

Biochemistry and cell biology

- Introduction
- Cells and tissues
- Biochemical pathways that affect ocular function
- The ocular surface
- Cornea and sclera
- Uveal tract
- Aqueous humour dynamics
- The lens
- The vitreous
- The retina
- The chemistry of the visual response
- Conclusion

Introduction

The eye is a miracle of self-organization. While many tissues such as muscle and kidney comprise predominantly a restricted set of more or less specialized cell types, almost all cell types are represented in the eye including secretory cells, neuronal cells, vascular cells, specialized fibroblasts, tissue myeloid cells, and supporting cells, and the matrices contain all of the molecular components found in other tissues. Uniquely, the cells and tissues have been customized for the eye.

Aristotle understood the phenomenon of self-organization as it applies to many organic and inorganic systems as ‘in the case of all things ... in which the totality is not, as it were a mere heap, but the whole is something besides the parts, there is a cause’ (Sasai, 2013). That the process of self-organization as it applies to the eye results in an organ specialized for the transmission, reception

and conversion of light energy into cellular signals remains a remarkable feat of differentiation and development. Even more remarkable is the observation that the forces leading to this event appear to be intrinsic to the cells, since optic cup and eye morphogenesis can be induced in embryonic stem cells *in vitro* given the right conditions (Fig. 4-1).

The unique feature of cells and tissues in the eye is that they are organized for the transmission, reception and conversion of light energy into cellular signals. Cells respond to stimuli in a remarkably similar manner. What differentiates one cell from another are the stimuli each cell responds to and the mechanisms it uses to respond. Cells have the genetic potential to express any type of receptor but their unique specialization is down to the limited set of membrane receptors they express. Through these receptors, cells respond to a specific stimulus by activating an intracellular second messenger system that has a limited generic range, i.e. the same set of signalling molecules is frequently activated by a wide range of ligand-receptor interactions: the specificity lies in the ligand binding. This produces a programmed response in the cell, resulting in an effect, e.g. aqueous secretion from acinar lacrimal gland cells after stimulation by adrenaline (epinephrine), an ocular muscle action potential after neurotransmitter release from nerve endings, or rhodopsin activation by a photon of light. Remarkably, the process of signal induction and transmission is based on a very limited set of biochemical reactions often involving an ATP-energy-driven mechanism, such as the addition or subtraction of a phosphate group to the signalling molecule, respectively via enzyme activity provided by a kinase or a phosphatase.

Cells are organized, singly or in groups (tissues), to receive information from the environment (via membrane receptors), to signal this information to

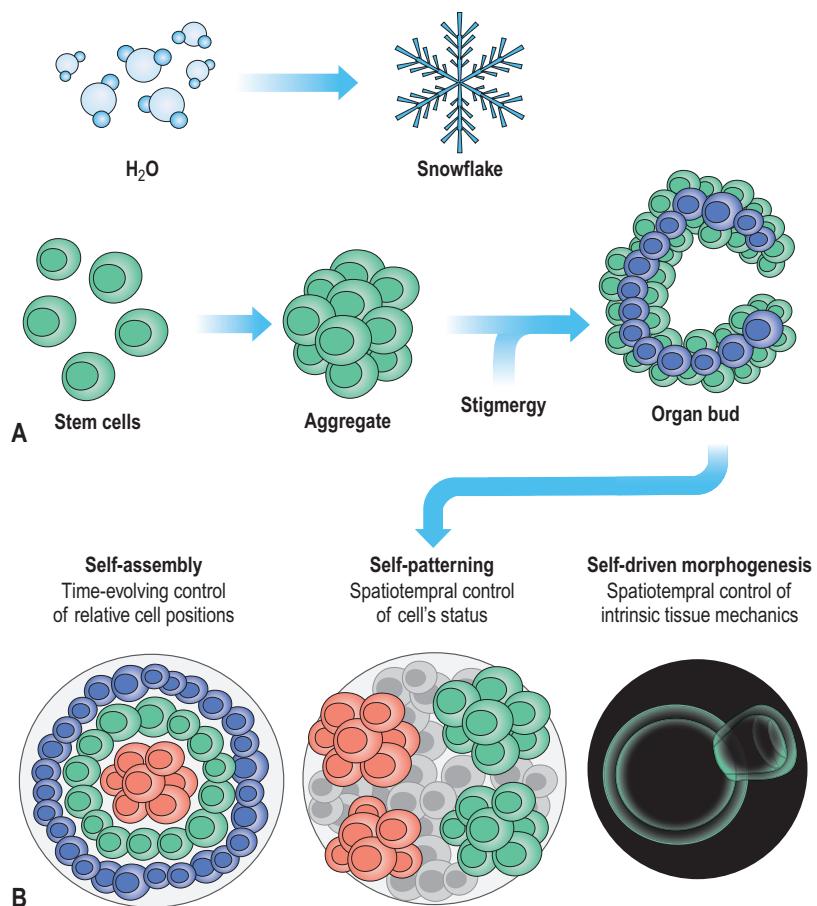


FIGURE 4-1 Self-assembly and self-organization is a general biological phenomenon. Individual particles aggregate as in water molecules transforming into snowflakes (A) or in stem cells which then develop by intrinsic forces into morphogenetically identifiable structures, a process that underpins embryogenesis (B). (Images from Sasai, 2013, with permission.)

the intracellular compartment (via signalling networks), to convert the message into cellular responses (gene activation and protein transcription) and to relay this information to the outside world (e.g. changes in cell behaviour, tissue function, secretion, etc.). Innumerable molecules and genes are involved in a single response by the cell through these networks, in which thousands of molecular interactions are connected through molecular ‘hubs’ (similar to hubs regulating aircraft traffic) (see next section). Cells may also respond simultaneously to several stimuli through several receptors, and controlling the flow of this information can be difficult. In recent years, this function seems to be attributed to that mysterious cellular constituent chromatin, whose histone tails

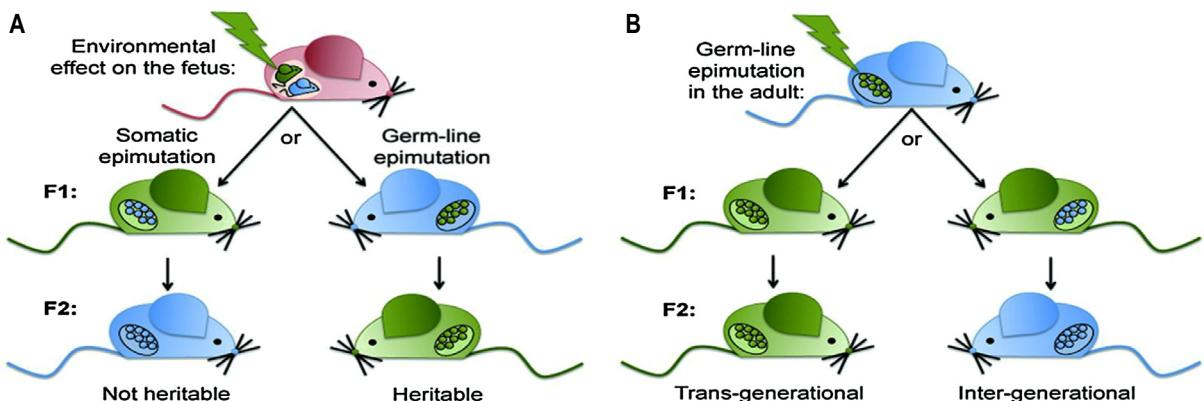
may act as a signal storage and converter device, similar to digital–analog converter devices in the electronic industry. In this way accumulated repeated rapid ON–OFF signals over time can induce epigenetic and indeed phenotypic change in cells and tissues, which not only influence cellular functions as they age but also can be transmitted to the next generation.

Additional content available at <https://expertconsult.inkling.com/>.

These concepts have been derived from the vast amount of information made available through full genetic analysis of various organisms (genomics) and the use of novel methods of investigation including microarray technology and informatics.

Studies in mice have shown that epigenetic changes to the gene pool of the fetus can lead to damage to either the somatic cells (genes not transmitted) or the germ-line cells (genes passed on). If the damage is not sufficient to kill the fetus the first-generation progeny (F1) will only pass on their mutated genes to the next generation (F2) if the germ-line genes have been affected (eFig. 4-1). If, however,

there is epigenetic germ-line damage and it is stably expressed in the adult, it may be passed on as part of the normal germ-line genes (i.e. is passed down several generations – transgenerational) unless reprogramming occurs in the embryo before implantation. Reprogramming can be viewed as a type of fail-safe repair mechanism.



eFIGURE 4-1 Epigenetic inheritance could occur through several mechanisms: (A) the fetus (F1) might be affected by environmental factors such as chemicals or drugs which would lead to either somatic (blue cells) or germ-line (green cells) mutations. In this case only the somatic mutations are heritable. (B) If a germ-line mutation is inherited, it can either be inherited over many generations or the germ-line cells can be reprogrammed back to normal cells and the genetic defect only lasts for one or two generations (inter-generational). In the figure, pink and blue mice are normal, green mice have the genetic defect.

(From Stringer et al., 2013.)

THE CONTINUING ADVANCE OF 'OMICs'

The development of systems biology, which is based on microarray techniques and high-throughput technology, has rendered the complexity of molecular interactions, such as those involved in signalling or in transcriptional regulation, amenable to analysis. This is the science of 'omics', a term applied to a body of work, knowledge or data, and includes genomics, transcriptomics, metabolomics, proteomics, signalomics and the microbiome. This has generated a vast amount of information leading to some further 'omic' subdivisions such as cancer genomics or toxicogenomics. Genomics examines the many genes which may be involved by increased or decreased expression, while transcriptomics studies the many transcription factors which may be activated or deactivated in any one cellular behaviour, such as cell division, and metabolomics investigates the many biochemical pathways which may be utilized, or not, in conversion of one molecular species to another in the process of energy generation and consumption. For the epigenetics researcher, there is even an epigenome, which provides information on DNA methylation, histone modifications and chromatin remodelling. Meanwhile, the microbiome is a term applied to the databank relating to gut commensals and has considerable influence on the immune system (see Ch. 7, p. 373). In several of these '-omes', molecular networks are entrained, which in themselves reveal the extensive interdependence that one system has on another. In addition, the notion of central ('hub'-based) molecular species without which the entire network would

collapse allows a hierarchy of importance to be applied to molecules. This is demonstrated in the genetic mutagenesis studies in which certain molecules, such as transforming growth factor- β , are lethal to the embryo when deleted, while others, such as plasminogen activator inhibitor 1, barely alter the murine phenotype.

Signalling networks are a prime example of how cellular information is transmitted. It is now recognized that there are hundreds to thousands of signalling receptors in the cell membrane interacting with around 10 second messenger 'hubs' in large interacting intracellular networks of several thousand cellular proteins (see eFig. 4-2 and eBox 4-1).

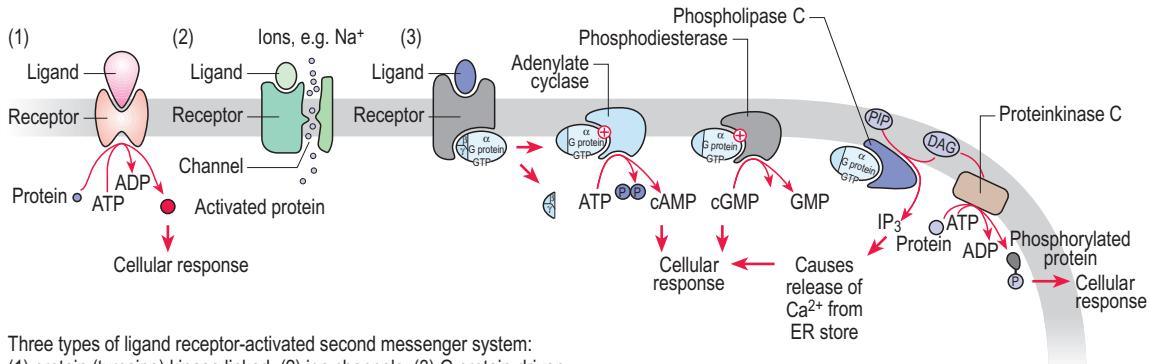
Additional content available at <https://expertconsult.inkling.com/>.

Examples of typical general second messenger systems include:

- receptor tyrosine kinase-linked receptor systems (RTK)
- ion channels and pumps
- G protein-driven messengers
- seven-transmembrane loops.

And there are several more generic types of receptor.

Each of these may interact with other intracellular signalling systems and the signalling systems themselves may be 'customized' to respond selectively depending on the conditions (Fig. 4-2). In addition, each receptor may be represented many times in a single cell (it is estimated that there are between 5000 and 10 000 major histocompatibility (MHC) class II molecules on a dendritic cell), while several different

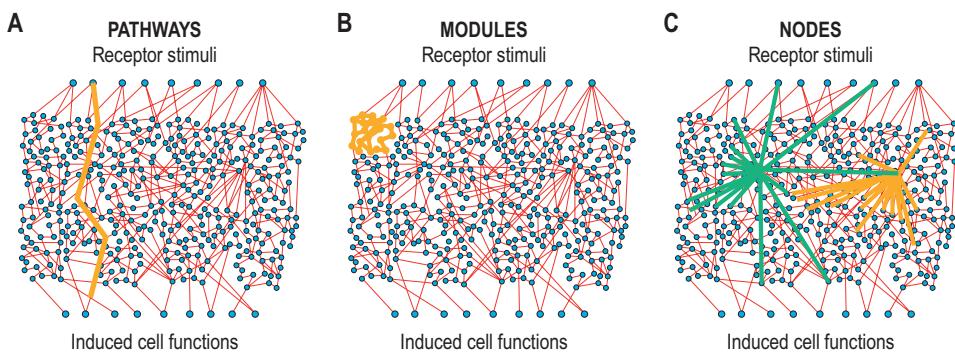


Three types of ligand receptor-activated second messenger system:
(1) protein (tyrosine) kinase linked, (2) ion channels, (3) G protein-driven

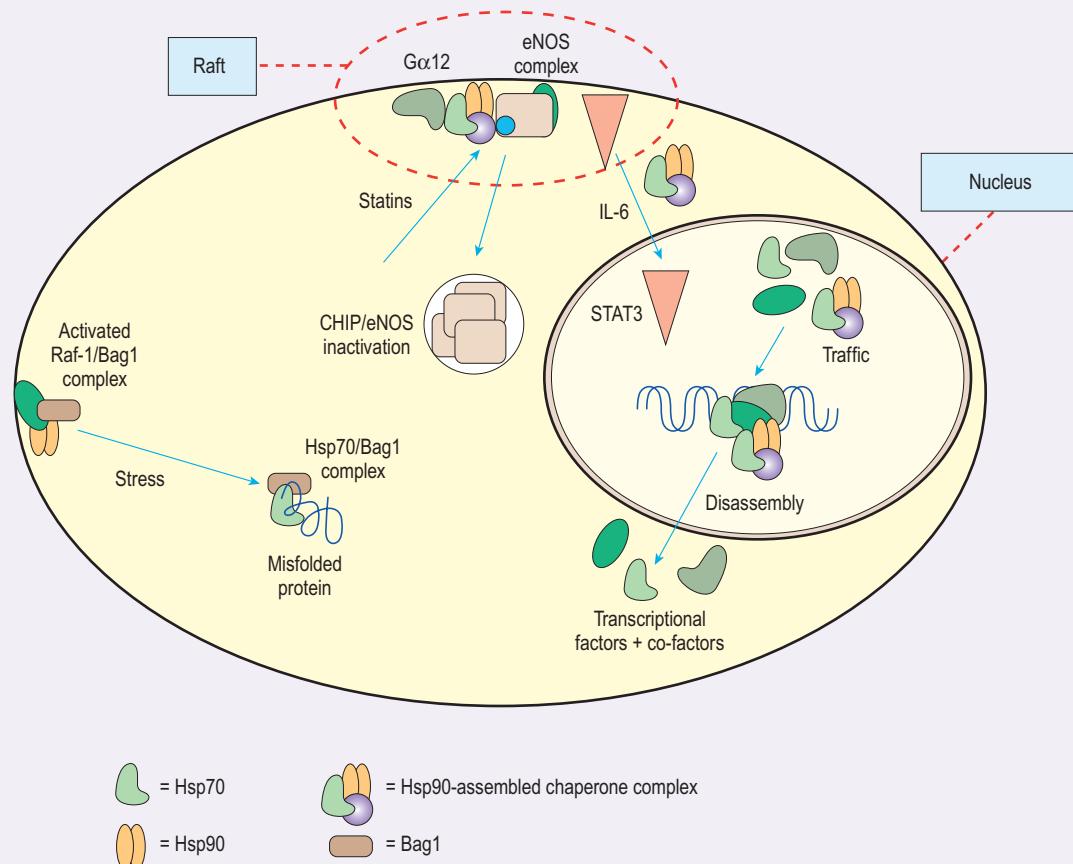
FIGURE 4-2 Ligand–receptor-activated second messenger systems.

Many of the thousands of molecules and genes are involved in a single response by a cell through intracellular networks; in this process immense numbers of molecular interactions are connected through a system in which some molecules form many interactions with others in ‘hubs’ while other molecules make very few interactions and are on the periphery of the network (eFig. 4-2). These concepts are derived from the vast amount of information made available through full genomic analysis of various organisms and the use of novel methods of investigation including microarray technology and informatics. Furthermore, hub molecules are frequently transient in activity,

one molecule acting as a hub during activation of one signalling pathway while the same molecule acts merely as a relay station during activity of another pathway. Information usually proceeds from ‘outside in’ to the cell, but on occasion information initiated outside the cell can be relayed back to extracellular targets (eFig. 4-2). Many of the cellular proteins may not be directly involved in signalling but may act as adaptors or amplify/diminish the overall response. In addition, other proteins act as ‘chaperones’ to protect proteins and signalling molecules for optimal function (eBox 4-1).



eFIGURE 4-2 Three concepts that are useful for describing signalling networks. Cell signalling is initiated by receptor stimuli. Each connection point reflects a signalling protein or second messenger, with lines indicating functional interactions. (A) Linear signalling pathways. (B) Modular structures within the network. (C) Nodes, which can be proteins or second messengers. Nodal points are regulated by many upstream events and/or regulate many downstream events. (From Meyer and Teruel, 2003, with permission from Elsevier.)

eBox 4-1**Molecular chaperones in the regulation of signalling:**

Chaperone proteins are everywhere in the cell, shepherding essential functional proteins such as enzymes and signalling molecules to ensure their proper functioning. Many different types of chaperone exist and some come into their own in certain circumstances, such as heat-shock proteins (Hsp), while others are constitutively functioning, such as lens crystallins (Fig. 4-2). Chaperones play an essential role in the activation of protein kinases: for instance, Bag1, the co-chaperone of Hsp70, which can activate the Hsp90-dependent process. Stress is known to inhibit cell

proliferation and sequestration of Bag1 may be how this occurs. Chaperones such as Bag1 and Hsp70 play an essential role in the maturation and activation of hundreds of protein kinases, regulating, for instance, cell proliferation in response to stress. Chaperones participate in raft-dependent signalling of molecules such as eNOS, G-proteins and STATs. Chaperones also help the subnuclear trafficking and disassembly of transcriptional factors and related complexes.

(From Sötő et al., 2005, with permission from Elsevier.)

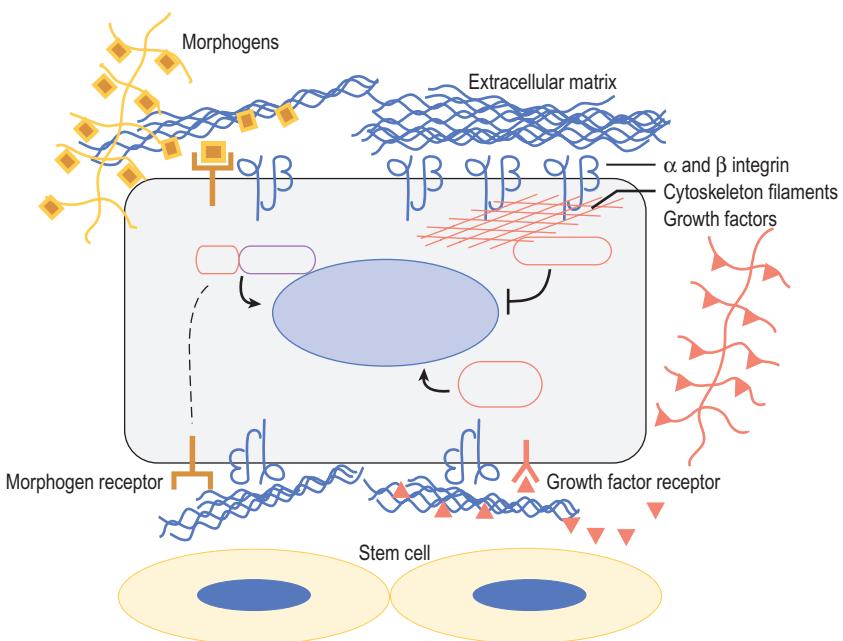


FIGURE 4-3 Suggested model for how matrix components (ECM), integrins and transmembrane receptors engage in 'cross-talk' in the stem cell niche. (1) The stem cell is physically anchored to the ECM, which activates growth factor receptors on the cell surface through the mediation of integrins; (2) the ECM also acts as a reservoir for growth factors and other mediators. Meanwhile the integrins also signal via the intracellular signalling molecules such as ERK 1/2/m Akt, and SMADs which retain the stem cell properties of the cell; (3) the ECM integrin coupling process allows the cells to sense biomechanical stiffness in the surrounds and transmits this to the cytoskeleton. Growth factors and their receptors; morphogens and their receptors; extracellular matrix; α and β integrin subunits; cytoskeleton filaments; SC, stem cell. (From Brizzi et al., 2012.)

ligand–receptor pairs may act in clusters at the cell surface, as for instance in the T-cell receptor synapse (see Ch. 7, p. 427).

Signalling networks behave similarly to other biological networks, such as metabolic and gene transcriptional networks, and probably represent a basic biological organizing system. Three basic concepts underpin a signalling network: signalling pathways, signalling modules and signalling nodes (eFig. 4-2).

In addition, receptors exist not only on the cell surface but intracellularly on endosomes and in the nucleus and ligands may have to be transported intracellularly to interact with their receptor. Information transmitted via ligand/receptor interactions can also be bidirectional, as for instance in the regulation of stem cells in stem cell niches through integrin molecule binding to extracellular matrix proteins (Fig. 4-3).

Cells and tissues

THE CELL

General structure

Technology drives science and there is no better example of this than the discovery of the cell as the basic unit of living organisms by Anton van Leeuwenhoek using a compound optical microscope. The basic structure of the mammalian cell can be illustrated by the retinal pigmented epithelial (RPE) cell (Fig. 4-4) as it contains most of the recognized cellular structures and intracellular organelles. While all cells have the potential and machinery for mitosis and motility, many adult tissue cells such as the RPE cell are considered terminally differentiated, non-motile cells, except under pathological conditions. The RPE cell is an example of a bidirectional transporting epithelial cell with polarity, i.e. an apical surface with

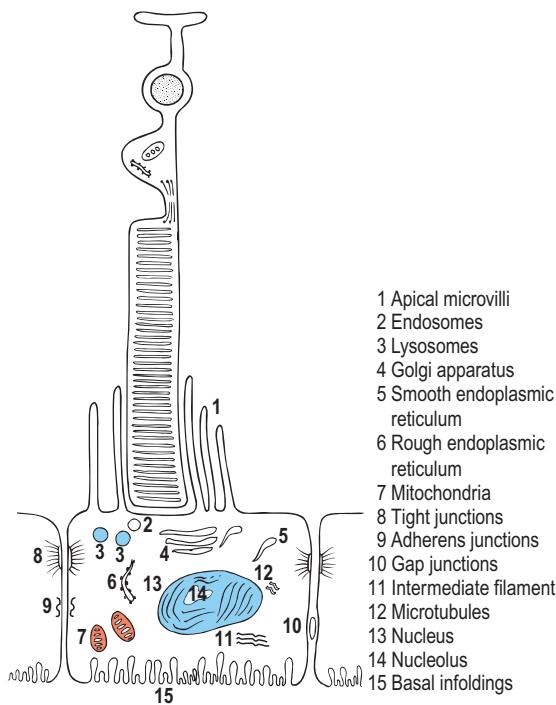


FIGURE 4-4 Diagram of RPE and photoreceptor cells. The photoreceptor outer segment lies in close apposition to the RPE cell, enclosed in a sheath of apical microvilli. The RPE cell is a terminally differentiated epithelial cell with several functions, one of which is to transport fluid across the cell towards the basal infoldings and into the choroid sink.

microvillous processes and a basal surface with numerous infoldings. The RPE is also an example of how the basic structure of the cell has been modified extensively, as in several types of specialized cells in the eye. Dysfunction of this critically important cell underlies the pathology of age-related macular degeneration (AMD), one of the commonest causes of blindness in developed nations (see Ch. 9, p. 513).

The plasma membrane

The plasma membrane, which surrounds all cells, is a selective two-way barrier to passive diffusion, which also has active transport mechanisms subserved by specialized proteins (for instance ion channels, pumps and suspended transporters) floating in a lipid bilayer, composed of phosphoglycerides, sphingolipids and sterols, which forms spontaneously due to its content of phospholipids. The barrier function of the plasma

membrane applies even to ions and is the basis of the electrical potential that occurs across cell membranes and which is energy-driven, for instance by which is energy-driven, for instance by ATPase enzymes. Other proteins are also suspended in the plasma membrane, such as receptors for hormones, neurotransmitters, viruses and other cells. Many of these receptors have a three-part structure with an extracellular, variably sized, component, a transmembrane component, and a short intracellular section coupled to the second messenger system. The plasma membrane is a variably thick lipid bilayer ‘crowded’ with many membrane proteins whose functions not only serve transmembrane traffic via receptors and transporters but also have a direct role to play in the physiology and indeed the survival of the cell: this is in part determined by the types of motion and tension which different membrane proteins exert (Box 4-1).

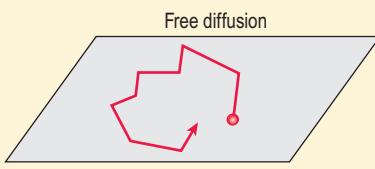
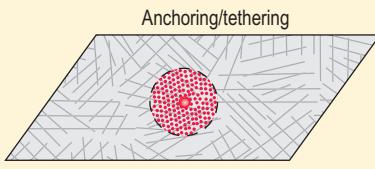
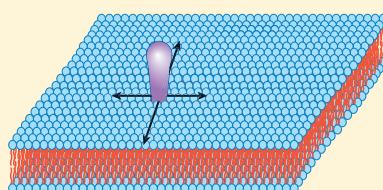
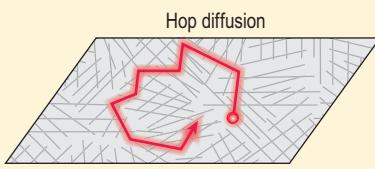
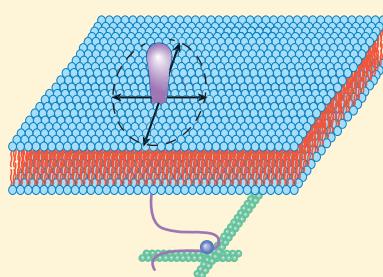
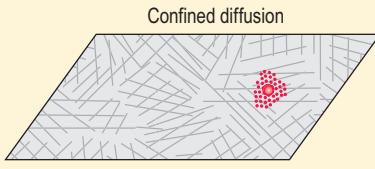
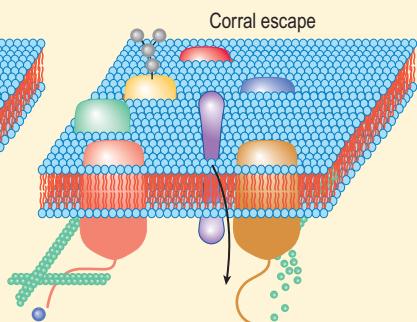
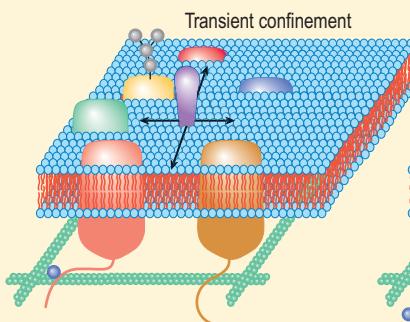
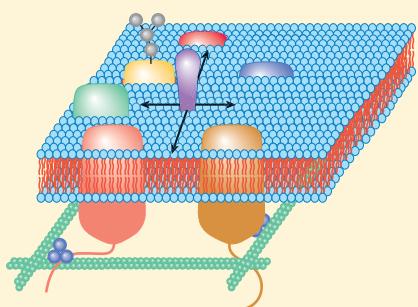
Many of these proteins are held in patchy distributions within the lipid bilayer, termed lipid (micro) domains, which themselves have variable constitution. The lipid domains also move within the membrane like rafts, and indeed if large enough are known as lipid rafts. Other microdomains decorate the cell surface in the shape of irregularly pitted invaginations and evaginations such as specialized structures for endocytosis (clathrin-coated pits) and caveolae, embedded in a glycoprotein-rich matrix (glycocalyx). These are also involved in a plethora of functions including cell signalling, protein trafficking, cell movement, waste disposal (exocytosis) and even cell survival (Fig. 4-5). Microdomains are frequently detergent-resistant and usually contain a specific protein such as caveolin in caveolae, or several proteins, as occur in lipid rafts, areas specialized for specific functions such as the immunological synapse in antigen-presenting cells (see Ch. 7, p. 421). Other microdomains include tiny domains (nanodomains), which contain GTP-binding protein (inhibitory) (GPI)-anchored proteins (important in some types of signalling) and glycosphingolipids, transient confined zones of varying size, and small regions composed of more fluid lipids.

Cells of the nervous system are especially rich in lipid rafts, which contain a high content of cholesterol, and sphingolipid rafts appear to have an organizational function either as discrete functional elements in which both the ligand and receptor are present, or

BOX 4-1 MEMBRANE PROTEINS HAVE MANY WAYS OF MOVING

Free diffusion (**A**) of proteins in the lipid bilayer allows random or directional movement depending on external triggers. Many proteins are, however, anchored or tethered either to cytoskeletal proteins or to extracellular matrix proteins (**B**). Examples include GPI-tethered proteins. Some proteins are only transiently tethered during a particular

function, for instance in the immunological synapse of immune cell activation (**C**). Some of the proteins can escape from the synapse or corral and become internalized. In contrast some proteins have limited movement and are confined to a region of the plasma membrane, as in confined diffusion (**D**).

**A****B****C****D**

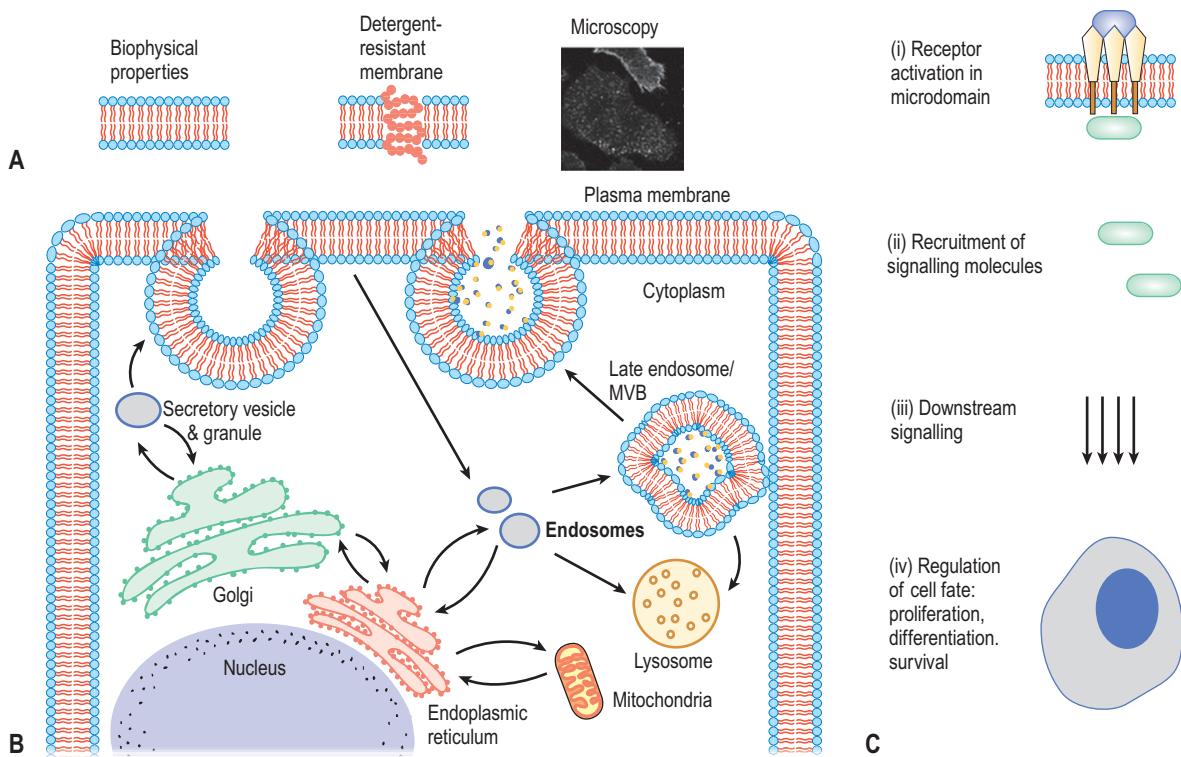


FIGURE 4-5 Plasma membrane microdomain dynamics. Membrane lipids such as phospholipids, sphingomyelin and cholesterol separate into two distinct phases: a highly mobile phase (L_d) allowing free diffusion and a highly ordered phase (L_o) greatly restricting their mobility. Sphingomyelin and cholesterol are in the latter group. These differences greatly affect membrane function such as endosome formation, cell proliferation and many other processes. The diagram in (A) shows the techniques involved in demonstrating lipid mobility in plasma membranes during different cell activities; (B) shows different types of membrane with different lipid membrane microdomains; (C) shows how separation of different microdomains yields information on the various protein receptors and signalling protein molecules which can be isolated from separated lipid membranes domains. (From Inder et al., 2013.)

where the activation of the receptor is dependent on recruitment to the raft of the effector ligand. It is likely that the enormous complexity associated with microdomain function will only be revealed using a systems biology approach such as a combination of proteomics and transcriptomics.

Many other specializations occur in the plasma membrane, depending on the cell type, such as junctional complexes, gap junctions, desmosomes, hemidesmosomes and contact sites with the basement membranes (see below). In the eye these membrane specializations are developed to a high level. For instance, the photoreceptor cell (Fig. 4-4) (see Ch. 1, p. 41) is a highly polarized structure comprising a receptor component, a nucleus and a synapse. The

rod photoreceptor (specialized for scotopic vision; see Ch. 9) develops as an evagination of the plasma membrane, which folds upon itself many times to form stacks of membranous disks by fusion of the peripheral disk membrane. The plasma membrane is typical of any cell, i.e. it comprises a lipid bilayer containing a high concentration of membrane proteins. The lipid bilayer is a self-assembling sheet of phospholipid that adopts the bilayer format because of the physicochemical properties of the polar phospholipids, ensuring that the polar groups are external and the hydrophobic groups form the inner layer of the leaflet. The photoreceptor can adopt this special arrangement because it has more cholesterol in its bilayer, not only making it less fluid but also

preventing crystallization of the membrane by inhibiting possible phase transition of the hydrocarbons. The synaptic terminal of the photoreceptor interacts with mobile Ca^{2+} channels in the bipolar cell which appear to function within lipid rafts confined in their mobility by attachments to the cytoskeleton (see Part B of the figure in Box 4-1).

Endoplasmic reticulum and Golgi apparatus

A wide variety of cell organelles are embedded in a cytoplasmic gel, which is traversed by a system of membranes, the endoplasmic reticulum (ER). The ER, a series of thin bilayered membranes, is a flowing dynamic system constantly forming and reforming. Cisternal, tubular and vesicular elements exist. The rough ER (RER) is distinguished from the smooth ER as a ribosome-studded structure that is highly developed in secretory cells such as the lacrimal gland acinar cell, and is specialized in other cells, e.g. the sarcoplasmic reticulum of striated (including extraocular) muscle. The RER is arranged *en face* in rows or rosettes of ribosomes (polysomes). Newly synthesized proteins come off the ribosomes and are threaded through the lipid bilayer into the interior of the ER where they are post-translationally folded, ready for secretion via the Golgi apparatus by vacuolar budding and fusion with the plasma membrane for exocytosis (Box 4-2). The smooth ER is also the site of synthesis of molecules such as lipids, triglycerides and steroids, and is prominent in cells such as the RPE and meibomian gland cells; the Golgi apparatus is a membranous stack of flattened cisternae which receives proteins, now equipped with a leader sequence required for secretion, for sorting and exocytosis from the smooth ER; the smooth ER also plays a role in lipid trafficking and modification.

The ER and the Golgi apparatus have other functions: they are involved in signalling, for instance during mitogen activation of cells for proliferation, via the small GTPases which are activated at the plasma membrane and also signal in the ER and Golgi body (Box 4-3). In fact, small GTPases and other molecules, such as phosphoinositides, provide a signature for each organelle and are involved in the specific lipid membrane folding that characterizes each organelle (Box 4-3). The ER also provides a regulatory role in ensuring quality control of good protein secretion (GPS). This is

regulated by a process of ubiquitylation by which the level of ubiquitin molecules added to the protein determines whether the protein is targeted for secretion or degradation in the proteasome (see eFig. 4-3).

Additional content available at <https://expertconsult.inkling.com/>



If proteins are not folded correctly they are unable to function and the ER generates a 'stress response', also known as the unfolded protein response (UPR), which is one form of ER stress. Together with a second fail-safe mechanism, mediated by a kinase known as the mammalian target of rapamycin (mTOR), the UPR and the mTOR pathways control many cellular processes including programmed cell death (apoptosis), protein translation, waste disposal (autophagy), energy supply (ATP), and response to inflammatory stimuli.

Additional content available at <https://expertconsult.inkling.com/>



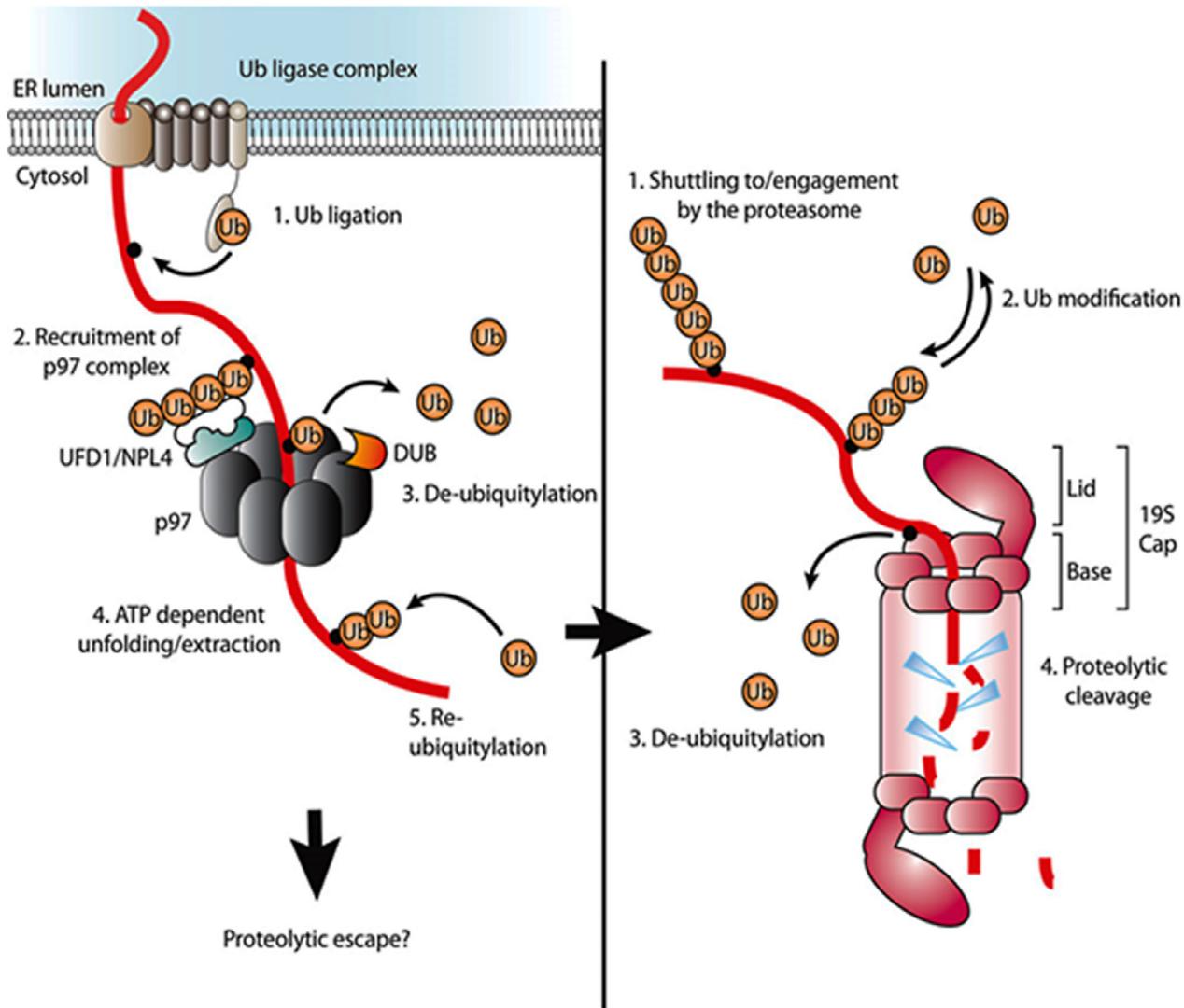
The ER also forms the nuclear envelope during telophase, when a series of flat vesicles surround the chromosomes and fuse at their edges. The envelope contains many *nuclear pores*, which are composed of eight cylindrical filamentous structures in a highly organized arrangement. Pores act as molecular sieves, permitting rapid passage of small 4.5 nm (4.5 kDa) particles and slower passage of larger molecules (12–70 kDa). The outer aspect of the nuclear envelope in secretory cells is lined with ribosomes and polyribosomes, while the inner surface is in contact with a nuclear filamentous matrix.

Mitochondria

Mitochondria are small (2 μm long) oval-shaped organelles comprising a two-membrane system of compartments, the inner one of which is composed of two domains, the inner boundary membrane (IBM) and the invaginations, folded into structures termed cristae (Fig. 4-6). At the cristae junction with the IBM, the mitochondrion inner membrane organizing system (MINOS) is located and separates the two populations of proteins: those in the IBM assist in protein movement (translocases) and those in the cristae contain the proteins involved in the respiratory chain, the F1FO-ATP synthase and carrier proteins for ADP/ATP (see below).

The intermembrane space contains carrier proteins that are responsible for the transport of metabolites

The way a protein is folded critically determines its function. This process is monitored by a ubiquitous cellular protein called ubiquitin (Ub) which is bound to the protein as it emerges from the endoplasmic reticulum. As more and more Ub molecules are added to the protein, it protects it from degradation, while as more Ub molecules are removed by the protein, it becomes susceptible to degradation and recycling/removal. This process is tightly regulated (see [eFig. 4-3](#)).



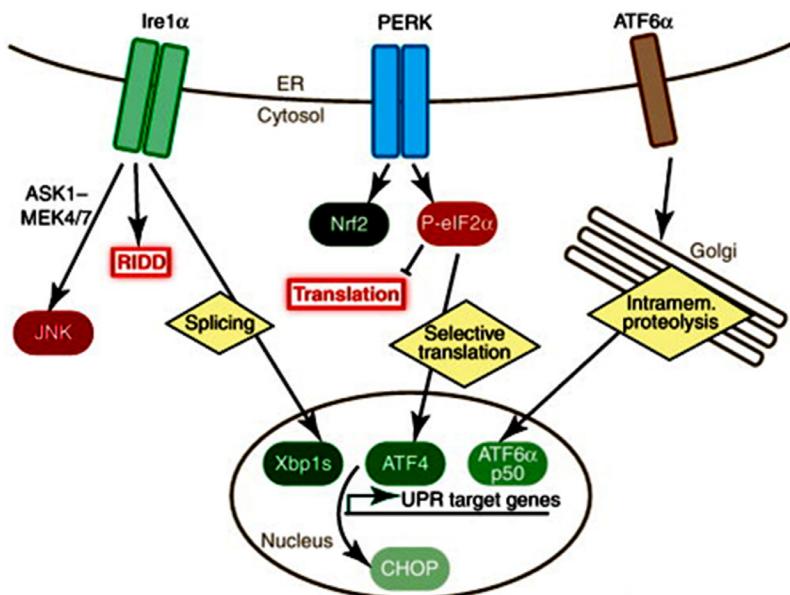
1. Ubiquitin ligase complex (see table 1)
2. UFD1/NPL4
3. YOD1, Ataxin 3, USP19
4. p97
5. Ubiquitin ligase complex? Ufd2?

1. Rad23, Dsk2, Ddi1, Rpn10, Rpn12, BAG6?
2. Uch37, Ubp6/USP14
3. Rpn11
4. Proteasome

eFIGURE 4-3 The function of a protein can be disabled both by dislocation and degradation. In the left panel a dislocated protein is ubiquitinated at the ER membrane and entered into a complex with p97 via NFD1/NPL4. Ubiquitin is cleaved, which allows the protein to be threaded through the central pore of the protein complex, p97. The protein is then re-ubiquitinated for proteasomal targeting and final degradation as shown in the right panel. (From Claessen et al., 2012.)

Cells respond to unfavourable conditions by demonstrating a stress response. For instance this occurs where there is an excess of free radical generation by the cell but can also occur when normal physiological mechanisms are tested, as in a condition known as ER stress. Stress is dealt with by the cell through a variety of

mechanisms, including the ER stress responses and a cell signalling system known as the mammalian target of rapamycin (mTOR) response, which is implicated in many conditions involving inflammatory responses in the eye (eFig. 4-4).



