid bilayer, while the hydrophilic ends are exposed to the aqueous environment both inside and outside the cell. Within the cytosol, the hydrophilic ends are kept sequestered away from water so that they are not dissolved in it in normal physiological states. Other proteins do not traverse the membrane bilayer entirely and they may be associated only with the inner lipid layer through covalent bonds or other fatty acid chain attachments. Membrane proteins can be broadly classified into

1. Integral membrane proteins, and
2. Peripheral membrane proteins.

Types of membrane proteins

Integral membrane proteins

These proteins traverse almost the entire length of the lipid bilayer and cannot be separated from the membrane without causing damage to the lipid structure. This is because of the strong union these transmembrane proteins form either with other proteins or lipids present in the membrane. Transmembrane proteins, e.g. the cell surface receptors, have polypeptide chains that run across the lipid bilayer and these chains protrude on either side of the membrane. The polypeptide chain present on either side of the lipid bilayer can function almost independently of each other, e.g. the cell surface proteins have the ligand binding site forming the exterior of the cell and serve to bind to the signals. The same protein has a cytosolic portion that extends into the cytoplasm which serves to activate the cell signaling pathways.

Transmembrane proteins are usually present in two forms- the α helix and the β sheet. The α helix may be a single pass

structure, i.e. they may cross the plasma membrane just once, e.g. receptor tyrosine kinases like the epithelial growth factor receptor, fibroblast growth factor receptor etc. They may otherwise be a multiple pass transmembrane structure like the G protein coupled receptors that run back and forth across the membrane seven times.

The β sheet proteins form transport channels that are mostly involved in transport of ions and other molecules across the cell. Transport proteins are classified into two main categories-
• The channel proteins, and
• The carrier proteins.

Channel proteins

These proteins are basically involved in passive transport of ions across the membrane along the concentration gradient. These channel proteins are formed as small aqueous pores within the lipid bilayer that allow ions to diffuse through them– a process that is described as facilitated diffusion.

Carrier proteins

These proteins are involved in active transport. They are able to carry ions across the concentration gradient, i.e., from a lower concentration to a higher concentration. This process of active transport is a more complicated system when compared to passive transport and requires energy. The active transport proteins are sub-classified into
(a) Coupled carriers
(b) ATP driven pumps.

Coupled carriers

These carrier proteins carry an ion from one side of the cell to the other. Uniporters are those proteins that are involved in transport

of one ion without involving other ions in the process. Symporters carry one ion along with another ion, e.g. Na^+ and Cl^- ions. Antiporters are those that are involved in transport of one ion from inside to outside the cell while at the same time carrying another protein from outside to inside the cell, e.g. the Na^+ and K^+ ions. These carrier proteins along with the ATP driven pumps are important for maintaining the osmolarity of the cell which is essential for homeostasis.

Mitochondria

All eukaryotic cells require energy for production of proteins, transport of proteins from nucleus to cytosol and transport of macromolecules into and outside the cell. Mitochondria is the power house of the cell that provides the ATP and GTP that helps meet the energy requirement of the cell. Of the two, most energy processes within the cell utilize ATP. These energy molecules are produced largely from carbohydrates and lipids obtained form dietary sources. Proteins present in the diet are broken down into amino acids that are usually utilized for protein formation for other purposes.

The most important source of ATP is the dietary carbohydrates. These carbohydrates, are broken down to smaller subunits before they can be utilized by the cell through various metabolic processes.

Carbohydrate metabolism differs according to the presence and absence of oxygen. In the absence of oxygen, they undergo a pathway called the anaerobic glycolysis, whereby glucose is broken down into acids such as lactic acid. This process is generally not a rich a source of ATP. Oxidative phosphorylation, on the other hand, results in metabolism of glucose with formation of pyruvate as an end product of this metabolism. Several ATP molecules are produced during these reactions and these can be utilized for energy dependent processes within the cell. Even this process is however, not entirely energy efficient, as the pyruvate that is formed is not normally utilized for other purposes. Utilization of pyruvate and production of further molecules is possible only through the presence of specialized structures in the cell called the mitochondria.