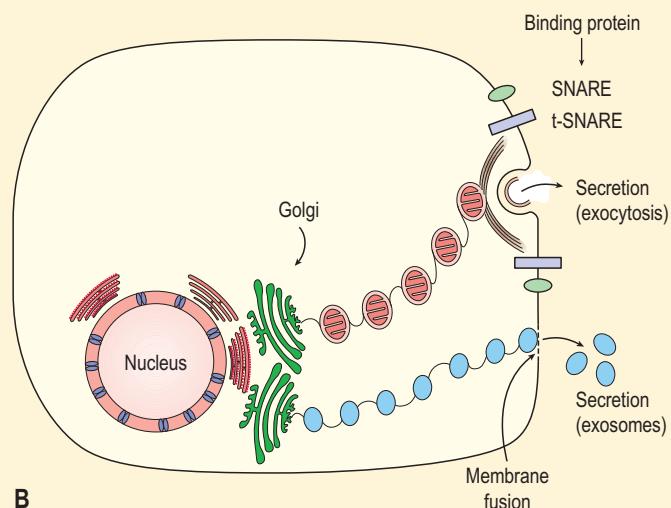
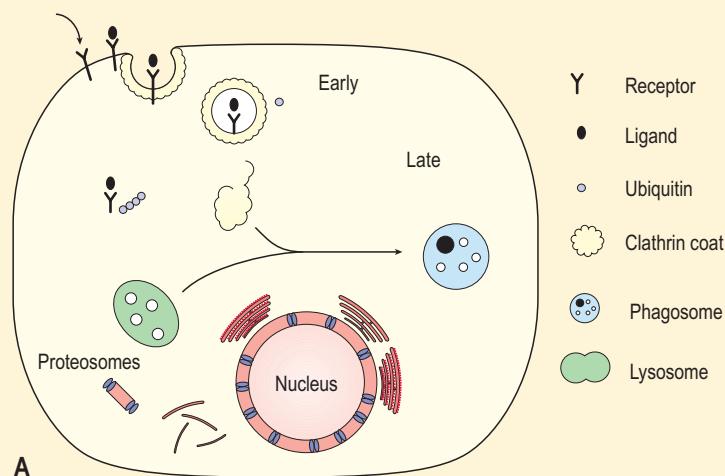
eFIGURE 4-4 IRE-1 α is an ER stress sensor which signals to the nucleus to activate the unfolded protein stress response (UPR) and maintain cellular physiology. However, if the cell fails to respond, convergent signals through PERK activate a nuclear transcription factor (CHOP) which initiates apoptosis. A similar signal can also be initiated via ATF6 α to activate CHOP

BOX 4-2 ENDOCYTOSIS, EXOCYTOSIS AND EXOSOMES

Endocytosis is generally achieved via incorporation of ligand–receptor complexes in clathrin-coated vesicles. This applies to soluble proteins and to small and large particles such as viruses, which frequently use constitutive cell surface receptors to enter cells.

Clathrin-coated vesicles start as small pits on the cell surface. When the vesicle is fully intracellular it loses its clathrin coat and becomes an endosome, which fuses with primary lysosomes that have a high content of acid hydrolases and other proteases. These lead to degradation of the ingested material, and further processing depending on the cell type. Certain cell surface receptors are recycled to the cell membrane during this process to engage further extracellular ligand (**A**). Clathrin-coated pits are normally restricted to the region of the plasma membrane by the cortical cytoplasm actin organization. Relaxation of this actin assembly by proteins such as latrunculin B allows movement of the coated pits.

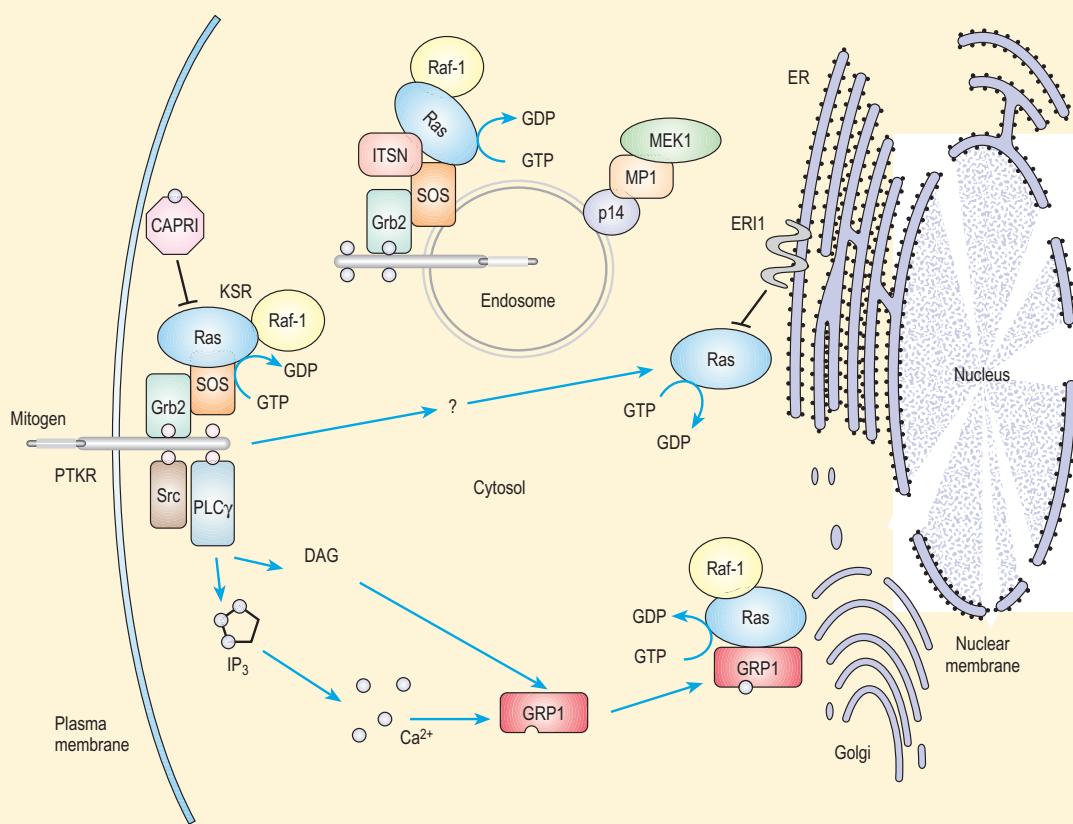
Exocytosis occurs by a similar process but in reverse. Cytoplasmic granules such as those in exocrine glands or in granulocytes are lipid vesicles containing material for extrusion. Secretory vesicles bud from the Golgi apparatus and are transported towards the plasma membrane by the cytoskeleton. There, they fuse in a lipid microdomain assisted by proteins such as SNARE and the SNARE-binding proteins in a ‘targeting patch’ (**B**). Fused secretory vesicle membranes act as targets for further secretory granule fusion in cells such as mast cells. In addition, vesicles termed exosomes may be ‘pinched’ off cells such as macrophages and immune dendritic cells in the same way as platelets are pinched off megakaryocytes. Exosomes convey material (information) from one cell to another and the nature of the information varies from cell to cell.



BOX 4-3 SCHEMATIC REPRESENTATION OF HOW MITOGENS ACTIVATE CELLS

Ligand binding to the tyrosine kinase receptor (PTKR) activates the small GTPases, Ras, through a complex of signalling molecules (Grb2/SOS). Activated Ras on endosomes from the plasma membrane induces signalling in this organelle as well as in the Golgi apparatus (indirectly via phospholipase C γ Ca and another protein known as GRPI) while inhibition of the small Ras occurs in the endoplasmic reticulum via an inhibitor protein ERII (see figure). In this

example of a signalling network, interstin and kinase suppressor of Ras serve as scaffold proteins while p14 acts as an adaptor protein in Ras-independent activation of the kinase, MEK-1 (mitogen-activated protein (extracellular signal-regulated (ER kinase) kinase), by the endosome. The integration of organelles and signalling networks with cytosolic proteins is thus central to proper functioning of the cell in response to an external stimulus such as a mitogen.



between the two compartments and also between the cytosol and the outer compartment. Their transport systems include antiport, aspartate/glutamate, ornithine/citrulline, maleate/citrate, symport, pyruvate/H⁺, and urea and porphyrin synthesis. Mitochondria are the powerhouses of the cell and have several essential metabolic functions as they contain all the elements for the respiratory assembly, for the citric acid cycle and for fatty acid metabolism. Their main functions therefore are to act as the site of energy-rich adenosine triphosphate (ATP)/guanosine triphosphate

(GTP) formation, to function as a calcium store mainly in the form of calcium phosphate, to engage in the uptake of energy-rich substances, and to facilitate the oxidative breakdown of ATP. Mitochondria and the ER form an integrated system known as the ER-mitochondria organizing network (ERMIONE), enabling the transport of lipids and calcium between the compartments.

Mitochondria are powerhouses of nutrient handling and energy storage, essential for cell growth and proliferation. Uncontrolled, this can lead to tumours,

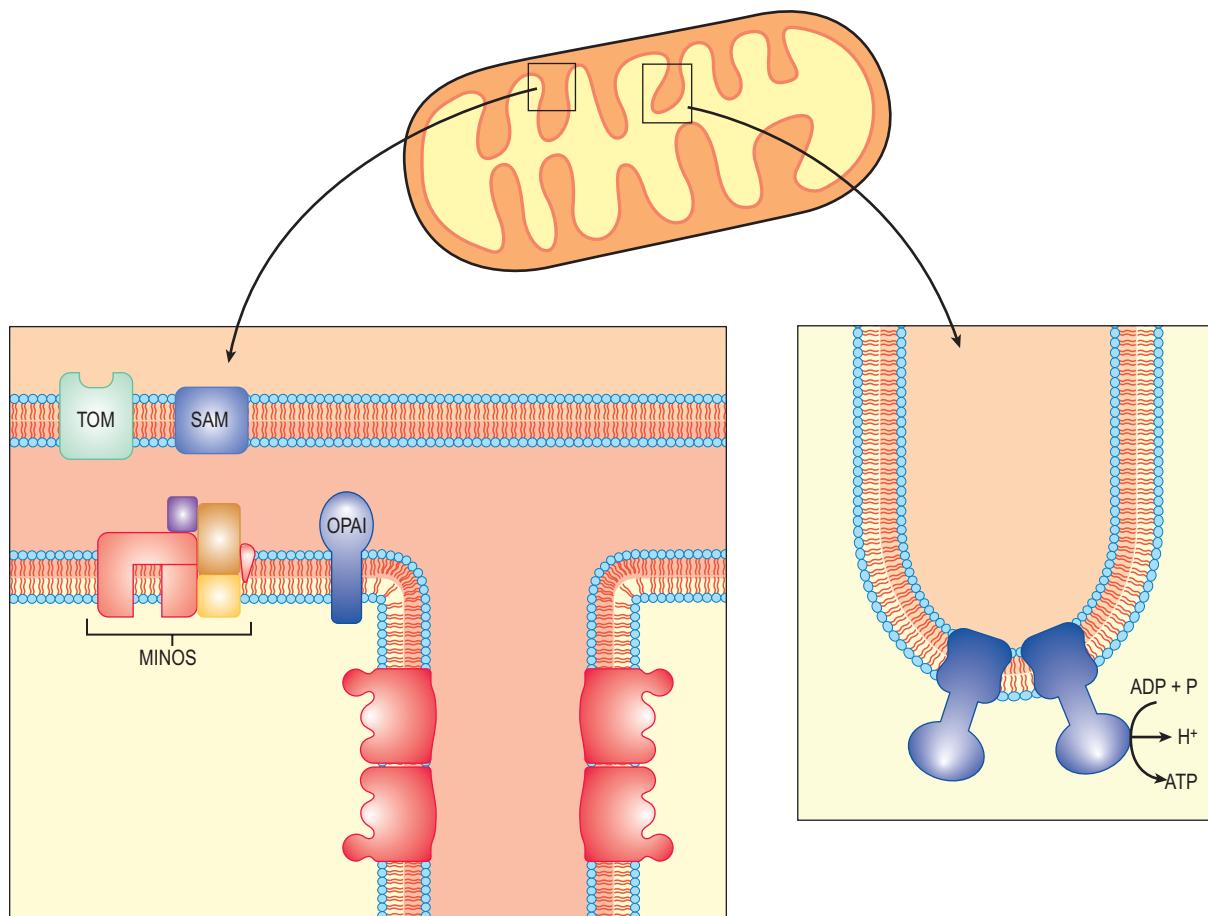


FIGURE 4-6 Mitochondrial membrane organization. Mitochondria are composed of an extensive intraorganelle membrane structure to maximize their numerous functions in metabolism, cell death, autophagy and energy control. Membrane organization is regulated by protein complexes such as MINOS, which occurs on the inner mitochondrial membrane, mostly at cristae junctions. MINOS is composed of several proteins and interacts with others such as the inner membrane morphology proteins OPA1, TOM and SAM.

and so a balance between homeostatic metabolism and cell death is essential. Mitochondria are therefore regulators of cell death through several routes (Box 4-4).

The central inducer of cell death is mitochondrial cytochrome c, which is released into the cytoplasm and complexes with Apaf-1 and caspase 9 (the apoptosome) and ultimately leads to caspase 3 and 7 as the final executioners. More recently, mitochondria have been recognized as sensors and regulators of cytosolic Ca^{2+} levels, through Ca^{2+} transporters which have central physiological roles in signalling, proliferation, metabolism and ultimately cell survival.

There are many ‘death receptors’ (receptors which initiate the cell programme leading to death of the

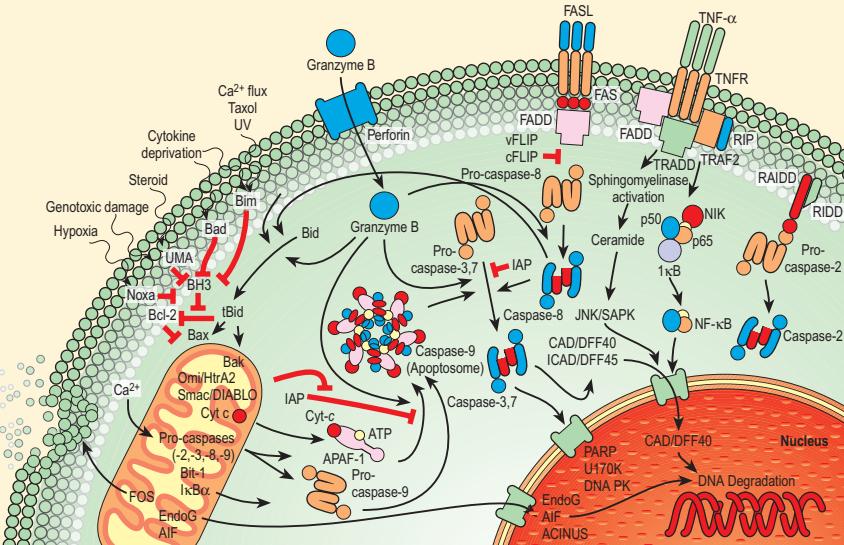
cell, i.e. apoptosis), including FasL, TNFR and granzyme B, as well as environmental conditions which promote cell death, such as hypoxia, genotoxic damage, cytokine or nutrient deprivation (linked to the tricarboxylic acid cycle and cytochrome c) (Fig. 4-7), excess steroid exposure, UV exposure and toxic drug exposure, and the balance between life and death is maintained by pro- and anti-apoptotic Bcl-2 proteins (see Ch. 7).

Mitochondria have their own complement of DNA (but no histones, and thus are not susceptible to epigenetic changes; see Fig. 4-1) as well as ribosomal RNA/transferring RNA, and generate a series of mitochondrion-specific proteins associated with mutations and a

BOX 4-4 MITOCHONDRIA AND APOPTOSIS: THE SIGNALLING PATHWAYS

Cytochrome c (red spots in Figure 4-7) in mitochondria controls many apoptosis pathways involving caspases, granzymes and perforin molecules as well as several

pro- and anti-apoptotic genes such as Bax, Bcl2, BH3, Bid and Bim. Many of these, plus numerous other proteins, form a complex known as the apoptosome.



(Figure reproduced with permission of EMD, Madison, WI, USA, www.calbiochem.com.)

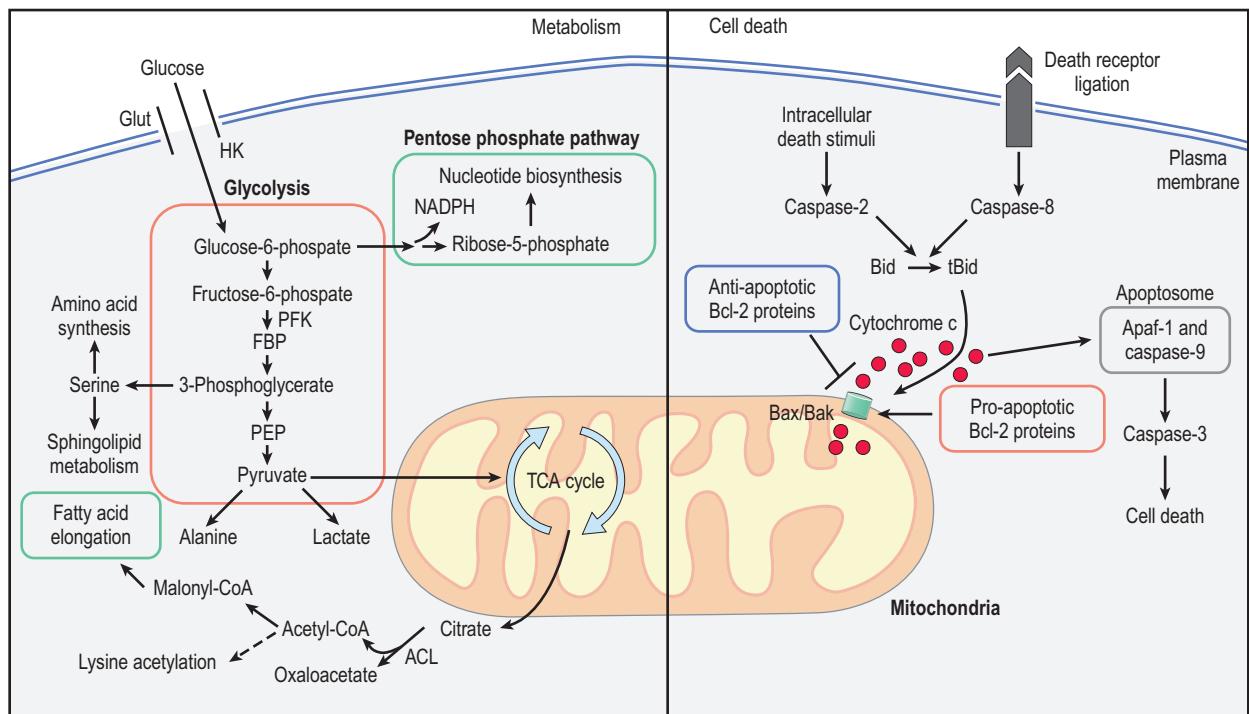


FIGURE 4-7 Cell metabolism and cell death (apoptosis) are intricately linked through the tricarboxylic acid cycle. Cross-talk between the signalling pathways for these two systems provides cross-regulation and determines whether a cell will live or die. Much of this cross-talk goes on in the mitochondria and is dependent critically on cytochrome c, which is central to both pathways in generation of ATP for energy and caspase-1 for apoptosis. (Andersen and Kornbluth, 2013)

number of discrete syndromes, some with ophthalmological consequences (see Ch. 3, p. 156). Since mitochondria originate only from ova, transmission of these genetic defects is purely maternal.

The nucleus

The nucleus is contained within a perforated sac, formed by pores (nuclear pore complexes). These allow transport of proteins and other signalling molecules passage between the transcriptional machinery and the appropriate instructions from outside. Both import and export of materials are receptor-mediated in an 'address-label'-like system based on small GTPases (see Box 4-3).

The main nuclear component is chromatin, a complex structure of highly extended DNA, RNA and protein in the interphase (non-dividing) cell, which becomes greatly condensed (by 400-fold) to form chromosomes during cell division. Packing of chromatin is achieved by interaction between negatively charged DNA and certain basic proteins (histones) which carry a positive charge at the pH of the cell; euchromatin is less packed than heterochromatin, the proportion of which varies from nucleus to nucleus and may be characteristic of certain cell types, e.g. the 'clock-face' heterochromatin of plasma cells detectable in histological sections. Genome stability is dependent on minimizing DNA damage, which might occur for instance during cytokinesis and chromatin condensation, and chromatin regulators exist to ensure stability. Most of these are histone-modifying enzymes such as acetyl and methyl transferases and deacetylases. Much of gene modification is mediated via reversible nucleotide methylation and acetylation.

The nuclear membrane also contains receptors for ligands, which may be synthesized in the cytoplasm or may have been endocytosed through plasma membrane receptors. Typical nuclear membrane receptors include steroids, growth factors such as fibroblast growth factor and novel groups of proteins known as the peroxisome proliferator-activated receptors (PPARs), which are involved in many cell processes such as lipid and glucose homeostasis, wound healing, and inflammation generally. PPARs are unique receptors that allow integration of signals mediated by lipophilic ligands with plasma membrane-derived

signals. Nuclear membrane receptors may be organized for induction or suppression of genes in a coordinated fashion (Fig. 4-8). In addition, a major function of the nucleus is to manage the packaging of mRNA into ribonucleoprotein particles called mRNPs. These are associated with very large numbers of proteins involved in the transcription of mRNA in the nucleus to translation and degradation of mRNA in the cytoplasm (Fig. 4-9).

The nucleolus is essentially composed of RNA and fibrillar material, and is the site of ribosomal RNA synthesis and intense transcriptional activity. In addition, it may contain non-coding regions of RNA involved in the stress response. The nucleolus develops during the late stages of mitosis in association with specific regions on the chromosomes, known as the nucleolar organizer centre.

The intracellular matrix

The cytoplasm is a highly viscous aqueous medium that has deformability (elasticity). Physically, it exists at different times as a gel or as a sol. The cortical cytoplasm (ectoplasm) is more akin to a gel structure, while the endoplasm is usually more fluid. Thus the cortical cytoplasm restricts movement of organelles such as coated vesicles (see Box 4-2). The gel-like properties of the cytoplasm are the result of the binding of 'structured' water molecules and Ca^{2+} ions to polymeric filaments; in fact, water, as the main constituent of cells, has a significant role even within the molecular interstices of cell membrane receptors where in its 'ordered' state it contributes to the tertiary structure of the molecule as shown by rhodopsin molecules in the photoreceptor disks (Fig. 4-10).

Three main types of intracellular filaments are described, microfilaments, such as actin; intermediate filaments; and group 3 cytoskeletal fibres, such as tubulin and myosin (see Box 4-5). More recently, a fourth type has been recognized as a cytoskeletal element, although this family of molecules was discovered over 40 years ago, namely septins.

Microfilaments. Microfilaments (5–7 nm), such as actin, tropomyosin and troponin, are universal constituents of cells and are involved in almost every cellular activity, including cell motility, contractility, endo- and exocytosis (see Box 4-2) and maintaining

BOX 4-5 DIAGRAM OF CELLULAR STRUCTURES INDICATING THE VARIOUS CYTOPLASMIC CONTENTS

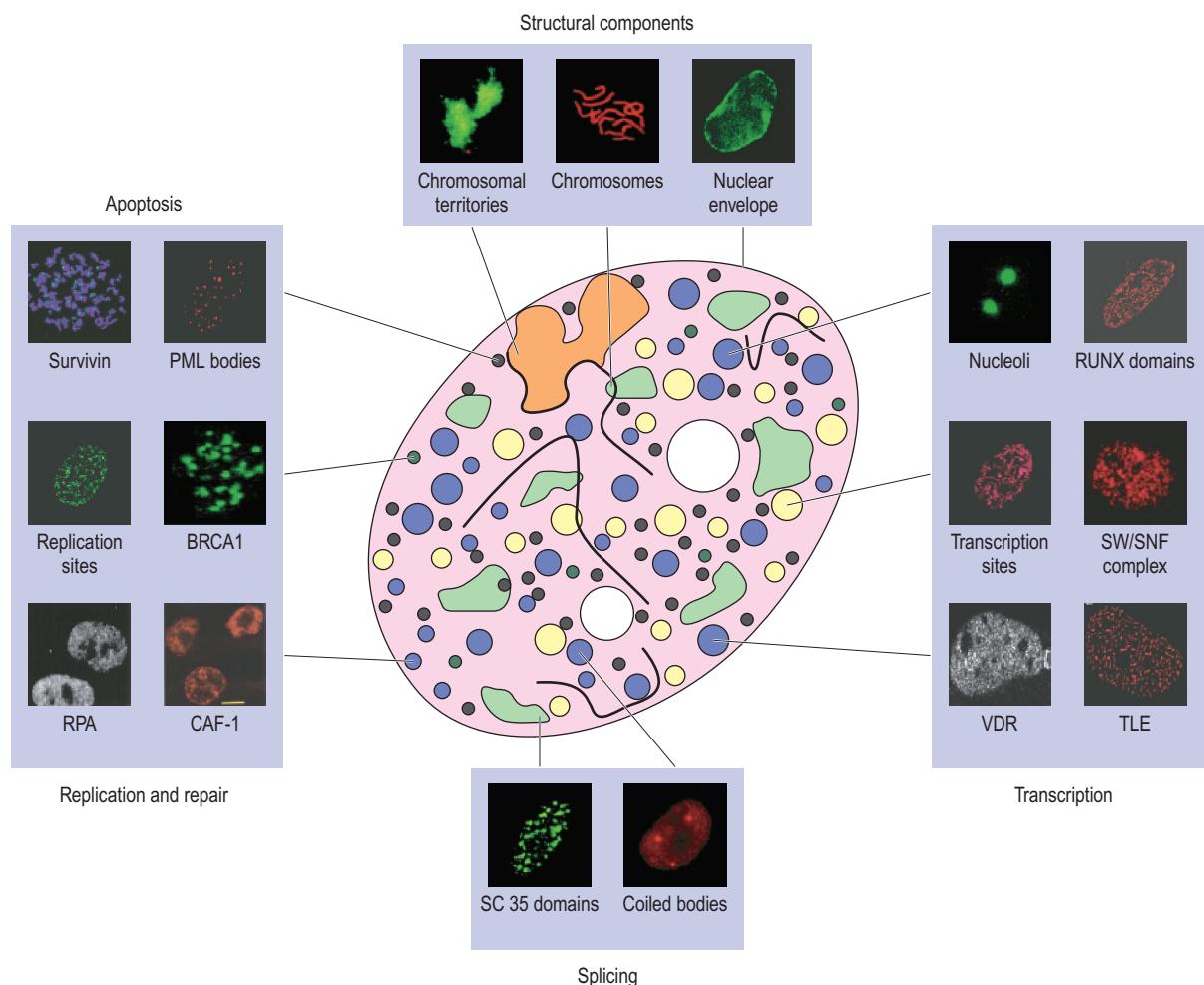
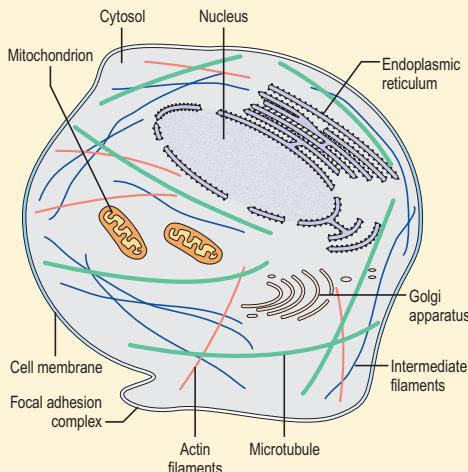


FIGURE 4-8 Subnuclear organisation of different nuclear structures. The nuclear architecture is functionally linked to the organization and sorting of regulatory information. Immunofluorescence microscopy of the nucleus *in situ* has revealed the distinct non-overlapping subnuclear distribution of vital nuclear processes. (From Stein et al., 2003, with permission from Elsevier.)

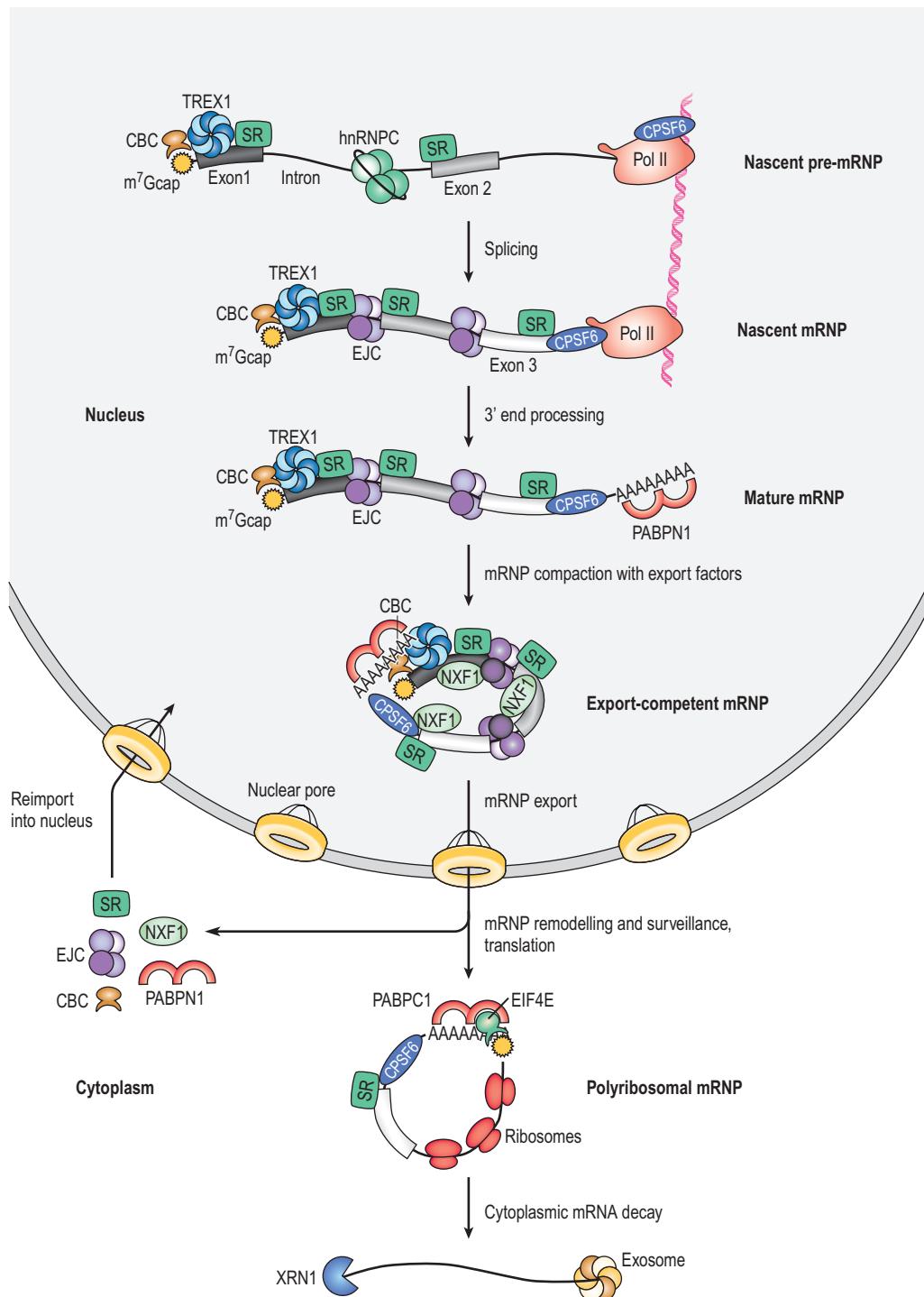


FIGURE 4-9 Summary diagram of how messenger ribonucleoprotein particles are assembled and remodelled. It is now realized that there is considerable diversity in how these molecules are generated. In the diagram one example is shown of an RNP cap binding complex (CBC) facilitating splicing of the 3'-end formation and export together with bound mRNAs through the nuclear pores into the cytoplasm. Finally, it is then bound to cytoplasmic proteins in ribosomes and becomes actively translated mRNA. (From Muller-McNicoll and Neugebauer, 2013.)

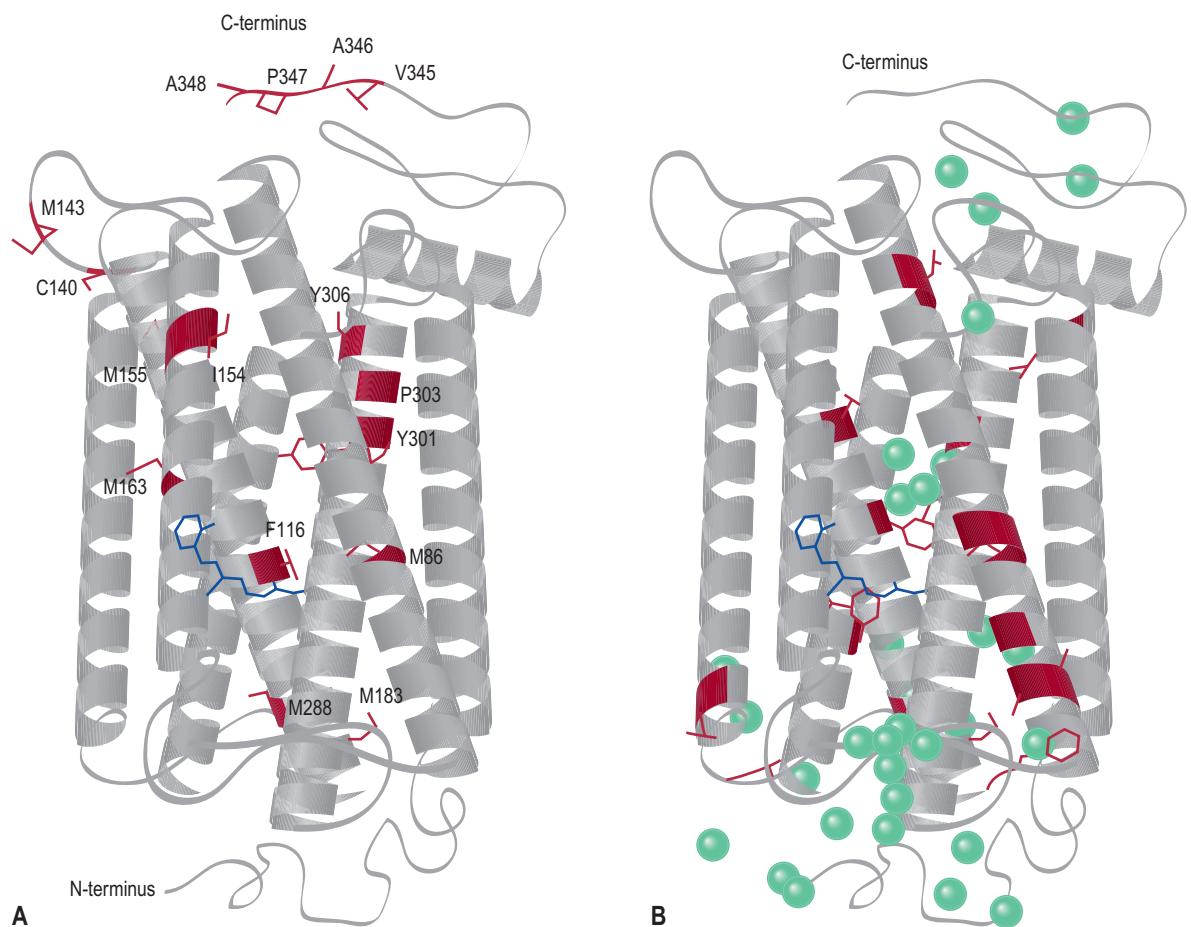


FIGURE 4-10 Ribbon diagram of the rhodopsin molecule as it flows in the photoreceptor disk membrane. Vitamin A bound to rhodopsin is shown in blue in (A). In (B), water molecules represented as green spheres, interact intimately with the binding of vitamin A to rhodopsin and are bound and released in concert with vitamin A as it is bound and released from the rhodopsin scaffold. It is believed that the water molecules modify, if not determine, how vitamin A interacts with rhodopsin. (From Orban et al., 2010)

structural integrity, where they insert into cell adhesion junctional complexes (see eBox 4-2).

Actin bundles are brought to a highly developed level in muscle cells. Actin occurs in several forms within the cell, depending on its associated protein, e.g. as a fine lattice meshwork or as a sheaf of fibres (stress fibres). Stress fibres are often the most prominent cytoskeletal features in a cell: they not only generate force but they respond to mechanical tension by reinforcement and in some respects are similar to sarcomeres of muscle cells.

The cellular distribution and, particularly, the degree of polymerization of microfilaments are determined

by the nature of proteins that bind to them, i.e. the microfilament-associated proteins (Table 4-1). Monomeric soluble actin (G-actin) is converted to gel-phase polymerized fibrils (F-actin) by association with certain proteins. In smooth muscle cells and fibroblasts, filamin assists polymerization; in contrast, in lymphocytes, profilin and thymosin maintain G-actin in the depolymerized state, presumably to facilitate flexibility in cell shape during rapid migration within tissues. The specificity of these actin-binding proteins is remarkable; examples include the ankyrin–spectrin–actin combination, which determines the red cell biconcave shape, and the spectrin–peripherin–actin

eBox 4-2**Filaments and junctions**

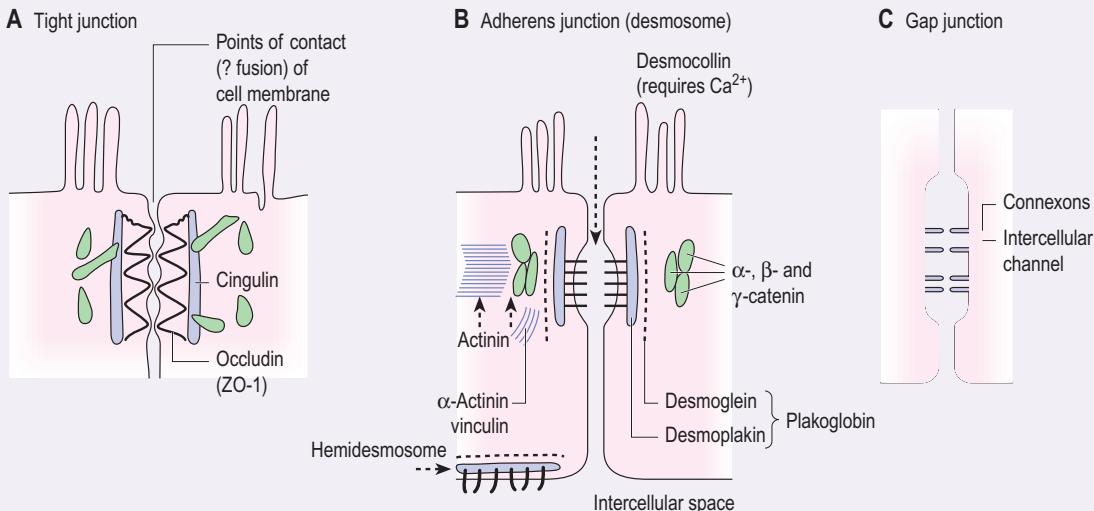
Intercellular connections or junctions are structures that make cells into tissues and tissues into organs. There are several types, each of which is composed of junction-specific proteins.

Tight junctions – have no detectable ‘space’ between the cell membranes. They are also known as zonulae occludens; they form a barrier to paracellular diffusion of all molecules, including water and ions. They are also involved in regulation of epithelial cell proliferation and differentiation. They occur at such sites as the blood–aqueous barrier of the ciliary body and the blood–retinal barrier at the apex

of the RPE cell. They consist of a system of ridges and grooves, as seen by freeze–fracture studies. There are four major classes of tight junction proteins:

- transmembrane proteins – occludin, claudin
- adaptors – ZO-1, cingulin, MUPPI
- transcriptional and post-transcriptional regulators – AP-1
- signalling proteins – α PKC, CDK4.

Claudins have unique abilities to selectively permit transport of charged ions and vary from tissue to tissue (A).



Desmosomes – form specialized ‘adherens’ junctions of 20 nm width between cells. Two types are described: spot desmosomes (at single site) and belt desmosomes (as a ring round the apex of the cell). The latter are also known as zonulae adherens. Their probable role is mechanical adhesion: cytoplasmic filaments insert into spot (cytokeratin) and belt (actin) forms (B). Several proteins are involved in different regions of the desmosome, including desmocollin, desmoglein and desmoplakin, forming a subfamily of cadherins. The cadherins bind to β -catenin and then to actin-based cytoskeletal proteins such as vinculin and α -actinin, ZO-1 and actin itself. The function of tight junctions (above) is dependent on the integrity of the adherens junctions. Hemidesmosomes have a similar plaque formed in this case by bullous pemphigoid antigen (BPAG1).

Gap junctions – so called because there is a 2 nm gap at this adhesion site between cells. Gap junctions occur in the basal regions of epithelial and other cells. They are a highly organized structure composed of ‘connexins’, plasma membrane domains containing connexins, which have a role in permitting the passage of larger ions such as Ca^{2+} , and other signalling molecules, so permitting a coordinated response by a group of cells, as in ocular or cardiac muscle (C).

Synapse – a specialized form of junction between nerves, or between nerves and muscles, and characterized by synaptic vesicles in the axon terminal and pre- and post-synaptic thickening of the plasma membrane (see Ch. 5).

TABLE 4-1 Function of actin-binding proteins

Function	Protein	Cell/structure
Gelation	Filamin	Smooth muscle/ fibroblasts
Bundling	α -actinin Fimbrin Talin	Muscle Microvilli All cells
Severing	Gelsolin Villin	Macrophages Microvilli
Depolymerizing	β -actinin Profilin Thymosin	Skeletal muscle Lymphocytes
	Actobinin	All cells
Membrane-binding	Vinculin Spectrin	Adhesion sites Red cells, photoreceptors
Receptor transport	Capping proteins	Leucocytes
Junctional complex	Radixin	Liver cells

combination between rod outer segment disks and plasma membrane. Actin polymerization and depolymerization is a highly regulated process requiring addition of actin monomers to one end of the microfilament and removal at the opposite end, each of which has separate K_{on} and K_{off} constants. This process is under the control of actin-depolymerization factor (cofilins) which alter these rate constants as necessary, leading to changes in the twist of the molecule and promoting severance of the actin filament.

Regulation of coordinated changes to the actin cytoskeleton is under the control of intracellular enzymes, particularly the Rho family of GTPases (this includes enzymes such as Rho, Rac and Cdc42, the last of which is a cell cycle-related protein). Certain kinases, known as the p-21 activated kinases (PAKs), are involved in regulating some of the diverse changes induced by Rac and Cdc42. PAKs may determine such cellular responses as polarity in epithelial cells and motility in fibroblasts. For example, coordinated functional regulation of the interaction between dynamin, cortactin, actin-binding protein 1, neuronal Wiskott–Aldrich syndrome protein 1 (N-Wasp-1), profilin and actin occurs during the pseudopodial extension and vesicle formation of migrating fibroblasts (Fig. 4-11). Central to this process is the ARP 2/3 complex which

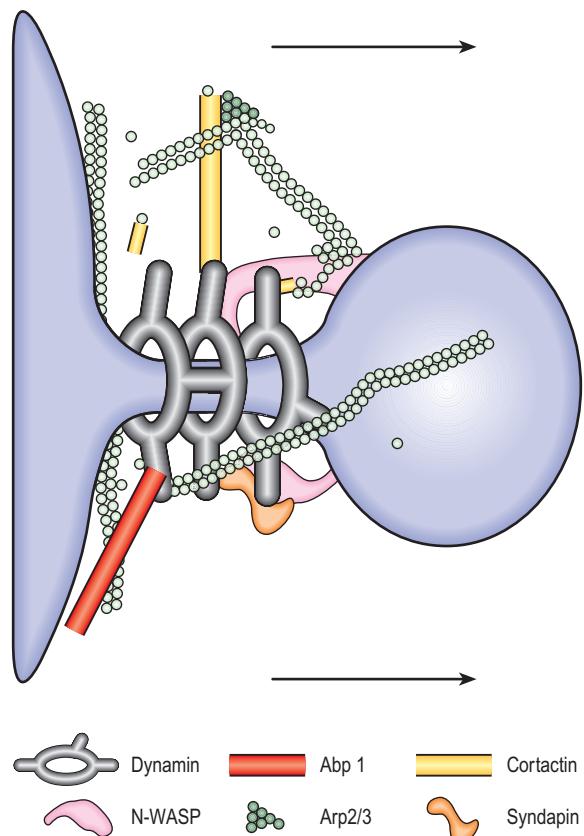


FIGURE 4-11 Dynamin-mediated tubulation and vesiculation of secretory and endocytic compartments. Model showing dynamin-mediated tubulation/vesiculation of a membrane as it might occur at the plasma membrane. Dynamin, its binding protein and associated proteins are likely to function together during vesicle formation. The molecular ‘pinchase’ activity of dynamin, together with the enhanced actin filament nucleation at the membrane interface, results in the tubulation and severing of the membranous vesicle necks (see text for details). Large black arrows indicate the generation of force and the movement of nascent vesicles. (From Orth and McNiven, 2003, with permission from Elsevier.)

is required for actin nucleation. For instance the Arp 2/3 complex is also involved in a relatively recently described family of actin-binding proteins, the coronins, which are involved in leucocyte migration.

Several isoforms and at least three families of actin exist (α , β , γ) and some of these are located in specific parts of the cell, e.g. β -actin in the cell cortex.

Intermediate filaments. Intermediate filaments (10–12 nm) are coiled α -helices which act as stretchable

components of the cytoskeleton scaffold. They occur in cell-, tissue- and differentiation-specific distribution in both cytoplasmic and nuclear compartments of the cell, and are classified into five groups depending on domain and sequence homology. Their major function is to protect cells from mechanical and non-mechanical damage (Fig. 4-12), and gene mutations account for around 30 different diseases in man, mostly related to skin, muscle and nerve dysfunction. In general, intermediate filaments are important for the correct positioning and function of cell organelles such as mitochondria and ER.

There are five classes of cytoplasmic intermediate filaments, often used to characterize cells in tissue culture or tumours, and one class of nuclear intermediate filaments:

- *keratins* – found in epithelial cells; over 50 individual members grouped into two types, I and II, exist as heterodimers
- *vimentin* – found in mesenchymal cells
- *desmin* – interconnects myofibrils of muscle cells, at site of Z disk and M line, thus maintaining their register
- *glial fibrillary acidic protein (glia)*
- *neurofilaments* – e.g. S100 protein in neuroectoderm; connect with microtubules via small projections
- *lamins* – line the inner surface of the nuclear envelope as a fibrous lattice (karyoskeleton); more than five types.

One function of intermediate filaments is to ensure normal cytoplasmic positioning and function of different organelles. Lamins undergo considerable molecular disruption during mitosis and may communicate with cytoplasmic intermediate filaments, but how this occurs is not clear. Others, such as keratins, provide mechanical strength to junctional structures such as desmosomes (see eBox 4-2).

 Cellular junctions are highly specialized: desmosomes and hemidesmosomes ('junctions' between the cell and basement membrane in epithelia) have some ultrastructural similarity, but at the molecular level there are clear differences between the constituent proteins. Desmosomes are formed by a series of proteins spanning the cell membrane and the intercellular space (desmoglobin, desmoplakin), while hemidesmosomes contain other proteins such as the $\alpha_6\beta_4$ integrin

receptor, which binds to laminin in the basement membrane, plus other proteins such as the bullous pemphigoid and the pemphigus antigens.

They may also have a function in the positioning of the nucleus in the cell in a cage-like bundle of fibrils. Intermediate filaments maintain cell homeostasis by dealing with stress in its various guises (Fig. 4-13), and the cell responds in a number of ways such as generating 'inclusion bodies', producing new and regenerating intermediate filaments, or by reorganizing the filaments into variously sized bundles.

Interactions between intermediate filaments and microfilaments are mediated by plectin, a >500 kDa dumb-bell-shaped protein that can self-associate and in addition can bind at both ends of the hemidesmosomal protein $\alpha_6\beta_4$ integrin and probably to other junctional proteins.

Group 3 cytoskeletal fibres. A third group of cytoskeletal fibrillar elements also exists but the fibres are less easy to categorize. Several thick filaments occur as part of the cytoskeleton, such as myosin (myosin comprises 25% of cytoplasmic protein in striated muscle) and microtubules. Microtubules are cylindrical structures about 24 nm wide, comprising 13 globular elements composed of the heterodimer ($\alpha\beta$) tubulin. Stable microtubules occur in flagellae and cilia, while labile microtubules are found in structures such as the muscle spindle. Microtubules are involved in movement and cell motility, including intracellular transport as in axoplasmic flow. They are often required to switch very rapidly from an extending to a shrinking fibril, as in processes such as cell division where a multiprotein structure termed the kinetochore engineers chromosome segregation by facilitating chromosome attachment to spindle microtubules. Rapid growth to shrinkage in the microtubule is achieved through GTP to GDP hydrolysis, mediated through the Rho family of small GTPases; this is known as 'catastrophe', while the reverse change is termed 'rescue' (Fig. 4-14).

These changes occur in any microtubule-related function, including cell movement, and drugs such as colchicine (used in Behçet's disease) and taxol (proposed as prophylaxis for proliferative vitreoretinopathy) disrupt microtubule organization and inhibit cell motility and cytokinesis.

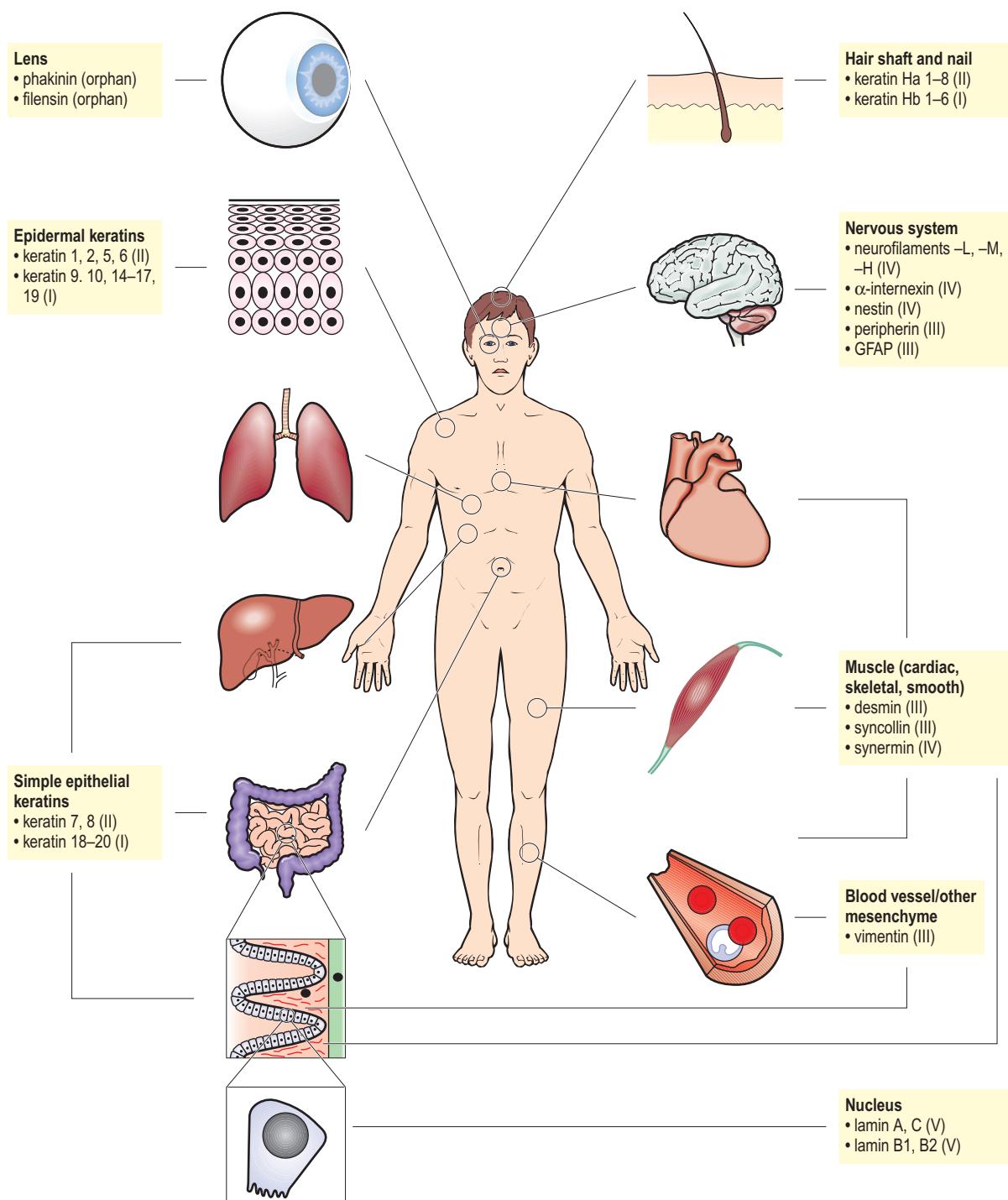


FIGURE 4-12 Distribution of intermediate filament (IF) proteins in the human body. IF proteins include five major types (I–V) and a separate 'orphan' category (IF type and category are listed in parentheses). Lamins (type V) are found in the nucleus of most mammalian cells, whereas the remaining IFs (types I–IV) are cytoplasmic and expressed in a cell-tissue-selective manner. For each tissue, representative major IFs are listed for the principal cell type. (From Toivola et al., 2005, with permission from Elsevier.)

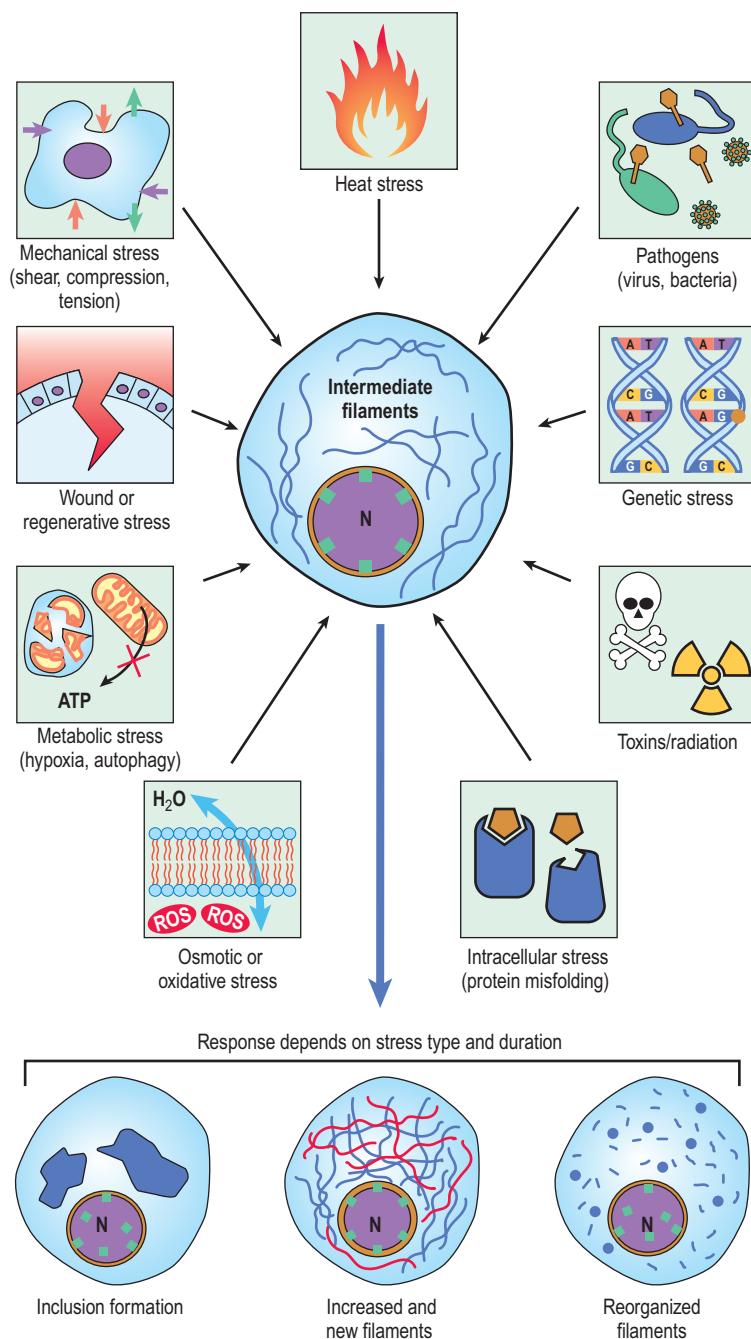


FIGURE 4-13 Cells respond to stress through their intermediate filaments by (a) forming inclusion bodies, (b) making new filaments or (c) reorganizing their filaments. (From Toivola et al., 2010.)

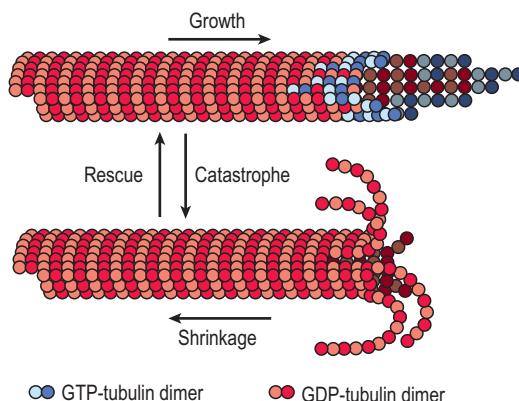


FIGURE 4-14 Microtubules switch between phases of growth and shrinkage generated by GDP-GTP-mediated incorporation and removal of tubulin dimers in the region of the tubulin cap. (From Bowne-Anderson et al., 2013.)

Group 3 fibres are thus a class of superfine filaments which combine to form an intracellular mesh-work in which proteins do not exist in solution as previously surmised, but are attached to and transported along the filaments with other structures such as 'free' ribosomes and small vesicles (polysomes). This arrangement has special relevance to highly organized cells such as lens fibres. Transport of molecules along microtubules involves two families of molecular motors, kinesins and dyneins.

Certain discrete cellular structures are composed of microtubules, such as the centriole (the microtubular organizing centre, a cylindrical structure comprising nine groups of triplet microtubules) and the mitotic spindle. Cilia are remarkably constant structures in eukaryotic cells, composed of nine peripheral and two central bundles of three fused microtubules. Movement occurs by sliding of the outer arm of dynein along the core of tubulin.

Septins. Septins are a family of proteins expressed in all cell types which are integrators of functions of other microfilaments such as the ATPase role of actin and the dynamic functions of microtubules. There are 13 septins in humans, mutations of which are known to cause specific diseases. Septins are important in cells with high migratory functions, such as lymphocytes. In addition, septin 8, which lies on chromosome 5q31, is associated with retinal degeneration.

Their main function in the cell appears to be supportive, e.g. as a scaffold for protein recruitment and as an intracellular compartment organizer, and they are called septins for this reason. For example, several septins in one cell combine to form a hexameric filament of around 25 nm long which acts as an intracellular 'septa' (barrier to diffusion) (Fig. 4-15).

In conclusion, therefore, there are extensive interconnections between the four filamentous systems orchestrating many fundamental types of cell behaviour. In addition, the cytoplasm also contains several storage products such as glycogen granules, lipid droplets and melanin in melanosomes, often inside endosomes, which, as indicated above, are transported around the cell by MT-associated proteins and made to function by active fibre contraction as in actin-mediated exocytosis (see Box 4-2). With age, some cells, such as the RPE cell, accumulate unwanted intracellular bodies such as lipofuscin granules.

Intracellular signalling mechanisms

As stated above, cells respond to external stimuli by means of cell surface receptors, which convert the external stimulus to a series of intracellular signals (second messengers) directed towards specific cellular functions such as protein transcription for growth control or ion-channel gating in neural responses (see Ch. 5, pp. 288–295).

Second messenger systems are based on a network of reactions involving an agonist, a receptor and an interacting set of coupled proteins. Cyclic adenosine monophosphate (cAMP) is the archetypal second messenger, and the result of such a response is the phosphorylation of a regulatory intracellular protein via a kinase, as for instance serine-threonine kinase or tyrosine protein kinases, which demonstrate great substrate specificity. In contrast the phosphatases, such as protein phosphatase-1 (PP1), which usually bring the kinase reaction to a close, are much more widespread in their range of activity and, consequently have many fewer genes expressed.

During the last 30 years, a family of ligand receptors known as WNTs has been shown to have widespread roles in embryonic development, cancer and wound healing. These molecules define the interaction between matrix and cells via intra- and inter-cell signalling and are essential for developmental processes

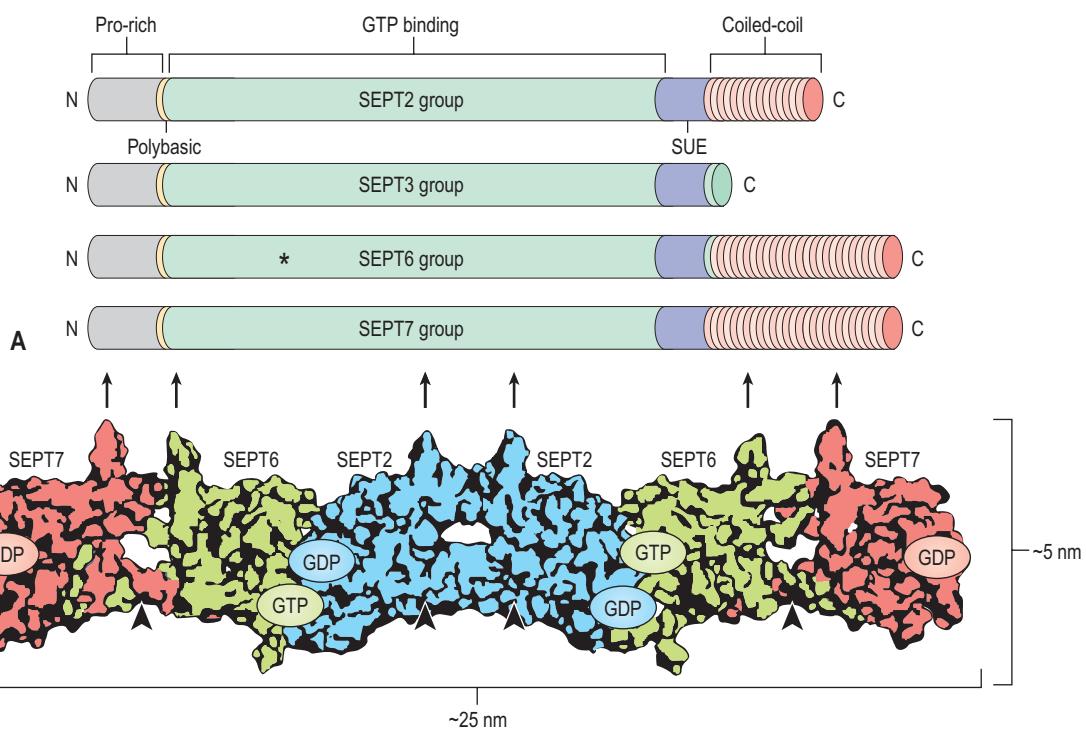


FIGURE 4-15 There are 13 human septins classified into four groups (SEPTS 2,3, 6 and 7). They have three conserved domains: a phosphoinositide-binding region; a GTP-binding region; and the septin unique element (SUE). (From Mostowy and Cossart, 2012.)

such as patterning. The name WNT derives from the discovery that the *int1* (integration gene) and the *(Wg)* Wingless gene in *Drosophila* were identical and there are now known to be several Wnt genes with multiple effects on extracellular matrix proteins.

Additional content available at <https://expertconsult.inkling.com/>.

THE EXTRACELLULAR MATRIX

Cells exist within a structural framework, the extracellular matrix (Box 4-6), which is secreted by the cells such as the myofibroblast during wound healing and consists of several classes of macromolecules, the most abundant of which is collagen (accounting for 30% of total protein in the organism) (Fig. 4-16 and Video 4-1).

Genetic and protein sequence analysis of extracellular proteins has shown that, despite the great variety of matrix molecules, extensive sequence homology exists between them with recurring structural motifs (Box 4-6). For instance, the 'epidermal growth factor'

motif appears to be present in several apparently different extracellular matrix proteins.

Extracellular matrix proteins determine the structural nature of the tissue

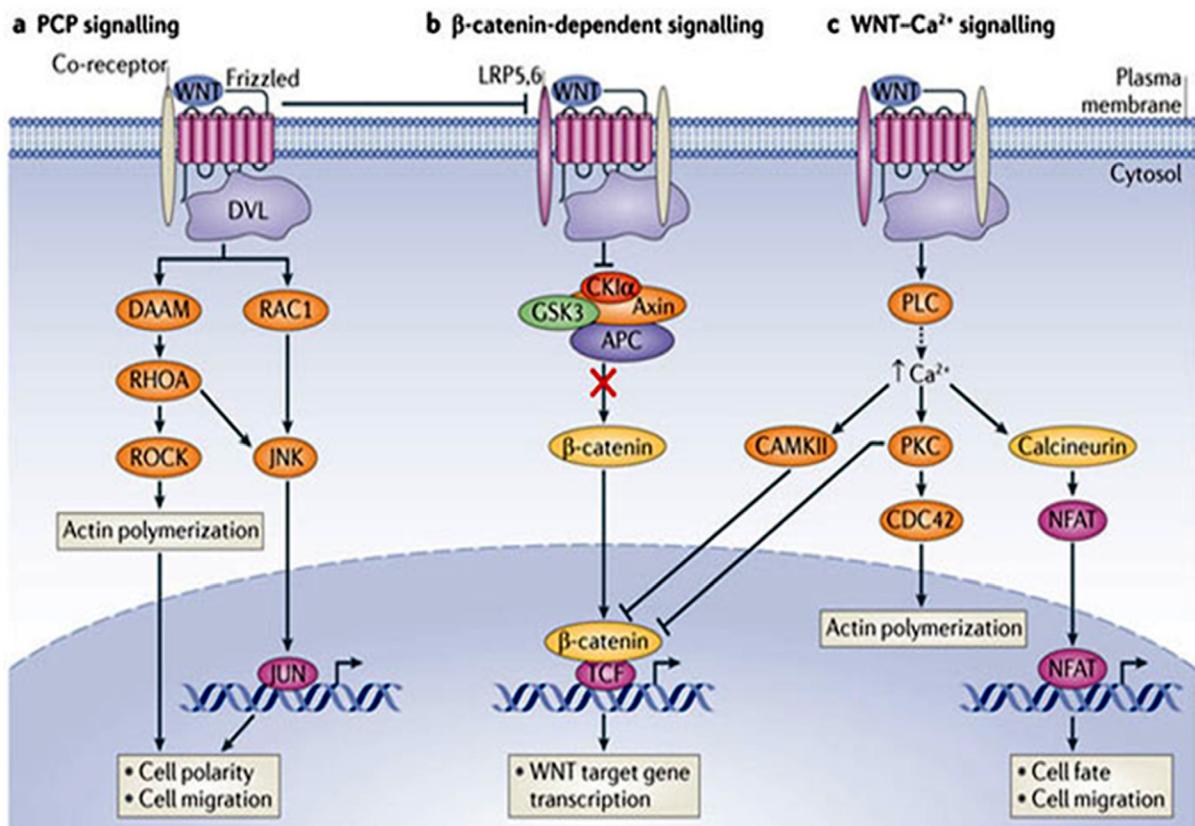
Tissues are defined by their extracellular matrix constituents. For instance, the cornea contains type I collagen filaments, which do not form fibrils of diameter greater than 5 nm; this is important in corneal transparency. The vitreous contains an isoform of type IX collagen, which is different in certain characteristics from cartilage type IX collagen.

Collagen fibres are composed of a triple helix of three polypeptide chains, of which there are 46 different types and which assemble into 29 distinct collagen molecules (Table 4-2).

At least 22 of these have been detected in the developing and adult eye. The eye and cartilaginous tissues share six types of collagen not found very frequently in other tissues; indeed, the collagen triple helix (Fig. 4-17 and Box 4-6) has been found as a domain of

WNT signalling pathways are central to many developmental and cell biological functions (eFig. 4-5), including planar cell polarity (PCP) mediated by Wnt /Frizzled interactions; β -catenin-dependent signalling which is extensively involved in cell–cell interaction and cell adhesion and includes interaction of Wnt with the low-density

lipoprotein receptor (LRP)5,6; and Wnt-calcium (Wnt-Ca^{2+}) signalling which is important calcineurin /NFAT signalling and is involved in cell fate and cell migration. This latter pathway is activated in dendritic cells but not macrophages and induces apoptosis in DC (see Ch. 7, p. 373–380).



eFIGURE 4-5 Wnt signalling involves the co-receptor Frizzled to promote actin polymerization and cell polarity (planar cell polarity, PCP), the low-density lipoprotein receptor (LRP) 5,6 to mediate β -catenin signalling to the nucleus, and Wnt- Ca^{2+} signalling, which activates the calcineurin-NFAT pathway determining cell fate. (From Niehrs, 2012.)

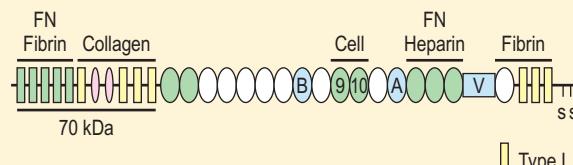
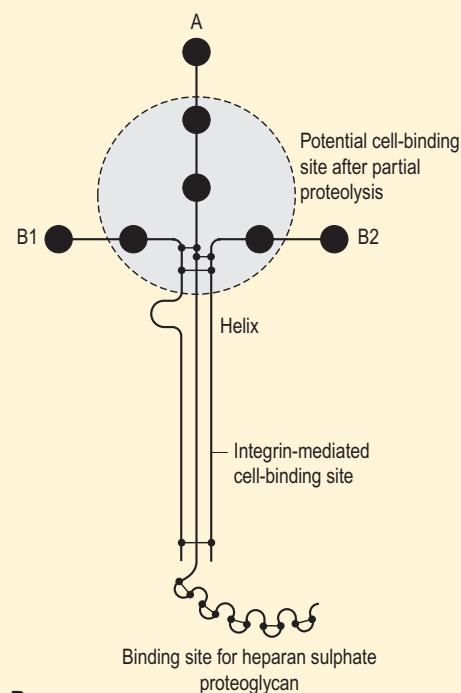
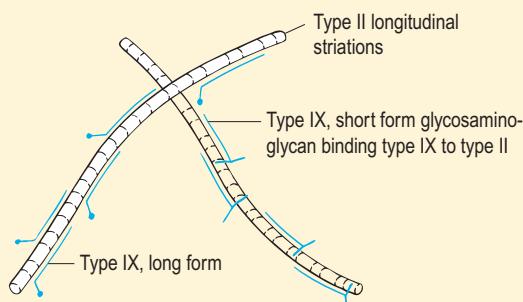
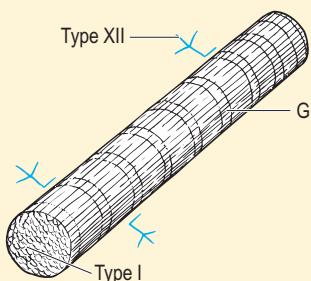
BOX 4-6 EXTRACELLULAR MATRIX PROTEINS

Extracellular matrix proteins appear to be made up of building blocks ('structural motifs'), which are protein domains with extensive homology to existing protein structures. For instance, three fibronectin domains are reproduced in many other proteins; the epidermal growth factor-like domain also appears in modular form in proteins such as plasminogen; and the arginine–glycine–aspartate (RGD) cell adhesion site is present in many molecules (**A**).

Fibronectin is a dimer containing discrete domains for the attachment of other molecules (**A**). The modular structure of fibronectin shows that it consists of 12 type I modules (rectangles), two type II modules (violet ovals) and 15–17

type III modules (ovals). The alternatively spliced domains IIIB, IIIA and the V region are shown in yellow. Binding domains for fibrin, collagen, cells and heparin are indicated; dimer forms via cysteine pair at the C-terminus (SS).

Laminin is a cross-shaped trimer in which the B1, B2 and A chains form a triple helix in the stem of the cross. Laminin also has several discrete domains for attachment of various molecules. In addition, it contains a cryptic cell adhesion-binding site, which becomes exposed after partial proteolysis (**B**). Fibrillar collagen is organized in tissues by association with smaller non-fibrillar collagens, e.g. type I with type XII collagen, and type II with type IX collagen (**C**).

**A****B****C**

(Part A reproduced from Mao and Schwarzauer, 2005, with permission from Elsevier; Parts B and C courtesy of Yamada and Miyamoto, 1995.)

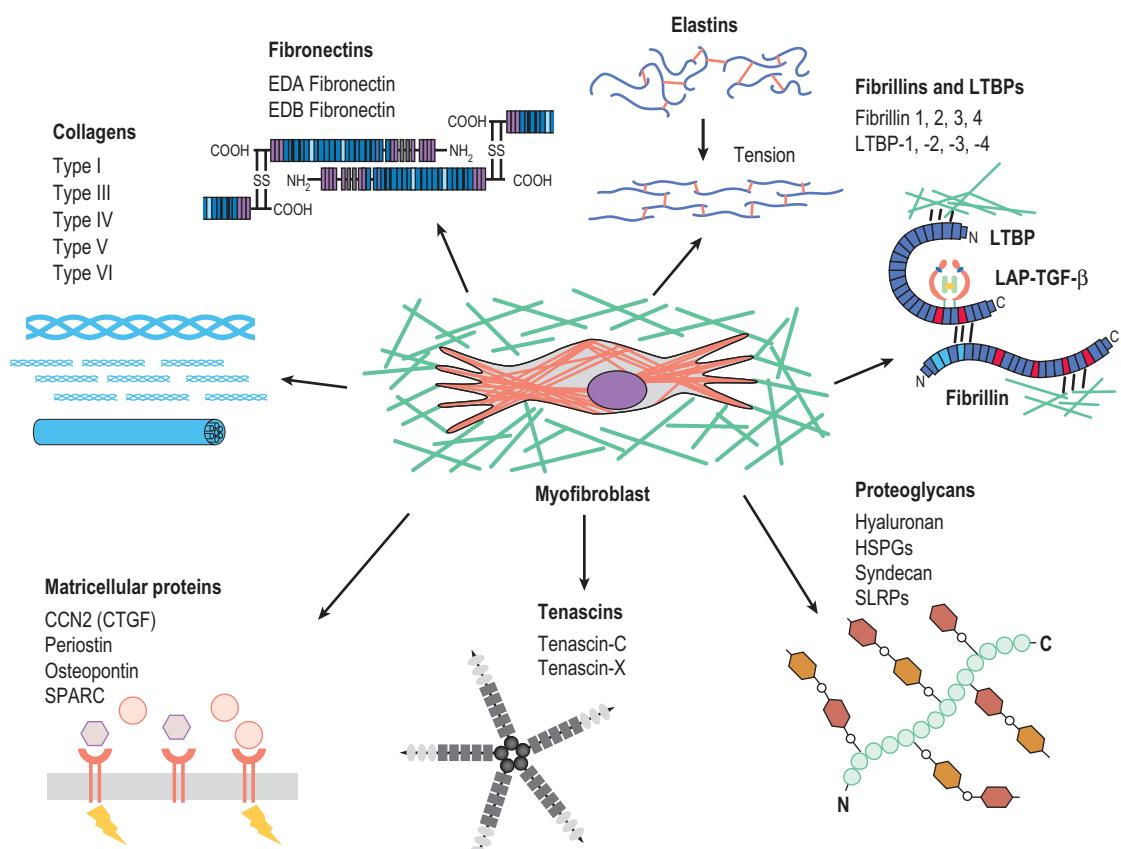


FIGURE 4-16 The myofibroblast matrix. Schematic of some of the ECM molecules relevant to tissue fibrosis. The myofibroblast (centre, with red stress fibres containing α -smooth muscle actin) lies enmeshed in its ECM (green). Components of the ECM are depicted (clockwise, from the 12 o'clock position): elastins, fibrillins and LTBPs, proteoglycans, tenascins, matricellular proteins, collagens and fibronectins. The myofibroblast encounters, signals, and modulates the expression of these various components as outlined in the text. (From Klingberg et al., 2013.)

TABLE 4-2 Some collagen types

Fibrillar	Anchoring	FACIT**	Transmembrane	Beaded filament	Network-forming	Multiplexin
I, II, III, V XI, XXIV XXVII	VII	IX, XII XIV, XVI XIX, XX XXI, XXII	XIII XVII XXIII XXV	VI, XXVI XXVIII	IV, VIII, X	XV, XXVI XXVIII

**FACIT, Fibril-associated collagen with interrupted triple helices.

many proteins and it has been suggested that all such proteins should be included in the collagen family. Collagen types are determined by the combination of the three types of chain forming the α helix core. For instance, type I collagen triple helix is made up of two unique α_1 chains and a unique α_2 chain, coded as

$[\alpha_1(I)]_2 \alpha_2(I)$; type II collagen is composed of three identical unique α_1 chains, $[\alpha_1(II)]_3$. Each collagen therefore has a set of unique chains that make up the triple helix.

In certain collagens, particularly the non-fibrillar collagens such as types IV and IX, the protein is

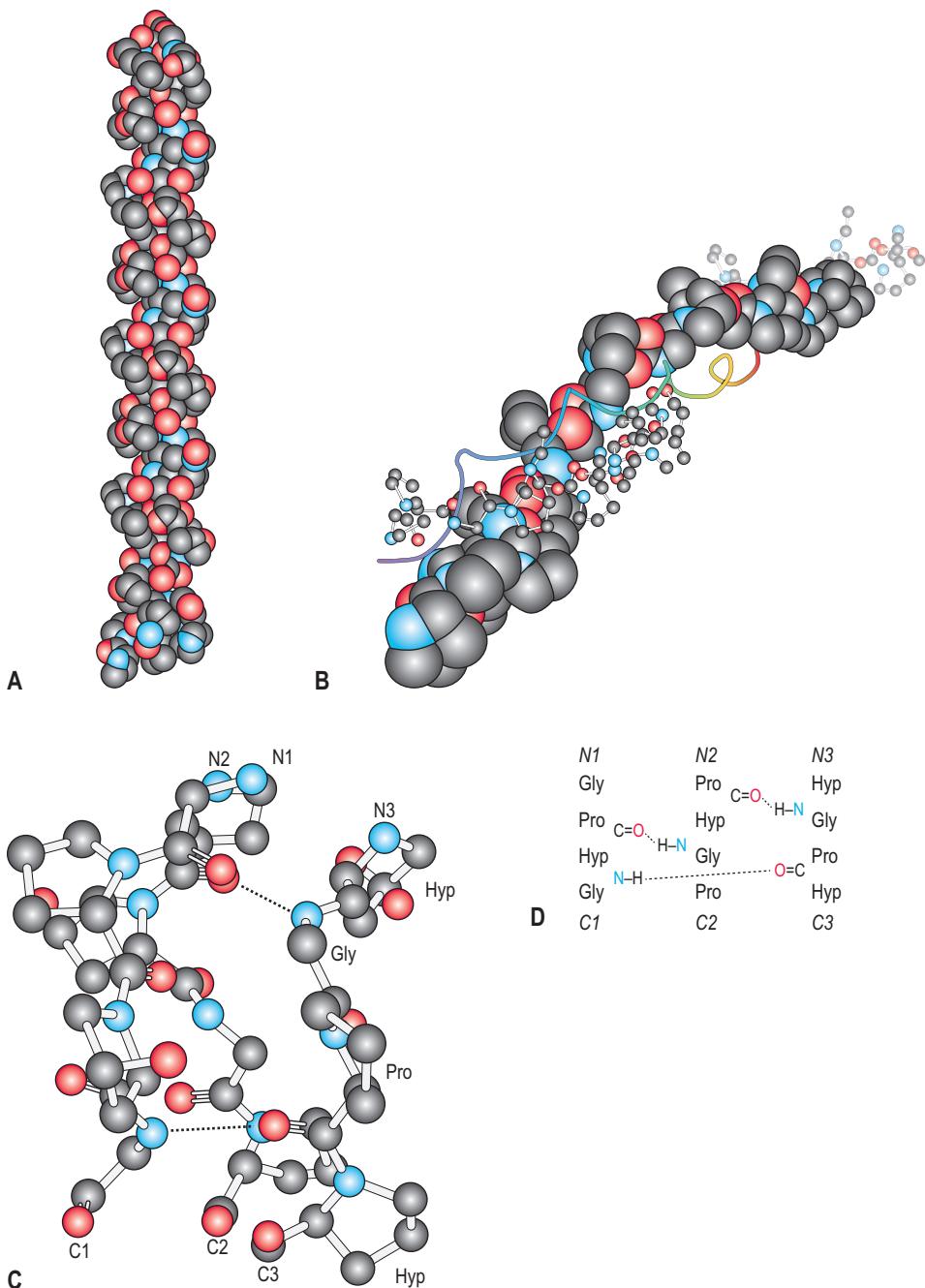


FIGURE 4-17 Overview of the collagen triple helix. **(A)** First high-resolution crystal structure of a collagen triple helix, formed from (Pro-HypGly)4-(ProHypAla)-(ProHypGly)5 [Protein Data Bank (PDB) entry 1cag (19)]. **(B)** View down the axis of a (ProProGly)10 triple helix [PDB entry 1k6f (7)] with the three strands depicted in space-filling, ball-and-stick, and ribbon representation. **(C)** Ball-and-stick image of a segment of collagen triple helix [PDB entry 1cag (19)], highlighting the ladder of interstrand hydrogen bonds. **(D)** Stagger of the three strands in the segment in panel **C**. (From Shoulders and Raines, 2009.)

composed of short segments of triple helix (COL1, -2, -3, etc.) interspersed with sections of non-collagenous (NC1, NC2, etc.) protein. These are also sometimes referred to as FACIT collagens (fibril-associated collagens with interrupted triple helices) (Table 4-2). These proteins usually act as bridges or networks for binding other proteins and forming complex protein aggregates such as basement membranes. Collagens in essence are the structural components that hold organs and tissues together and the mechanism by which they form is interesting: the three polypeptide chains form a monomer by self-assembly from a small nucleus in a zipper-like fashion, much in the way that crystallization occurs, and the formation of fibrils from monomers is also entropy driven. Indeed the semi-crystalline packing of collagen fibres, achieved through lysine hydroxylysine cross-links, confers tensile strength to tissues.

A subcategory of collagenous proteins is included as components of transmembrane proteins, including types XIII, XVII, XXIII and XXV (Table 4-2), and remain following cleavage of the soluble fragment of the cytokine or adhesion molecule by enzymes known as sheddases. They exist as homotrimers of a collagen-specific α -chain. Some transmembrane collagen-like molecules occur as specific receptors such as the macrophage scavenger receptors. Sheddases are of several types and include the ADAMTs (proteinases of the adisintegrin and metalloproteinase family), which are involved for instance in the shedding of molecules such as soluble tumour necrosis factor- α -converting enzyme (TACE) from leucocytes (see Ch. 7). This general process is important in establishing the soluble regulatory constituents of particular extracellular matrices.

The transmembrane collagen XVII (originally described as bullous pemphigoid antigen 180, BP180) is important in cellular adhesion because it binds α_6 integrin and laminin 5 extracellularly and β_4 integrin, plectin and BP230 intracellularly. Degradation products of collagens, such as the non-fibrillar collagens XVIII and XV, which yield the anti-angiogenic products endostatin and restin after cleavage, have major roles in regulating cell function through inhibition of matrix metalloproteinases.

The eye contains a wide variety of different collagens. For instance, the cornea contains types I, V and VI in the stroma, while types IV and VII are present

in the subepithelial layer. In addition, type XVI transmembrane linker collagen has been found in the basal epithelial cell matrix. The iris contains collagen types I, III and IV, while the zonule contains type IV. The lens contains only type IV, while the vitreous contains collagens II, IX and XI, complexed to the extracellular matrix protein fibrillin. This complex has an important role in vitreous matrix organization.

Certain types of collagen are unique to ocular tissue in that their structure has been modified. Examples include type VIII in Deszemet's membrane, types II and IX in vitreous (both similar but not identical to cartilage collagens) and type III in the distensible tissue of the choroid. Several of the newer collagens contain domains similar to type IX, and it has been suggested that these may represent a subfamily of type IX collagens (Table 4-2). The more recently described types XV, XVIII and XIX are located in basement membranes and may have a role in the formation of blood vessels.

Collagen does not simply fill the space between cells but engages four types of specific receptors on cells: integrins, discoidin domain receptors (DDRs 1 and 2, a subfamily of receptor tyrosine kinases involved in signalling to the cell), glycoprotein VI (GPVI) receptor (on platelets) and leucocyte-associated immunoglobulin-like receptor-1 (LAIR-1). DDs and integrins are exquisitely specific in the collagen domain to which they bind and their main function is to maintain tissue stability during intra-tissue cell migration, while GPVI and LAIR-1 are involved in haemostasis after tissue injury.

Elastin is the second major insoluble protein of the extracellular matrix. While collagen occurs in all tissues, elastin is present only in deformable tissues such as blood vessel walls, lung parenchyma and the zonule of the lens. Unlike collagen, elastin does not contain any methionine residues and is therefore separable from collagen after digestion with cyanogen bromide. Elastin is formed from soluble tropoelastin monomers, secreted from fibroblasts and smooth muscle cells, which initially self-associate through hydrophobic domains in a process termed co-acervation *in vitro* which *in vivo* corresponds to coalescence into spherical globules of 1–6 μm size. This process of self association aligns lysine residues to form cross-links, via allysine intermediates, desmosine and iso-desmosine, a process which is facilitated by the elastin

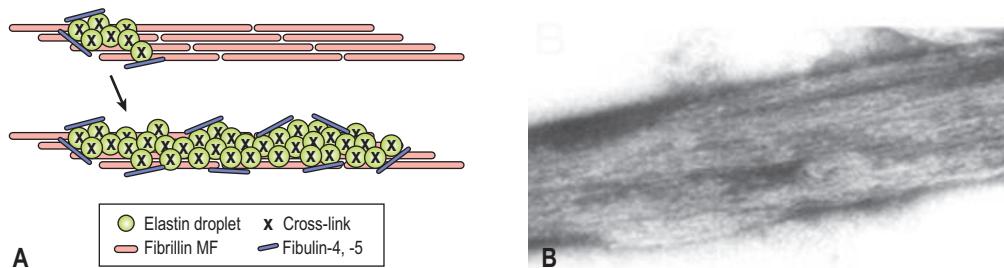


FIGURE 4-18 Elastin ‘droplets’ are deposited on fibrillin microfibrils and cross-linked to the fibrillin molecule via lysyl oxidase in association with fibulin molecules. (From Muiznieks and Keeley, 2012.)

co-acervated droplets depositing onto fibrillin microfibrils together with fibulin (see below) (Fig. 4-18). The final fibre is 90% elastin and 10% fibrillin and cross-links are mediated via a set of lysyl oxidase enzymes, mutations in which have been associated with the pseudo-exfoliation syndrome, the commonest cause of secondary glaucoma and a risk factor for successful cataract surgery due to the role of elastin in formation of the zonule. Elastin has little order to its tertiary structure; instead, it forms a random coil, which tends to become more ordered during deformation.

As for collagen, elastin degradation products have a role in regulating cell behaviour in the matrix and under certain circumstances may be pathogenic, e.g. in promoting tumour invasion and angiogenesis. Even smaller molecules derived from a range of matrix proteins (matrikines) have regulatory activity for connective tissue cells; for instance, the tripeptide GHK, which has stimulatory activity for several cell types and promotes collagen biosynthesis and wound healing overall.

Fibrillins are a family of proteins serving as components of extracellular elastin microfibrils that are responsible for the biomechanical properties of tissues. Cysteine-rich glycoproteins, composed of multiple repeats of a Ca^{2+} -binding epidermal growth factor-like domain, are secreted in a proform and polymerize extracellularly. They are present in vitreous (see above) and are an important component of the zonule. Fibrillins have a structural role in long-range elasticity of tissues and are a component of elastic fibres (see above). They also have a role in the fine-tuning of growth factor signals such as those involving transforming growth factor- β (TGF- β) and particularly latent TGF- β -binding proteins (LTBP) involved in

morphogenesis. Mutations in *FN1* cause a dominant form of Marfan syndrome.

Tenascins are extracellular matrix proteins best described as adhesion modulating, matricellular proteins which do not form major fibrillary structures such as collagen or elastin. They are module-built proteins comprising EGF-like repeats, fibronectin-type III repeats and fibrinogen domains (Fig. 4-19). Several forms are described (tenascin-C, -X, -W, etc.) and phenotypes in knockout mice are broadly normal although there may be some subtle defects in wound healing. Tenascin-Y appears to be restricted to neural tissue, while mutations in tenascin-X have been linked to Ehlers–Danlos syndrome, in conjunction with known defects in pro-collagen and elastin biosynthesis. Tenascins are widely distributed and appear to have an anti-adhesive role, particularly antagonizing the effects of fibronectin. Interestingly, fibronectin is found in the anterior lens capsule, while tenascin is found in the posterior capsule.

Laminins are an integral part of basement membranes and have a characteristic hetero trimeric cross-shaped structure composed of an α chain, a β and a γ chain constructed to shape and an α coiled rod (see Box 4.6 and eFig. 4-6).

There are 16 isoforms variously made up for one α ($\alpha_1\text{--}\alpha_5$), 3β ($\beta_1\text{--}\beta_3$) and 3γ ($\gamma_1\text{--}\gamma_3$) chains that are specific for different cell types (e.g. kalinin in epithelial cells) and are named according to their chain components. For instance laminin-332 (i.e. α_3 , β_3 and γ_2 chains) is specific for epithelial cells and binds to the $\alpha_3\beta_1$ and $\alpha_6\beta_4$ integrins.

Additional content available at <https://expertconsult.inkling.com/>.

The infrastructure of the basement membranes is composed of type IV collagen in a highly organized

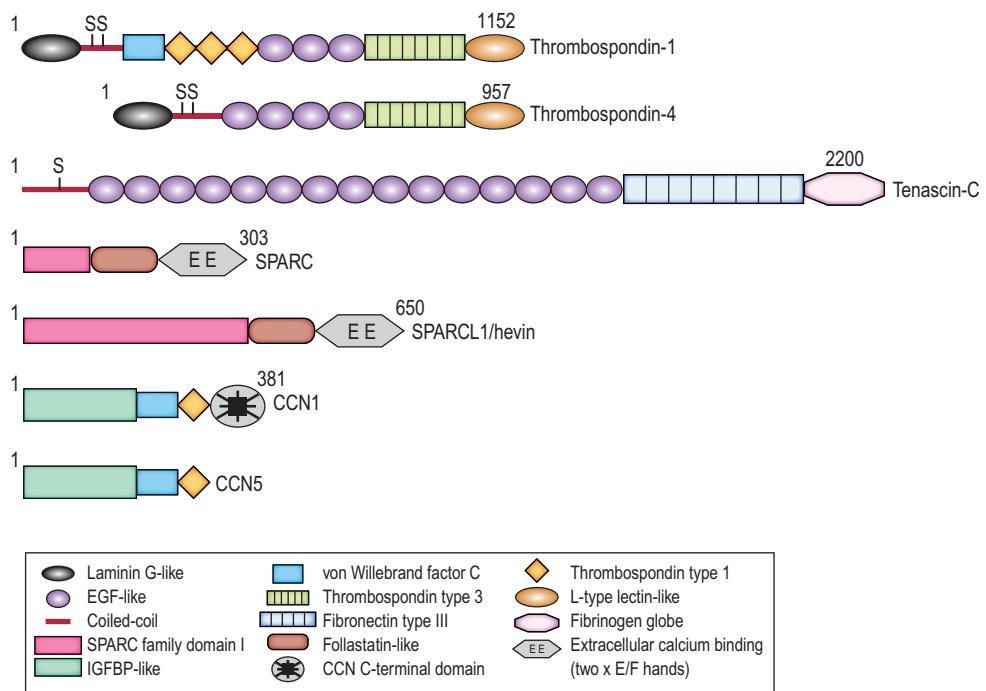


FIGURE 4-19 Schematic diagrams of the domain organization of representative members of the protein families discussed in this chapter. Not to scale. (From Mosher and Adams, 2012.)

lattice network to which the complex of laminin–nidogen is also bound. The proteoglycan then acts as a space-filling molecule in the basement membrane (Fig. 4-20). Cells are bound to the basement membrane via anchoring fibrils containing type VII collagen and transmembrane proteins such as types XI and XVIII collagen.