Structure

Mitochondria are among the larger organelles present in the cell and is visible even through the light microscope. Structurally it has been described as resembling bacteria with extensions in its outer wall. Under higher magnification, it was realized that these extensions seen on mitochondria were not rigid structures. Mitochondria were a plastic structure which demonstrated the ability to change their surface characteristics with great ease and this was the reason for extensions observed under the light microscope. Mitochondria are thus able to change shape, fuse with each other and to other intracellular organelles such as the microtubule assembly. The number of mitochondria depends on the state of the cell, i.e. a cell that is more actively involved in protein synthesis tends to demonstrate a greater number of mito-chondria. For example, a liver cell which is actively involved in synthesis, demonstrates about 100-200 mitochondria.

Ultrastructurally, the mitochondria have two membranes called the inner and outer mitochondrial membrane with an intervening space labelled as the membrane space. The inner mitochondrial membrane encloses the mitochondrial matrix. The outer membrane is, by and large, a smooth structure but it is intersperced by large aqueous channels at

several points. These large channels that are formed are called *porins* (Fig. 1.3). Porins are freely permeable to transport of most proteins that are of a relatively low molecular *weight*, i.e. lesser than 5000 Da. These proteins freely cross the outer membrane but are trapped within the membrane space.

The inner membrane is not a smooth structure like that of the outer membrane. They are thrown into several infoldings called the cristae. The inner membrane and the mitochondrial matrix are the structurally important regions of the mitochondria. The structural relevance of the matrix results from the fact that it is the site of the 'citric acid cycle' or 'Kreb cycle'. Kreb cycle is, perhaps, the most important carbohydrate utilization process of the cell resulting in the formation of the carbon dioxide and water. During this process, several molecules of ATP are generated and this is then utilized for the energy requirement of the cell. .

The other important feature of mitochondria is the fact that it is an important source of extra nuclear genetic material. While the overwhelming majority of the genetic

material is stored in the nucleus, eukaryotic cells can exhibit DNA in other intracellular organelles. Among these organelles, mitochondria store a large amount of DNA, RNA polymerases and other nuclear proteins. The reason for such a large presence of genetic material in the mitochondria is currently unknown. However, it has been well characterized that inheritance of mitochondrial DNA does not follow mendelian laws like the nuclear DNA. Because of its nature of inheritance, the mitochondrial DNA *lead to* progeny with same haploid characteristics as that of the parent. This feature has been used to identify early genetic damage that occurs as a result of exogenous damage through radiation or chemicals. Oxidative damage, that is cellular damage that results from excessive production of reactive oxygen species, can be first recognized from mitochondrial DNA. Mitochondria are thus, not only the energy power house of the cell, but also an important source of extra nuclear genetic material.

Nucleus

It is the largest organelle in the cell and is the major store house of genetic material of the cell. The genetic basis of protein formation and the various genetic mechanisms that are important for the regulation of periodontium in health and disease are discussed in detail in later chapters. This important characteristic of the nucleus is not discussed here in this chapter.

The functions of the nucleus and the cytosol are, interdependent on each other. Optimal function of one organelle is dependent on proteins/macromolecules present in the other. In order to maintain homeostasis, there is a need for constant communication between the two
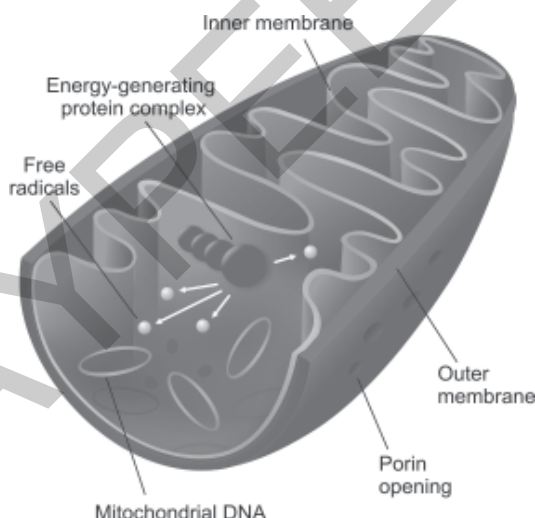