Most laminins are produced by the cells adhering to the basement membrane in which they are found; interestingly, the laminins of the internal limiting membrane (ILM) of the retina are synthesized by ciliary body and lens cells. ILM laminin and fibronectin are substrates for ocriplasmin, a therapeutic enzyme with potential for use in vitreoretinal disease.

Non-structural proteins

Many other proteins are distributed throughout the extracellular matrix, which, although not having a direct structural role, have important functions in cell–matrix and cell–cell interactions. Many of these proteins are engaged in self-organization with other ECM proteins (see p. 178) to form fibrillar networks.

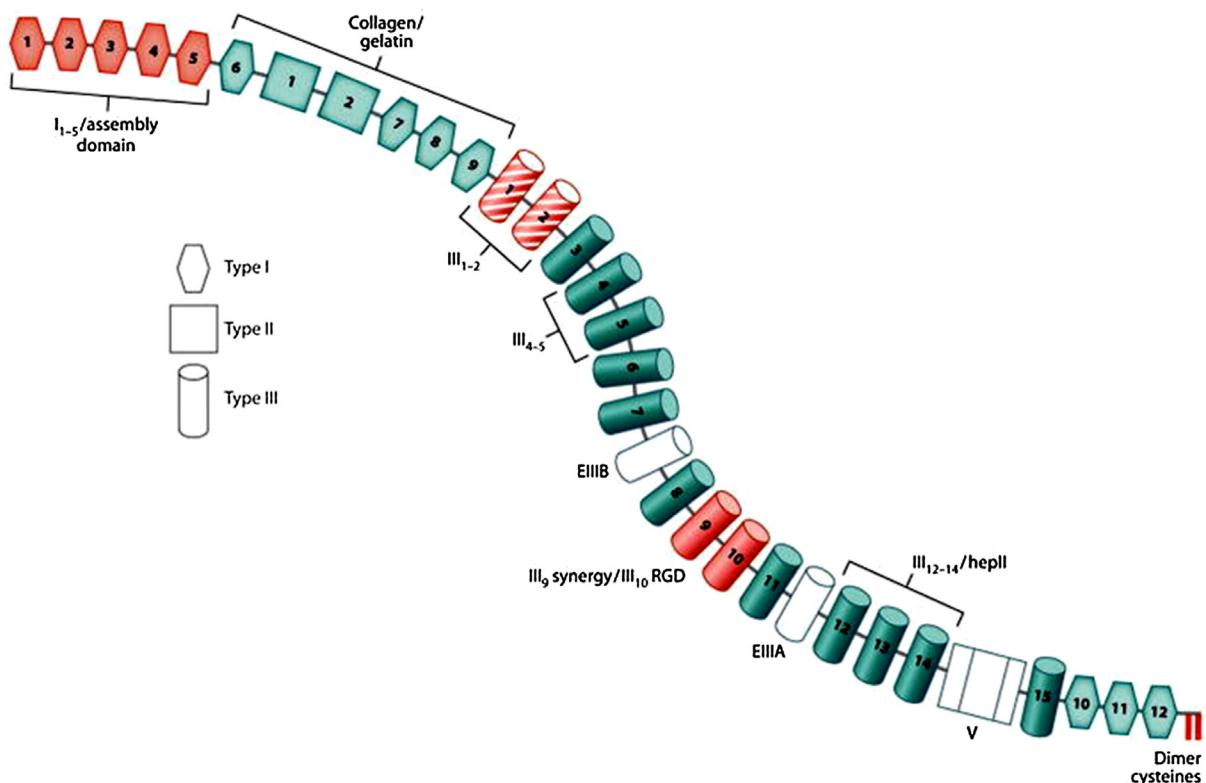
Probably the best known of these is fibronectin, a 250 kDa heterodimer that has multiple cell and molecule binding domains (see Box 4-5).

Additional content available at <https://expertconsult.inkling.com/>.

For instance, one of the type III fibronectin domains contains the ubiquitous cell adhesion domain Arg-Gly-Asp-Ser (RGDS in single-letter amino acid nomenclature), found in many other proteins. Such domains bind to other proteins which traverse the cell membrane, anchoring the cell to the matrix, including the integrins. Multiple forms of fibronectin occur by alternate splicing. Two major forms exist: plasma fibronectin, secreted by hepatocytes, and cellular fibronectin, secreted by fibroblasts and forming the ECM fibrillar network. Fibronectin may be involved in reverse integrin-mediated cell signalling and, through the actin-binding protein profilin, may regulate stress fibre formation in endothelial cells and fibroblasts. Fibronectin also has high-affinity binding sites for fibrin(ogen), thus promoting incorporation of fibrin into the extracellular matrix during wound healing. Fibrin binds

Fibronectin. This protein probably initiates the process by binding integrin receptors on the cell surface and entraining the mechanical forces of the cell through transmembrane connections with the dynamic acting cytoskeleton. Matrix assembly is critically dependent on interactions between matrix proteins and the cells which produce them. Such proteins self-organize (see p. 158) through specific

domains on cell surface protein receptors such as $\alpha_5\beta_1$ integrin which connect to the intracellular cytoskeleton proteins such as actin via linking proteins such as talin and vinculin. Cell contractility then fashions the shape, contour and architecture of the matrix. Once in place, fibronectin then contributes to the assembly of other matrix proteins through its many molecule-specific domains (eFig. 4-7).



eFIGURE 4-7 The fibronectin molecule: Each subunit comprises three types of repeat involved in Fn assembly (including the cell-binding RGD domain), the collagen-binding domains, and glycosaminoglycan-binding domains (heparin and syndecan). (From Singh et al., 2010.)

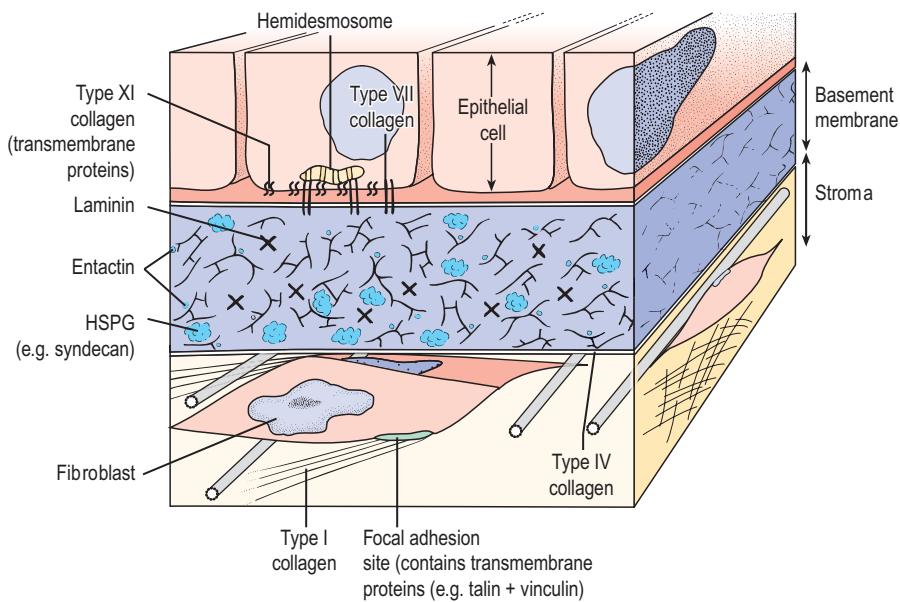


FIGURE 4-20 Structure of basement membrane.

growth factors such as fibroblast growth factor 2 and vascular endothelial cell growth factor, providing sources for angiogenic stimulation.

Thrombospondins (Fig. 4-19) are a group of extracellular matrix proteins that were first identified as platelet release proteins but that are now known to be secreted by endothelial cells and other cell types. Together with SPARC (secreted protein, acidic and rich in cysteine), tenascins and CCN proteins they are considered to be adhesion-modulating proteins. Five forms of thrombospondin exist, two of which appear to be alternately spliced forms of the parent molecule. Like extracellular matrix proteins, thrombospondins are composed of building blocks, each with specific cellular and molecular adhesive properties. Thrombospondins are involved in the regulation of angiogenesis, for instance in preventing angiogenesis associated with ocular inflammatory disease (see Ch. 9). In this regard, they form complexes with a ubiquitous plasma protein, histidine-rich glycoprotein (HRG), which has many regulatory functions in wound healing, cell migration and immune cell function. In addition, thrombospondins are better regarded as matricellular proteins that regulate a variety of processes, including cell adhesion and collagen fibrillogenesis. SPARC (Fig. 4-19) is a component of

basement membranes but appears to be essential only for normal lens physiology since SPARC-deficient mice develop age-related cataract and lens rupture at 6 months of age but have no other phenotype. Less is known about CCN (for CYR61/CTGF/NOV) proteins (Fig. 4-19), apart from von Willibrand factor, which is involved in haemostasis.

Several other proteins are present in the extracellular matrix, such as proteases (e.g. plasminogen) and their inhibitors (plasminogen activator inhibitor 1 (PAI-1), α_2 -macroglobulin, etc.). PAI-1 is present at high concentrations around various cells in the quiescent state and is considered important in the regulation of cell migration by controlling the level of cell-associated plasminogen activator required to initiate the degradation of basement membrane proteins. Other important proteins include the matrix metalloproteinases (MMPs) and their inhibitors (TIMP-1 to -3). Some of these proteins are secreted by the cells themselves, while others are synthesized predominantly in the liver and reach the extracellular matrix via the plasma.

Some proteins have their action at a distance from the cell, such as fibrillin, a 350 kDa microfibrillar protein that is involved in the assembly of elastin fibrils (Fig. 4-18). Fibrillin is found in the tertiary

vitreous and the zonule (see previous section). Mutations in fibrillin are found in Marfan syndrome, a disease of elastic tissue in which dislocation of the lens is a central feature.

Glycosaminoglycans occur in the extracellular matrix bound to core proteins as proteoglycans

Glycosaminoglycans (GAGs) occur in a variety of forms, essentially based on a repeating disaccharide structure (Box 4-7).

The prototype proteoglycan was described in relation to cartilage, in which a series of proteoglycans are linked to a hyaluronic acid backbone (Fig. 4-21). This, however, is not relevant to ocular proteoglycans; for instance, hyaluronic acid in the vitreous is not associated with other GAG-containing proteoglycans but is linked to collagen type IX, which in this situation is regarded as a proteoglycan.

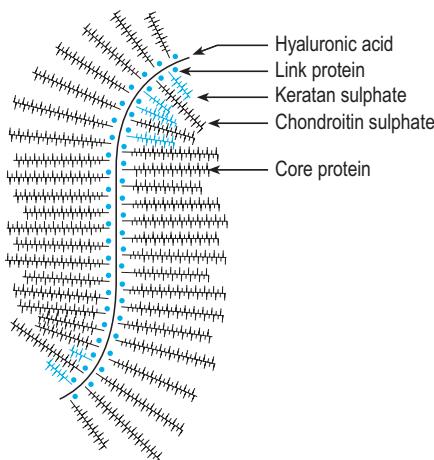


FIGURE 4-21 Artist's impression of hyaluronic acid molecule. (After Rosenberg, 1975.)

In addition, keratan sulphate proteoglycan occurs in the cornea in the absence of hyaluronan, where it appears to regulate collagen fibril diameter (see below). In other tissues, hyaluronan has both structural and cell-regulatory activities. For instance, two receptors of the LINK protein family, LYVE-1 and stabilin-1, are important in specific functions of lymphoid cells and endothelial cells, respectively. The main cell surface receptor for hyaluronan is CD44 and after internalization hyaluronan may even have some intracellular functions.

The number of proteoglycans that have been described in relation to other cellular functions has increased greatly (Table 4-3). Proteoglycans (PGs) are thus now described in three families of proteins: transmembrane, hyaluronan-binding and collagen fibril-regulating proteoglycans. Transmembrane PGs have a direct role in specific intercellular interactions, hyaluronan-binding PGs function as links for space-filling effects (e.g. in the vitreous cavity), while collagen fibril-regulating PGs have specific roles, e.g. force-generating in tendons, light transmission in the cornea, mechanical support in bone and deformability in heart muscle. In the cornea, four PGs (decorin, lumican, keratocan and mimecan) are the major keratan sulphate moieties that, together with collagens type VI and XII, are essential for maintaining corneal transparency.

Extracellular matrix molecules are intimately involved in cell adhesion

Some proteoglycans such as the syndecans participate constitutively in cellular adhesion and its associated cell signalling, acting as co-receptors with other receptors such as integrins in the receptor cluster, and thus

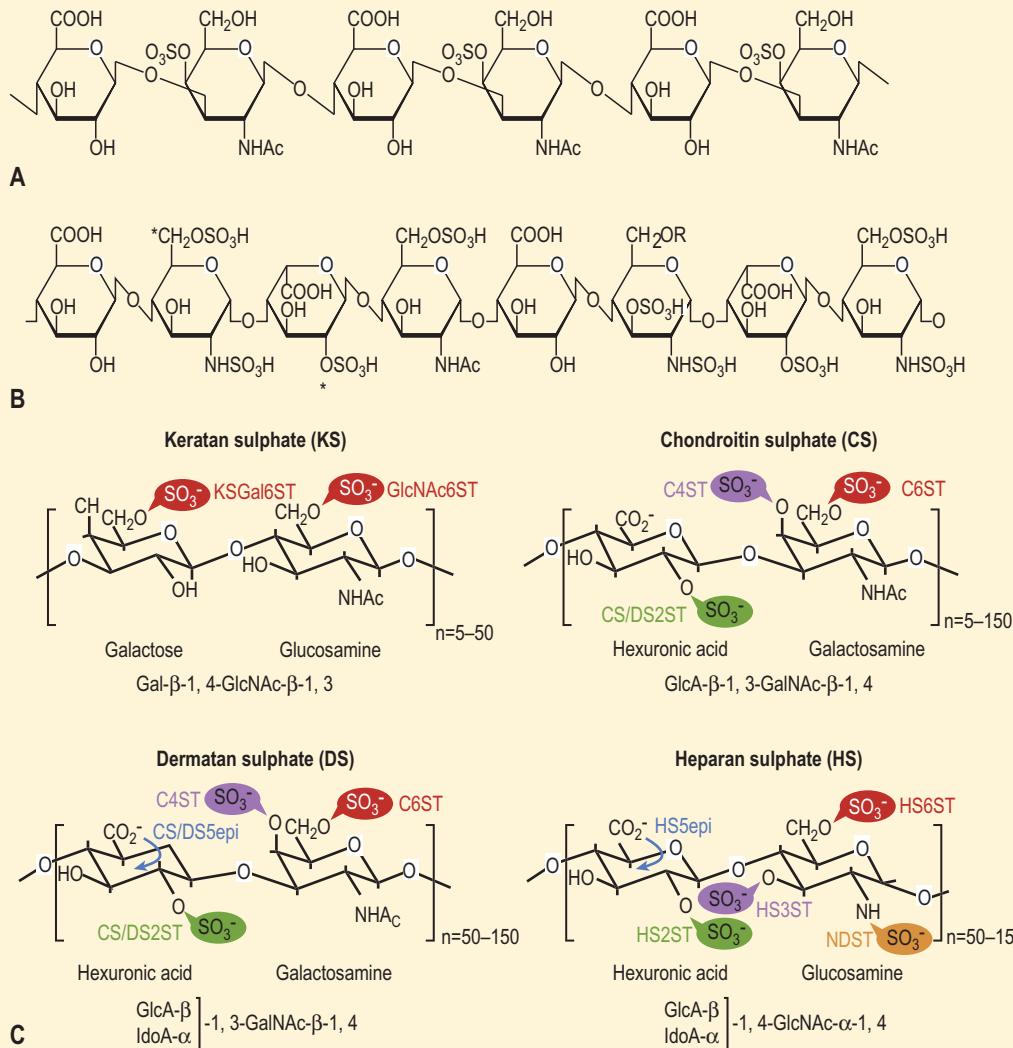
TABLE 4-3 Some tissue proteoglycans

Proteoglycan	GAG	Tissue	Function
Aggrecan	CS, KS	Cartilage	Structural support
Versican	CS	Fibroblasts	Cell migration, support
Decorin	CS/DS	Fibroblasts	Fibrillogenesis
Fibromodulin	KS	? Keratocytes	Fibrillogenesis
α_2 (IX) collagen	CS	Vitreous, cartilage	Collagen binding
Syndecan	CS, HS	Epithelia, fibroblasts	Morphogenesis
Basement membrane	HS	Basement membrane	Support
CD44	CS	Lymphocytes, epithelia, retina	Cell–cell interactions

BOX 4-7 STRUCTURE OF GLYCOSAMINOGLYCANs

Glycosaminoglycans are long chains of repeating disaccharides based on a common structure: (A) heparan sulphate; (B) chondroitin 4,6-sulphate. Chondroitin 6- and 4,6-sulphates have additional sulphate groups at the appropriate C atoms (*); dermatan sulphate has the GlcA

(glycosamino-) residue replaced in variable lengths of the chain by IdoA (iduronic acid). Heparan has many more sulphate groups and a higher content of IdoA than heparin. Keratan sulphate has galactose instead of glucose, and hyaluronic acid has no sulphate groups.



Variations in the common structure of disaccharide units of glycosaminoglycans (GAGs) underpin the different GAGs. Dermatan sulphate contains iduronic acid, while chondroitin sulphate contains glucuronic acid. C4ST, CS-40-sulphotransferase; C6ST, CS-60-sulphotransferase; CS/DS2ST, CS/DS-20-sulphotransferase; Gal, galactose; GalNAc, N-acetyl-galactosamine; GlcA, glucuronic acid; GlcNAc, N-acetyl-glucosamine; GlcNAc6ST, N-acetyl-glucosamine-60-sulphotransferase; HS2ST, HS-20-sulphotransferase; HS3ST, HS-30-sulphotransferase; HS6ST, HS-60-sulphotransferase; IdoA, iduronic acid; KSGal6ST, KS-galactose-60-sulfotransferase; NDST: N-deacetylase-N-sulfotransferase (Adapted from Bulow and Hobert, 2006.)

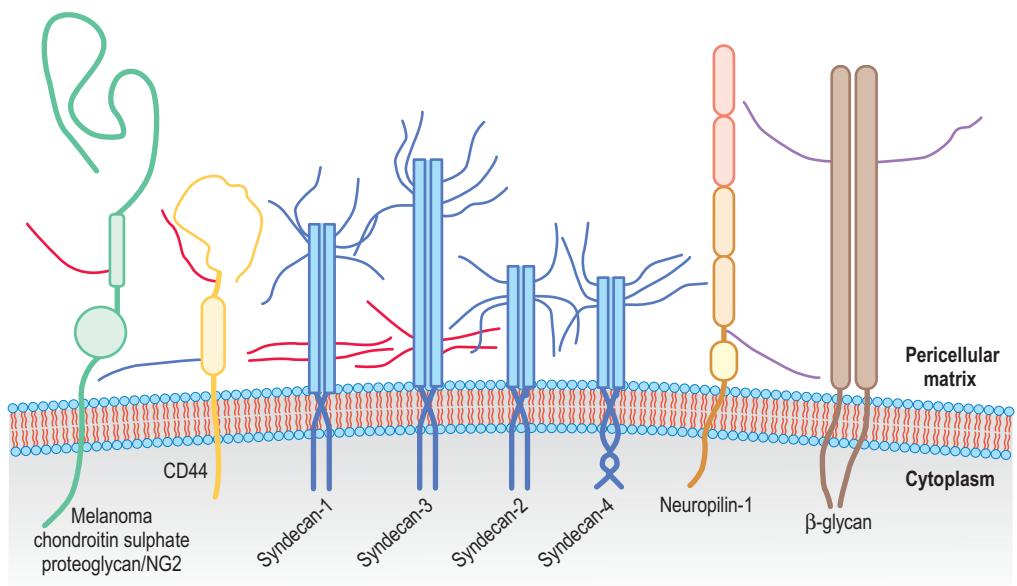


FIGURE 4-22 The transmembrane proteoglycans. The four syndecans are usually substituted with heparan sulphate chains (dark blue), while syndecans-1 and -3 have additional chondroitin sulphate chains (pink). The melanoma chondroitin sulphate proteoglycan/NG2 (green) has one chondroitin sulphate chain. The other proteoglycans also have other functions and are considered ‘part time’ proteoglycans. (From Couchman, 2010.)

connect through to the cell cytoskeleton (Fig. 4-22). Other proteoglycans interact variably with glycosaminoglycans such as CD44 and neuropilin, both of which are involved in leucocyte–matrix interactions and may be dependent on the state of leucocyte activation.

Cell adhesion is fundamental to many biological processes, such as morphogenesis, development, immune reactions to foreign proteins and many other processes. Cells adhere both to the matrix and to other cells, and usually do so via specific receptors in the cell membrane, i.e. integrins. Integrins are heterodimeric proteins (they have α and β chains, some of which are common to more than one integrin type), and are described in terms of their chain composition, e.g. $\alpha_5\beta_1$ and $\alpha_1\beta_6$ integrins (Table 4-4; see also Ch. 9 and the role of integrin receptors in leucocyte adhesion). Each cell type adheres preferentially to particular extracellular matrix proteins, depending on the type of integrin receptor it happens to express (see Table 4-4). In addition, cells may express different integrins at different times, depending on their state

TABLE 4-4 Integrin binding to extracellular matrix proteins

- Integrins $\alpha_1\beta_1$, $\alpha_2\beta_1$, $\alpha_6\beta_1$, $\alpha_7\beta_1$, $\alpha_4\beta_4$, $\alpha_6\beta_4$ bind to laminin
- Integrins $\alpha_4\beta_1$, $\alpha_5\beta_1$, $\alpha_v\beta_1$, $\alpha_v\beta_3$, $\alpha_6\beta_4$ bind to fibronectin
- Only integrin $\alpha_3\beta_1$ binds to both laminin and fibronectin

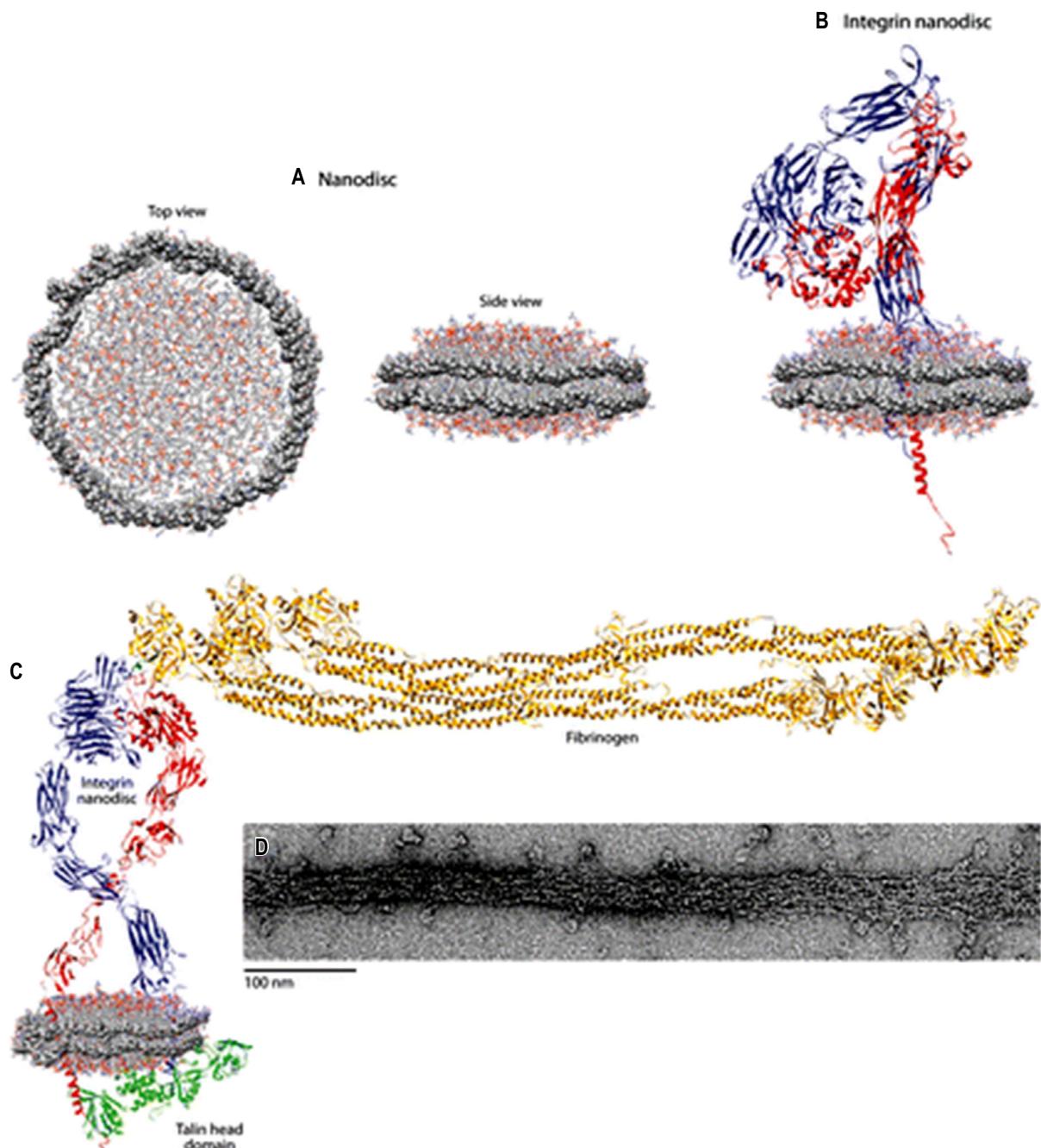
of activation, as, for instance, with the (myo)fibroblast during wound healing (see below). Differential adhesion by cells is shown clearly in the lens where anterior epithelial cells use the $\alpha_5\beta_1$ integrin to bind fibronectin, while the equatorial and posterior fibre cells express the $\alpha_6\beta_1$ integrin.

Binding of the cell to the matrix via integrin receptors not only has a structural role but also is involved in transmembrane signalling, which may be two-way, i.e. the matrix may modify the behaviour of the cell and the cell may transmit information to other cells via the matrix (inside-out signalling).

Additional content available at <https://expertconsult.inkling.com/>.



This is an exquisitely sensitive system and is tunable, i.e. the integrins can vary their affinity for adhesion to matrix proteins, as directed by cellular inside-out signalling processes. The molecular subtlety is so exquisite that integrins embedded in discoid membrane structures (nanodiscs) are bent and become extended when they bind ligand. Integrins are kept in a loosely bound state by ‘clasps’ on either side to the membrane. The orientation (tilt) of the integrin molecule precisely determines the interaction with ligand such as fibrinogen/fibrin. Intracellular talin mediates inside-out signalling, which is regulated by kindlins under the control of the small signalling molecule Rap1 ([eFig. 4-8](#)).



eFIGURE 4-8 (A) Nanodiscs with lipid molecules are shown (coloured) and membrane scaffolding proteins (grey) are shown in solid surface view. The nanodiscs range from 10 to 13 nm in diameter. (B) Reconstituted integrin nanodisc containing an integrin molecule (ribbon diagram, α subunit in blue and β subunit in red). (C) The head of a talin domain (green), integrin nanodisc (red and blue), and an integrin ligand (in this case, fibrinogen, yellow). (D) A negatively stained electron microscopy image of the reconstituted inside-out integrin activation system using fibrin as a ligand. (From Kim, et al., 2011)

TABLE 4-5 Proteins associated with cell junctions

Adherens junction	Desmosome
α , β , γ Catenin	Plakoglobin
Plakoglobin	Desmoplakin I and II
Vinculin	Desmoplakin IV
α Actinin	Desmocollin
Tenuin	Lamin B-like protein
Plectin	Plectin
Radixin	Desmoglein

Epithelial and endothelial cells bind via transmembrane complexes to each other and to the basement membrane

Epithelial and endothelial cells rest on a highly organized basement membrane (see above). Binding of epithelial-type cells occurs predominantly via adherens-type junctions and desmosomes/hemidesmosomes, in which several distinct proteins have been identified (Table 4-5). Some of these proteins are members of what is known as the cadherin family of proteins (cell adhesion proteins), a group of transmembrane proteins that regulate intercellular adhesion (see eBox 4-2).

These proteins are not only important in the mechanical support of intercellular interactions but also play a role in inter- and intracellular signalling. Gap junctions in particular facilitate and indeed may permit amplification of signalling events which are important in coordinated cell behaviour, as for instance in an epithelial cell sheet.

Mesenchymal cells bind to the matrix via focal adhesion sites

Mesenchymal cells, such as fibroblasts, keratocytes, chondrocytes and stromal cells generally bind to the extracellular matrix via specific focal adhesion sites that contain transmembrane cytoskeletal proteins. Actin stress fibres bind via α -actinin and talin to the cytoplasmic side of the integrin receptor and to vinculin, while the extracellular component of the integrin receptor binds to extracellular matrix proteins such as collagen and fibronectin (see Fig. 4-20 and eFig. 4-8).

Direct reverse signalling takes place through these sites using the signalling protein, focal adhesion kinase, one of the protein tyrosine kinases. Also involved is a further intracellular protein termed VASP

(vasodilator-stimulated phosphoprotein) which integrates profilin–actin binding with talin, vinculin, F-actin and the integrin signalling complex. Together with known ion channel-dependent mechanosensors, focal adhesion proteins such as talin act as mechanosensors responding by induced unfolding of their tertiary structures. Similarly, cofilin acts to sever actin filaments in a negative-dependent fashion based on tension in the actin filament at focal adhesion sites. Certain other mesenchymal cells, such as muscle, have specific proteins in their focal adhesion sites such as dystrophin and paxillin, not only at the site of attachment of the extracellular matrix but also at the neuromuscular junction. A mutation in the dystrophin gene has been identified in patients with muscular dystrophy. An integrin-linked kinase appears to be central to regulation of outside-in and inside-out signalling in adherent cells.

Biochemical pathways that affect ocular function

The eye, particularly the outer retina, is highly metabolically active and requires large amounts of ATP, the universal energy storage molecule, for this purpose. Cells cash in on this energy bank by hydrolysing ATP and coupling this event to cell-specific enzymic reactions that are otherwise energetically unfavourable. ATP is generated by oxidative metabolism, particularly of glucose but also of other molecules such as fats and proteins.

Hydrolysis of ATP is not without risk to the cell: free electrons are produced (H^+), which are normally mopped up by nicotinamide adenine dinucleotide (NAD). Indeed, NADH is the main electron carrier in the oxidation of glucose and other molecules, while NADPH (see below) is used to reduce certain molecules such as free fatty acids to permit them to enter the metabolic pathways.

Oxidative consumption of glucose requires coenzyme A, which is a carrier of acyl groups. Other coenzymes are also required for active metabolism, many of which are derived from vitamins.

GLUCOSE METABOLISM AND TISSUE GLYCATION

Glycolysis is the conversion of glucose to pyruvate in the absence of oxygen, and is accompanied by a net

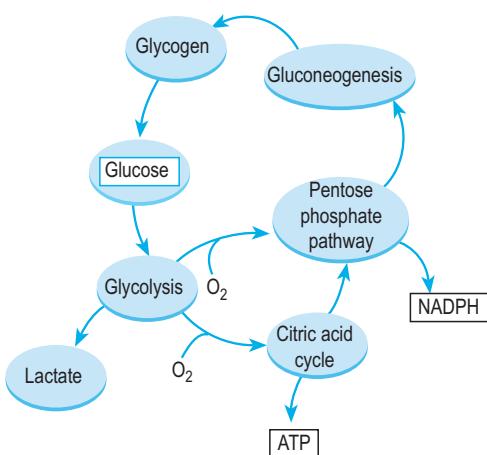


FIGURE 4-23 Summary of glucose pathways.

gain of two molecules of ATP. In the presence of oxygen, pyruvate enters the citric acid cycle with the production of 24 molecules of ATP. NAD⁺ is regenerated in the mitochondrial electron transport chain. Glucose and other fuel molecules entering the citric acid cycle may thus be consumed to provide energy for synthesis of the building materials for other molecules such as amino acids.

Not all the fuel molecules are utilized immediately, but instead they are diverted to produce molecules that act as sources of power rather than energy. This is achieved via the pentose phosphate pathway, in which NADPH and ribose are produced, the former for use in reductive processes and the latter in the biosynthesis of nucleotides for RNA and DNA. These different pathways are briefly summarized in Figure 4-23.

The pentose phosphate pathway also permits glucose formation from unrelated precursors (gluconeogenesis) but does not occur in the brain owing to lack of glucose-6-phosphatase; this probably also applies to the retina. However, the pentose phosphate pathway is active in the cornea and lens (see below).

Finally, glucose may be stored in the liver and muscle as glycogen, whence it can be retrieved for energy purposes by glycogenolysis. The Müller cells in the retina also contain stores of glycogen, which may be essential to maintain retinal function (see Chs 1 and 5).

Energy metabolism is controlled globally by nutrient intake, use and storage and is regulated by a

range of signalling pathways targeting food excess such as insulin, insulin-like growth factor-1 and mammalian target of rapamycin (mTOR); food restriction is likewise controlled by a set of pathways including the ATP-consuming anabolic pathways and ATP-generating catabolic pathways, which are under strict regulatory control by a ‘master-switch’ enzyme system, adenosine monophosphate-activated protein kinase (AMPK), which controls the overall whole body energy metabolism and is involved in loss of neuroprotection in the brain, and possibly the retina, in ischaemic states. In addition, sirtuins, a family of seven histone-deacetylase proteins in mammals, serve as sensors of energy (ATP) levels and also have wider transcriptional and other regulatory roles such as protection from toxicity of reactive oxygen species via upregulation of AMPK.

Glucose enters cells by facilitated diffusion

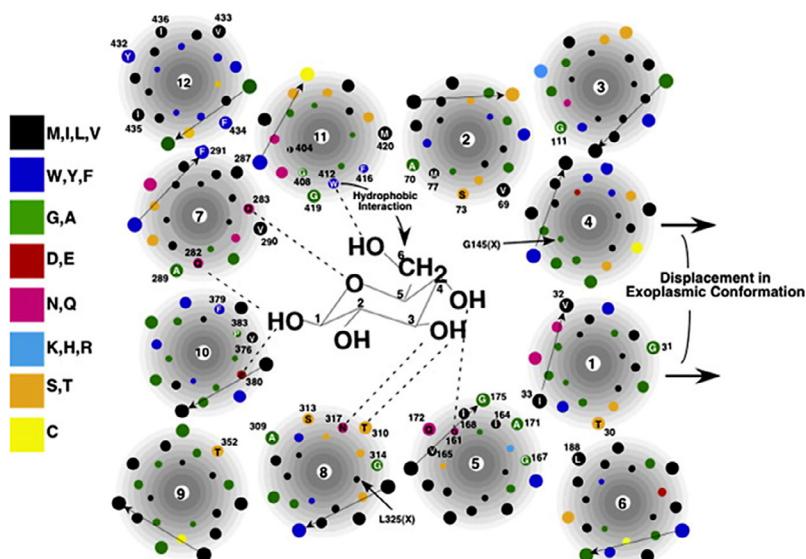
Glucose transporters (GLUTs 1–14) are members of the SLC2 (solute carrier 2) gene family and are membrane proteins which permit the facilitated diffusion of hexoses and polyols, including myoinositol, glucosamine and ascorbate, into and out of cells down their concentration gradient. Different cells and tissues have different GLUTs, which is relevant to how each tissue handles glucose (eFigs 4-9 and 4-10).

Additional content available at <https://expertcon.sult.inking.com/>.

Thus skeletal muscle utilizes GLUT 4, which is dependent for its function in the plasma membrane on insulin; in contrast, brain and retina, which are considered to be insulin-independent tissues, express GLUTs 1 and 3. The liver and adipose tissue also have GLUTs 2 and 7, with several of the other transporters. GLUTs 1, 3 and 4, particularly GLUT 1, can be upregulated in response to hypoxia via the gene hypoxia-inducible factor-1, which also induces other genes such as vascular endothelial cell growth factor, which is important in retinal vessel permeability and new vessel growth. GLUT 4 in muscle is critical for whole body glucose homeostasis; GLUT 7 is involved in gluconeogenesis and glycogenolysis in the liver, where it is located in the endoplasmic reticulum in association with glucose-6-phosphatase, the essential enzyme for these reactions.

The glucose transporters have a closely defined mechanism for shifting glucose molecules across the cell membrane (eFig. 4-9) and are specific to tissues and cells (eFig. 4-10). Glucose is transported across the membrane in aqueous phase propelled by hydrophobic interactions between glucose molecules and residues on the amino acids in the transporter protein which maintain phase separation of the molecules, while other amino acids are dedicated to promoting binding and thus carriage of the

molecule across the glucose gradients that exist. About one-third of the glucose is transported through the gut via Glu2 and Glut 5 (for fructose) and is carried via red blood cells due to their very high concentration of GLUT1. In the resting state most of the glucose is utilized by the brain and nervous tissues while during exercise muscle uses most of the circulating glucose. Adipose tissue regulates glucose utilization via production of hormones termed adipokines.



eFIGURE 4-9 Model of the exoplasmic substrate-binding site of GLUT1. The glucose molecule in the centre of the diagram is not drawn to scale. The helices surrounding amino acids in the glucose transporter are shown in a simplistic fashion for clarity. Amino acid residues that are in contact with solvent in the aqueous cavity are numbered and identified by the single-letter code. Dotted lines represent putative hydrogen bonds. (From Mueckler and Thorens, 2013.)

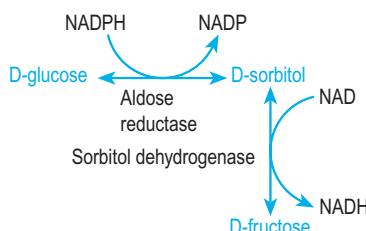


FIGURE 4-24 Aldose reductase pathway.

Excessive levels of glucose may impair cellular metabolism

Glucose metabolism is tightly regulated by insulin, IGF-1 and glucagon release from the pancreatic β cells. In diabetes mellitus, in which there is impaired secretion or utilization of insulin, hyperglycaemia leads to excessive uptake of glucose into cells, despite negative feedback on the expression of GLUTs on the cell surface. This has the effect of overloading the metabolic pathways with activation of alternative routes for glucose handling such as the aldose reductase pathway (Fig. 4-24).

In certain tissues, such as the lens, the effect of increased sorbitol may be to cause damage by osmotic dysregulation because sorbitol cannot be transported out of the cell easily. In this case, even high glucose can have direct osmotic damaging effects on the cell owing to raised intracellular $[Ca^{2+}]_i$, perhaps as a result of cell shrinkage with consequent activation of the stretch receptors. Alternatively, the excessive utilization of NADPH in this pathway might have deleterious effects on the levels of myoinositol, which is required for intracellular signalling, or on the increased generation of reactive oxygen species directly via activation of the phosphoinositol pathway.

It has therefore been suggested that activation of the aldose reductase pathway is not the direct cause of cellular damage but is merely a coincidental perturbation in the cell; instead, excessive production of free radicals may be important during oxidation of the high concentrations of glucose. Interestingly, aldose reductase has been implicated in inflammatory processes by promoting lipid peroxidation and free radical generation (see below). Whatever the mechanism, aldose reductase is likely to be involved in the pathophysiology of the lens at least, because inhibitors of this enzyme prevent the development of cataracts.

Chronic hyperglycaemia has also been shown to alter cellular metabolism via modulating insulin post-receptor intracellular signalling, involving the cascade insulin receptor substrate 1 (IRS-1)/phosphatidyl-inositol 3-kinase (PI3K)/Akt, as the result of production of high levels of hexosamine in the cell. This may be one mechanism of induction of insulin resistance in diabetes mellitus. A more general mechanism for the effects of high ambient glucose concentration on cell behaviour has been the induction of the cytokine TGF- β with its widespread effects on cell function, particularly increased proteoglycan synthesis and consequent basement membrane thickening. Patients with type II diabetes are known to have increased serum levels of TGF- β .

Excessive levels of glucose lead to glycation of proteins

Addition of sugar moieties to proteins can occur by enzymatic conjugation (termed glycosylation) or non-enzymatic conjugation (termed glycation). Glycation of proteins is classically considered to occur in the presence of high concentrations of glucose in two phases: an early reversible phase during which the protein forms a Schiff base and Amadori products, followed by a later irreversible phase in which advanced glycation end products (AGEs) appear (Fig. 4-25). However, AGEs can be produced in the early phase because free glucose degrades to α -oxoaldehydes, which are potent glycation agents. AGEs can thus be formed at any stage in the process of glycation, including through binding of Schiff bases and fructosamine. In addition, α -oxoaldehydes such as glyoxal, methyl glyoxal and 3-deoxyglucosone are produced as glycolytic intermediates and also during lipid peroxidation (see below), and lead directly to AGE formation (Fig. 4-25).

Glycation of proteins occurs at lysine, arginine and cysteine amino acids, thus affecting proteins such as collagen (pentosidine cross-links) and haemoglobin and occurs as part of ageing and pathologically in diabetes mellitus; in addition, important extracellular matrix proteins, such as PAI-1 (see above) may be glycated, leading to defective control of cell behaviour, including cell migration and activation. Glycation of cell membrane proteins such as Ca^{2+} channels in pericytes may impair their function, thus rendering them

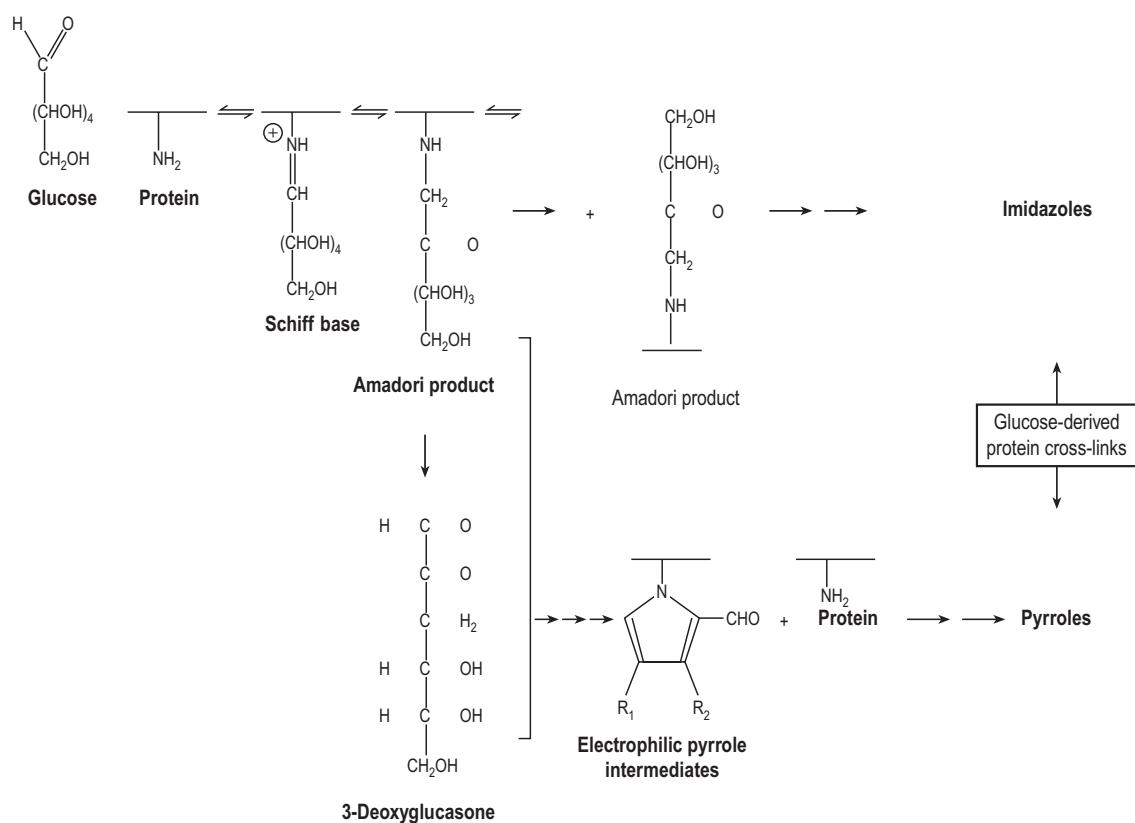
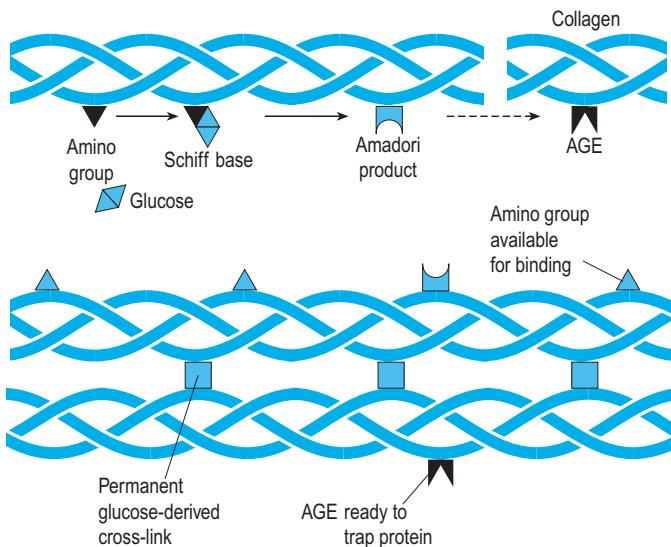
**A****B**

FIGURE 4-25 Process of non-enzymatic glycation. (A) Biochemical pathway via Schiff base and Amadori products leading to glucose-derived cross-links and ultimately advanced glycation end products (AGES). (B) Diagrammatic representation of molecular interactions involved in AGE-mediated collagen cross-links. (Courtesy of A. Ceramini and the publishers of Scientific American.)

less responsive to endothelin-1-induced contractility. The two processes of non-enzymatic glycation and auto-oxidation of proteins are also directly interrelated and may be important in glucose-derived oxidative stress. This last effect is a direct inducer of apoptosis and mediates neuronal and endothelial cell death in diabetic neuropathy and vasculopathy, through binding of AGEs to a specific receptor, RAGE.

A number of enzymes are known to repair proteins in the early stages of glycation, including fructosamine-3-kinase and glyoxalase and the term 'carbonyl stress' is used to describe imbalance in glycation versus deglycation.

Interestingly, the free radical scavenger vitamin B₆ may inhibit AGE production via the inhibition of oxidative degradation of Amadori intermediates and the trapping of reactive oxygen products (see below).

Glucose and lipid metabolism

Glucose and lipid metabolism are also intimately linked via the metabolite acetyl coenzyme A. In the presence of excess glucose, production of fatty acids and cholesterol through acetyl coenzyme A is increased with consequent increases in phospholipids (see below) and circulating levels of very low-density lipoproteins. While this has well-known implications for the development of atherosclerosis, more recently this form of metabolic dysfunction has been suggested to underlie pathologies such as age-related macular degeneration, not simply through the production of abnormal lipid deposits in areas where their removal is difficult, such as the subretinal space, but also by a concomitant increase in lipid-based free radicals (see below).

OXIDATION/REDUCTION AND FREE RADICAL PRODUCTION

Oxidative metabolism and the generation of ATP storage energy molecules are conducted via the cytochrome enzyme system. However, during the process of oxygen consumption, a small amount of oxygen (<5%) is metabolized by alternative pathways. Univalent reduction of oxygen produces highly reactive free radicals, namely the superoxide anion and the hydroxyl radical, and the toxic molecule hydrogen peroxide (Fig. 4-26).

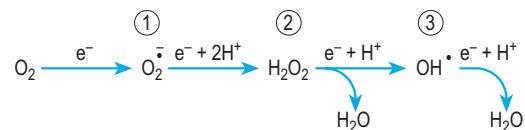


FIGURE 4-26 Free radical production: (1) superoxide anion; (2) hydrogen peroxide; (3) hydroxyl radical.

TABLE 4-6 Detoxification of free radicals

Mechanism	Agent
Superoxide anion degrading enzymes	Superoxide dismutases
Antioxidants	Ascorbate
Free radical scavengers	Vitamins A and E
H ₂ O ₂ degrading enzymes	Catalase, peroxidase

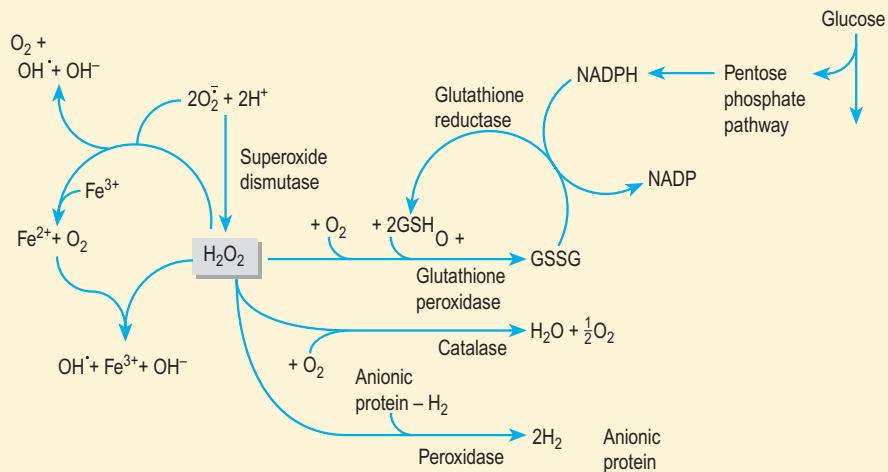
The superoxide anion is generated in mitochondria in the ubiquinone cytochrome b system and in other membranes by auto-oxidation via the cytochrome P₄₅₀-linked reductases (also part of the drug-metabolizing system), which are rich in the vitamin flavoprotein. Superoxide is then converted by superoxide dismutase to hydrogen peroxide (H₂O₂), which diffuses into the cytosol. Superoxide is also generated in the cytosol itself via recycling of redox enzymes such as xanthine oxidase; conversion to H₂O₂ in the cytosol is rapid. Xanthine, in particular, accumulates in certain tissues during ischaemia, and subsequent reperfusion leads to massive release of free radicals, producing extensive tissue damage. This can be prevented by free radical scavengers.

Free radicals can be generated by other mechanisms. Reduction of Fe³⁺ to Fe²⁺, by reducing agents such as ascorbate, catalyses the conversion of H₂O₂ to OH[•], and auto-oxidation of other compounds such as thiols and catecholamines produces free radicals. The Fe²⁺ ion has particular relevance to the problem of retained intraocular metallic foreign bodies. Tissue damage during inflammation is in a large part attributable to the production of free radicals by phagocytic cells during the respiratory burst (see Ch. 7). These interactions are illustrated in Box 4-8.

Free radicals are detoxified by a variety of enzymatic and non-enzymatic mechanisms (Table 4-6). In

BOX 4-8 GENERATION OF FREE RADICALS FROM HYDROGEN PEROXIDE

The oxidation of hydrogen peroxide (H_2O_2) leads to glutathione consumption via the pentose phosphate pathway and requires a supply of glucose to maintain homeostasis.



In addition, a critical redox regulatory system involving glutathione and the enzyme glutathione S-transferase is central to cellular homeostasis (Fig. 4-27). Superoxide dismutases require divalent ions such as Mn²⁺, Cu²⁺ and/or Zn²⁺ for normal activity; some of these have been demonstrated in ocular tissues. Further reduction of H₂O₂ is either by enzymatic or non-enzymatic means, and may involve several mechanisms directed towards removal of reactive oxygen species (see Box 4-8).

The mechanism of cell damage varies with each molecular species. Chelated metal ions are important in non-enzymatic degradation of H₂O₂, producing the highly reactive OH[·] radical, which is particularly damaging to cell membranes. H₂O₂ causes damage by inhibiting glycolysis and glucose uptake and is the most stable of the reactive oxygen species as well as being generated by several intracellular processes (see eFigs 4-10 and 4-11).



Additional content available at <https://expertcon.sultinkling.com/>.

Oxidative stress is considered one of the major mechanisms underlying DNA damage and has been correlated with changes in telomere length associated

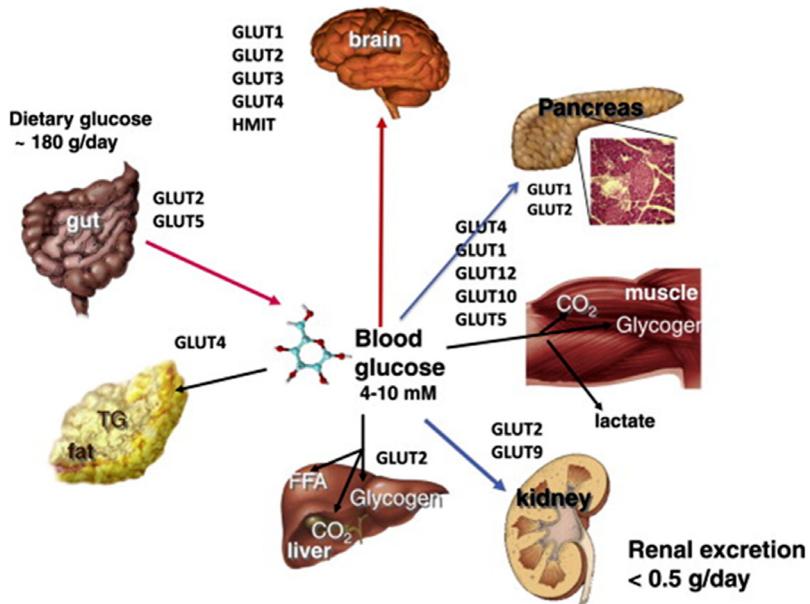
with ageing. Cells respond to DNA damage using a protein termed protein kinase ataxia telangiectasia mutated (ATM) which mediates DNA repair, cell cycle arrest and apoptosis. Oxidative stress can directly activate dimeric ATM (usually the monomer is the active agent), which seems to act as a cellular sensor for potential oxidative damage.

LIPIDS AND LIPID PEROXIDATION

Lipids are the structural basis on which cell membranes are built and are generally composed of a hydrophobic tail of two fatty acid chains bound to a hydrophilic (polar) head (Box 4-9). Fatty acids, thus, have at least three roles:

- as integral components of phospholipids and glycolipids
- as functional molecules, e.g. hormones and second messengers
- as energy stores in the form of triacylglycerols.

In addition to phospholipids and glycolipids, cholesterol is the third major lipid component of eukaryotic cell membranes; cholesterol is predominantly hydrophobic but has a hydrophilic group on carbon 3



eFIGURE 4-10 Role of GLUT proteins in the maintenance of glucose homeostasis. The various GLUT proteins expressed by different tissues are shown. (Mueckler and Thorens, 2013.)

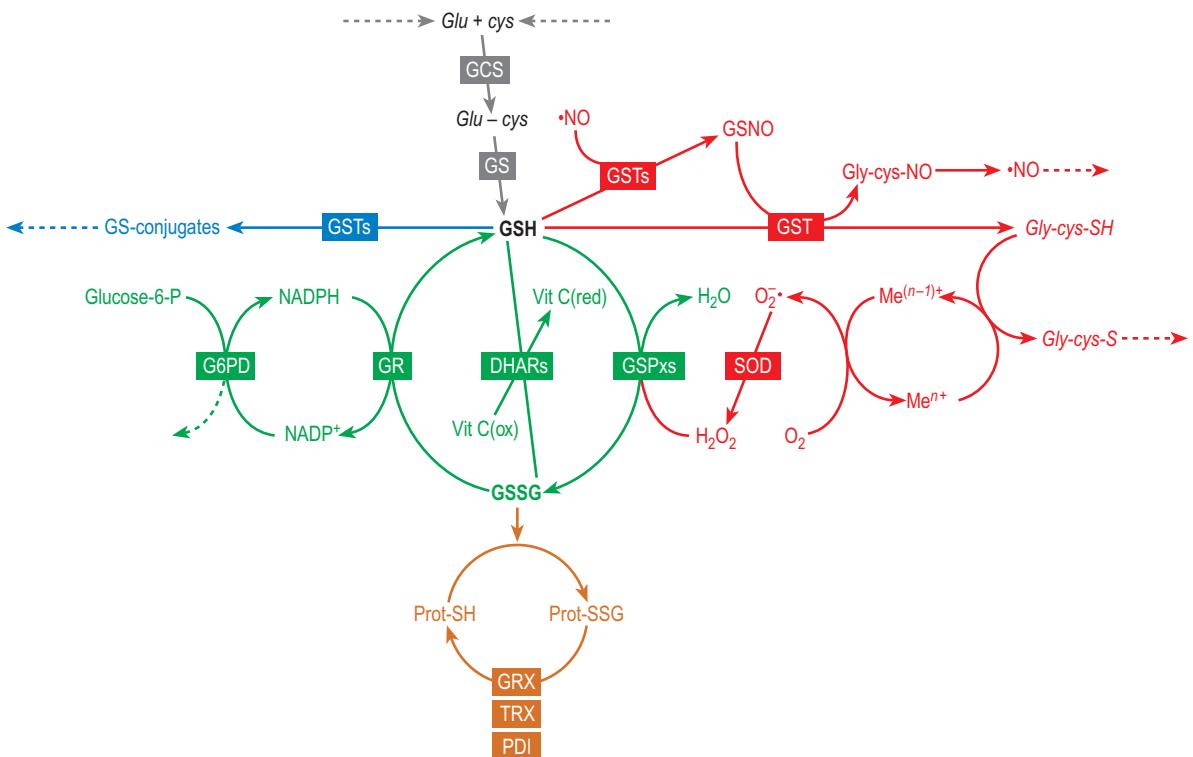


FIGURE 4-27 Outline of the interrelations connecting the various roles played by GSH in cellular homeostasis: antitoxic (blue), antioxidant (green), pro-oxidant (red), modulator (yellow). γ -GCS, γ -glutamyl-cysteine synthetase; DHARs, dehydroascorbate reductases; G6PD, glucose-6-phosphate dehydrogenase; GPxs, glutathione peroxidases; GR, glutathione reductase; GRX, glutaredoxin; GS, glutathione synthetase; GSNO, S-nitrosoglutathione; GSTs, glutathione S-transferases; Me, metal; PDI, protein disulphide isomerase; SOD, superoxide dismutase; TRX, thioredoxin. (From Pompella et al., 2003, with permission from Elsevier.)

(Fig. 4-28). In an aqueous medium, lipids arrange themselves in such a way that the polar (hydrophilic) groups face the aqueous phase, whereas the hydrophobic groups face each other (a micelle). However, owing to the bulky nature of the two chains of fatty acids, membrane lipids do not readily form micelles but group together as a lipid bilayer (Fig. 4-28). Phospholipid bilayers can reach macroscopic dimensions and are barriers to the diffusion of aqueous solutes but remain quite fluid themselves. Thus, they form an ideal material to act as biological membranes. The fluidity of lipid membranes is related to the length of the fatty acid chains, the number and nature of double bonds in the chain and, in eukaryotes, the content of cholesterol. Proteins suspended in lipid bilayers, such as rhodopsin, have very rapid lateral mobility within the membrane unless they are anchored across the

membrane to cytoskeletal and/or matrix proteins; in contrast the polarity of the protein in the membrane is fixed.

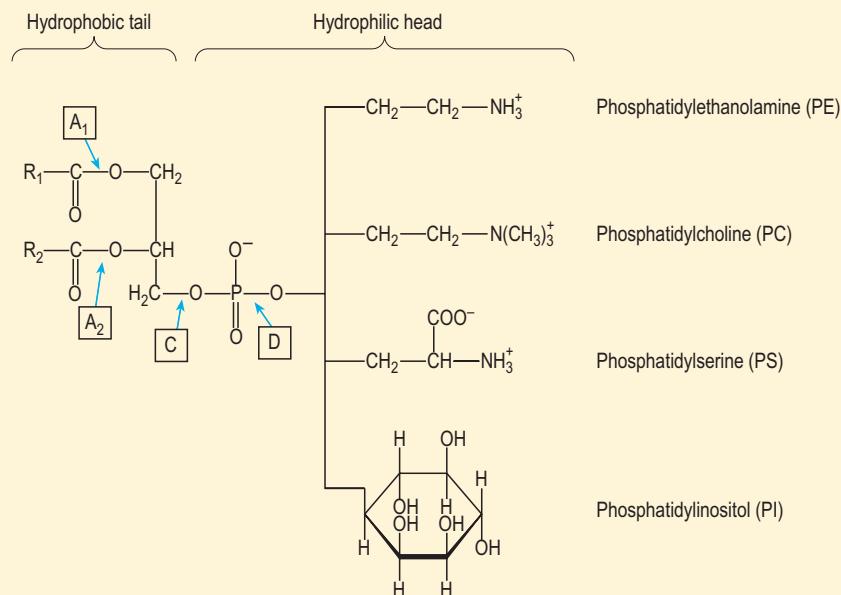
The formation of lipid bilayers is a spontaneous event, due to the physical hydrophobic interactions between the hydrocarbon tails, assisted by polar interactions between the aqueous phase and the hydrophilic head, and between adjacent polar groups. Furthermore, lipid bilayers form closed compartments in which defects are self-sealing, even after disruption. In many membranes, however, proteins, especially receptors and co-receptors, are distributed in clusters while the lipids form lipid rafts which differ in their lipid composition from adjacent regions (see p. 162).

Fatty acids undergo physiological degradation during energy consumption by oxidation and polyunsaturated fatty acids (PUFAs) are particularly

BOX 4-9 GENERAL STRUCTURE OF PHOSPHOLIPIDS

The general composition of lipids is a hydrophobic tail consisting of fatty acids bound to a hydrophilic head, which characterizes the molecule (**A**). Glycolipids differ from

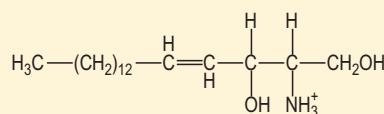
phospholipids in having a sugar moiety rather than a phosphorylcholine linked to the fatty acid chain. Sphingomyelin is intermediate between these (**B**).



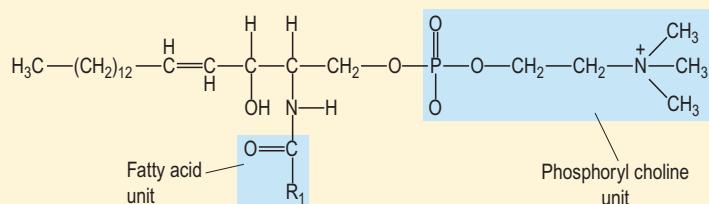
R_1 and R_2 : Fatty acids (usually saturated and unsaturated, respectively)

A₁ **A₂** **C** **D**: Sites of phospholipase activity

A



Sphingosine



Sphingomyelin

B

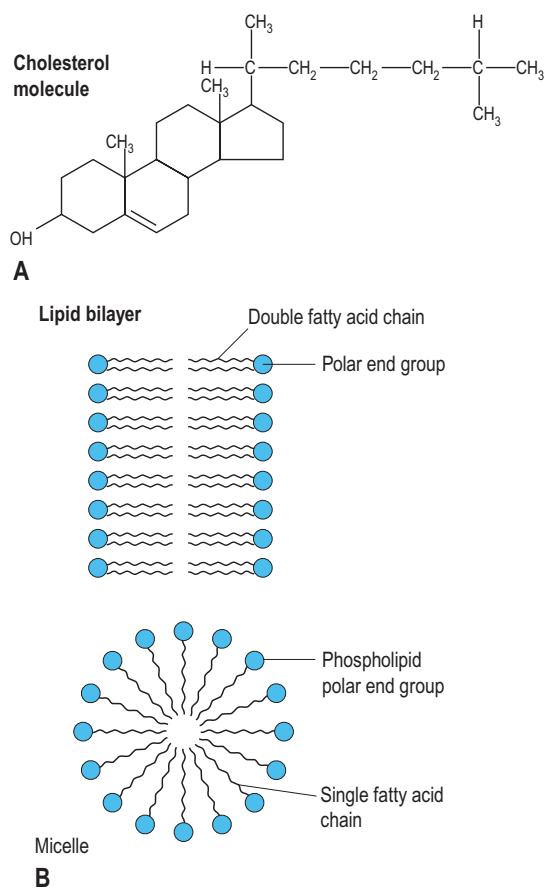


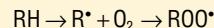
FIGURE 4-28 Structure of the cholesterol molecule (A) and the lipid bilayer (B).

susceptible. Similarly, lipids in cell membranes can be oxidized. Indeed, peroxidation of lipids is one of the main sources of cell damage, and polyunsaturated fatty acids are particularly susceptible to this form of non-enzymatic damage.

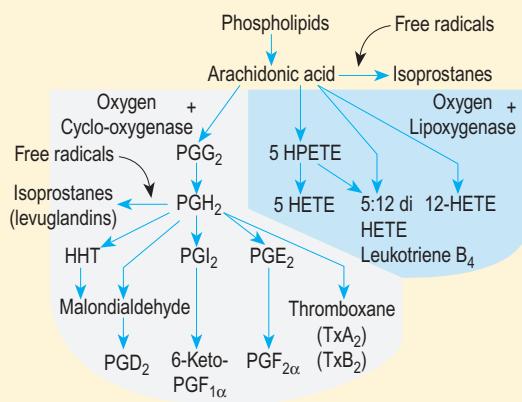
The lipid peroxides oxidize neighbouring fatty acids and set up chain reactions in the membrane. Phospholipase A₂, by releasing free fatty acids from the membrane, is thought to detoxify this reaction. However, the arachidonate released is then converted to prostaglandins and other eicosanoids (Box 4-10; see also Ch. 7, p. 382). Arachidonates may also be modified by interaction with free radicals in a non-enzymatic reaction. This produces a series of prostanoids, prostaglandin-like chemicals termed isoprostanes. In

BOX 4-10 OXIDATION OF LIPID MEMBRANES

Phospholipids, especially those containing arachidonic acid (20:4) and docosahexanoic acid (22:6), a fatty acid abundant in photoreceptor cells, are oxidized to lipid free radicals, which are then conjugated to form dienes. These are then converted to lipid peroxides.



Peroxidation of lipids in cell membranes leads to the release of large quantities of arachidonate, which acts as a substrate for eicosanoid production.



fact, isoprostanes are produced in much larger quantities than prostaglandins and are detectable *in vivo* as a measure of lipid peroxidation, and thus indirectly tissue damage. Isoprostanes occur at low levels in normal healthy individuals, thus reflecting a level of oxidative injury not completely suppressed by natural antioxidants. However, as biomarkers of disease hydroxyoctadecadienoic acids (HODEs), hydroxyeicosatetraenoic acids (HETEs), and hydroxycholesterols appear to be sufficiently stable end products of lipid peroxidation, as are isoprostanes and neuroprostanes. Isoprostanes have biological and potentially pathological effects on cell function, thus directly contributing to disease.

Release of carbonyl compounds from both carbohydrates and lipids can lead to protein modification by glycation (AGEs, see above) and by lipoxidation (ALEs, advanced lipo-oxygenation end products). These are seen especially in uraemic patients on dialysis. Vitamin B₆ (pyridoxamine) may also inhibit ALE production as well as AGE production (see above).

Lipid peroxidation can be limited by vitamin E, a lipid-soluble free radical scavenger that is present within the bilayer (especially photoreceptors) and reacts directly with the lipid free radical to produce the phenoxy radical, or with ascorbic acid in the aqueous phase (see above).

Lipids also occur extracellularly and are of particular importance as lipoprotein carriers in the circulation, and also as lubricants on surfaces. This is particularly important for the ocular surface.

The ocular surface

The ocular surface comprises the conjunctival and corneal non-keratinized epithelium and is bathed by the tear film. However, integrity of the surface depends on other structures such as the apposition of the eyelid margins, the meibomian gland and the lacrimal gland secretions. Collectively, this is termed the lacrimal functional unit (LFU). Evaporation of the tear film is clinically measurable as the tear film break-up time, and tear film integrity is restored by blinking. Blink rate and break-up time are therefore linked. Tears provide lubrication for lid closure, assist in smoothing out the irregularities in the ocular surface (which otherwise may have transient effects on light transmission), and also have an antibacterial function.

THE TEAR FILM

The tear film is a protective covering for the cornea composed of three layers: a surface oily layer, an aqueous layer and a deep mucous layer. Previous estimates of tear film thickness suggested that the aqueous layer comprised more than 95% of the tear film, but this has been reduced to 60%, with a larger component being provided by the mucous layer based on evidence from *in vivo* studies using the confocal corneal microscope. In addition, clinical evaluation of the tear film involves an assessment of the tear meniscus height (TMH). The TMH reflects the volume of tears that collects at the contact line between the eyelid margin and the bulbar conjunctiva and has several other names, including the inferior marginal strip and the tear prism or rivus.

The cornea presents a hydrophobic non-wettable surface, which is made wettable by possessing a layer of mucus on its surface. This is overlaid by the aqueous

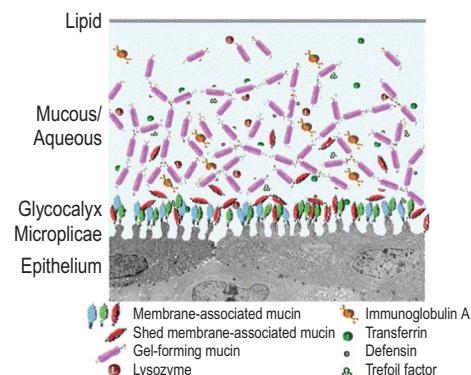


FIGURE 4-29 Diagram of the tear film–glycocalyx interface demonstrating tear components and the apical surface glycocalyx with its membrane-tethered mucins. (From Govindarajan and Gipson, 2010.)

component of the tear film and lastly by a layer of lipid, which prevents evaporation of the tears (Fig. 4-29). Soluble mucus in the aqueous layer and meibomian lipid combine to ensure stability of the tear film by lowering the surface tension and permitting spreading of the tear film on blinking. The break-up time is therefore thought to represent the time it takes for aqueous to evaporate and meibomian lipid to come in contact with the hydrophobic epithelial cell layer.

The blink reflex removes up to 70% of the surface aqueous–mucous layer while the remaining 30–40% thick mucous layer in contact with the cornea is not wiped away on blinking. Instead, it is thinned by blinking and may take 30 minutes to reconstitute itself. The mucous layer is in part composed of the glycocalyx of the epithelial cells and also by an additional layer of tear mucins produced by the conjunctival goblet cells. Reduced tear film break-up time in dry eye disorders of various types therefore reflects a disturbance in tear mucin/aqueous protein interaction and may be associated with a reduction in goblet cell density.

Tear film lipids have unique characteristics

The lipid layer of the tear film is derived from meibomian gland secretions and is very thin (0.1 µm). It is composed of a mixture of polar and neutral lipids (Box 4-9) with a melting point (35°C) that ensures it is always fluid on the ocular surface. The polar lipids are in contact with the aqueous phase of the tear film and

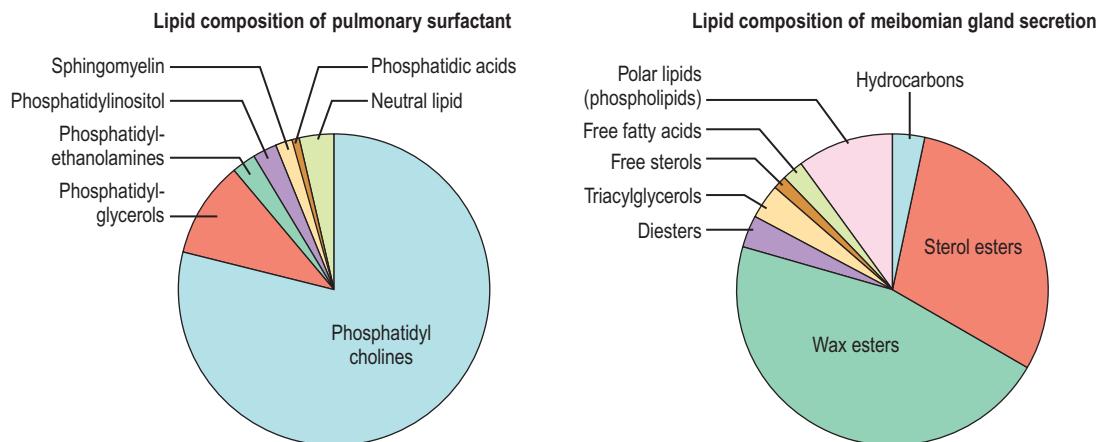


FIGURE 4-30 Proportion of different lipid moieties compared between composition of pulmonary surfactant, a lipid-rich surface layer covering the lung mucosa, and the meibomian gland secretion, the major contribution to tear film lipid. Note the diversity of lipids in the tear film, particularly the wax esters. (From Panaser and Tighe, 2012)

TABLE 4-7 Composition of the tear film			
Physical properties	Solutes ($\mu\text{mol/L}$)	Proteins	Enzymes/inhibitors
98% H_2O_2	Na 120–160	Lysozyme	Glycolytic
6–9 μL volume	Cl 118–135	Lactoferrin	Amylase
pH 7.5	HCO_3 20–25	Tear-specific prealbumin (lipocalin)	Plasminogen activator
310–334 mOsm	K 20–42	G protein	(α_2 -macroglobulin)
	Mg 0.7–0.9	(IgA, IgG)	(α_1 -antitrypsin)
	Ca 0.5–1.1	(Ceruloplasmin)	
	Glucose 0.5–0.7	(Albumin)	
	Retinol	(Orosomucoid)	
	Urea		

provide structural stability to the tear film, while the non-polar lipids are at the air interface and provide barrier function and thixotropic properties. The lipids include unsaturated and branched-chain fatty acids and alcohols, 8–32 carbon chains in length. Tear lipids are under study by way of lipidomics and different sets of wax esters, oleates and cholesterol esters have been identified. Interestingly, in comparison with lung surfactant, there is a more heterogeneous composition with prominence of wax esters (Fig. 4-30).

Tear lipids promote movement of water into the aqueous phase during formation of the tear film, where they bond to tear lipocalins. The functions of the meibomian lipid layer are:

- to prevent evaporation of tears
- to prevent spillover of tears at the lid margin

- to prevent migration of skin lipid onto the ocular surface
- to provide a clear optical medium.

Lacrimal gland secretion provides the aqueous component of tears

The lacrimal gland and its accessory glands (see Ch. 1, p. 89) are classic exocrine acinar glands secreting a dilute aqueous solution containing proteins and small molecular weight components and electrolytes (Table 4-7). Although over 1500 proteins have been identified in tear fluid by proteome analysis, the principal proteins in tears are immunoglobulin A (IgA), lactoferrin, G protein, tear-specific prealbumin (lipocalin) and lysozyme (Fig. 4-31). Tear-specific prealbumin is a member of the lipocalin superfamily and together with

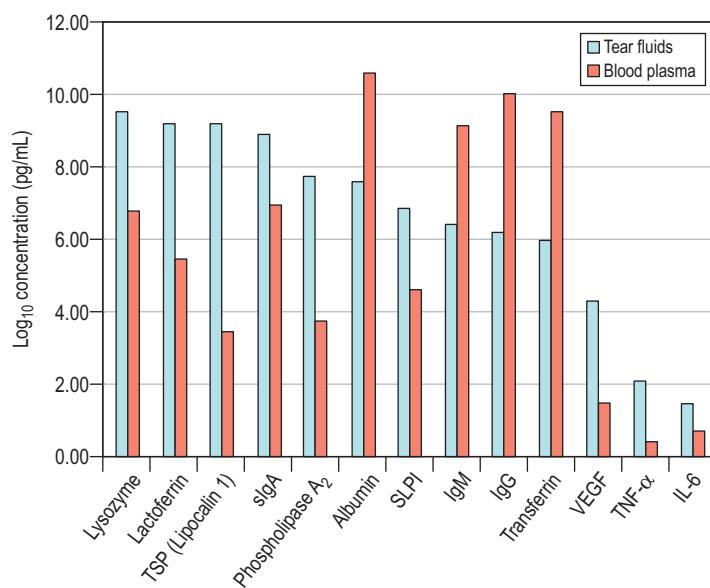


FIGURE 4-31 Comparison of protein concentrations in human tear fluid and plasma to show the remarkable difference between tear fluid and plasma. The proteins shown in this figure represent a wide dynamic range (from mg/mL to pg/mL). (From Zhou, 2012.)

a further lipocalin, apolipoprotein D, is secreted by the lacrimal gland. The function of tear lipocalin is to interact with meibomian gland lipid and induce surface lipid spreading. It may also have a role in removing harmful lipophilic molecules.

Tear secretion by the lacrimal gland is under neural control: basal tear secretion occurs at a rate of 1.2 μ L/min, but massive tear production can be induced by a variety of mechanical and psychophysical stimuli. Recent studies in mice also show that tears contain the first discovered soluble male pheromone. Neural control is mediated by the autonomic nervous system

(see eFig. 4-11).

Additional content available at <https://expertconsult.sult.inkling.com/>.

The aqueous component contains several proteins with an antibacterial activity (e.g. lactoferrin and lysozyme), and the high levels of immunoglobulins may have an immunological role (see Ch. 7). Lactoferrin synergizes with lysozyme in its antibacterial action by binding to lipoteichoic acid on the bacterial surface and allowing access of lysozyme to the peptidoglycan. The aqueous component also has anti-adhesive and lubricant properties possibly attributable to lipocalin, thus ensuring that protein and debris generally do not adhere to the corneal surface

(e.g. during prolonged periods of lid closure while sleeping).

The mucous layer stabilizes the aqueous layer by providing a hydrophilic contact surface

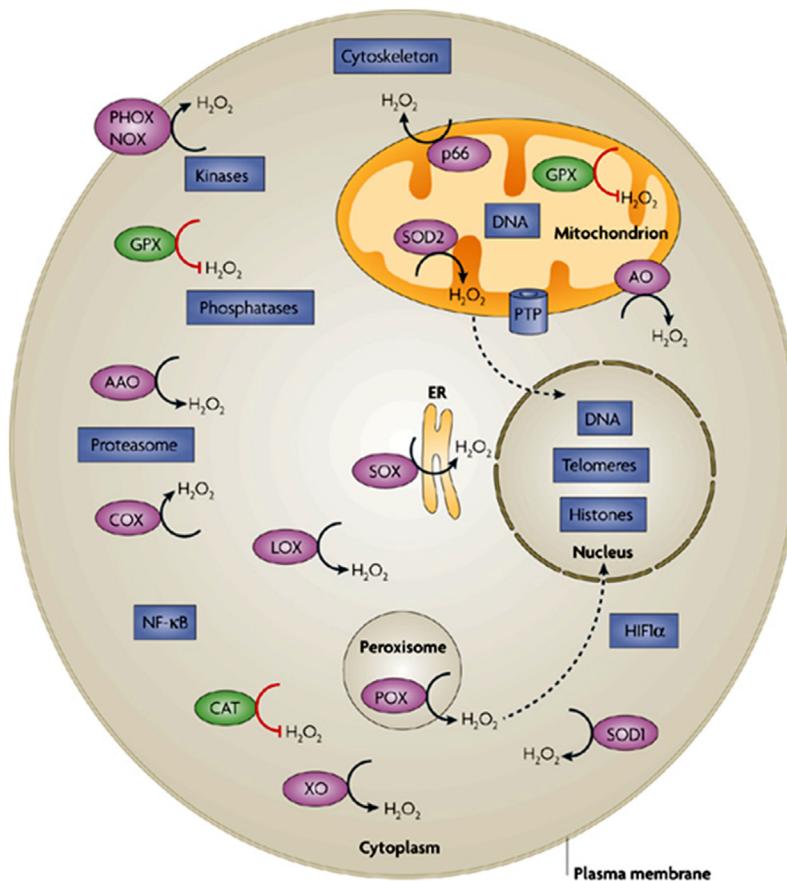
The mucous layer is composed of the glycocalyx of the epithelial cell surface and an additional layer of tear-specific mucoproteins (Fig. 4-29). Further tear mucins are secreted by the conjunctival goblet cells.

Mucus imparts viscosity to the tear film and is an inclusive term for the entire secretion from the goblet cells (i.e. glycoproteins, proteins and lipoproteins). Mucins are the glycoprotein components of mucus and vary greatly in molecular size (up to 50×10^6 kDa). The molecular structure of mucin has been likened to a bottle-brush, where the hairs of the brush are represented by multiple O-glycosylated short oligosaccharide chains on an elongated protein 'handle' containing many threonine and serine residues (Fig. 4-32). This structure has the capacity to form many interactions with other hydrophobic (lipid) as well as hydrophilic (protein) and charged molecules, thus forming mucus.

There are many mucins produced by cells of mucosal surfaces and there is some tissue specificity concerning the type of mucin on each surface. Mucins are classified

Moreover, it is likely that free radicals, and in particular H_2O_2 , involved in cell damage in various ways. They are not only a cause of acute damage and the stress response but also have a cumulative effect that may contribute to ageing. This may be more than simply a by-product of

damage: some of the mitochondrial production of H_2O_2 is generated by an enzyme involved in the apoptosis pathway which induces oxidative stress and may accelerate ageing (eFig. 4-11). Thus the genes that control production may have a say in determining lifespan.



eFIGURE 4-11 (1) H_2O_2 is produced by several enzymatic systems (indicated in purple) which generate H_2O_2 in different cellular compartments including phagocytic oxidases (PHOX) and NADP/H oxidases (NOX) in the plasma membrane; superoxide dismutases (SOD2), mitochondrial p66^{Shc} (p66) and amine oxidase (AO) in mitochondria; peroxisomal oxidases (POX) in peroxisomes; sulphhydryl oxidase (SOX) in the endoplasmic reticulum (ER); and amino acid oxidases (AAO); cyclooxygenase (COX), lipid oxygenase (LOX) and xanthine oxidase (XO) and superoxide dismutases (SOD1) in the cytosol. (2) H_2O_2 disrupts cellular homeostasis by targeting regulatory macromolecules (in blue) including actin, myosin, tubulin (cytoskeleton), different kinases, several Ser/Thr and Tyr phosphatases, the proteasome, the mitochondrial permeability transition pore (PTP), mitochondrial and nuclear DNA, transcription factors such as HIF1 or nuclear factor (NF)-B, and histones and telomeres. Catalase (Cat) and glutathione peroxidase (GPX) enzymes buffer H_2O_2 to avoid cumulative oxidative events. (From Giorgio et al., 2007.)

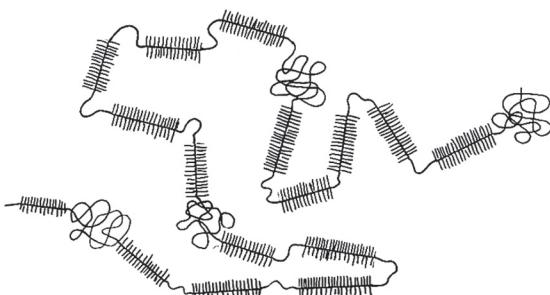


FIGURE 4-32 Bottle-brush mucin. (Courtesy of J Tiffany.)

according to whether they are secreted or membrane tethered: in tears, the main secreted mucins are produced by goblet cells (MUC5AC), by lacrimal acinar cells (MUC7) and by corneal and conjunctival epithelium. Thus, MUC5AC is secreted along with trefoil peptides TFF1 and TFF3. In contrast, MUC1, MUC4 and MUC16 and the sialomucin complex are associated with the glycocalyx and, although regarded as membrane mucins, they may be released into the aqueous phase of tears through activity of ADAM-TS-1 (a disintegrin-like metalloproteinase with thrombospondin type 1 motif) metalloproteases. The ocular surface membrane-tethered mucins are considered to have anti-adhesive properties, particular MUC16 for bacterial adherence. The importance of mucin in tears has been emphasized by the realization that tears are not predominantly aqueous but are probably a type of mucin gel, formed especially by MUC5AC.

An interesting physiological feature of the tear meniscus occurs at Marx's line, a lissamine green and fluorescein staining border of the tear meniscus at the mucocutaneous junction between the conjunctiva and the eyelid skin. Evaluation of this line is assuming greater importance in dry eye disease and it has been suggested that this line represents a zone of tear hyperosmolarity due to the relative immaturity of rapidly proliferating conjunctival epithelial cells, induced at this site by the mechanical forces generated by the repetitive action of eyelid closure. Such cells may produce less tear mucins such as MUC16, which weakens the barrier to penetration by dyes such as lissamine green.

Tear secretion is under psychoneuroendocrine control

The regulation of tear secretion is shown in Figure 4-33. Most of the control is through the autonomic

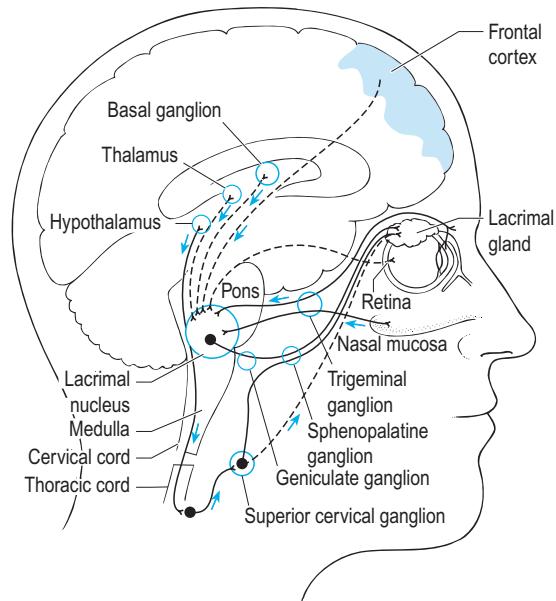


FIGURE 4-33 Neural control of tear secretion.

system and thus tear secretion is affected by instillation of drugs that modulate this system (e.g. pilocarpine, atropine). Apart from direct effects on the acinar cells (see above, intracellular second messengers), the parasympathetic system can markedly increase tear flow via its effects on the myoepithelial cells surrounding the acinar cells (see Ch. 1 and eFig. 4-13).

Hormonal control of tear secretion is well recognized but not clearly defined. Reduction in tear flow occurs in women after the menopause, while testosterone stimulates secretion of certain tear components, such as IgA.

THE CONJUNCTIVA

The conjunctiva is richly endowed with a variety of specialized cells that allow it to act as a base for and the source of many of the constituents of the tear film. These include immune cells (T and B cells, mast cells, dendritic cells; see Ch. 7) and specialized mucus-secreting goblet cells.

The epithelium is more than a simple covering layer for the conjunctiva

The conjunctival epithelium can be regarded as intermediate in type between the keratinized squamous

epithelium of skin and typical non-keratinized columnar mucosal epithelia, for instance of the respiratory and gastrointestinal tracts. However, it resembles the latter more closely and may require the multifunctional protein clusterin to inhibit keratin production. Clusterin is found in all body fluids including tears and on the surface of cells lining body cavities. It is involved in transport of lipoproteins, inhibiting complement-mediated lysis, and in modulation of cell–cell interactions.

Although the conjunctiva is non-keratinized, keratins are expressed by the conjunctival epithelium in a typical paired combination (K3/K12). However, there is much more K3/K12 in corneal than in conjunctival epithelium. Several other cytokeratins present in non-keratinized stratified (K4 and K13) or simple (K8 and K19) epithelia are also found.

The epithelium contains numerous innate immune cells (macrophages, mast cells, NK cells and dendritic cells) but also has a rich population of intraepithelial and stromal T cells organized to form the conjunctiva-associated lymphoid tissue (CALT), similar to the mucosa-associated lymphoid tissue (MALT) of other organs (see Chs 2 and 6). Even within the CALT, the distribution of lymphocyte subsets varies from tarsal to bulbar to fornical conjunctival regions (see Ch. 1). In addition to its function as a barrier to external organisms, the epithelium is a major source of tear mucins derived from the intraepithelial goblet cells, which, like the lacrimal gland, are under neuroendocrine control (see eFigs 4.12 and 4.13).



Additional content available at <https://expertconsult.sut.inkling.com/>.



The turnover and health of the epithelial and goblet cells are markedly dependent on vitamin A and the retinoids, and become abnormal in vitamin deficiencies, leading to a severe form of dry eye syndrome. In addition, certain accumulations of goblet cells occur, e.g. on the tarsal (lid) conjunctiva where they occur in crypts (Henle's crypts) and on the bulbar conjunctiva a few millimetres nasal of the limbus (Manz's glands).

In addition to goblet cells there are other types of conjunctival epithelium, distinguished by ultrastructural appearances: secretory epithelia appear in mature and immature forms depending on their content of secretory granules and the presence of a Golgi complex; in contrast, other cells with a high content of RER

and/or mitochondria are presumably involved in transport and epithelial regeneration.

The conjunctival stroma is highly vascular and contains aqueous veins

The stroma of the conjunctiva has a superficial 'lymphoid' layer and a deep layer containing a rich plexus of vessels, including lymphatic vessels, that acts as a watershed between the intraocular circulation and the external circulation of the eye and lids. Through these vessels waste materials from the anterior chamber of the eye are transported to the pre-auricular draining lymph nodes and venous drainage systems in the neck.

THE LIDS

The lids function to protect the cornea and adnexal structures and have a highly specialized structure that ensures they are properly apposed to the surface of the globe (see Ch. 1, p. 82). Indeed, defects in lid apposition, as occur in diseases such as trachoma where there is scarring and deformation of the lids, lead to significant corneal exposure, ulceration and blindness (see Ch. 9).

Closure of the lids leads to compression of the lipid layer of the tears such that it increases in thickness to about 1.0 mm at the lid margin. When the eyes open, the lipids are dispersed to form a lipid bilayer from the lower lid upwards as the eyelid opens, with the hydrophilic groups on the phospholipid molecules interacting with the aqueous compartment.

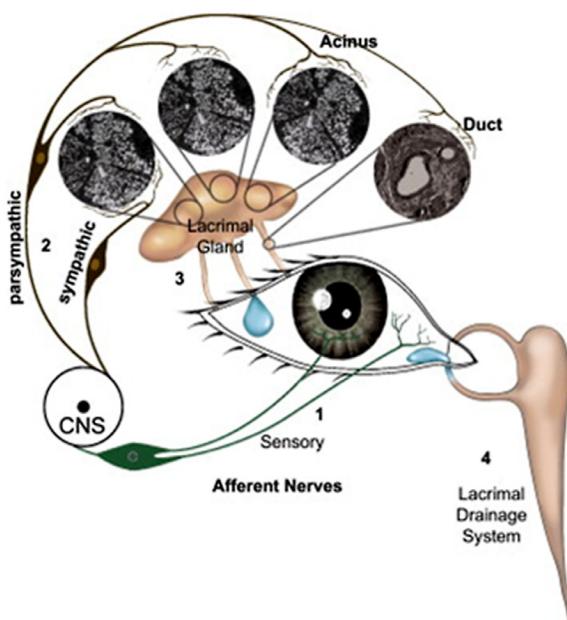
Control of lid movement is via the VII nerve for motor function and the V nerve for afferent input via mechanisms such as the blink reflex (see Ch. 1, pp. 77–78).

The lid also contains accessory lacrimal glands (glands of Krause in the fornix and glands of Wolfring in the tarsal lid margin), specialized mucus-secreting glands (they secrete a different type of mucin, rMUC4, important in eyelid opening during development, from that secreted by the goblet cells (rMUC5AC, see above) in the conjunctiva) and oil-secreting glands (meibomian glands) that contribute to the makeup of the tear film. Lashes and their hair follicles, as well as sweat glands, are also important specialized structures in the lid, where they function to protect the eye from foreign particles.

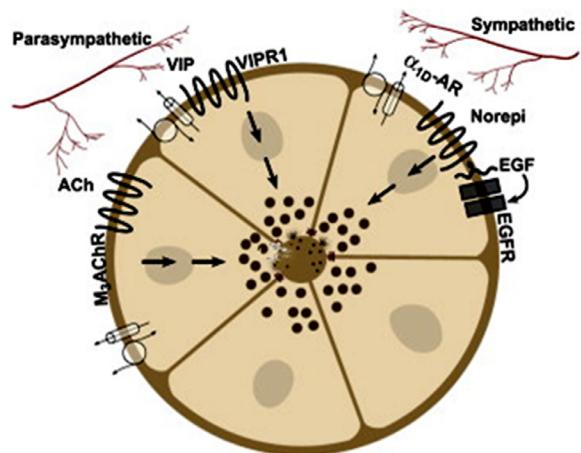
Tight neural control of lacrimal secretion is essential to healthy functioning of the tear film. The three major components of the aqueous secretion (water, electrolytes and proteins) are each secreted through different mechanisms, but there is overall neural regulation of the final secreted product so that a coordinated response can be achieved.

Sensory input comes via the trigeminal nerve endings in the cornea and conjunctivae to induce efferent parasympathetic and sympathetic nerves that innervate the lacrimal gland (eFig. 4-12) to release neurotransmitters which effect the release of the tear fluid (eFig. 4-13).