Fig. 1.3: Mitochondria *(For color version, see Plate 1)*

compartments. Therefore, the nucleus and the cytosol are always organized in a sort of two way traffic system. The importance of this two-way communication can be illustrated from the following examples. Important proteins like histones and RNA polymerases that are required for nucleic acid regulation within the nucleus are normally present in the cytosol. Similarly, protein synthesis begins in the nucleus and is completed in the cytoplasm. This bidirectional trafficking is made possible through a network of open channels. Transport, both in and out of the nucleus occurs through an active transport process.

The nucleus is covered by the nuclear envelop that separates the nucleus and cytosolic components into two. The nuclear envelope has an outer nuclear membrane and an inner nuclear membrane which are structurally dissimilar to each other (Fig. 1.4). The outer nuclear membrane is continuous with the membrane of the endoplasmic reticulum at various points. The outer nuclear membrane has numerous ribosomes embedded on its surface. Inner nuclear membrane, on the other hand, has specific proteins that can bind to genetic material and transport them to the cytosol. The space between the inner and outer nuclear membrane, called the perinuclear space is continuous with the lumen of the endoplasmic reticulum.

The nuclear membrane is lined by large open structures called the 'nuclear pore complexes'. These nuclear pore complexes are built by proteins called nucleoporins that are arranged in a symmetrical octagonal manner. Usually, the greater the transcriptional activity of the cell, the greater the number of pores in the nuclear envelop. Each nuclear pore is composed of one or more aqueous channels.
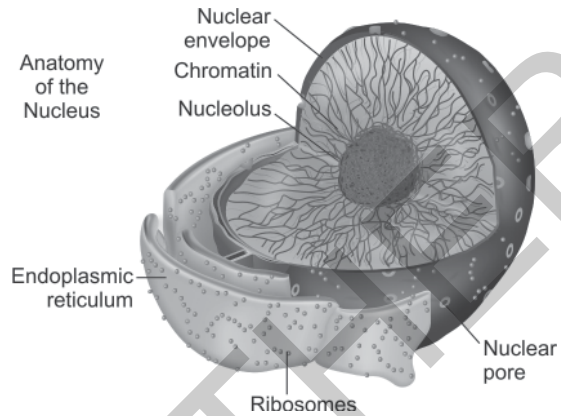

Fig. 1.4: Nucleus *(For color version, see Plate 2)*

These channels allow smaller molecules (less than 5000 Da) to pass through freely. This passive diffusion allows molecules such as Histones to pass into the nucleus. Histones, as we shall see in detail later, are required to pack DNA to form chromatin. This passive diffusion allows a large number of histones to be transported into the nucleus in a short period of time. In a cell that is undergoing active synthesis, about 100 histones must be transported into the nucleus every minute. The larger molecules, however, cannot use this transport mechanism as the small pores do not allow them to pass freely from one side to the other, e.g. the RNA polymerases that have to enter the nucleus and the large ribosome bound proteins are far too large to passively diffuse through these pores. As these macromolecules are important for the normal functioning of the genetic transfer and protein synthesis, this requires an alternate transport mechanism. This is achieved through an active transport process.

Active transport

Active transport process involves specific proteins that bind to and carry macromolecules in and out of the nucleus.

This is made possible through the presence of carrier proteins that traverse the lipid bilayer of the nuclear membrane. The large aqueous channels in the nuclear pores allow the proteins to pass through without unfolding ie., they are transported in the same state in which they were synthesized. This is in contrast to other organelles where proteins have to be unfolded before they can be transported into or out of their barrier membranes. Membranes that are transported across the nucleus are recognized by what has been termed the nuclear localization signals. These signals, normally consists of short sequences of amino acids rich in lysine and arginine. These signals are in turn recognized by receptors present in the nuclear envelope called the nuclear import receptors, one of which binds to the signal and the other to nuleoporins. These nucleoporins also exhibit specific sites that can be utilized for binding to the import protein. The commonly used binding sites are called 'FG" repeat areas, rich in phenylalanine and glycine which then recognize and bind to the import proteins. In some cases, the import proteins do not bind directly to the signal. Instead, signal binding is mediated by a set of adaptor proteins which serve as a bridge between the import proteins and the signal.