Tear secretion is dependent on several autonomic receptors and ligands apart from the more well-recognized adrenergic and cholinergic mediators (eFig. 4-13).



eFIGURE 4-12 The neural anatomy and physiology of lacrimal gland tear secretion. (From Dartt, 2009.)



eFIGURE 4-13 Release of acetylcholine occurs from parasympathetic nerve endings as well as vasoactive intestinal peptide (VIP) while noradrenalin is released by sympathetic nerves. Additional mediators such as EGF and α 1d-AR contribute to the overall response. (From Dartt, 2009)

Incomplete eyelid closure may be physiological during sleep. Control of eyelid closure is under both reflex supranuclear and learned/conditioned (cerebellar) control.

Cornea and sclera

The outer coat of the eye (cornea/sclera) is a tough non-compressible layer of connective tissue that can withstand considerable deformation and pressure.

THE CORNEA

The clear cornea functions as the main optical lens of the eye, focusing transmitted light rays onto the retina. Its cellular and extracellular matrix components are of the same basic chemical composition as other opaque tissues in the body which scatter, rather than transmit, light rays. Scattering of light in opaque tissues is the result of the large disparity in refractive index (RI) between matrix components such as collagen (RI = 1.55 in the dry state) and glycosaminoglycans (RI = 1.35). The cornea's ability to transmit light is, in essence, a function of how the cells and matrix components are organized within the tissue to reduce this RI disparity.

Corneal transparency is a function of its relative acellularity and matrix structure

The epithelium. The six-cell-thick stratified layer that is the corneal epithelium (see Ch. 1, p. 14) presents the first refracting interface to transmitted light. Most of the light-absorbing properties of the cornea take

place in this layer, mainly for short-wavelength light. However, the majority of light on the visible spectrum is transmitted through the epithelium.

The cells are typical keratin-expressing epithelial cells containing integrin receptors for basement membrane components such as fibronectin, laminin and collagen. Corneal epithelial cells express a particular combination of paired 55/64 kDa keratins (keratin 3/keratin 12). The 54 kDa protein may have a role in inflammatory eye disease, while the 64 kDa protein appears to be useful as a marker for differentiating cells of the central cornea from limbal stem cells (see below). Keratin 12 may be important in corneal epithelial junctions because K-12 knockout mice are prone to recurrent erosion (epithelial cell loss).

The cells are organized to present few interfaces, the most prominent being at the interface between the basal cells and the basement membrane. Hemidesmosomes affect the adhesion between these cells and the basement membrane. The hemidesmosome is bound to the anterior corneal stroma through a band of anchoring fibrils, which pass through the lamina densa of the basement membrane proper into a densely woven network of collagen fibres (see Box 4-6 and Fig. 4-20) known as Bowman's layer (see Ch. 1, p. 16) (Fig. 4-34). Bowman's layer is about 12 µm thick and composed of type VII collagen. In addition, trans-plasma membrane collagen type XVI supports firm adhesion in these basal cells.

The epithelium presents an effective barrier to fluid transport, which is achieved by extensive close contacts and tight junctional complexes eBox 4.2. The site

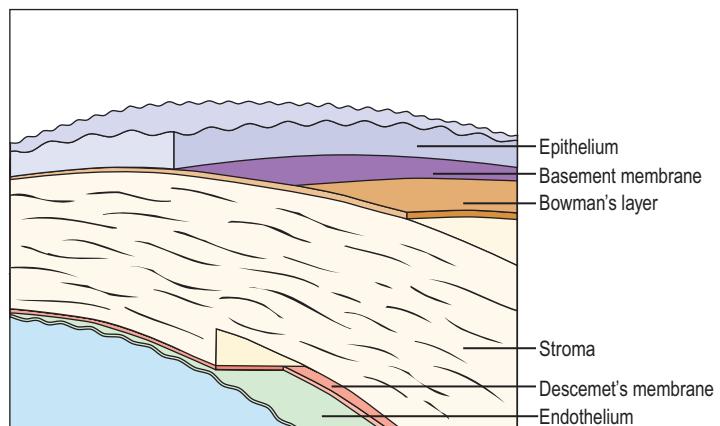


FIGURE 4-34 Diagram demonstrating the layers of the human cornea: the anterior basement membrane, Bowman's layer, the stroma, Descemet's membrane and the endothelium. (From Last et al., 2009.)

of the barrier is in the suprabasal epithelium and is mediated by high expression of the tight-junction protein claudin. Spot desmosomes are numerous and differences in the content of desmosomal proteins have been observed depending on the site. For instance, desmoglein and desmocollin are absent from basal limbal epithelial cells, which may have functional significance in their role as putative stem cells. Limbal stem cells arise from pouches or stem cell niches in the peripheral corneal epithelium.

Matrix factors affecting transparency: collagen. The stroma in the human cornea accounts for 90% of the corneal thickness and thus contributes most to the optical function of the tissue. Several different types of collagen are present in the cornea. In addition to normal basement membrane types IV and VIII collagens at the epithelial and endothelial cell layers, the two specialized corneal regions, Bowman's layer and Descemet's membrane, contain collagens not normally found in other matrices. Bowman's layer is a condensation of type I/VI with a high proportion of type III in a matrix containing chondroitin and dermatan sulphate, while Descemet's membrane contains high levels of novel collagens (types V, VIII, IX and XII) organized in a lattice arrangement. This provides elasticity and deformability to the cornea while maintaining high levels of light transmission. Descemet's membrane also imparts strength and resilience to the

corneal stroma and is the main resistance to normal intraocular pressure.

The orthogonal lamellar arrangement of stromal type I collagen (which accounts for 50–55% of stromal collagen; see Ch. 1) fibrils is considered to be important in determining corneal transparency. Transparency was initially attributed by Maurice (1957) to 'destructive interference' in which light is scattered by neighbouring fibrils in predictable and opposing directions, which tend to cancel each other out except along the primary visual axis. However, this concept cannot apply to light transmission in Bowman's layer where the fibrils are irregularly displayed, suggesting that the arrangement of the fibrils is less important than their size. An alternative view therefore is that significant light scatter does not occur within the cornea because the fibril diameter does not exceed 30 nm and the interfibrillar distance is around 55 nm. It is only when the distance between the regions of different refractive index becomes greater than 200 nm that light scatter occurs, as for instance when corneal swelling occurs. In one sense this is essentially a paraphrase of Maurice's theory because in both situations the critical factor is interfibrillar distance.

Type V (approximately 10%) and some type III (1–2%) collagen also exist in the corneal stroma, while the remainder is made up of type VI collagen. Types I, III and V collagen are fibrillar collagens, but type VI has large non-helical globular polypeptides at both the

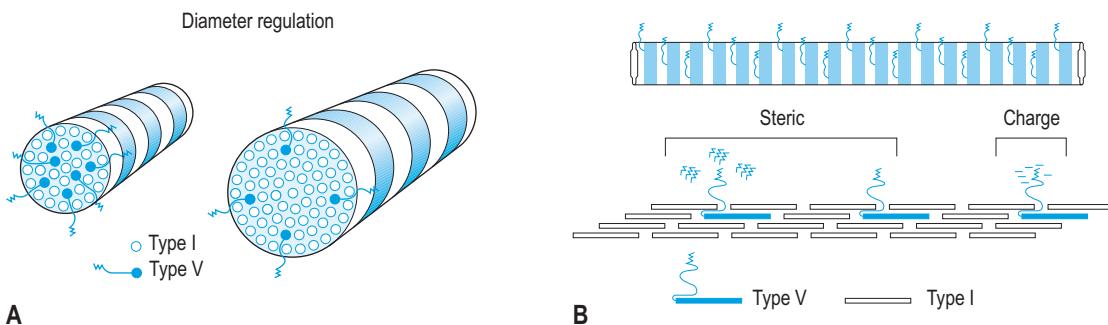


FIGURE 4-35 Model for type V regulation of collagen fibril diameter. Collagen fibrils within the cornea contain a high percentage of type V collagen and have small diameters (**A**), while fibrils in other tissues possessing low levels of type V collagen have a large diameter (**A**). The experimental reduction of type V collagen generates fibrils that have the characteristics of those found in tissues with low type V levels. A mechanism by which type V collagen may limit fibril growth is shown in (**B**). Type V N-terminal domains project onto the fibril surface, and when sufficient numbers have accumulated, they block further accretion of collagen monomers and thereby limit growth in diameter. The N-terminal domain is large and possesses a number of acidic residues, and so may affect this block using steric and/or electrostatic hindrance. (Courtesy of David E. Birk.)

C- and N-termini of the helical protein backbone (see Table 4-2). Collagen fibrils are heterotypic (i.e. they contain both type I and V collagen) and type V is thought to initiate fibril formation and regulate fibril thickness (Fig. 4-35). However, fibril thickness is also dependent on the nature of the stromal glycosaminoglycans and particularly on the specific proteoglycan (see below).

The collagen fibril lamellae are arranged in parallel, running at oblique angles to each other (see Ch. 1, p. 17). Apart from their small size, their uniformity of thickness is likely to be a major factor in light transmission. The parallel arrangement of the central corneal fibrils extends to the periphery where the fibrils adopt a concentric configuration to form a ‘weave’ at the limbus with some transversely running fibrils fusing with the circumferential collagen fibrils. More recently, it has been proposed that scleral collagen fibrils take a curved orientation across the peripheral cornea, thus acting as anchoring fibrils which flatten the peripheral cornea at this point (Fig. 4-36). This imparts considerable strength to the peripheral cornea and permits it to maintain its curvature and thus its optical properties.

Glycosaminoglycans. The corneal stroma is unusual in that it contains no hyaluronan, except at the limbus where there is a gradual increase in concentration towards the sclera. The major corneal glycosaminoglycan is keratan sulphate; in the central cornea non-sulphated chondroitin is also present, while towards the periphery chondroitin sulphate is the second major GAG. Chondroitin-4-sulphate and dermatan sulphate are almost identical (see Box 4-7) and many believe that the second major GAG is not chondroitin but dermatan sulphate.

Corneal GAGs exist in the native state as proteoglycans (PGs); four major forms exist in the cornea: decorin, lumican, keratocan and mimecan. Decorin- and lumican-deficient mice have reduced corneal transparency due to disorganization of fibril arrangement, while keratocan maintains overall corneal thickness and less so mimecan. Both corneal dermatan sulphate and keratan sulphate proteoglycans are considered to belong to the class of small non-aggregating PGs (small leucine-rich PGs known also as SLRs).

The critical region in proteoglycans is their linkage sites; for keratan sulphate the link is an *N*-glycosidic

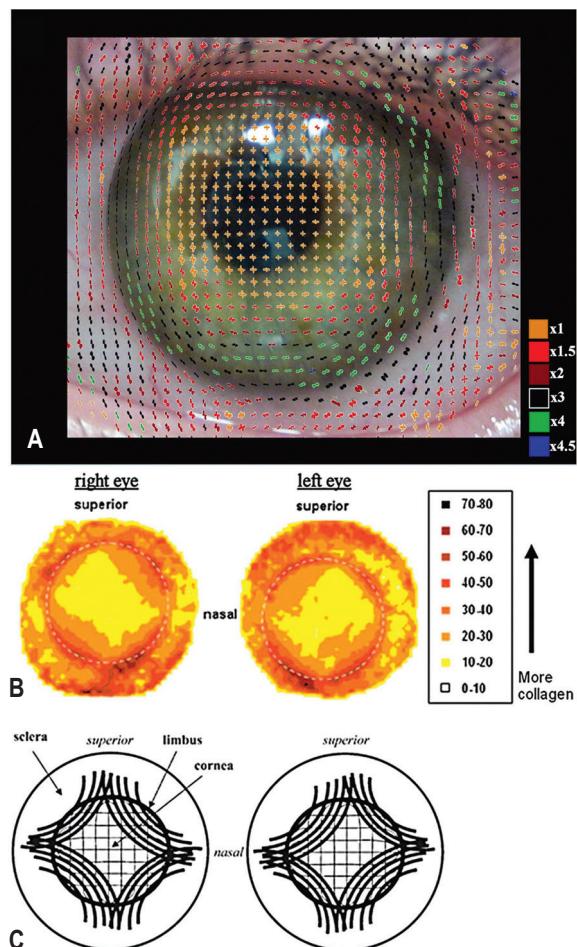


FIGURE 4-36 (A) X-ray diffraction data showing the preferred directions of stromal collagen lamellae across the human cornea, limbus and anterior sclera. The scale of the X-ray vector plots (indicated in the colour key) reflects the degree of collagen alignment. (B) Maps of X-ray scatter intensity showing the distribution of preferentially aligned lamellae in a pair of eyes from the same human donor. The limbus is denoted by a broken line. Note the symmetry between left and right eyes. (C) Theoretical model showing the net course of lamellae and based on the data shown in A and B. Lamellae are thought to change direction in the corneal periphery by a process of splitting and interweaving. (Figure modified from Meek and Boote, 2009.)

bond between *N*-acetylglicosamine and asparagine in the core protein. The terminal sites of the branched oligosaccharide structures contain fucose or mannose, while chondroitin sulphate contains xylose residues.

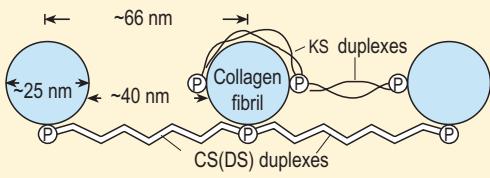
The interaction between proteoglycans and collagen fibrils has been elegantly demonstrated using cupromeronic blue and MgCl₂ at a tightly controlled

(3.0 mM) concentration (the ‘critical electrolyte concentration’). Both dermatan sulphate proteoglycan and keratan sulphate proteoglycan bind to the collagen arrays at specific binding sites (one proteoglycan to one binding site), suggesting that these sites are essential to the spacing of the fibrils and to the thickness of the interfibrillar space. Keratan sulphate proteoglycans appear to bind to the step regions of the fibrils while dermatan sulphate proteoglycans bind to the gap (Box 4-11).

BOX 4-11 ORGANIZATION OF TISSUE

PROTEOGLYCANS

The organization of proteoglycans in the collagen matrix is probably as shown below: KS-PG (keratan sulphate proteoglycan) double complexes maintain the lateral interfibrillar distance, while CS-PGs (chondroitin sulphate proteoglycans) regulate the overall longitudinal arrangement of the fibrils by spanning three fibrils (**A**). Proteoglycans bind directly to the collagen fibrils, probably through the ‘minor’ collagens such as V or VI that co-distribute with the type I fibrils and have non-collagenous polypeptide domains to interact with the proteoglycans (**A** and **B**).

**A****B**

(Figure courtesy of J Scott and IRL Press.)

Different binding ‘maps’ occur in different species, such as the mouse. Therefore, several variations on the theme of proteoglycan–collagen interaction appear to be compatible with transparency. However, SLRs appear to be essential. Studies in human tissue developed this model to reveal a regular hexagonal arrangement of six proteoglycans per collagen fibril interacting with the ‘next but one’ fibril (Fig. 4-37).

Swelling pressure versus hydration. The cornea is about 80% hydrated. This is higher than other tissues such as the sclera, which is about 70% hydrated. Despite this, the corneal stroma ‘imbibes’ water if it is placed in a solution of saline (this is well demonstrated by the injured, lacerated cornea which swells and becomes opaque); the water-attracting ‘hygroscopic’ properties of the cornea are the result of its high content of GAGs. The cornea has been described as ‘a

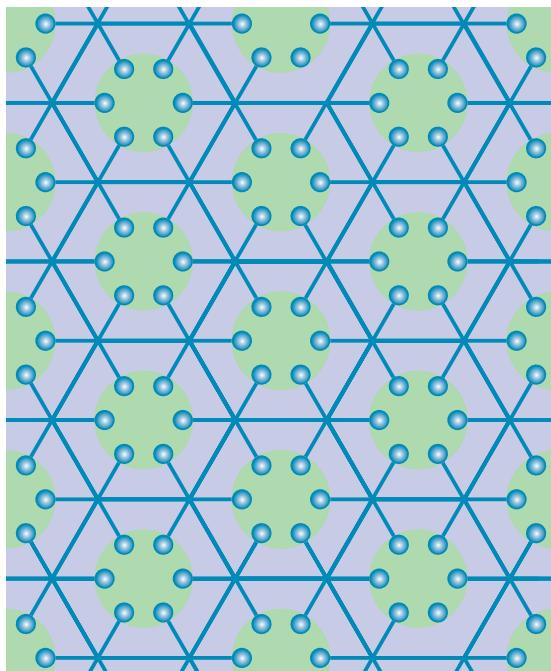


FIGURE 4-37 Schematic visualization of the basic components of a new model for the corneal collagen lattice. At equidistant sites along their circumference, six core proteins of proteoglycans are attached to the hexagonally arranged collagen fibrils. GAGs of the proteoglycans as stained by CB are connecting their next nearest neighbour collagen fibrils and form a ring-like structure around each collagen fibril. (From Muller et al., 2004, with permission from Elsevier.)

slice of water stabilized in three dimensions by a meshwork of fibrils and soluble polymers'. The cornea thus has a swelling pressure and a metabolic pump (the endothelium) designed to maintain it. The swelling pressure generates a level of interfibrillar tension and this may be the biophysical mechanism whereby the fibrils are maintained in their normal arrangement. In addition, the swelling pressure itself may reciprocally activate chloride channels and other transporters that maintain a balance of excess ions in the aqueous humour.

The swelling pressure of the cornea is an important clinical concept and attempts have been made to measure swelling pressure using instruments such as the Ocular Response Analyzer® (ORA), an instrument similar to an applanation tonometer used for measuring intraocular pressure. The ORA measures corneal hysteresis, which is a function of biomechanical strength of the corneal tissue related to the viscosity of the matrix and its response to deformation. However, whether the ORA truly measures hysteresis independently of ocular pressure is still unclear, despite its considerable relevance for corneal refractive surgical procedures.

Cellular factors affecting transparency: (1) keratocytes. Corneal fibroblasts (keratocytes) are important in maintaining transparency because they are the source of stromal collagens and proteoglycans. Although most of the changes that occur in the assembly of the matrix are post-translational, the enzymes that promote these changes are present in the keratocytes, in which the essential specific genes have been induced (specific enzyme defects are associated with corneal opacification as in the mucopolysaccharidoses). Keratocyte dysfunction may also underlie the corneal haze seen after corneal refractive surgery.

Collagen turnover in early postnatal life is about 24–50 hours, but there is little information on adult collagen metabolism. For both collagen and GAGs, studies on cultured keratocytes have not been very informative because these cells produce a range of GAGs not found *in vivo*. In contrast, organ cultures of cornea produce a panel of GAGs more akin to that found *in vivo*. The preferential production of KS-PG to CS/DS-PG (dermatan sulphate proteoglycan) in corneal cells from different species has been attributed

to the relatively hypoxic conditions and to anaerobic glycolysis, which favours the former. In rabbit cornea, the development of transparency correlates with a dramatic increase in the concentration of KS-PG in the early postnatal period.

Keratocytes also express a range of corneal 'crystallins', so called because they are thought to play a role in light transparency by reducing light scatter and not because of homology to lens crystallins (see below). Corneal crystallins include trans-ketolase and aldehyde dehydrogenase and expression varies from species to species.

Cellular factors affecting transparency: (2) leucocytes. The cornea also contains a population of resident stromal and intraepithelial leucocytes which are more frequently found in the peripheral cornea (see Chs 1 and 7). These cells include macrophages and dendritic cells which are thought to be important in the maintenance of ocular immune privilege (see Ch. 7), as well as rare haemopoietic stem cells. These are distinct from limbal corneal stem cells of mesenchymal origin, which reside in epithelial stem cell niches (see below).

Cellular factors affecting transparency: (3) the endothelium. The endothelial pump determines the level of hydration of the GAGs and thus transparency. Despite the greater than normal level of hydration of the corneal stroma, its water binding is unsaturated, a condition achieved by an endothelial pump (Box 4-12), which transports water out of the cornea towards the anterior chamber. This is known as the pump-leak mechanism and is an energy-driven ATPase-dependent mechanism (see p. 208). Transport of Cl^- ion in this model probably depends on the cystic fibrosis transmembrane conductance regulator (CFTR), a Cl^- channel which is expressed in many fluid transporting epithelia in the body.

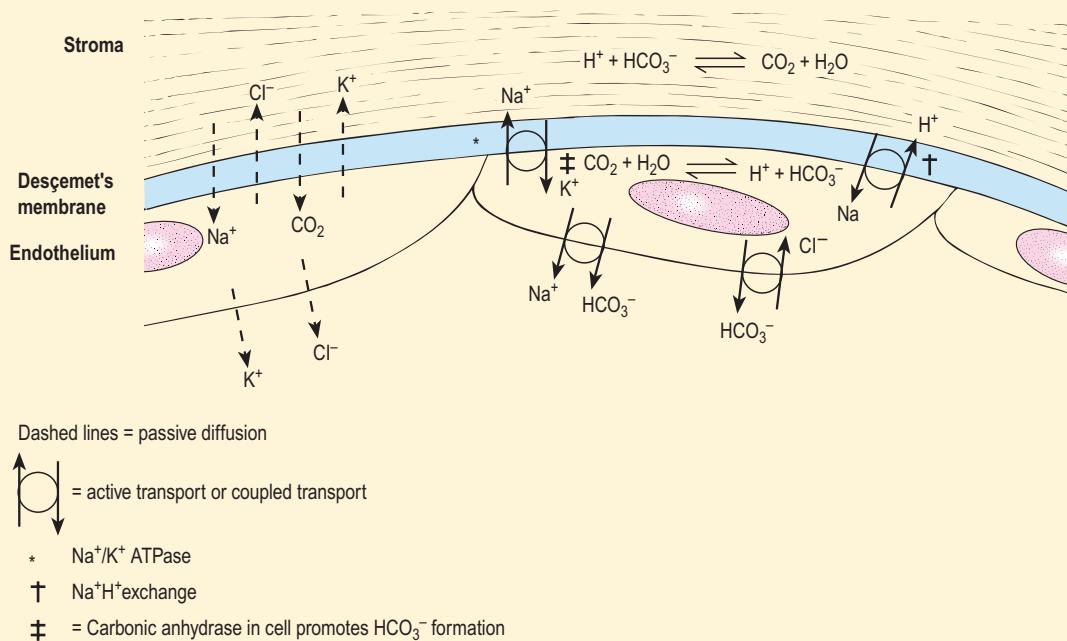
Other mechanisms may be also be operative in the transport of fluid across the endothelium. These include an Na^+/H^+ exchanger protein which drives an electrogenic coupling between Na^+ and HCO_3^- ions. This 'antiport' is essential for maintenance of the intracellular pH by exchanging Na^+ (in) with H^+ (out) in the cell (Box 4-12). This may be one component of the electro-osmosis concept for fluid transport in the

BOX 4-12 CORNEAL ENDOTHELIAL CELL PUMP

The corneal endothelium transports water out of the stroma by an ATP-driven ion pump mechanism.

Na^+ and HCO_3^- are transported across the endothelium from the stroma to the aqueous, mediated by a Na^+/K^+ -dependent ATPase and a HCO_3^- -dependent ATPase, probably involving carbonic anhydrase. The Na^+/K^+ ATPase is

located in the plasma membranes but the HCO_3^- -dependent ATPase is in the mitochondria, where its major role in ion transport may be to generate the ATP required for the Na^+/K^+ ATPase. Carbonic anhydrase may also be involved in the Na^+/H^+ antiport for maintaining intracellular pH.



cornea. In addition, the corneal endothelial cell membrane contains a water transporter/channel, namely aquaporin 1, which is involved in bulk transport of water molecules (see below under Lens) and perhaps also in CO_2 transport. This, however, seems to be mainly a passive transporter, responding to sudden changes in stromal water content and endothelial cell volume rather than having a constitutive physiological role.

Recently, a metabolic component to fluid transport across the cornea has been suggested based on the high content of lactate in the stroma and the steep gradient towards the anterior chamber. A bulk movement of lactate and water could be facilitated via transcellular monocarboxylate co-transporters in the endothelium.

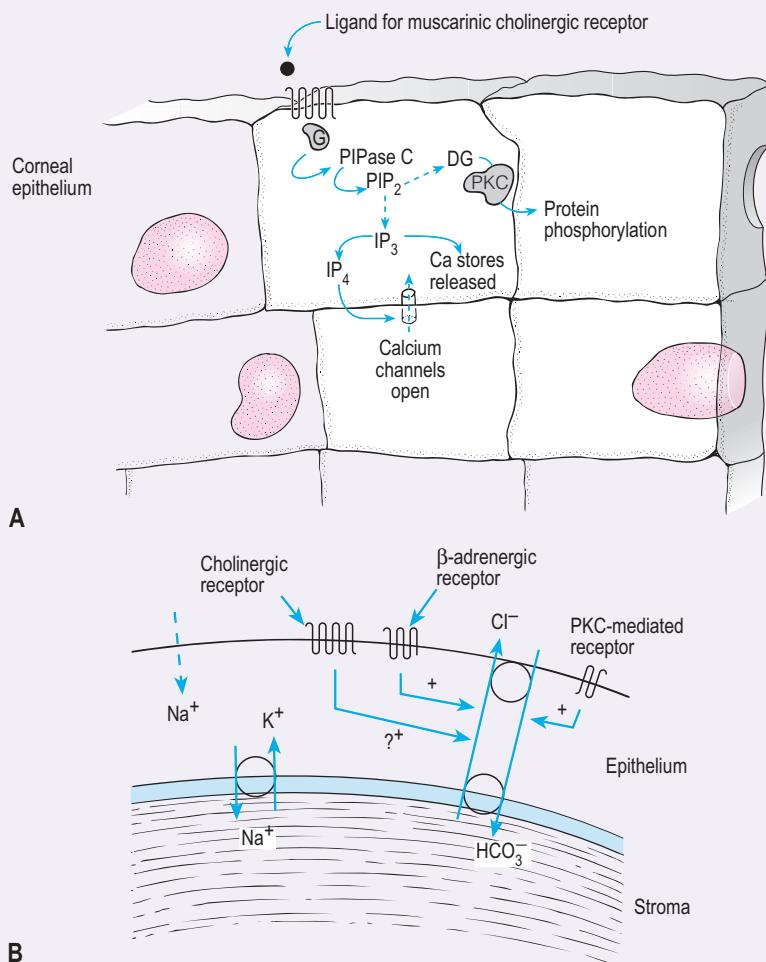
Transport of water out of the cornea is a dual process. In addition to the transport of water from the stroma to the aqueous humour, there is a net flux of ions and water towards the epithelium and the tears. For instance, there is a Cl^- pump which appears to be modulated by several receptors including β -adrenergic and serotonergic receptors coupled to the adenylate cyclase and Ca^{2+} second messenger systems, and receptors that involve protein kinase C. Dopamine and α -adrenergic receptors may also be involved in Cl^- transport, but importantly many of these receptor systems are coupled to the CFTR Cl^- channel expressed in corneal and conjunctival epithelia (see eBox 4.3).

Ion transport into the cornea mediated by Na^+/K^+ ATPase and $\text{Ca}^{2+}/\text{Mg}^{2+}$ ATPase in the basolateral plasma

eBox 4-3**Agonist receptors in the corneal epithelium**

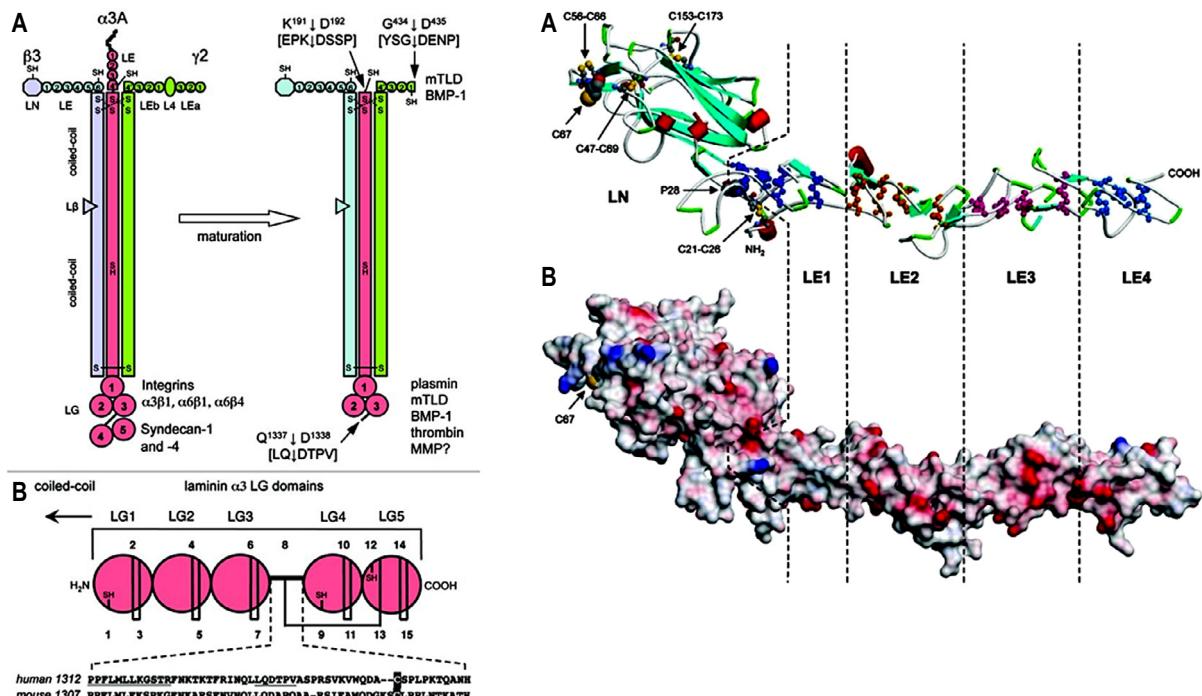
Adrenergic and cholinergic receptors are present in the corneal epithelium and are coupled to a variety of second messenger systems including cAMP, phosphatidyl inositol phosphate 2 (PIP₂), and protein kinase C (PKC) (A). They are involved in the transport of fluid out of the cornea in an anterior direction by enhancing the function of the HCO₃⁻/Cl⁻ pump (B).

Active pumps for outward extrusion of Cl⁻ (activated by β-adrenergic and other receptors) and inward transport of Na⁺ and HCO₃⁻ are present. Dashed line, passive transport; circles, coupled/active transport. There is also a separate water transporter, aquaporin 5, in the corneal epithelium, which is important for the maintenance of normal corneal stromal thickness.



Laminin-332 (eFig. 4-6) is specific to the epithelial basement membrane and is central to epithelial sheets bound to mesenchyme which have to withstand external forces as in the skin and other mucosal layers. Matrix metalloproteinases are involved in post-translational processing of laminins as they incorporate themselves into the basement membrane to form anchoring adhesions and reciprocally, MMPs (MMP-3, -12, -19 and -20) are activated by laminin

degradation products to process the $\gamma 2$ chain and induce epithelial migration during wounding. Laminin in the basement membrane is tightly bound to nidogen (entactin), a protein that mediates the binding of laminin to heparin-containing proteoglycans (heparin sulphate proteoglycan) such as perlecan and syndecan in the basement membrane matrix.



eFIGURE 4-6 Structure of human laminin. Human laminin is known as laminin 332 on the basis of its three subunit chains ($\alpha 3A$, $\beta 3$ and $\gamma 2$). Each chain is composed of different domains that are indicated in (A). An odd number of cysteine groups allows SH bonds to form in the L domains after maturation of the molecule. The first three LG domains (LG1–3) interact with $\alpha 3\beta 1$, $\alpha 6\beta 1$ and $\alpha 6\beta 4$ integrins while the last two (LG4,5) contain binding sites for syndecan-1 and -4. Newly synthesized laminin 332 undergoes maturation by proteolytic processing at the $\alpha 3A$ chain N- and C-terminus as well as at the $\gamma 2$ chain N-terminal extremity. A schematic structure of laminin domains is shown in (B), with numbered cysteine groups and the disulphide bridges.

membrane of the epithelium is also operational in corneal ion shifts. Na^+ movement from the tears into the epithelium is by passive diffusion down a concentration gradient, but from the epithelium into the stroma active transport is required via the Na^+/K^+ ATPase to which Cl^- transport in the opposite direction is coupled, again by CFTR channels. This transport system is sensitive to the eicosanoid metabolite 12(R)HETE (compound C) (see Box 4-10). In addition, this electrolyte flux across the epithelium accounts for the electrical potential difference from (−) outside to (+) inside the cornea of ~25–40 μV .

Optical factors affecting image formation. The curvature of the cornea plays a major part in refracting and focusing light to produce an image on the retina. Even with a completely clear cornea, the image can be distorted by abnormalities in curvature, producing the various forms of regular and irregular astigmatism. A certain degree of astigmatism is present in many eyes since the corneal curvature is rarely perfectly spherical. These account for some refractive errors which are common in the healthy population. Refractive errors can also be caused by variations in the overall ocular dimensions of the eye.

Additional content available at <https://expertconsult.inkling.com/>.

Severe degrees of astigmatism can be caused by disease of the cornea such as keratoconus ('cone-shaped cornea'), and the late effects of scarring from wounds such as corneal incisions following cataract surgery, when it can be very difficult to restore preoperative curvature. While these conditions do not directly affect the transparency of the cornea, they can increase the amount of spherical and chromatic aberration, and diffraction, thus degrading the image.

Metabolism of corneal cells

Oxidative metabolism and glucose utilization. The epithelium takes up most of its glucose from the stroma and converts it to glucose-6-phosphate, after which 85% is metabolized via the glycolytic pathway to pyruvate. The bulk of this is then metabolized to lactic acid, but some is diverted into the citric acid cycle to produce ATP as an energy store. The pentose phosphate pathway accounts for the remainder of

glucose utilization by the epithelium, producing an important resource for free radical control, namely NADPH (see Fig. 4-23). This is the main mechanism of generation of reducing agents such as glutathione and ascorbic acid. The corneal epithelium has also developed a unique nuclear ferritin-based mechanism to minimize DNA damage from ultraviolet (UV) light-induced free radical damage. Lactoferrin in tears may also assist in this process.

The metabolism of keratocytes is mostly concerned with generating sufficient energy to produce and maintain stromal components (see above). In the steady state, these cells are not highly metabolically active and there is a high stromal lactate production, possibly contributing to fluid transport across the endothelium (see above).

The endothelium has large energy requirements to sustain its ATPase-dependent pump mechanism and is about five times as active as the epithelium. The major metabolic pathway in the endothelium is anaerobic glycolysis, with the citric acid cycle and the pentose phosphate pathway also playing a significant role.

Oxygen handling by the cornea. The epithelium obtains its oxygen from the preocular tear film at a rate of 3.5–4.0 μL per cm^2 per hour. The endothelium, however, and the keratocytes in the deep stroma receive their oxygen supply from the circulation via the aqueous humour. Corneal function and health are therefore dependent on local conditions at the surface of the eye and on systemic factors such as cardiopulmonary capacity.

Oxygen is consumed in the citric acid cycle, generating 36–38 molecules of ATP per molecule of glucose. The utilization of the citric acid cycle versus the glycolytic pathway is determined by the energy demands of the tissue, specifically the need for ATP. Thus the endothelium makes greater use of the citric acid cycle than the epithelium. In addition, oxygen consumption by the cornea increases almost twofold when acidosis prevails, as occurs in contact lens wear. This is in part the result of the activation of pH regulatory mechanism, including Na^+/H^+ exchange, which then stimulates Na^+/K^+ ATPase activity.

As discussed above, excess oxygen can be detrimental to the organism if it is converted to the

Briefly, a ‘normal-sized’ eye, anteroposterior dimension 22–24 mm, is termed an emmetropic eye and is one in which the image is focused sharply on the retina by the combined refracting properties of the cornea and the lens. Myopia or short-sightedness occurs in eyes with above average length and the image therefore is focused in front of the retina; conversely, long-sighted eyes have a shorter than normal length and the image is focused behind the retina. Myopia can be corrected by placing a convex lens in front of the cornea, which thus causes some divergence of the light rays and with the correct lens, the image can be formed on the retina correctly. Myopia can also be corrected by ‘reshaping’ the cornea (see p. 213) to cause some degree of ‘flattening’ of its curvature and thus reduce its refractive power. Conversely, hyperopia can be corrected using a convex lens and similarly corneal surgery can be performed to correct hyperopia, although it is generally less successful. Further information on refraction with links to optometry literature is given in Chapter 5 (see eFig. 5-4).

superoxide radical and then to hydrogen peroxide (see Box 4-8 and Fig. 4-26). In both the epithelium and the endothelium, redox systems involving glutathione and its two enzymes (glutathione reductase and glutathione peroxidase) depend on the generation of NADPH and thus on a supply of glucose. When the intracellular levels of glutathione are reduced in the cornea by one-third, the clarity of the cornea and its ability to pump fluid decline dramatically.

The aqueous contains high levels of H₂O₂, perhaps by virtue of the reduction of oxygen in the aqueous via ascorbate usage. Free radical damage to the corneal endothelium induces apoptosis and thus may account for the progressive endothelial cell loss associated with age.

Free radical damage to the cornea can also be induced by therapeutic interventions such as that incurred during phacoemulsification for cataract and by the use of UV radiation to perform corneal collagen cross-linking as part of refractive surgery (see p. 213).

Several receptors for neurotransmitters and other agonists are present in the corneal epithelium. These are probably involved in nutrient handling and regulation. In addition, since the cornea is richly innervated, they probably contribute to a healthy nutritive neurogenic environment, preventing neuropathic keratopathy.

Effects of contact lens wear on corneal physiology. Contact lenses are common optical devices which correct the refractive errors of the eye to achieve emmetropia. The tear film bathes both surfaces of the contact lens (Fig. 4-38), but corneal epithelial function is still relatively compromised. The corneal epithelium layer receives its oxygen from the tears and its glucose from the circulation via the aqueous and the limbal vessels (see above). Contact lenses reduce the direct availability of oxygen to the epithelium, thus shifting the balance from aerobic to anaerobic metabolism. The already high lactate levels in the cornea are doubled with contact lens wear and carbon dioxide production is increased. The induced acidosis has a direct effect on stromal hydration by impairing deturgescence mechanisms (see above).

Hard (rigid) contact lenses are usually made from polymethylmethacrylate (PMMA) and have the greatest effect on corneal function; in addition to restricting oxygen availability, hard lenses deplete glycogen

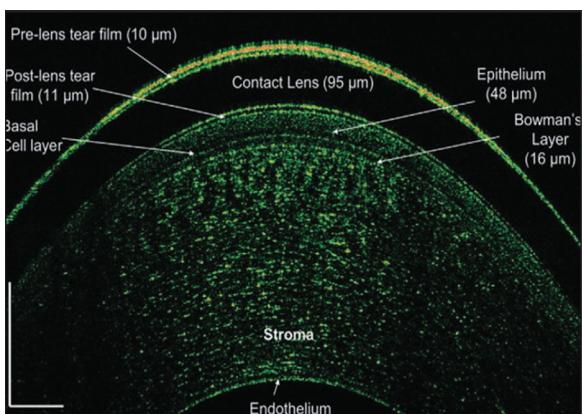


FIGURE 4-38 Ultra-high resolution optical coherence tomography image of the central cornea with a PureVision (Bausch & Lomb, Rochester, NY) lens after instillation of artificial tears. The central cornea was imaged with 6 mm scan on the horizontal meridian. The image was taken immediately after lens insertion and instillation of one drop of artificial tears. The epithelium, including the basal cell layer, and Bowman's layer, are evident in addition to the pre-lens and post-lens tear films. Total corneal thickness was measured at 526 μm. Bars = 250 μm. (Reprinted with permission from Wang J, Jiao S, Ruggeri M, Shousha MA, Chen Q. In situ visualization of tears on contact lens using ultra-high resolution optical coherence tomography. *Eye & Contact Lens* 2009;2:44–49.) (Wang, 2011)

stores, even though the level of glucose availability is not reduced. It has been suggested that hard lens-induced inhibition of aerobic enzymes such as hexokinase reduces direct glucose utilization by the cornea. Prolonged wear of hard contact lenses is therefore not possible, owing to the damaging effect on corneal transparency induced by the disturbed metabolism. Soft contact lenses are made from polymers of hydroxyethyl methacrylate (HEMA), poly(HEMA/vinylpyrrolidones), silicone or other similar materials, and permit extended wear of the lens owing to their permeability to oxygen and carbon dioxide. However, there is still some degree of lactate accumulation with soft lenses and prolonged use appears to affect the function of the endothelium. HEMA-based lenses are hydrophilic, while silicone-based lenses are hydrophobic: accordingly, there is less protein deposition on the latter but greater levels of denatured proteins. However, corneal inflammatory episodes are if anything more frequent with silicone lenses. Manufacturers of contact lenses continually produce new ‘biomimetic’-type lenses with increasing water content (up to 59%) in attempts to support normal corneal physiology.



(hydrogel lenses). In addition, incorporating other material into contact lenses such as polyethylene glycol and cross-linked hyaluronan are other possibilities under consideration to improve biocompatibility.

A popular compromise in contact lens type is the gas-permeable rigid lens, which combines the reduced toxicity of PMMA with high gas-transfer capability. The wide variety of lens types and materials has led to their being characterized on the basis of their oxygen flux, defined as the *DK* value:

$$\text{Oxygen flux} = DK/L \times \Delta P$$

where *D* is the diffusion coefficient, *K* is the solubility, and *L* is the thickness of the lens material. ΔP is the change in the partial pressure of oxygen across the material. HEMA and PMMA have a low oxygen flux, while hydrogels and silicones have a high flux. Both the thickness of the lens and the *DK* value determine its suitability for use in terms of its gas permeability. The actual amount of oxygen that reaches the cornea is the most important factor in the design of a contact lens, and most practitioners describe contact lenses in terms of their equivalent oxygen performance (EOP).

Contact lenses may have deleterious effects on the epithelium, causing thinning, reduction in the hemidesmosome density and the number of anchoring fibrils, and reduced adhesion of the epithelium to the basement membrane. This may be a direct effect of low O₂-transmitting lenses on basal epithelial cell proliferation. This is especially true of extended-wear hydrogel lenses. In severe cases, excessive use of contact lenses produces epithelial oedema and keratopathy in the form of punctate epithelial erosions. Rigid contact lenses also produce tear film instability by causing damage to the epithelium and the mucin layer in particular.

Contact lens wear may also induce changes in the corneal stroma (thickening) and the endothelium (polymegathism).

Cell turnover and wound healing in the cornea

The epithelium. The epithelium is constantly being regenerated by mitotic activity in the basal layer of cells. However, after epithelial abrasion, the initial response of the basal epithelium at the edge of the defect is to migrate as a flattened sheet of single-cell thickness across the stroma. These cells adhere by forming hemidesmosomes and intercellular contacts

and more superficially placed cells tumble over the adherent cells into the available space, leading to rapid filling of the epithelial defect (see Video 4-1).

Additional content available at <https://expertconsult.inkling.com/>.

Further proliferation of the basal cells, combined with reorientation of nerve endings into the wound edge, leads to differentiation of the newly divided cells into the five- to seven-cell layered mature epithelium. Closure of corneal epithelial defects occurs in a predictable manner which can be visualized clinically using fluorescein staining of the cornea; here, the initial wound edges, in geometric patterns are filled in and meet in the centre of the cornea.

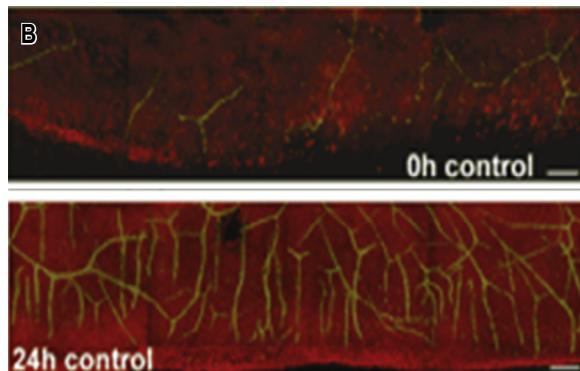
Migration of epithelial cells is achieved by cytoskeletal and cell-shape changes involving redistribution of actin-myosin fibrils and changes in actin-binding proteins (e.g. fodrin cell adhesion molecules such as E-cadherin), under genetic regulation via growth factors. Migration of the cells also depends on outside-in intracellular signalling via matrix components such as fibronectin/fibrin, laminin and collagen peptides through cell surface integrins (see pp. 178–183). The role of fibronectin/fibrin in corneal epithelial resurfacing may be to facilitate healing where the normal basement membrane and, in particular, its laminin component have been lost but are not essential for wound healing.

Adhesion of epithelium to the basement membrane and Bowman's layer is normally mediated via hemidesmosomes, the lamina densa and the anchoring type VII collagen fibrils (see Fig. 4-20). However, while hemidesmosomes form during the early stages of re-epithelialization (18 hours), many days elapse before anchoring fibrils reappear, and many months pass before full ultrastructural integrity is restored. This may explain in part the phenomenon of recurrent erosion where there has been damage to Bowman's superficial stromal layers of the cornea. Proteolytic activity in repairing epithelial defects is also important – both urokinase-type plasminogen activator and matrix metalloproteinases have been implicated.

Limbal corneal stem cells. The above process describes corneal epithelial repair after abrasion. However, the epithelium is constantly being renewed (in the mouse the entire corneal epithelium is renewed in 7 days) under the control of stem cells located in



This has been visualized directly in cultures of corneal epithelium (see [Video 4-1](#)) in which superficial epithelial cells appear to rush to fill the defect ahead of the basal more slowly centrally migrating epithelial cells. In addition, the differentiation of the corneal epithelium into a multilayered structure after wounding requires neural input from the corneal nerve endings which orient themselves perpendicular to the wound edge after the initial single layer of cells has closed the defect and are probably required to promote full differentiation of the six to ten layers of cells ([eFig. 4-14](#)). Thus, in conditions of neuropathy or when the nerves fail to extend their axons, as after herpes infections of the cornea, full epithelial healing frequently fails and recurrent breakdown of the defect occurs.



eFIGURE 4-14 (A) Time-lapse video sequence of corneal epithelial wound healing showing ‘tumbling’ of superficial cells to fill the defect; (B) orientation of regenerating nerve endings towards the wound edge (dark area in lower section) as the wound closes. (From [Song et al., 2004](#).)

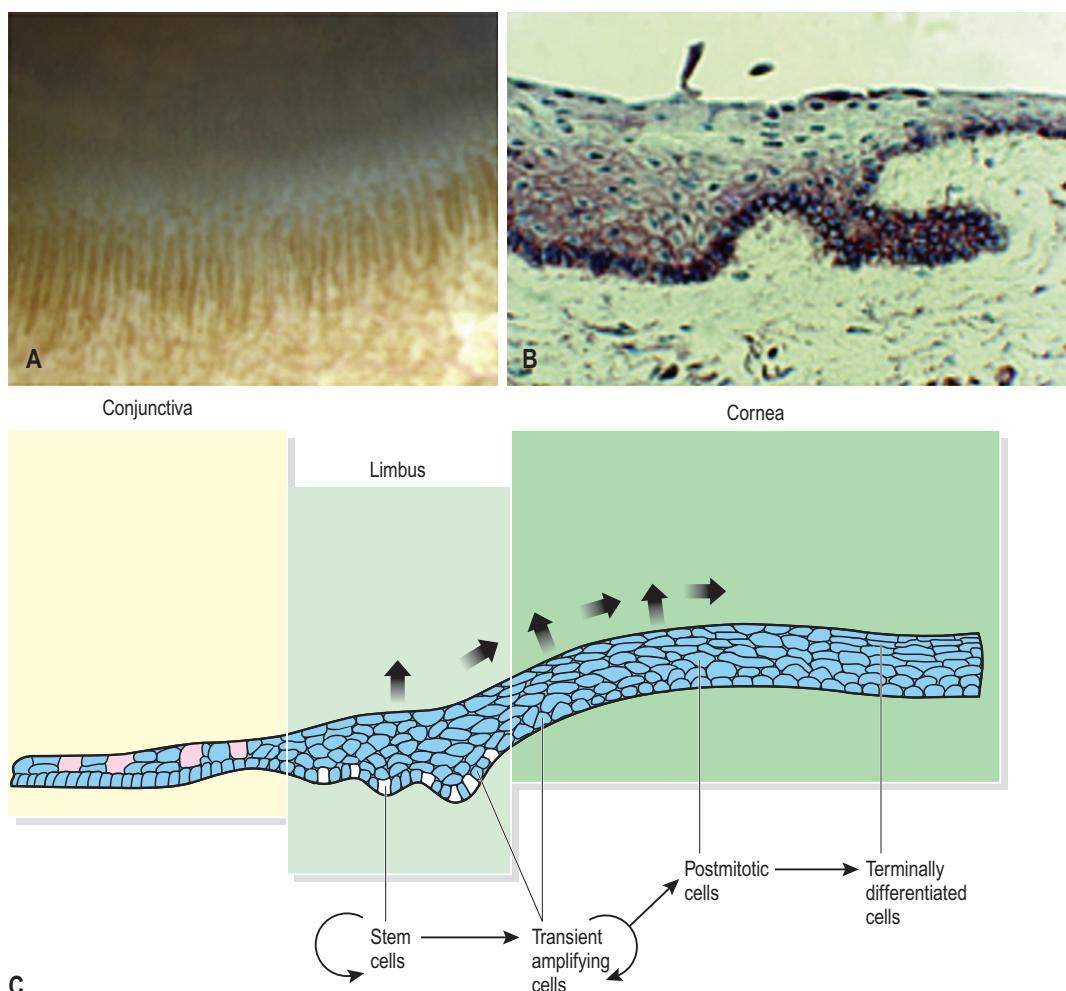


FIGURE 4-39 Concept of limbal location of corneal stem cells and transient amplifying cells. Stem cells (white) are exclusively located in the basal limbal epithelium at the bottom of the epithelial papillae forming the palisades of Vogt. Transient amplifying cells occur in the basal epithelia of limbus and peripheral cornea. Postmitotic and terminally differentiated cells make up the suprabasal and superficial layers. (From Schlötzer-Schreiber and Kruse, 2005, with permission from Elsevier.)

an epithelial ‘stem cell niche’ at the limbus in the palisades of Vogt (Fig. 4-39). While some renewal of epithelial cells takes place from the basal layer to the surface throughout the cornea, the main source of cell renewal is limbal stem cells. Patients who have severe damage to the limbal region of the cornea, as for instance with chemical burns, suffer from stem cell deficiency and are prone to conjunctivization of their cornea in which the conjunctival epithelium migrates onto the cornea to fill the defect, bringing with it stromal tissue and blood vessels. The outcome is

corneal opacification and blindness. Attempts to promote corneal epithelial wound healing using growth-promoting agents such as epidermal growth factor and retinoic acid have not met with great success.

The stroma. Incisional wounds of the cornea that involve the stroma may be accidental or intentional. The immediate effect is to cause wound gape and imbibition of water from the tears by corneal GAGs (see p. 186). This causes localized opacification (light scatter) and initiates a series of events in the

cornea directed at closing the wound. These include deposition of fibrin within the wound, rapid epithelialization of the wound incision, and activation of the keratocytes to divide and synthesize collagen and GAGs. During the early phase of corneal wound healing, there is loss of specialization in the keratocytes such that they revert to a fibroblast-like function and lay down collagen and GAGs found in any typical wound: e.g. hyaluronan acid, type I and type III collagen, and matrix glycoproteins. In addition, the size and arrangement of the fibrils are not regular, further contributing to the corneal opacity. In extensive wounds, this opacification remains permanently; however, in smaller, well-defined wounds there is an attempt by the cornea to restore clarity by producing normal corneal matrix components.

Surgical wounds to the cornea aim to minimize the risk not only of causing wound-related corneal opacities but also of inducing shape change to the cornea and thus astigmatism. Accordingly, cataract surgeons are using increasingly small wounds to remove the lens, the most recent innovation being optical coherence tomography (OCT)-guided femtosecond laser surgery, in this case where the laser is used to perform the incision in the lens capsule rather than the cornea. Since the corneal curvature is a function of tension in its circumferential fibres (see Fig. 4-36), restoration of the normal curvature will not be achieved unless the edges of the wound are apposed by surgical reconstruction. This is the basis of refractive surgery where, initially, partial thickness radial keratotomy was performed by making 'relaxing' incisions in the peripheral cornea to release the circumferential tension and thus 'flatten' the corneal curvature. The wounds are intentionally left to heal in a gaping configuration. Various types of laser surgery, such as argon-F1 (excimer = excited dimer), ultraviolet laser energy and more recently femtosecond laser, are used to produce precise customized 'ablation' of the anterior stroma performed directly on the exposed tissue after lifting a central, hinged flap of stroma. Ablation is thought to be caused by photon–photon interactions derived from thermal reactions or directly by photoablation, whereby covalent cross-links in the collagen fibrils are disrupted. This is termed laser *in situ* keratomileusis (LASIK) in which the surface of the cornea is reshaped (usually flattened) by raising a corneal flap, laser treating the

exposed stromal bed and restoring the corneal flap without sutures. Both these and conventional surgical corneal incisions are fully epithelialized in the normal manner, with epithelial migration into the depths of the wound sometimes producing excessive layers of cells.

Refractive surgery continues to expand both in quantity and in novelty of technique, combined with greater precision using methods such as eye-tracking to control the incision. Wavefront-guided surgery combined with anterior segment OCT imaging are further innovations in this ever-expanding field. Wavefront technology is a term used to describe the fundamental quality of an optical system, for instance in the field of astronomy, in terms of wavefronts of higher and lower order, and the eye lends itself well to such analysis. For instance, images formed by the healthy eye may be blurred for three main reasons: light scatter, diffraction and optical aberrations, e.g. chromatic or spherical. Using wavefront technology, optical aberrations from monochromatic sources such as myopia are selectively addressed and treated with wavefront-based LASIK.

Refractive surgery, including photorefractive keratectomy (PRK), LASIK and full-thickness penetrating corneal graft, has complications including sometimes severe residual astigmatism and even progressive ectasia (widening) of the initial wound. Alternative approaches have been tried to restore normal corneal curvature; these include topography-guided laser surgery using keratometry, sometimes including restoration of collagen cross-linking induced by riboflavin and UV light. Collagen cross-linking helps to 'stiffen' the stromal bed (Fig. 4-40).

The endothelium. The corneal endothelium does not normally undergo mitosis in humans even after direct injury as in a perforating corneal wound. With age there is a decline in the number of endothelial cells with an increase in their size and variable morphology (polymegathism). The response to direct wounding is to undergo enlargement and 'cell slide', as occurs in the epithelium in the early stages of migration. If sufficient numbers of endothelial cells are lost, the cell layer cannot perform its pumping action and the cornea imbibes water (decompensates) and becomes opaque.

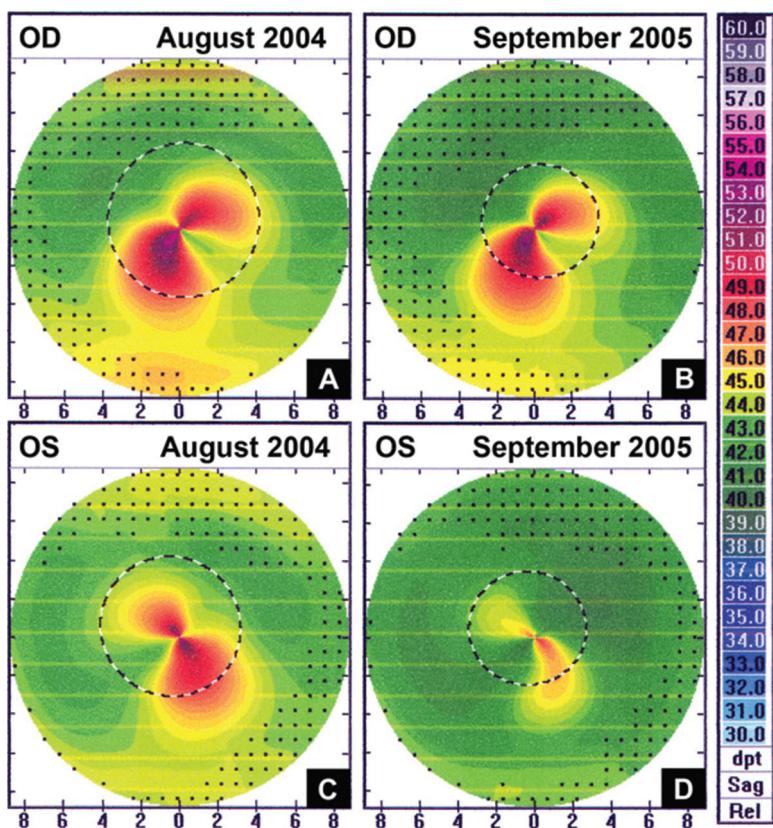


FIGURE 4-40 Corneal topographic maps showing the stabilizing effect of corneal cross-linking on corneal curvature (compare lower right and left panels). (From Hafezi et al., 2007.)

Vascularization. The normal cornea is avascular, although a few vessels may be found at the limbus. Blood vessels from the conjunctiva or the deep episcleral plexus may invade the periphery of the cornea beyond the limbus during healing of wounds or infected ulcers. When corneal epithelial or stromal defects fail to close promptly, often as a result of infection or during the severe inflammatory response of chemical injury, the continued release of proteolytic enzymes from inflammatory cells as well as damaged corneal cells, causes degradation of the stroma and increases the risk of spontaneous perforation. Matrix metalloproteinases such as matrilysin and stromelysin and MMP-9 as well as plasminogen activators (uPA and tPA) and pro-inflammatory cytokines, such as interleukin 1 (IL-1), IL-6 and IL-8, tumour necrosis factor- α and TGF- β , macrophage inflammatory proteins (MIP) 1 α and β , and granulocyte-macrophage

colony-stimulating factor, stimulate further ingress of inflammatory cells and initiate a vascularization response (see Ch. 7). Growth factors such as VEGF, FGF and HGF (hepatocyte growth factor) are also released, of which VEGF is considered most active. Vessels advance across the cornea to the site of injury or infection and contribute to the eventual opaque ‘leucoma’ of the healed cornea.

Inflammation also induces new lymphatic vessels from the limbal regions, from lymph vessel precursors in the conjunctiva, especially after herpes simplex virus infections of the cornea, which participate in the overall immune response. Lymphatic vessels are induced by the isoform VEGF-C. Inhibitors of angiogenesis are co-released during the process of inflammation, and include endostatin, a degradation product of collagen XVIII, and other products of collagen, fibronectin and even KI5, which is a fragment of

plasminogen itself. In addition, thrombospondins (1 and 2) in synergy with a scavenger receptor CD36 are important anti-angiogenic factors present in the normal corneal stroma. The lack of corneal vascularity is determined during development and appears to be under the control of the transcription factor FoxC1.

Vitamin A and the cornea. Deficiency of vitamin A leads to impaired corneal and conjunctival epithelial function, with loss of corneal lustre, Bitot's spots, punctate erosions and xerophthalmia, partly as a result of loss of goblet cells in the conjunctiva. Vitamin A or retinol is required for control of epithelial keratin expression and the synthesis of cell surface glycoproteins involved in the glycocalyx. Deficiency of vitamin A leads to a form of keratinization of the corneal epithelium.

Vitamin A is also essential for normal corneal wound healing. A simple abrasion or ulcer that would be dealt with rapidly by a healthy cornea is likely to be complicated by a stromal melting response (keratomalacia) in vitamin A-deficient humans and animals. Experimentally it has been shown that topical retinoic acid can reverse the effects of vitamin A deficiency. In addition, retinol (Vitamin A) itself promotes the synthesis of the α -1 proteinase inhibitor, which inhibits a wide range of proteolytic enzymes. Vitamin A has also been shown to have protective antioxidant effects on corneal endothelial cells in culture while retinoic acid is important in promotion of T regulatory cells for control of immune, especially autoimmune, responses.

Vitamin A can be converted from traditional β -carotenoids taken in developing countries but is less efficiently converted to vitamin A than from carotenoids in other material such as lycopene, lutein and zeaxanthin.

THE SCLERA

The sclera is non-transparent and tough because of its acellularity and matrix components.

Matrix factors. The sclera is essentially acellular, containing only a few fibroblasts and non-branching traversing vessels. Recent studies have shown that there are some contractile fibroblasts (myofibroblasts)

in the sclera and choroid that may have a role in refractive properties of the eye. It is opaque for the opposite reasons that the cornea is transparent, i.e. that the type I and III collagen fibres are of variable diameter and their distribution is irregular. There are also several other minor collagens in sclera (types V, VI, VIII, XII, XIII). The proteoglycans are predominantly proteodermatan and proteochondroitin sulphate of the SLR type (see p. 186) and they are localized to the collagen fibres in a similar manner to corneal proteoglycans. However, there are no proteokeratan sulphates. Other proteoglycans present in sclera include aggrecan, PRELP (proline-arginine-rich and leucine-rich repeat), decorin and biglycan among others. In addition, the amount of proteoglycan in the sclera is considerably less than that in the cornea, with the effect that it is much less hydrated (70%). The sclera, unlike the cornea, also contains elastic fibres around a fibrillin core, accounting for about 2% of total fibril content in the adult.

The sclera also contains a certain amount of large aggregating proteoglycans, such as versican, neurocan and brevican, combined with hyaluronan. There is a considerable turnover of extracellular matrix constituents in the sclera, and this may determine the shape and size of the eye and thus refraction itself.

Increases in the anteroposterior dimension of the globe occur with age, and the adult globe size is reached around age 7–10 years. Continuing increases in the size of the globe underlie the development of myopia (short-sightedness) and considerable research has been devoted to understanding this phenomenon. Recently it has been suggested that the choroid may serve as a paracrine tissue, regulating collagen and proteoglycan synthesis, particularly hyaluronic acid, in the sclera and consequently its overall dimensions.

Bulk fluid transport and the uveal effusion syndrome. Although most of the bulk transport of fluid out of the eye takes place through the anterior chamber drainage angle and/or the uveoscleral meshwork (see Ch. 1, p. 21), there is appreciable transretinal transport of fluid towards the choroid. Some of this is drained via the normal choroidal vessels (10% of the ocular fluid is drained via the vortex veins) but a proportion is drained directly trans-sclerally. The effect of this trans-scleral flow is to 'suction on' the

retina to its adjoining RPE layer and maintain retinal apposition.

Fluid flowing across the sclera is absorbed by the matrix proteoglycans. Thus, the sclera is maintained in its normal state by having proteoglycans with a low water-binding capacity. In some conditions, such as the rare uveal effusion syndrome, and in nanophthalmia, the sclera contains high levels of abnormal proteoglycans, especially dermatan-sulphate-containing proteoglycans, which bind and trap large volumes of water. Thus the sclera thickens and may secondarily obstruct the choroidal venous drainage, causing further swelling and water retention.

Uveal tract

The uveal tract, comprising the iris, the ciliary body and the choroid, is a continuous layer of which the major functions are to regulate the pupil size for optimal visual function and to act as the lymphovascular tissue of the eye. Each component, however, has several other functions.

THE IRIS

Physiology

The iris is derived from neuroectodermal and mesodermal tissue and is designed to function as the lens aperture of the eye. This is achieved by the opposing actions of the sphincter pupillae and the dilator pupillae muscles (see Ch. 1, p. 26). The sphincter is an annular band of true smooth muscle that inserts close to the pupil margin at the pigment epithelium. The dilator is a highly unique series of myoepithelial cells representing the continuation of the outer layer of ciliary body pigmented epithelial cells. Some consider these cells rather to be myofibroblasts which do not have full complement of muscle proteins including desmin. The non-pigmented ciliary body epithelial cells are continuous with the posterior pigmented iris epithelium (see Ch. 1). Between the origin at the ciliary body and the insertion near the sphincter pupillae, the dilator has several side insertions into the stroma of the iris which allow it to mobilize the iris during dilation.

The functions of the pupil are:

- to regulate the amount of light entering the eye (it increases 16-fold on dilation of the pupil from 2 to 8 mm)

- to increase the depth of focus for near vision
- to minimize optical aberrations.

These functions are mediated by light and near reflexes, whose neural pathways involve autonomic parasympathetic (constriction) and the sympathetic (dilation) mechanisms (see Ch. 6). Unusually large pupil diameters have been linked to the development of myopia through increased optical aberrations.

The neuromuscular junctions of the iris are susceptible to direct pharmacological manipulation by agents that induce miosis (cholinergic agents, sympathetic antagonists) and mydriasis (anticholinergic agents, sympathomimetic agents) (see Ch. 6). In addition, pharmacological agents that induce the release of neurotransmitters from synaptic terminals (e.g. substance P is released after nitrogen mustard exposure) can have a marked effect on pupil responses. There is some evidence that dual innervation exists for iris muscles with excitatory and inhibitory input to each; thus the action of any individual drug may not be entirely predictable, depending on the state of activity at the time of administration of the drug.

Sympathetic activity in the dilator muscles appears to be mediated mainly by α -adrenergic receptors because the effects can be blocked by phenoxybenzene, but some β activity also exists. In some species, such as the cat, the action of sympathetic agents on the sphincter muscle appears to be mediated by β receptors only; in the monkey the action appears to be strongly α -mediated in the sphincter and dilator, thus producing antagonism. Studies on isolated human iris dilator indicate that the excitatory innervation is α -adrenergic while the inhibitor is cholinergically mediated.

Pigmentation of the iris contributes to iris colour, as does backscatter of short-wavelength light reflected into the iris stroma; thus, iris thickness is a significant determinant of iris colour.

Blood flow in the iris

About 5% of the total ocular blood flows through the iris. Iris blood vessels, derived from the major vessel circle, are contained as radial coils within tube-like formations of the stromal tissue, an arrangement that allows them to remain patent when the iris is fully dilated. Iris vessels have tight junctions and lack fenestrations; this renders them relatively impermeable to large molecules, as demonstrated by anterior segment

fluorescein angiography. They constitute a second component of the blood–aqueous barrier (see below). Imaging techniques using high-resolution ultrasound allow visualization of iris and ciliary body vessels and indicate that flow velocities as low as 0.6 mm/second can be detected (Fig. 4-41).

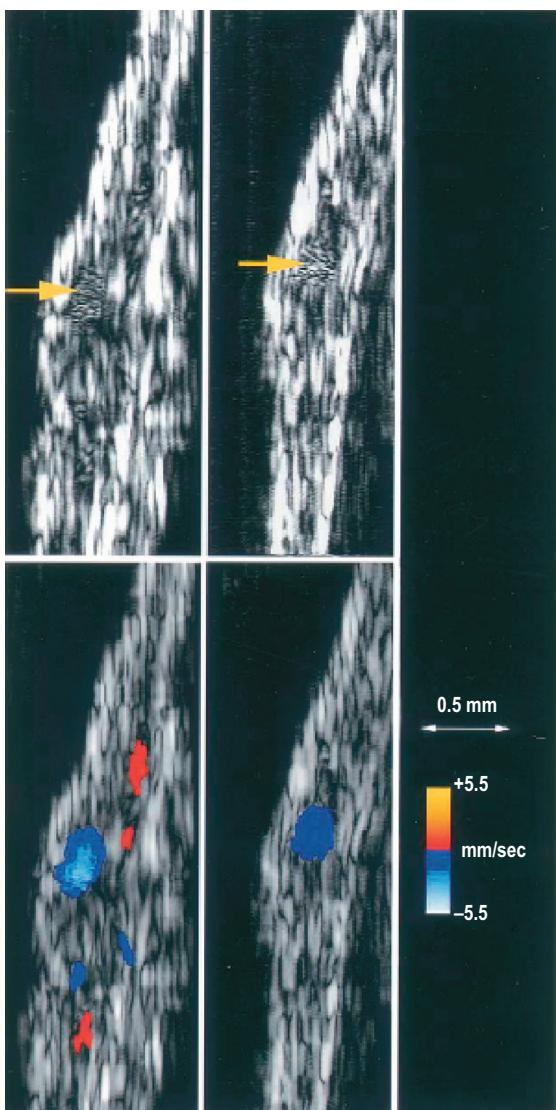


FIGURE 4-41 Top panels: swept-mode images of iris and ciliary processes at 37°C (left) and 4°C (right). Arrows indicate position of major arterial circle. Bottom panels: colour flow images derived from swept-mode data shown above. (From Silverman et al., 2002, with permission from the World Federation for Ultrasound in Medicine and Biology.)

THE CILIARY BODY

Functions of the ciliary body

The ciliary body has multiple functions:

- It provides the blood and nerve supply to the anterior segment.
- It maintains intraocular pressure by secretion of aqueous.
- It constitutes the major portion of the blood–aqueous barrier.
- Its musculature underlies the process of accommodation.

Blood flow in the ciliary body

The ciliary body receives blood vessels from the long posterior ciliary arteries and the major iris circle (see Ch. 1, p. 28). Blood flow through the ciliary body is about 7% of total ocular flow. The vessels are highly fenestrated, leaking most of their plasma components into the stroma. Blood flow in the iris and ciliary body is autoregulated like that of the retina (i.e. it does not alter significantly with changes in perfusion pressure), but it is also under autonomic control and can be modified by a variety of adrenergic and muscarinic inputs. Under normal circumstances, aqueous production is independent of ciliary body blood flow until the latter declines to <75% of normal.

Ciliary muscle and accommodation

In humans, relaxation of the zonule is induced by contraction of the ciliary muscle, which moves forward, thereby allowing the lens to adopt a more spherical shape owing to the elasticity of the lens capsule (Fig. 4-42). Accommodative range in emmetropic, non-presbyopic (see next paragraph) individuals is 5–6 dioptres. There has also been some evidence that contraction of the ciliary muscle steepens the corneal curvature, thus increasing its refractive power, as occurs in lower vertebrates. The parasympathetic neurones mediating this response are carried in the III cranial nerve via the long ciliary nerves (see Ch. 1, p. 30). It is not clear which of the three sets of ciliary muscle fibres is responsible for the major action in inducing this forward movement but, as for the iris muscles, the effects can be blocked by anticholinergic drugs. There is also a small sympathetic inhibitory component and this is increased in late-onset myopes. Accommodation produces greater ability to focus on

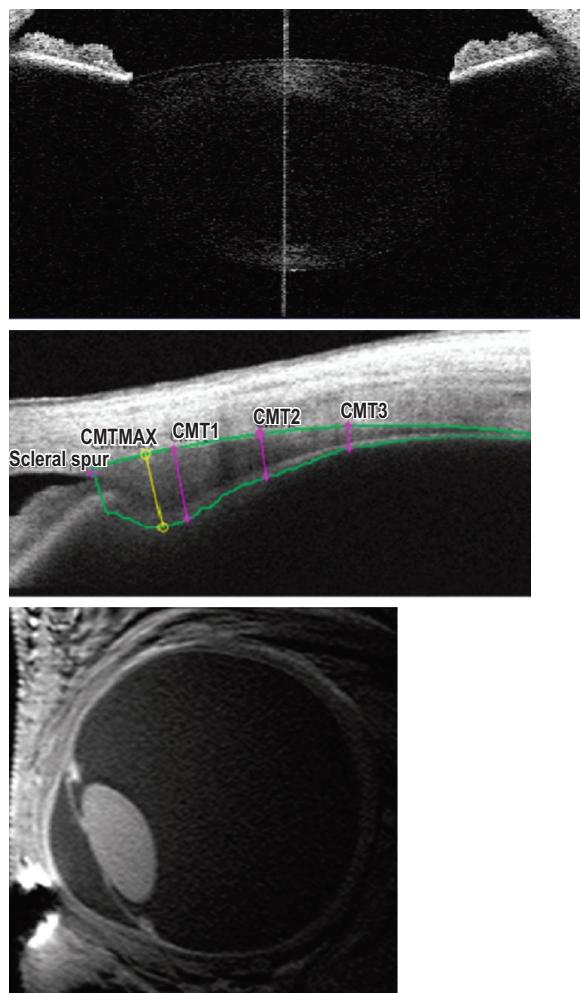


FIGURE 4-42 OCT images of the crystalline lens (top) and ciliary muscle (middle), and MR imaging of eye (bottom). Ciliary muscle image analysis shows cross-sectional ciliary muscle thickness (CMT) at 1, 2, and 3 mm posterior to the scleral spur as well as maximum thickness. (From Richdale et al., 2013.)

near objects owing to the increased refractive (dioptic) power of the lens. Epidemiological and theoretical quantitative analyses have shown that intense near work for prolonged periods disrupts emmetropization associated with eye growth and induces myopia. In addition, young children sleeping in dimly lit, as opposed to completely dark, rooms are at risk of developing myopia because of the persistence of poorly focused images through thin eyelid skin.

With age, accommodative ability declines due to changes in the deformability of the lens, while the

ciliary muscle remains fully contractile. Reduced lens deformability is due to increases in lens size as well as changes in crystallin protein, combining to produce a 'hardened' lens (beginning stages of cataract formation). It has been estimated that the lens equatorial diameter decreases by 0.055 mm for every dioptre decline in accommodative ability.

Blood–aqueous barrier

Aqueous humour, secreted by non-pigmented ciliary epithelial cells, is derived from plasma but contains different concentrations of electrolytes and other small molecules and a restricted set of proteins in low concentration. These differences have led to the concept that a barrier exists between the plasma transudate in the ciliary body stroma and the aqueous in the posterior chamber of the eye which prevents the free diffusion of molecules over a certain size from gaining access to the posterior and anterior chambers which freely communicate via the pupil. The barrier is erected by the tight junctions between non-pigmented ciliary epithelial cells (see Ch. 1, p. 30). In contrast, extensive gap junctions between pigmented and non-pigmented cells allow the two layers of the ciliary epithelium to act as a metabolic and transport syncytium. However, in the iris, where tight junctions between the epithelial cells do not exist, it has been assumed that the barrier is formed by tight junctions between the vascular endothelial cells. Recently, this concept has been challenged. The differences in protein composition between aqueous and plasma are not absolute and thus the barrier is leaky, since some high molecular weight proteins are present in aqueous. Instead there is evidence that the protein transudate in the ciliary body stroma diffuses anteriorly into the iris stroma and thence into the anterior chamber down a concentration gradient (Fig. 4-43). Thus, aqueous in the anterior chamber always contains a small amount of protein, while aqueous in the posterior chamber is protein-free (Fig. 4-44).

Breakdown of the blood–aqueous barrier occurs in many conditions, including inflammation and vascular disease. In these circumstances the ciliary body/iris vessels become highly permeable and the stromal transudate increases with a great excess of proteins diffusing into the aqueous. The aqueous humour becomes visibly cloudy (seen as 'flare' by slit lamp

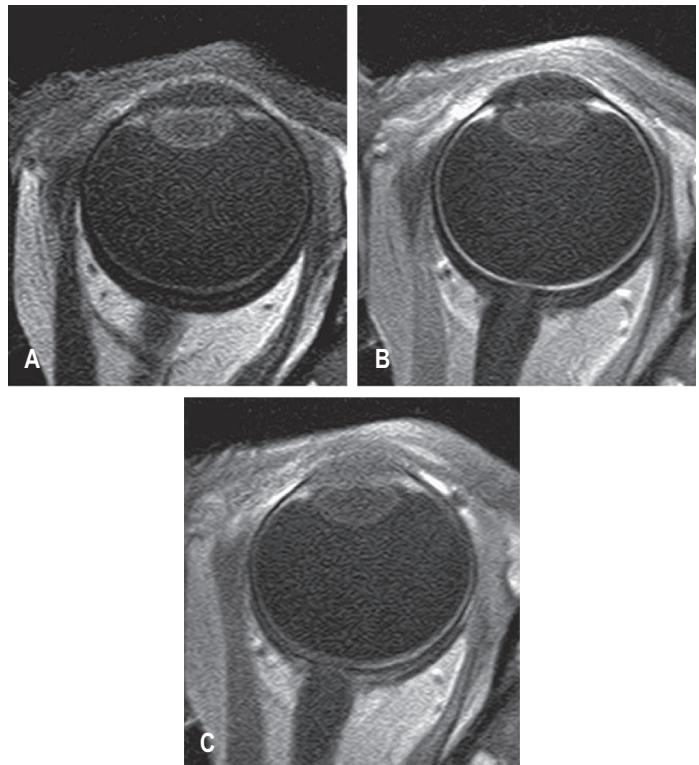


FIGURE 4-43 Contrast-enhanced MRI images of the eye. (A) Pre-contrast image shows details of tissues in the anterior segment of the eye, including iris, ciliary body and both the anterior and posterior chambers. (B) Within 2 minutes of contrast infusion, there is clear enhancement of the ciliary body and the choroid, but the anterior chamber, posterior chamber and vitreous body show no enhancement. (C) After 90 minutes, the enhancement in the ciliary body and choroid has begun to diminish. There is clear enhancement in the anterior chamber but the posterior chamber and the vitreous remain unchanged from A and B. (From Freddo, 2013.)

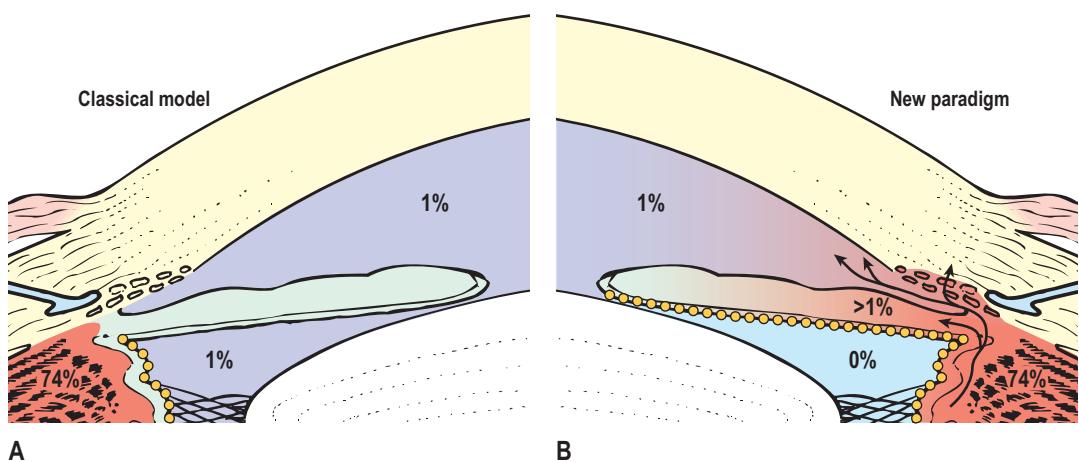


FIGURE 4-44 (A) In the classical model, the tight junctions of the non-pigmented ciliary epithelium and of the iris vascular endothelium are considered to be the main components of the blood–aqueous barrier, by preventing the passage of molecules greater than 10 kDa into the anterior and posterior chambers. The iris stroma is presumed to be free of plasma proteins and the concentration of plasma proteins in the aqueous is uniform throughout the anterior and posterior chambers. Increases in aqueous humour plasma protein concentrations are considered the result only of an increase in barrier permeability. (B) In the new concept (paradigm) of blood–aqueous barrier physiology, the small amount of plasma-derived protein present in aqueous humour diffuses from the ciliary body stroma to the root of the iris, accumulates in the iris stroma and is then released into the aqueous humour of the anterior chamber only (arrows). Thus, the posterior chamber is free of protein. Some of the protein delivered to the iris root immediately enters the trabecular outflow pathways (uveo-scleral outflow, arrows). The tight junctions of the non-pigmented ciliary epithelium and of the iris vasculature endothelium still provide the main barrier function. However, an additional key element becomes the tight junctions of the posterior iris epithelium, which prevent the protein in the iris stroma from diffusing posteriorly, when combined with the one-way valve created by the pupil resting on the anterior lens capsule, and the continuous forward flow of aqueous humour through the pupil. (From Freddo, 2013.)

biomicroscopy) because of plasma proteins in anterior chambers – it may even become ‘plasmoid’ owing to the presence of fibrinogen and other proteins. Inflammatory cells are also likely to be present when the blood–aqueous barrier breaks down. If clotting occurs, as in severe uveitis, the aqueous becomes ‘plastic’.

Eicosanoids in the iris/ciliary body

Prostaglandins were first discovered in the eye in 1957 by Ambache, who demonstrated the biological activity in aqueous and named the factor ‘irin’. Eicosanoids is the generic term to describe prostaglandins (PGs) and leukotrienes, both of which are metabolites of arachidonic acid (see p. 197). Prostaglandins are synthesized in large amounts after trauma or inflammation involving the iris/ciliary body, from arachidonic acid released from esterified sites in membrane phospholipids. Other neuropeptides are involved in this response. For instance, release of substance P from the iris leads to receptor-mediated breakdown of PIP₂ (see Ch. 6, p. 362) and the formation of large amounts of arachidonic acid in the iris sphincter and synthesis of PGE₂. In the ciliary body, the cyclo-oxygenase pathway is also active in microsomes. PGE₂ is involved in miosis, while PGF_{2α} is involved in the control of intraocular pressure. Interestingly, breakdown of the blood–aqueous barrier in response to PGE₂ agonists is impaired in PGE-receptor knockout mice. PGF_{2α} is also known to increase vasodilatation and capillary permeabilization in the anterior segment of the eye. Many other peptides are present in the iris/ciliary body and the aqueous, including neuropeptide Y, vasoactive intestinal peptide, somatostatin and calcitonin gene-related peptide (CGRP). Nitric oxide is also released during activation of iris/ciliary body tissues. Many of these mediators modulate normal iris/ciliary body functions such as miosis and aqueous humour production. For instance, CGRP relaxes iris dilator smooth muscle via cAMP mechanisms. They also have other functions such as the immunosuppressive role of vasoactive intestinal peptide in ocular immune privilege (see Ch. 7, p. 457).

Drugs that inhibit the cyclo-oxygenase pathway (see p. 197), such as indomethacin and aspirin, may be useful in ocular inflammation. However, steroids act at the level of phospholipase A₂ and may have a more global effect on the response (see Ch. 6, p. 363).

In addition, the lipoxygenase pathway is active in the anterior uvea with synthesis of leukotrienes B₄, C₄ and D₄, and the chemotaxis of polymorphonuclear leucocytes (see Ch. 7). Penetration of drugs into the intraocular environment after topical application occurs more readily through the conjunctiva and the sclera than the cornea, partly as a result of the numerous transport mechanisms available in the conjunctival epithelium but also because of the greater transepithelial permeability of the conjunctiva compared to cornea, which is impermeable even to low molecular weight compounds (<1000 kDa).

Detoxification and antioxidation in the anterior segment

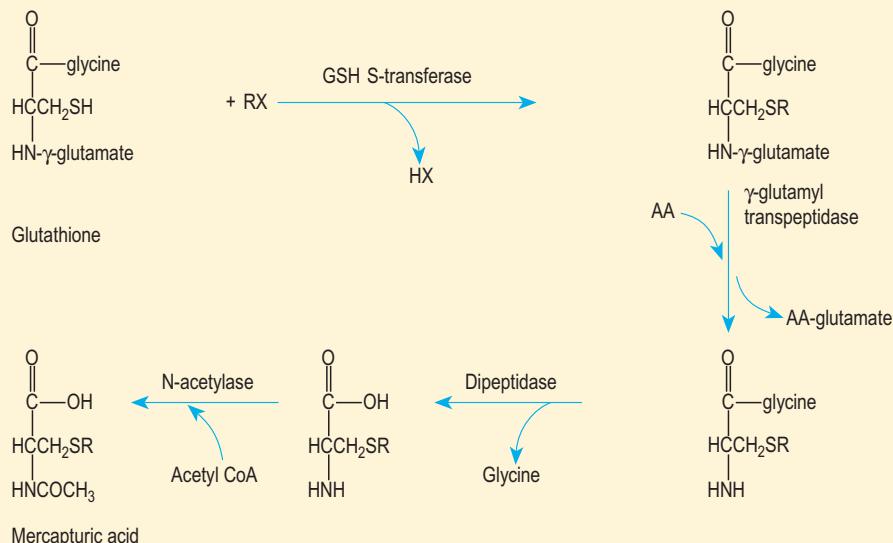
The cytochrome P₄₅₀ system is the major drug detoxification (CYP) system in the eye. Microsomes contain a group of proteins known as the cytochrome P₄₅₀ proteins, which catalyse the transfer of a single oxygen atom to endogenous and exogenous substances destined for excretion and/or detoxification, such as steroids, phenobarbital, etc. (see Ch. 6, p. 344). Their main effect is to convert hydrophobic compounds to hydroxylated hydrophilic compounds, which are then more easily metabolized.

The cytochrome P₄₅₀ system is present in the ciliary body (at about 5% of the concentration in liver) where it acts to detoxify many compounds. It does this by either converting the hydroxylated, highly reactive compound to a glucuronide via UDP-glucuronyl transferase or by conjugating it to glutathione via glutathione S-transferase (Box 4-13). Several CYP family enzymes have been identified and/or purified from the ciliary body, in particular the non-pigmented epithelium. For instance the enzyme CYP2D6 is important for metabolizing the topical anti-glaucoma β-adrenergic blocker timolol. There is considerable genetic variation in the induction of the cytochrome P₄₅₀ system in the eye, perhaps explaining the variable toxic effects of drugs in individuals.

The ciliary body is the main source of antioxidant systems in the anterior segment. Although antioxidant systems exist in the lens (see below) and the cornea (see above), the ciliary body is especially rich in antioxidant systems with the highest concentrations of catalase, superoxide dismutase, and glutathione peroxidase types I and II. Type I is selenium-dependent

BOX 4-13 CYTOCHROME P₄₅₀ AND DRUG DETOXIFICATION

The cytochrome P₄₅₀ system detoxifies compounds by utilizing the glutathione S-transferase system and degrading compounds to mercapturic acid.



while type II is selenium-independent. Type I is closely linked to glutathione reductase whose main function is the reduction of oxidized glutathione (GSSH) produced by the detoxification of peroxides (see Box 4-13).

Hydrogen peroxide (H₂O₂) is present in normal aqueous, most of it derived from the non-enzymatic interaction between reduced ascorbate and molecular oxygen, and it is reduced to H₂O by glutathione secreted by the ciliary epithelium. Most of these studies have been performed in experimental animals and it is not clear how relevant they are to the human eye. It has been suggested that oxidized ascorbate (via the superoxide anion) is more important in degrading H₂O₂ in humans. Melatonin, a neuropeptide involved in biological circadian rhythms, is also an H₂O₂ scavenger. A role for xanthine oxidase has also been suggested. H₂O₂ can induce noradrenaline (norepinephrine) release from the iris/ciliary body in the aqueous and has recently been implicated in cataract formation.

Ciliary body tissue also contains a peroxiredoxin, a constituent of a widely distributed family of antioxidant enzymes, whose amino acid sequence and tissue

distribution are now known. Their role is to degrade H₂O₂ and alkyl peroxides.

THE CHOROID

Functions of the choroid

The function of the choroid is to act as the lymphovascular supply to the posterior segment of the eye.

Vascular function. The choroid is almost entirely composed of vessels embedded in a loose connective tissue matrix which has a high content of type III collagen, typical of an expansile or spongy tissue. The blood supply to the choroid has several interesting features:

- Some 98% of the blood to the eye passes through the uveal tract, of which 85% is through the choroid.
- Blood flow occurs at a rate of 1400 mL/min per 100 g tissue, which is higher than the perfusion of blood through the kidney.
- The choriocapillaris is organized in a lobular architecture, collecting into larger vessels and finally into four vortex veins, one in each quadrant of the globe (see Ch. 1).

- Venous blood draining from the choroid is not desaturated, only 5–10% oxygen having been extracted during passage through the eye. The choroid supplies the outer retina, where the partial pressure of oxygen (P_{O_2}) is highest, rapidly falling towards the retinal inner segments and then rising again, less so, towards the inner retina.
- Blood vessels in the choroid are highly fenestrated and leaky, like ciliary body vessels.
- Choroid and ciliary body blood vessels are sensitive to the P_{O_2} and P_{CO_2} ; in conditions of high P_{CO_2} the vessels expand greatly in a forward direction, altering the forward position of the retina, and exerting pressure on structures such as the vitreous gel and lens/iris diaphragm.
- Although previously considered to have considerable autoregulation in relation to perfusion pressure, choroidal blood flow, especially around the optic nerve, is sensitive to the effects of nitric oxide and endothelin and other as-yet unidentified vasoconstrictors.

Non-vascular functions. The choroid has additional functions:

- It is involved in ocular temperature control by dissipating heat from the eye.
- It secretes growth factors which control scleral thickness and may have a role in emmetropization (see eFig. 4-13).
- It drains aqueous fluid from the anterior segment of the eye via uveoscleral outflow, which may be as much as 40% of aqueous outflow in humans (see p. 211).
- Higher primates, including man, have intrinsic choroidal neurones which may have a role via nitric oxide in controlling vascular diameter and blood flow to the choroid.
- In humans and other primates, non-vascular smooth muscle cells may be found in some cases forming discrete rings around blood vessels and even in the region of the fovea.

Lymphoid function. Intraocular structures lack a recognized lymphatic system. However, through the uveoscleral outflow, drainage of intraocular fluid into conjunctival (aqueous veins) and orbital lymphatics occurs. Some reports of suprachoroidal lymphatic

lacunae have been documented. The choroid contains a rich network of immune cells including mast cells, macrophages and dendritic cells which adopt this migration route to the draining lymph node, which in the human are the pre-auricular and submandibular lymph nodes of the eye (see Ch. 1). This tissue can respond massively to intraocular inflammation (see Ch. 7, p. 459). In addition, choroidal and ciliary body/iris melanocytes are potential antigenic targets for autoimmune disease (see Ch. 7).

Aqueous humour dynamics

A fundamental physiological function of the eye is to maintain an intraocular pressure (IOP) between 10 and 20 mmHg. This is achieved by the circulation of aqueous humour secreted by the ciliary body into the posterior chamber and circulated through the pupil towards the anterior chamber angle where it drains via the outflow apparatus into the episcleral veins (see Ch. 1). Factors affecting IOP include:

- circadian rhythms
- episcleral venous pressure
- rate of secretion and flow of aqueous humour
- neural (cranial nerves V and VII) and hormonal influences.

The intraocular pressure, as measured clinically, actually represents the balance between the inflow and outflow of aqueous and is altered by changes in the gradient of pressure between the posterior chamber and the anterior chamber, and eventually by the episcleral pressure. The uveoscleral outflow is greatly affected by alterations in this gradient of pressure as appears to occur in glaucoma (Fig. 4-45).

AQUEOUS HUMOUR IS SECRETED BY THE CILIARY BODY EPITHELIUM

The rate of aqueous humour formation is about 2–3 $\mu\text{L}/\text{min}$. Aqueous humour is formed by the transport of water and electrolytes from the leaky fenestrated capillaries of the ciliary processes to the epithelial syncytium and thence across the plasma membrane of the non-pigmented epithelium (see Box 4-14).

Composition of aqueous humour

The aqueous humour is composed predominantly of electrolytes and low molecular weight compounds

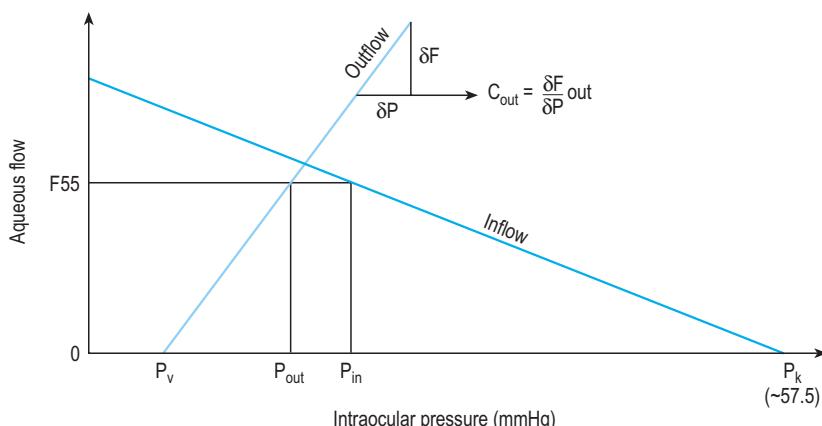


FIGURE 4-45 Aqueous inflow declines as intraocular pressure (IOP) rises towards P_k (about 57 mmHg). Goldmann estimates pseudofacility as $P_k = 0.58 \times$ brachial BP. The gradient for inflow is $(F_{55}/P_k - F_{55}) = C_{in} = C_{ps}$ where F_{55} is steady-static flow. C_{ps} is the pseudofacility. (Courtesy of D. Woodhouse.)

with some protein, and there are significant differences from plasma in several of the components. Aqueous receives contributions from a variety of sources, including the corneal endothelium and the iris/lens (see p. 211), in addition to active secretion from the ciliary body. Several trace compounds are also present in aqueous humour, including steroid sex hormones, enzymes such as carbonic anhydrase, lysozyme and plasminogen activator, and cytokines such as basic fibroblast growth factor (bFGF) and TGF- β (see Ch. 7, p. 457). It is likely that most of the high molecular weight components are present only in the anterior chamber. Low levels of catecholamines (adrenaline, noradrenaline and dopamine), prostaglandins and cyclic nucleotides are present in normal aqueous, but the source of these compounds is uncertain.