Similarly transport of proteins outside the nucleus occurs though nuclear export proteins. These proteins are encoded by a set of genes that exhibit structural homology to that of the import proteins. Functionally, too, they are very similar to import proteins and the only difference they exhibit is in the direction of movement, i.e., they move molecules from inside to outside the nucleus. This bidirectional active transport is an energy dependent process that requires activation of GTP/GDP. This energy is produced by a molecule called Ras. Ras is a monomeric GTPase, i.e., it can split GTP to form GDP and Pi and in the process releases energy. Ras is present in both cytosol and nucleus and is essential for active transport across the nucleus. Ras is present as bound to GTP or GDP and like other signal molecules, alternates between conversion from an active to inactive state. This conversion is brought about by GAPs that convert Ras-GDP to Ras-GTP. GAP's are present in significantly higher concentration in the cytosol. In contrast, the GEF's convert the GTP-Ras to GDP-Ras and is present more in the nucleus. They promote the import and export in the appropriate direction.

Golgi complex

The Golgi complex is one of the largest and most regularly outlined structure of the cell. The Golgi complex was identified even with light microscope. The Golgi complex is particularly prominent in cells that are undergoing active synthesis. The endoplasmic reticulum and Golgi complex are important for transport of proteins from nucleus to other parts of the cell. The Golgi complex is located close to the nucleus near the centrosome of the cell.

Structure

The Golgi complex consists of a number of flattened membrane bound structures called cisternae. Normally, eukaryotic cells contain about six or seven cisternae that are bound together through tubular connections such that they form a single complex. The cisternae and the tubules together form the structural units of Golgi (Fig. 1.5).

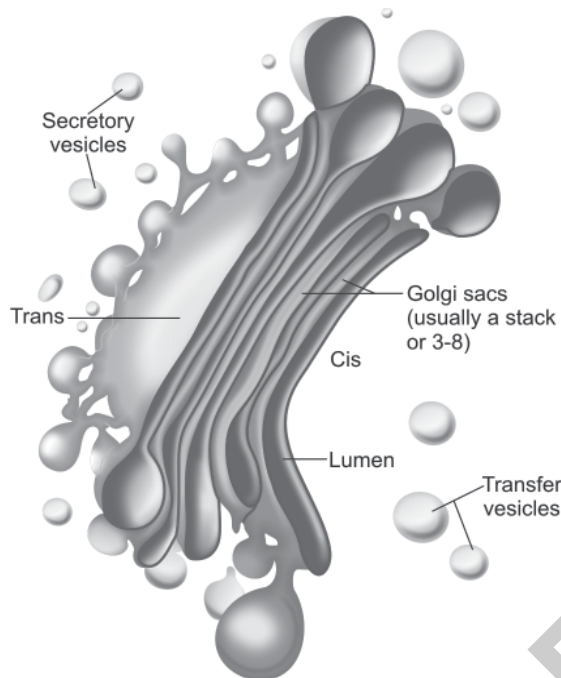For practical purposes, the Golgi complex may be divided into two cis face and a trans

Fig. 1.5: Golgi apparatus
(For color version, see Plate 2)

face both of which are divided into two distinct compartments. Each compartment has its assembly of cisternae and tubular structures. The cis face represents the areas through which proteins and lipids enter the Golgi, while the trans face is the area through which they exit.

Functional significance

Several proteins that are required for important biochemical reactions within and outside the cell are glycoprotiens and proteoglycans. Both classes of proteins require addition of carbohydrates to the proteins that are secreted by the nucleus- ER/ribosome complex. This addition of carbohydrates to the protein skeleton is accomplished in the Golgi complex.

Glycoprotein processing

The Golgi complex is involved in the addition of oligosaccharide chains to the glycoproteins. Oligosaccharide processing occurs in an orderly manner within the cisternal stacks starting from the cis face and ending in the trans face. This processing involves both removal and addition of sugars when complex oligosaccharides are added to the proteins. Otherwise, this process involves merely removal of sugars such as that seen in high mannose oligosaccharides.

Proteoglycan processing

Proteoglycans are also processed in Golgi complex as the glycosylation of the core proteins occur in the Golgi complex. In addition to this, the glycosaminoglycan side chains of the proteoglycans are sulfated in the Golgi complex. Sulfation confers negative charge to the proteoglycan and thus helps in hydration and maintenance of the extracellular matrix. Glycosylation of the glycoproteins and proteoglycans is an important step in protein processing. Generally, glycosylation prevents digestion of proteins by the proteases present within the cell. In addition, it may serve specific functions such as–
- *Adhesion in E-cadherin*
- *Cell signaling*

Process of glycosylation

Golgi complex is divided into cis, medial and trans compartments wherein there is an orderly glycosylation of the proteins. Modification of proteins occurs in each compartment in separate phases. This process has been proposed to occur through two mechanisms- vesicular transport and cisternal maturation. Vesicular transport mechanism

occurs as a result of protein being transported along the Golgi through its tubular network. In this mechanism, it is assumed that the cisternae are immobile and remains in their space. According to the cisternal maturation theory, the cisternae are thought to be dynamic and move from one end of the complex to the other. As a result of this cisternal transport, the glycosylated proteins enter from the cis face and exit from the trans face after completion of their processing.

Lysosomes

After protein processing is completed in the Golgi, it is transported by the lysosomes to the exterior of the cell. Lysosomes are vesicles that contain lysosomal enzymes that are used to digest macromolecules (Fig. 1.6). Among the various enzymes present are nucleases, lipases, phospholipases etc. that are used to digest extracellular components. All these enzymes are acid hydrolases that function at a pH of 5 or less. If these hydrolytic enzymes were to gain entry into the cytosol, they would be capable of causing considerable damage to the intracellular structures. To prevent this form happening, the lysosomes are bound by a firm, non-porous membrane that prevents leakage of these enzymes into the cytosol. Additionally, the acid hydrolases function best in the acidic environment of the lysosomes and not in the alkaline environment of the cytosol. The glycosylated proteins are prevented from digestion by the lysosomal enzymes due to the presence of sugars bound to the protein.

Functional significance

Lysosomes are involved in endocytosis and exocytosis, which is the transport of macromolecules into and out of the cytosol respectively. In addition to this, the lysosomes
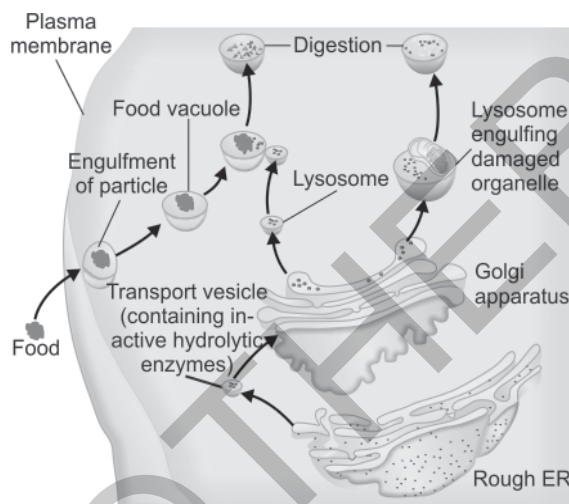

Fig. 1.6: Lysosome
(For color version, see Plate 2)

are also involved in autophagy, i.e. digestion of intracellular macromolecules and sometimes organelles that have either outlived their usefulness or are malformed. Lysosomes thus provide the scavenger apparatus that is required to cleanse the cell from any undesirable material.

Endoplasmic reticulum

Endoplasmic reticulum (ER) is an important component of the intracellular organelle assembly. Its importance stems from the fact that the endoplasmic reticulum is an essential requisite for protein synthesis and transport process. For efficient functioning of this process, the ER membrane comprises about 50 percent of the total volume of the cell.

Structure

Endoplasmic reticulum consists of a labyrinthine network of tubules and sacs which are interconnected with each other (Fig. 1.7). As a result, it is thought to have a highly convoluted lumen. This lumen is called
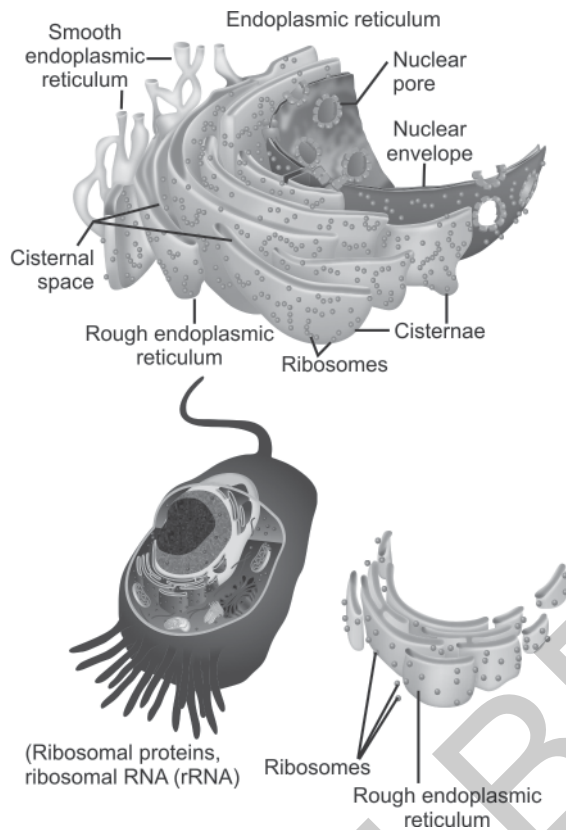
Fig. 1.7: Endoplasmic reticulum
(For color version, see Plate 3)

the cisternal space or the endoplasmic reticulum lumen. The cisternal space is separated from the cytosol by the endoplasmic reticulum membrane which acts as a selective barrier preventing the ingress and egress of molecules.

Functional relevance

Endoplasmic reticulum plays an essential role in synthesis of proteins and lipids. Most transmembrane proteins are synthesized in the endoplasmic reticulum. Similarly, the lipids that are an integral part of the

membranes of other organelles such as mitochondria are also synthesized in the endoplasmic reticulum. The proteins and lipids are synthesized in various regions of the endoplasmic reticulum. Based on their structure they have been divided into two regions- rough endoplasmic reticulum (RER) and smooth endoplasmic reticulum (SER). Rough endoplasmic reticulum is so designated due to the presence of ribosomes attached to the endoplasmic reticulum. The ribosomes or the rough endoplasmic reticulum complex is entirely involved in protein synthesis. Areas of the endoplasmic reticulum that do not have ribosomes attached to its membrane are called the smooth endoplasmic reticulum and these are involved in lipid synthesis.

Protein synthesis begins in the nucleus and they are then secreted into the cytosol through the nuclear pores. The endoplasmic reticulum selectively captures these secreted proteins from the cytosol and internalizes them for further processing. These proteins are of two types, the transmembrane proteins and the water soluble proteins. The transmembrane proteins remain attached to the membrane of the endoplasmic reticulum, whereas the water soluble proteins cross the *membrane* barrier and enter the lumen. Functionally, these proteins differ in that the transmembrane proteins reside either in the endoplasmic reticulum itself or become an integral part of the membrane of other organelles, while the water soluble proteins remain in the lumen of other organelles or are secreted outside the cell.

Normally most proteins are transported into organelles after the process of translation is completed. Otherwise, the enzymes of the cytosol are capable of proteolysis of the polypeptide chains. In order to avoid this,

Molecular Biology of Periodontium

This book is primarily meant to help readers gain an insight into the otherwise complex processes that regulate the periodontium in health and disease. Most modern diagnostic techniques and the newer therapeutic approaches utilize information that we have obtained from the study of various intracellular events. The knowledge of molecular biology is essential for students and clinicians alike for not only a deeper appreciation of Periodontics but also for integrating future treatment strategies into routine practice. This book provides a stepping stone in this direction.

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