The protein content of aqueous is very low (about 1/500 of plasma), and the major species is albumin. Since immunoglobulins are relatively large molecules, it is unlikely that they gain access to the aqueous via the ciliary body epithelium; rather, diffusion through the ciliary body /iris stroma is more plausible; alternatively, local production via iris lymphocytes or plasma cells is a possible source since local antibody production in the eye has been well recorded. Small amounts of fibronectin are also produced locally.

Aqueous contains detectable amounts of hyaluronic acid, derived as breakdown oligomers from vitreous hyaluronic acid during the normal process of GAG

renewal (see p. 187). However, some of the aqueous hyaluronan is of higher molecular weight than vitreous, suggesting that it is produced in the anterior segment. Aqueous hyaluronic acid may have a role to play in regulation of IOP because perfusion of the anterior chamber with hyaluronidase leads to a marked drop in pressure. However, it is also possible that intracameral hyaluronidase affects trabecular meshwork cells and extracellular matrix, thus leading to a lowering of the IOP.

NEURAL/AUTONOMIC CONTROL OF AQUEOUS SECRETION

Adrenergic and cholinergic agonists and receptors are present in the iris and ciliary body, and autonomic innervation of this tissue occurs in muscle, vessels and epithelial cells. Adrenergic receptors are present in the ciliary epithelium and regulate IOP via the adenylate cyclase system. β -Adrenergic antagonists and α_2 -selective adrenergic agonists both suppress aqueous flow. Muscarinic receptors linked to the PIP₂ second messenger system are also present in the ciliary epithelium.

Cholinergic mechanisms are not involved in control of intraocular pressure

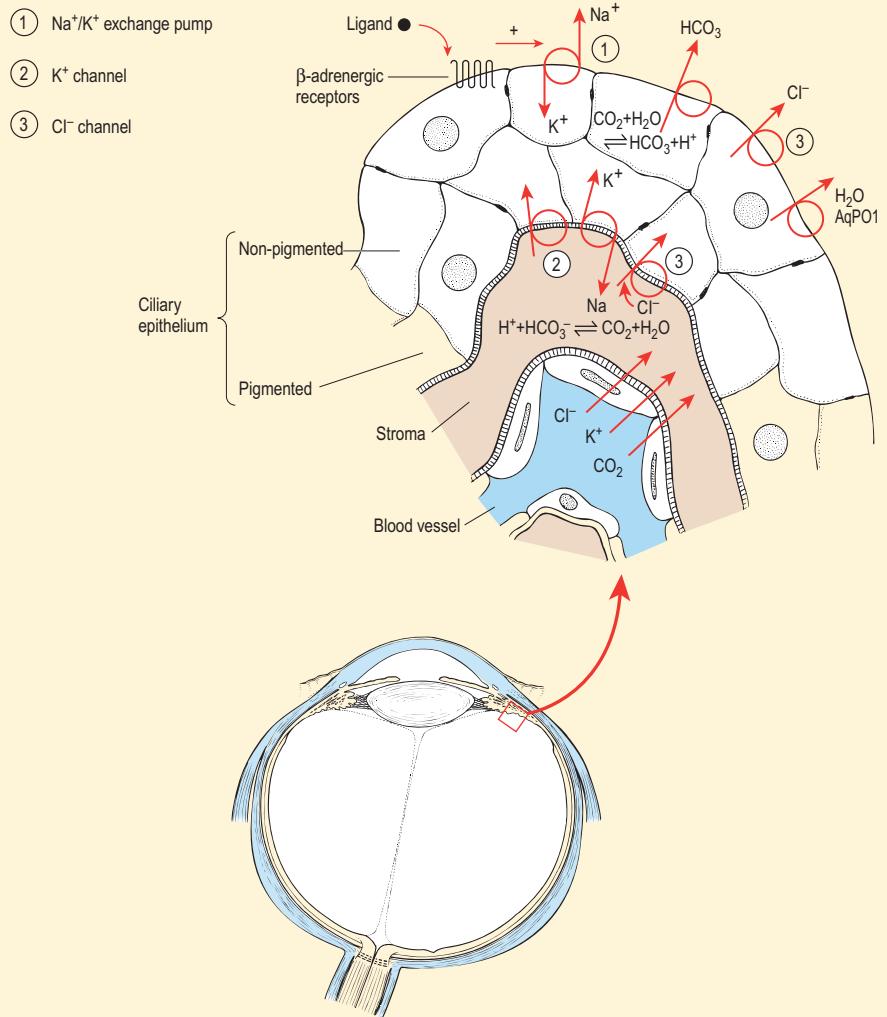
M₃ muscarinic receptors in the ciliary epithelium linked to phosphatidylinositol in the cell membrane have been identified, but they do not appear to have a significant role in IOP control. Despite this,

BOX 4-14 SECRETION OF AQUEOUS HUMOUR

Classic theory suggests that passive diffusion of water and ions from the fenestrated vessels of the ciliary body is followed by active transport of Na^+ and Cl^- across the ciliary body syncytium. This is an active secretory process involving Na^+/K^+ ATPase and carbonic anhydrase type II activity. In some respects this has been viewed as ultrafiltration of ions and water and eventually leads to the formation of aqueous humour, which is secreted into the posterior chamber. However, the oncotic pressure of the ciliary stroma is greater than the hydrostatic pressure difference across the ciliary epithelium, so tending towards absorption of water into the ciliary body from the posterior chamber. Thus, active transport of ions in the opposite direction is the main mechanism of aqueous humour formation. This process is also under adrenergic receptor control at the level of the ciliary epithelial cells and possibly also by regulation of blood flow to the ciliary body. The possibility has been raised that

H_2O transport may also be achieved by aquaporins. The ciliary epithelium has been shown to express two aquaporins, AQP1 and AQP2, but these do not seem to play a major role in aqueous humour production. In addition, some of the K^+ channels are Ca^{2+} -sensitive and can be activated by Ca^{2+} entry via Ca^{2+} channels. More recently, the role of the Cl^- ion in aqueous humour formation has assumed greater importance through its transport via the cystic fibrosis transmembrane receptor. This may play a greater role than the HCO_3^- carbonic anhydrase-mediated mechanisms and may also be facilitated by adenosine receptors.

Aquaporin water channels are also present in both the secretory machinery (AqPO4 and AqPO1) and the outflow channel (trabecular meshwork endothelium) and appear to contribute to bulk flow of water because intraocular pressure is reduced in AqPO4/1 knockout mice.



cholinergic agents such as pilocarpine are thought to have some action via reduction in aqueous secretion, although the experimental evidence is weak. Most of the action of pilocarpine appears to be mediated via its effect on outflow resistance and uveoscleral flow (see below).

Adrenergic receptors regulate IOP via adenylate cyclase

The majority of α receptors in the ciliary body are α_2 , while more than 90% of β receptors are β_2 . Stimulation of α_2 receptors lowers the IOP via a reduction in aqueous humour production through inhibition of adenylate cyclase. Adrenaline, a preferential α -adrenergic agonist, stimulates prostaglandin synthesis, particularly that of PGE₂ and PGF_{2 α} , the latter having potent ocular hypotensive activity.

Stimulation of β receptors, particularly β_2 receptors, also leads to an increase in aqueous secretion via activation of adenylate cyclase.

The dual control of aqueous secretion through activation (β) or inhibition (α) of adenylate cyclase is mediated by their respective stimulatory and inhibitory G proteins (see Ch. 6, p. 345). Thus, IOP can be lowered by α_2 agonists (e.g. clonidine) or β_2 antagonists (β -blockers, e.g. timolol). The α_2 receptors are also linked to vasoactive intestinal peptide receptors, which are co-stimulated and lead to a reduction in cAMP levels, which in itself may also lower IOP. β antagonists have no effect on aqueous flow when aqueous production is at its lowest, whereas α_2 agonists and carbonic anhydrase inhibitors do.

The mechanism whereby changes in intracellular cAMP levels alter aqueous secretion is not known but appears to involve transport of the HCO₃⁻ ion across the cells. In addition, a number of other components appear to be involved in aqueous secretion, such as protein kinase C, which is linked to adenylate cyclase activation and thus may act as part of an intracellular signalling network connecting the two main second messenger systems (see Ch. 6, p. 345).

Nucleotides and nucleotide receptors

Low molecular weight nucleotides such as adenosine are present in aqueous humour in significant quantities (4⁻⁴ μ M) and are likely to play a role in IOP control. The wide-ranging effects of nucleotides,

particularly adenosine, are mediated via receptors present on nerve endings, including PTX and PTY receptors. Topical application of some nucleotides to the eye leads to lowering of the IOP.

Interestingly, melatonin has some IOP-lowering effects and the melatonin analogue 5-methoxycarbonylaminoo-N-acetyltryptamine (5-MCA-NAT) has similar effects which are thought to be due to induction/modulation of adrenergic receptors in the eye.

Circadian regulation of aqueous humour formation

It has long been known that there is a diurnal variation in IOP, possibly due to melatonin-based mechanisms. This is in part the result of a circadian regulation of aqueous humour secretion. Secretion in humans occurs at a rate of 2.6 mL/min during the day and falls to 1.0 mL/min at night. Both β -adrenergic receptor-mediated and neuropeptide-mediated mechanisms, particularly that of vasoactive intestinal peptide, are involved. Activation of G protein-coupled adenylate cyclase leads to cAMP production, which activates protein kinase A, thus regulating the cation channels. The process is terminated by hydrolysis of cAMP by phosphodiesterase.

Is guanylate cyclase involved in intraocular pressure control?

Large amounts of brain natriuretic peptide (BNP) as well as atrial natriuretic peptide (ANP) are found in the iris/ciliary body and in the aqueous humour of rabbits and humans. The receptor for ANP is linked to membrane-bound guanylate cyclase, and *in vitro* studies have shown that this enzyme can be stimulated by ANP in the ciliary body. In the rabbit this is accompanied by a reduction in IOP but its relevance in humans is not clear because similar levels of peptides are found in both normal subjects and in glaucoma patients.

AQUEOUS HUMOUR OUTFLOW FROM THE EYE

Flow of aqueous humour from the eye is controlled at several different levels, including at the site of the 'conventional' (C_c) outflow pathway via the trabecular meshwork, at the uveoscleral (C_u) outflow system, and, outside the globe, at the site of the episcleral veins (see Ch. 1).

Control of outflow at the trabecular meshwork

Resistance to the outflow of aqueous occurs at the level of the endothelium covering the trabecular meshwork, which is an extension of the corneal endothelium, but also within the matrix of the meshwork itself. The hydraulic conductivity at this site is around $10^{-7} \text{ cm}^{-2} \text{ s}^{-1} \text{ g}^{-1}$, which is several times greater than for other lining endothelia and has been attributed to a process of water transport termed 'transcytosis', in which membrane-bound water-containing vesicles are transported through micron-sized pores in the cell, across the meshwork. Formation of transcellular pores may be a pressure gradient-induced mechanoreceptor-based mechanism involving the actin-myosin cytoskeleton and appears less effective when the cells are stiffened, as occurs with agents which increase outflow resistance such as thrombin and sphingosine-1-PO₄.

Despite the potential contribution of the trabecular endothelial cell, the juxtaganular cribriform meshwork is considered to account for much of the resistance to aqueous flow into Schlemm's canal. This has been attributed to matrix components, especially the GAGs, present in this region. The trabecular beams, composed of type I collagen, with a significant proportion of type III and IV collagen, and other matrix constituents such as laminin, fibronectin and elastin, are separated by GAG-filled spaces, particularly hyaluronan (Fig. 4-46) that retard the flow of fluid by virtue of hydrophilic properties and large hydrodynamic volume. A wide range of GAGs have been identified in the trabecular meshwork matrix—namely, hyaluronan, chondroitin sulphate, dermatan sulphate, keratan sulphate and heparan sulphate—with significant variation between species. Some unidentified proteoglycan material has also been detected. Trace amounts of types V and VII collagen have also been detected in the trabecular meshwork.

Apart from the mechanosensor or stretch receptor function of trabecular meshwork (TM) endothelial cells discussed above, TM endothelial cells have special characteristics: namely, active phagocytic properties, high levels of cytoskeletal actin, which in cultured cells is particularly sensitive to cytochalasin B, and lower levels of microtubules, which appear to be relatively non-responsive to colchicine. These cells also contain vimentin and desmin, thus showing some

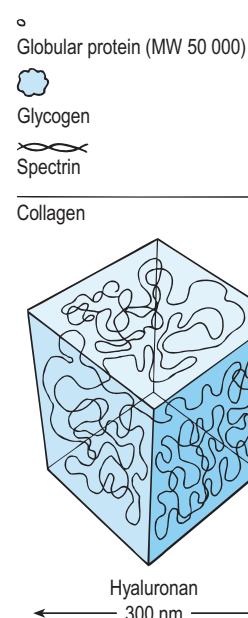


FIGURE 4-46 Model of hyaluronan, showing size compared to other 'typical' molecules. (Courtesy of J. Alberts.)

similarity to smooth muscle cells. Taken together, these findings suggest that trabecular meshwork endothelial cells are specialized for both endocytic transport of water and solutes, and contractility. Actin mobilization appears to be mediated via adrenergic receptors, probably of the β_2 type, which are highly responsive to adrenaline. Energy metabolism in the trabecular meshwork endothelium is predominantly glycolytic rather than oxidative, although both enzyme systems are present and functional. Transport of water may be achieved not merely by passive transportation of H₂O packets but by activation of a water channel protein, aquaporin-1 (AQP01), found in corneal endothelial cells.

Metabolism of trabecular meshwork cells

The matrix components in the trabecular meshwork are thus synthesized and degraded by the endothelial cells. In addition, these cells have high levels of surface tissue plasminogen activator (tPA), higher even than in vascular endothelial cells, and this is likely to play a role in maintaining patency and reducing the resistance of the outflow passages. Phagocytic activity of trabecular meshwork cells is associated with several

other enzymatic activities such as GAG-degrading enzymes and acid phosphatase.

Trabecular meshwork cells have receptors for a variety of agents including adrenaline (β_2 adrenergic receptors, decrease phagocytosis) and glucocorticoids. Both steroids and oxidative damage induce the expression of the trabecular meshwork inducible glucocorticoid response (TIGR) protein. Mutations in the *TIGR* gene, now known as the myocillin gene, have been found in patients with glaucoma. Myocillin is found widely in ocular tissues and may be a component of exosomes (see p. 165) which could potentially obstruct TM outflow if released in excess or not removed after release. Other susceptibility genes such as the optineurin and the WD repeat domain (*WDR36*) genes have been found but account for only a small proportion of patients with primary open angle glaucoma and the *CYP1B1* gene (drug detoxifying gene, see p. 221) in congenital glaucoma. In addition, TGF- β appears to be involved in the glucocorticoid cell response and in the production of trabecular meshwork extracellular material. Steroids inhibit prostaglandin by trabecular meshwork cells at concentrations as low as 10^{-8} mol/L. Prostaglandin synthesis by trabecular meshwork accounts for a significant proportion of its arachidonic acid metabolism (70% compared with less than 5% in other cells), suggesting that prostaglandins play a major role in trabecular meshwork cell physiology. In addition to substantial amounts of PGE and PGF_{2 α} , leukotriene B₄ appears to be produced in high amounts.

Trabecular meshwork cells contain the free radical and hydrogen peroxide detoxifying enzyme systems present in other tissues such as the ciliary body (see above). Both a catalase and a glutathione-dependent system are active in handling hydrogen peroxide, which can reach levels as high as 25 μ mol/L in the aqueous humour. Ageing is associated with increasing free radical damage and mitochondrial dysfunction and clearance of waste products by the ubiquitin-proteasome system, and other lysosomal and non-lysosomal enzyme systems such as the calpains of TM cells becomes less efficient. Waste products thus accumulate in the TM itself ('the garbage catastrophe theory of ageing') and contribute to increased outflow resistance, ocular hypertension and glaucoma. This

age-related process will also be affected by genetic susceptibility as shown in the recent identification of two mutations in the lysyl oxidase gene in patients with secondary glaucoma associated with pseudoexfoliation syndrome. Lysyl oxidase is required for elastin production (see p. 183) and presumably vitreous/zonule components are more readily degraded and washed through to the TM in this syndrome.

Uveoscleral drainage

A variable proportion of aqueous (up to 40%) drains directly into the anterior uvea at the ciliary body immediately posterior to the cornea and thence into the suprachoroidal space and towards the posterior pole of the eye (see Ch. 1). The anterior uvea at this point is incompletely lined with endothelial cells. In addition, the localization of MMP-1 suggests a role for this enzyme in uveoscleral outflow. Uveoscleral drainage is possible because the pressure in the suprachoroid is 2–4 mmHg lower than in the anterior chamber; this can be reversed after trabeculectomy and can lead to choroidal effusions. This pressure differential is also less with age, leading to greater risk of choroidal effusion in such patients. Prostaglandins may decrease the intraocular pressure by increasing the uveoscleral outflow. Several possible mechanisms have been proposed, including relaxation of the ciliary muscle, cell shape changes, cytoskeletal rearrangements or compaction of the trabecular meshwork matrix.

Episcleral circulation

It was shown many years ago that dye-stained aqueous fluid would not drain out of the eye into the episcleral veins if the IOP was less than 15 mmHg. Thus, this represents the combined episcleral venous pressure and the oncotic pressure in the perivenous tissues of the episcleral veins. Aqueous humour draining via the canal of Schlemm into the aqueous veins does so by passing through large transcellular channels and giant vacuoles on the meshwork side of the canal. The canal has direct vascular communications on its outer wall with a network of intrascleral collector channels that drain into the scleral veins (see Ch. 1). An alternative model for aqueous outflow has been proposed which involves a mechanical pumping mechanism generated in response to small changes in IOP and linked to the

ocular pulse. Pumping of aqueous from Schlemm's canal into the collecting veins and episcleral veins is assisted by small valves in this model.

The episcleral venous pressure can be measured using a non-invasive manometer (the EV-310) and is normally around 8–10 mmHg.

An intraocular pressure-independent link between aqueous production and aqueous outflow?

Bestrophins are anion channels known to be involved in fluid transport in the gut. Recently, similar proteins have been identified in the ciliary body, including the HCO_3^- channel bestrophin-2 which is selectively expressed in non-pigmented epithelial cells. Unexpectedly, bestrophin-2-deficient (*best2* $-/-$) mice have a higher rate of aqueous inflow but a reduced IOP. This appears to be due to the very high level of a soluble adenylate cyclase, in the non-pigmented epithelium, which is acutely sensitive to HCO_3^- , but is absent from the outflow pathway cells. This suggests that the ciliary body may produce substances via HCO_3^- -sensitive soluble adenylate cyclase, which directly influences outflow independent of pressure (Lee, 2011).

Does aqueous contain components that contribute to flow resistance?

Although aqueous has the same viscosity as isotonic saline, its passage through microporous filters *in vitro* is slower than that of saline. This effect can be abolished by proteolytic agents and detergents, but not by hyaluronidase. It has therefore been suggested that some forms of glaucoma may be caused by a build-up of a surfactant-like material with age.

The lens

The transparency of the lens is a function of the highly ordered state of its cells and extracellular matrix. In essence, the extracellular matrix of the lens is confined to its capsule, while the cells form a syncytium with interlocking cellular processes. The syncytial arrangement is brought about by the extensive communicating gap junctions between the cells, but the cells are not fully apposed, leaving a nanometer-sized space between cells which permits an important and essential circulation within the lens.

LENS TRANSPARENCY

The light transmission properties of the ocular media vary to some degree depending on the nature and the age of the tissue. The cornea, aqueous humour and lens all transmit long-wavelength light well above the limit of visible light (about 720 nm). However, short wavelengths below 300 nm are absorbed by the cornea but wavelengths above 300 nm are transmitted through the aqueous; the lens further filters the majority of the short wavelengths below 360 nm and is an absolute barrier to light below 300 nm (Fig. 4-47).

The epithelium

The single-celled epithelium of the lens and its capsule do not scatter or reflect light, essentially because the combined refractive index is the same as that of the aqueous humour (1.336). However, the epithelium is of great importance in the maintenance of fluid and electrolyte balance of the lens syncytium via ion-pump mechanisms (see below). Thus, any agent that disturbs epithelial function and/or viability (such as ionizing radiation to the lens bow region) will have significant effects on lens clarity. This applies to all aspects of lens structure and function.

The organization of the lens fibre cells underpins the transmission properties of the lens

It might be expected that the plasma membranes of the lens fibres would produce interference diffraction patterns that would affect the ability of the lens to

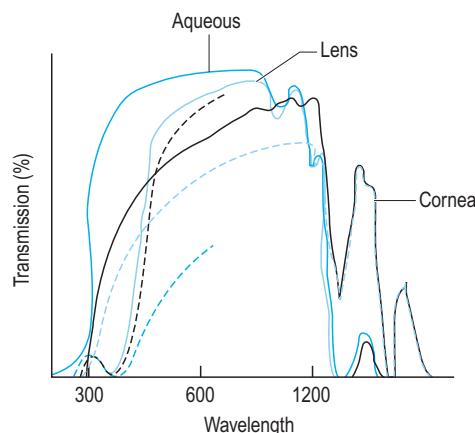


FIGURE 4-47 Optical density curves for various ocular components.
(With permission of Drs Jaffe and Horwitz and JB Lippincott.)

transmit light. However, it has been shown that the weak diffraction rings that are produced occur in a repeating pattern with a period of the same dimension as the thickness of an individual lens fibre in the anteroposterior axis. This reduces any scatter by the plasma membranes of the normal epithelium; it has been estimated that the amount of scatter by the epithelium in the human lens is about 5% of the transmitted light.

Lens fibres differentiate from mitotic equatorial lens epithelial cells, which migrate posteriorly and centrally as new fibre cells are generated. This produces a zone of differentiating fibre cells which not only lose their nuclei but also most of their intracellular organelles as they become mature cells towards the lens centre (nucleus) (Fig. 4-48). This loss of cellular

organelles as well as their anteroposterior orientation assist in allowing light transmission. The fibres are organized in a densely packed cellular arrangement, with interdigitations like pieces in a three-dimensional jigsaw puzzle (see Ch. 1, p. 32) in which extensive intercellular communication exists via the lens gap-junction, generated by a set of heterodimeric proteins composed of connexins (Cx43, Cx46 and Cx50) (Fig. 4-49) as well as the gap-junction-like protein, aquaporin-0 (AQPO). Lens fibres essentially become crystallin-replete (over 90% of the total cellular protein) sacks in which crystallins are embedded within a complex cytoskeletal matrix, some components of which are also lens-specific (e.g. the beaded intermediate filament protein). The polydisperse nature of the crystallins prevents spontaneous crystallization in the packed arrangement. The high refractive index (RI) of the lens is caused by the crystallins; at the periphery of the lens the RI is slightly less (1.38) than at the nucleus (1.41). The water content of the lens is also greater at the periphery (75–80%) than at the lens nucleus (68%).

As the lens fibres differentiate towards the centre (lens nucleus), this packing arrangement becomes modified and less compact (Fig. 4-50). The presence of the crystallins is in itself insufficient to explain the transparency of the lens. Transparency is predominantly the result of the packing of the crystallins in very high concentration such that they resemble a dense liquid or a glass because of the high level of ‘short-range spatial order’; this means that the scatter of light from each individual molecule is related to the scatter from its immediate neighbours and that they tend to cancel each other out. At a macroscopic level, the arrangement of the crescent-like fibre cells in end-to-end concentric shells around a polar axis provides a highly ordered architecture. A series of coaxial refractive surfaces, thus created, promotes transparency of the multicellular structure. However, this general view does not apply to all species and in most there are two types of fibre cell, an S-shaped cell and a concentric cell. Overlapping tails of S-shaped cells form the lens sutures, which paradoxically lie along the visual axis and can affect optical quality and produce variability in focus.

In addition, the circulation within the lens provided for by the nanometer space between the gap

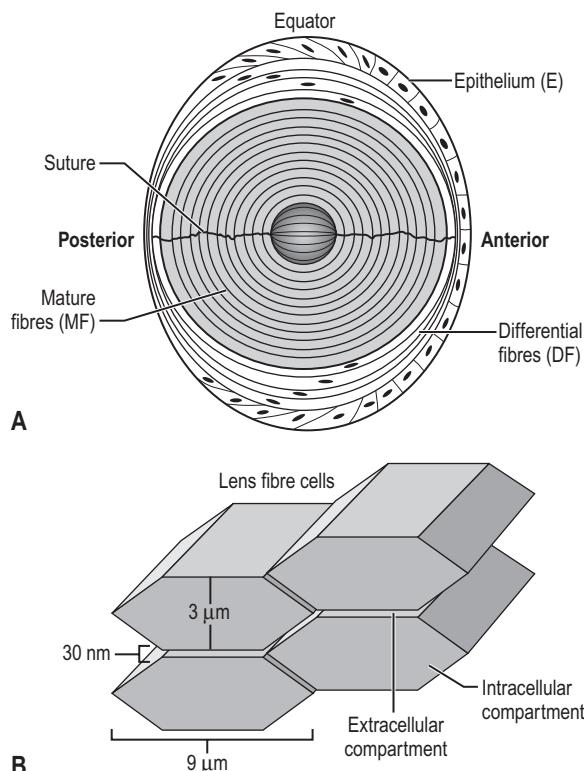


FIGURE 4-48 (A) Three zones comprise the lens structure: the epithelium (function: transport of proteins), the anucleate mature lens fibres (function: light transmission) and the differentiating fibres (retain some organelles: function: nutrient transport link between epithelium and lens fibres). (B) The lens fibres appear as flattened hexagons when cut in cross-section. (From Mathias et al., 2010.)

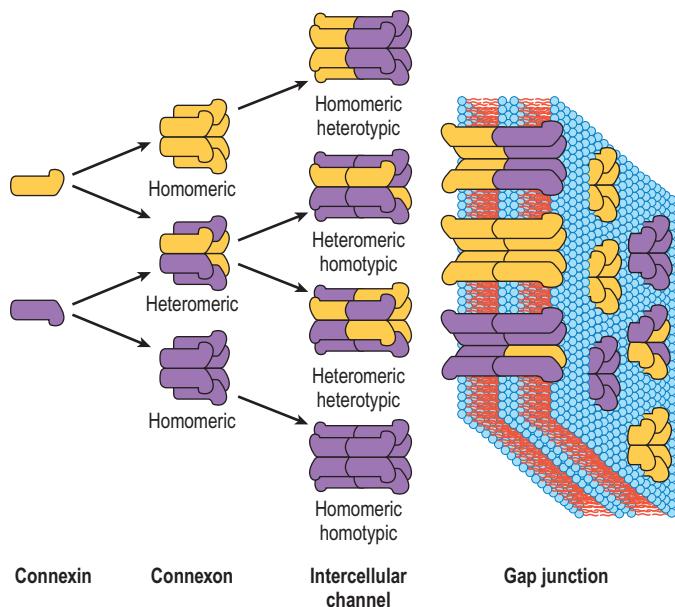


FIGURE 4-49 Gap junctions are a major feature of cellular connections in the lens: they comprise many closely packed channels each formed by two hemichannels (connexons), one in each of the connecting cells. (From Mathias et al., 2010.)

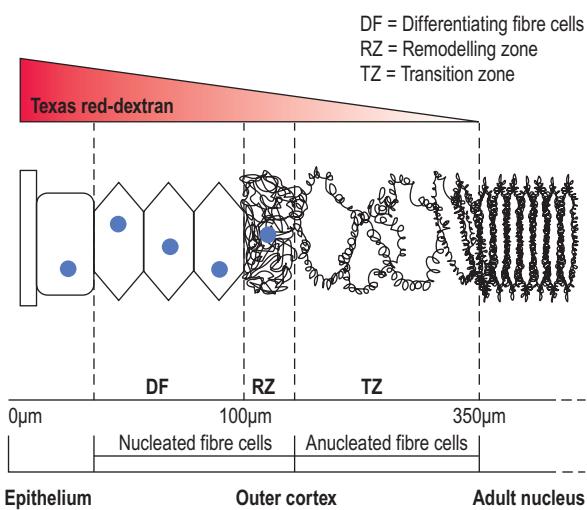


FIGURE 4-50 Summary diagram of the changes in the lens as adult lens fibres differentiate from lens epithelium. (From Borchman and Yappert, 2010.)

junctions in fibre cells is essential for lens fibre metabolism even at its basal level. Mutations in gap junction connexin proteins account for many different types of congenital cataract.

The crystallins

Crystallins make up 90% of the water-soluble proteins of the lens; there are three types in mammals (see eBox 4-4).

Some crystallin protein also coextracts with the urea-soluble protein, indicating that a fraction of the crystallins is strongly bound to the cytoskeletal (urea-soluble) proteins (5% total protein). The water/urea-insoluble protein represents membrane protein (2% total protein) and some crystallin is also found in this fraction when it is solubilized in detergent.

The α A and α B crystallins show about 50% sequence homology. The molecules exist as polydisperse globular proteins in aggregates organized in three concentric layers or as a protein ‘micelle’. However, their true quaternary structure is unknown. α A and α B crystallins belong to the family of small heat-shock proteins and display chaperone-like activity. Studies in knockout mice have shown that α A lens-specific crystallin is required for normal lens differentiation and transparency, while α B, which is expressed in neural tissue and upregulated under conditions of stress, is not essential for lens transparency. Homology of lens crystallins to certain enzymes such as aldehyde dehydrogenase class 3 enzymes in corneal epithelial cells suggests that this form of ‘gene sharing’ is quite widespread.

The crystal structure of some small invertebrate heat-shock proteins such as Hsp16.5 has allowed a model for lens α A crystallin micelles to be developed (Fig. 4-51). In this model it has been shown that neither α A or α B is necessary to develop the molecular

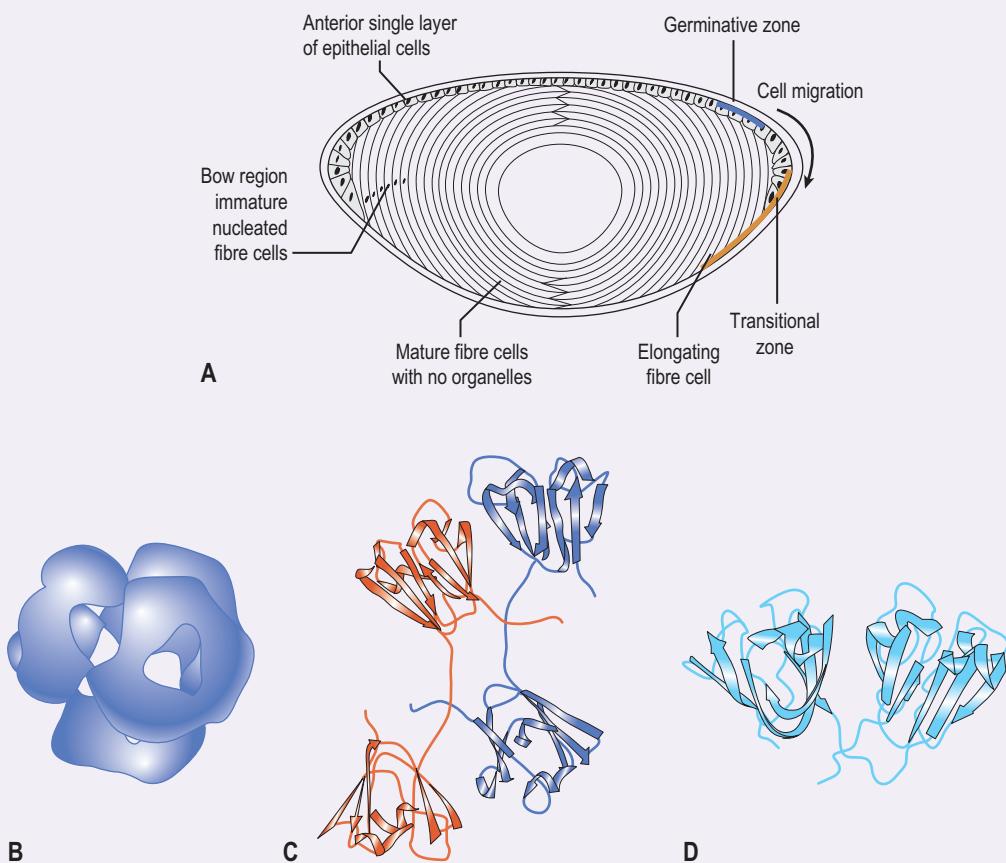
eBox 4-4**The crystallins**

Three types of crystallin have been identified in mammals, α , β and γ , mainly on the basis of molecular weight (see figure). The δ crystallins have also been detected in birds. Several other 'taxon-specific' crystallins (ϵ , τ , ρ , χ , μ , λ , ζ , SIII) are recognized in other species, based on the criterion that they account for at least 10% of the total water-soluble protein. More recent data suggest that in vertebrates there are only two classes of crystallins, α and $\gamma\beta$. Native α crystallin is of two types, αA and αB , each with a molecular weight of about 20 kDa. In the native state, however, the α crystallins form large multimeric aggregates of 300–1200 kDa (average 800 kDa), held together by non-covalent interactions.

β Crystallins range in molecular weight from 23 to 35 kDa and occur in several subtypes: βB_1 , βB_2 and βB_3 ;

βA_2 , βA_3 and βB_4 . Mixed aggregates of between 50 and 200 kDa occur naturally. The γ crystallins are monomeric in the native state; there are six types (* γA –E, and γS (formerly known as βS)), differentiated by charge. Not all types are present in human lenses at all ages, some such as γS and γC being present at higher concentrations in fetal than in adult lenses. The relative amounts of $\alpha:\beta:\gamma$ crystallin also vary greatly depending on age and other factors; in the 'typical' lens the ratio of $\alpha:\beta:\gamma$ is of the order 40:35:25. Protein sequence analysis has shown homology between the β and γ crystallins; in addition, all three proteins exist as β -pleated sheets.

The structure of the human lens (A) and the major soluble lens crystallins (α , B), (β , C) and (γ , D) is shown below.



(From Moreau and King, 2012.)

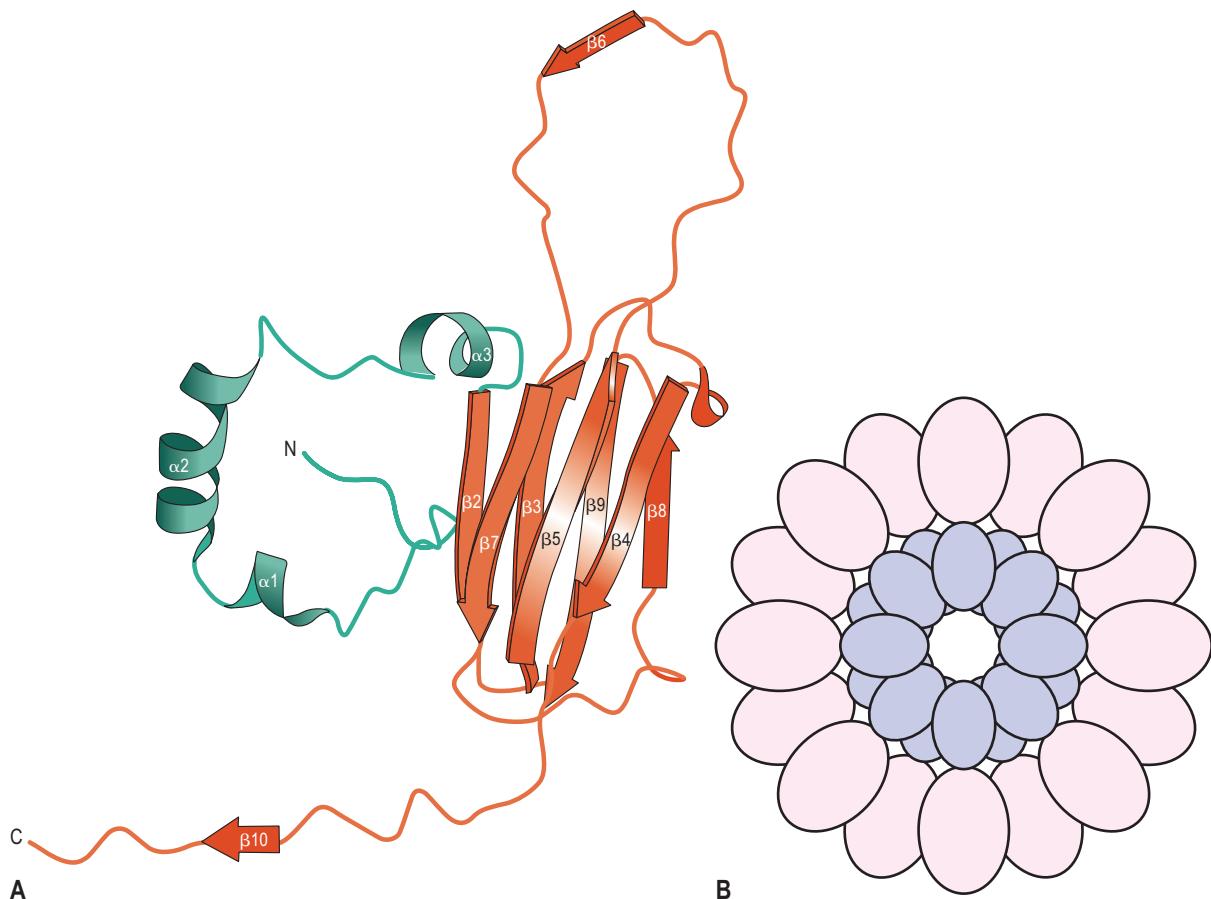


FIGURE 4-51 (A) Secondary structure of the Hsp16.9 subunit with an ordered N-terminus. The N-terminal domain (green) contains three helical segments, as shown, in half of the subunits. The remaining subunits in Hsp16.9 and all subunits in Hsp16.5 have unstructured N-termini. The α crystallin domain (brown) consists of seven-stranded β -sandwich, an interdomain loop containing one β -strand (β_6 at the top) and a C-terminal extension (at the bottom), which is largely unstructured except for the short V10-strand. (B) A possible micelle-like structure for α crystallin. The subunits contain two domains and assemble into large aggregates through interactions between their hydrophobic N-terminal domains (lilac), which are located in the centre of the aggregate. The hydrophilic C-terminal (α crystallin) domains (not pink) are on the surface of the assembly. (Part A is reproduced with permission from Nature Publishing Group. Part B is from Augusteyn, 2004, with permission from the Optometrists Association of Australia.)

arrangement but that some combination of either will suffice. However, the tertiary and quaternary structure of human crystallins remains elusive due to expected difficulties in achieving crystallization.

Phosphorylation of the αA_2 and the αB_2 chains produces the αA_1 and the αB_1 chains. Spontaneous non-enzymatic cleavage of the molecules also occurs, as does high molecular weight aggregation, especially with age. The α crystallins, acting as molecular chaperones, ‘trap’ other crystallins and proteins such as intermediate filaments, which may

be undergoing denaturation and unfolding. In this way they maintain lens transparency by preventing disruption of the highly ordered structure of the crystallin packing.

The $\gamma\beta$ crystallins are thought to have a similar structure: four repeating antiparallel β sheets in the form of ‘Greek key’ motifs. γ Crystallin is a highly stable molecule, attributed to its extensive internal symmetry; recent studies have revealed a link between mutations in congenital cataract and cataract of old age (Fig. 4-52).

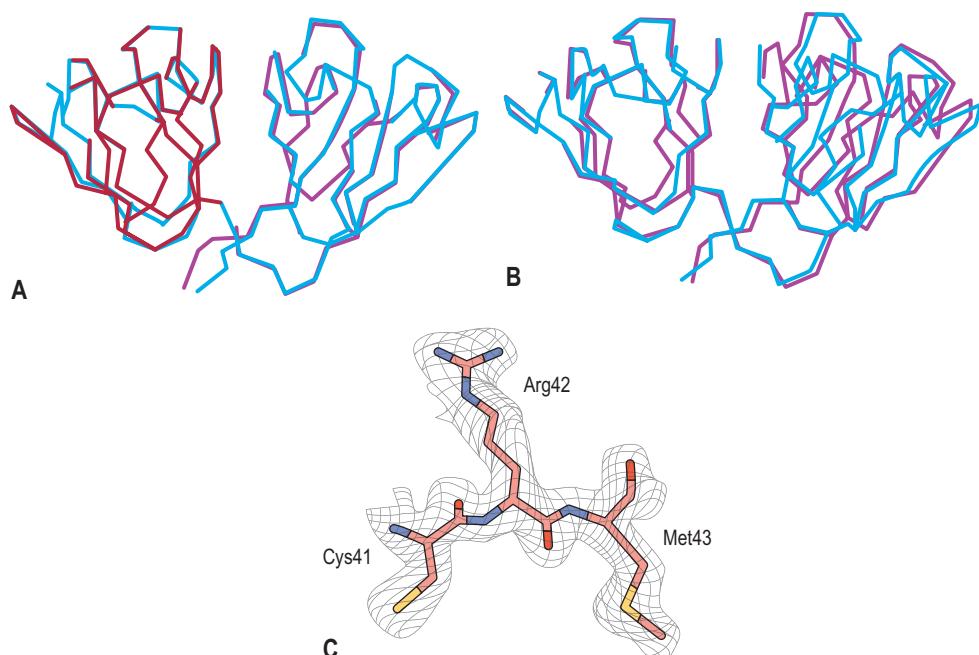


FIGURE 4-52 Crystal structure of human W42R γ D-crystallin, a mutant form of human γ crystallin which has only minor differences to normal γ crystallin but is protease-sensitive and is associated with certain forms of congenital cataract. (A) Superposition of the normal and mutated forms of γ crystallin. (B) Best fit superposition. (C) Electron density of residue Arg42 mutated from Trp42 in the normal γ D crystallin. (From Ji et al., 2013.)

Multimeric complexes of β crystallins tend to form between the acidic molecules (βA_2 , βA_3 and βA_4) and the basic molecules (βB_1 , βB_2 and βB_3), followed by association between similar heterodimers. Homology among the various β crystallins both within and between species is quite variable and sequence analysis is still in progress for most of the human proteins. However, on the basis of $\gamma\beta$ sequence homology a predicted structure of β crystallin has been suggested and X-ray crystallography has shown it to have some basis. In this model, the two γ -like structures are joined by a connecting peptide.

Although the molecular packing of the crystallins and their high refractive index contribute extensively to lens transparency, biophysical studies of molecular interactions between proteins indicate that the crystallins in themselves are not essential – proteins that can adopt the correct state of phase transition and osmotic pressure would do equally well. In essence it is related to the volume exclusion properties of proteins which are at their highest with γ D crystallins, the most abundant crystallin in the lens.

Cytoskeletal proteins of the lens

Cytoskeletal proteins are usually to be found in the urea-extractable fraction of lens proteins. In addition to the usual complement of microfilaments, such as actin, vimentin and spectrin, and intermediate filaments (see section on cells and tissues above), there are certain lens-specific intermediate filaments such as beaded filaments. Vimentin is the major intermediate filament in the lens cell, and is present in epithelial and cortical fibre cells but not in nuclear fibre cells. A similar distribution has been found for microtubules in lens cells. Cytokeratins are not found in the adult lens. Some differences occur in relative proportions of cytoskeletal elements. Thus, talin, α -actinin and the signalling proteins are at high concentration in lens equatorial epithelium, while vinculin is prominent in stable fibre cells with strong cell–cell contacts.

Beaded filaments are specific to the lens fibre. Two main species have been identified: beaded filament-specific proteins 1 and 2 (BFSP1, BFSP2, also known as filensin and CY49, respectively). In addition, there

are link proteins, the plakins, which together are likely to be involved in crystallin packing and density distribution, perhaps by offering attachment sites for crystallin molecules. CP49 is also known as phakinin. Both proteins co-assemble with α crystallin but not with vimentin.

Lenses with targeted deletions of phakinin and filensin are opaque even though lens fibre morphology is normal, indicating that these two proteins, which co-assemble to form beaded filaments, are essential for lens transparency through lens fibre cytoskeletal organization.

Membrane lipids and proteins

Membrane lipids in lens cells are highly saturated, the main phospholipid being the extremely stable dihydrophosphingomyelin. In cell membranes they are densely populated with proteins, thus restricting their mobility. Cholesterol is also abundant, which in view of its exclusion from protein 'rafts' (see p. 162) leads to patches of pure cholesterol in the membrane (cholesterol bilipid domains). Lens cell membrane proteins are extractable from lens membranes in detergents such as sodium dodecyl sulphate (SDS), a major protein being lens fibre cell-specific junctional complex protein aquaporin-0 (AQP0), previously known as major lens intrinsic protein 26. In fact this was the

first aquaporin (or water channel) identified and they act as osmoreceptors or cell volume regulators. At least 100 of these genes have been described, 11 of which are present in mammalian systems and five involved in fluid transport through various ocular tissues in and out of the eye (Fig. 4-53).

The *AQP0* gene is located on the cen-q14 region of the long arm of chromosome 12. The protein is not only involved in water transport but also in intercellular communication and movements of ions as part of the microcirculation of the lens. In the closed configuration it acts as an adhesive protein for the posterior lens capsule, maintaining the lens electrical dipole. AQP0 is important for lens transparency; mutations in this gene causing cataracts. AQP0 is absent from lens epithelial cells and its relationship to gap junction proteins in other cells is unclear. However, AQP1 is present in lens epithelium.

Other membrane proteins include numerous enzymes such as ATPases, and cytoskeletally attached proteins such as calpastatin-1 and N-cadherin. Several other high molecular weight proteins also exist in the lens fibre plasma membrane such as plakoglobin, plectin, periplakin and desmocollin, members of the spectrin family with a role in maintaining cell shape as for spectrin in red cells. Lensin is a lens-specific cytoskeletal protein linking beaded filaments to cell membrane.

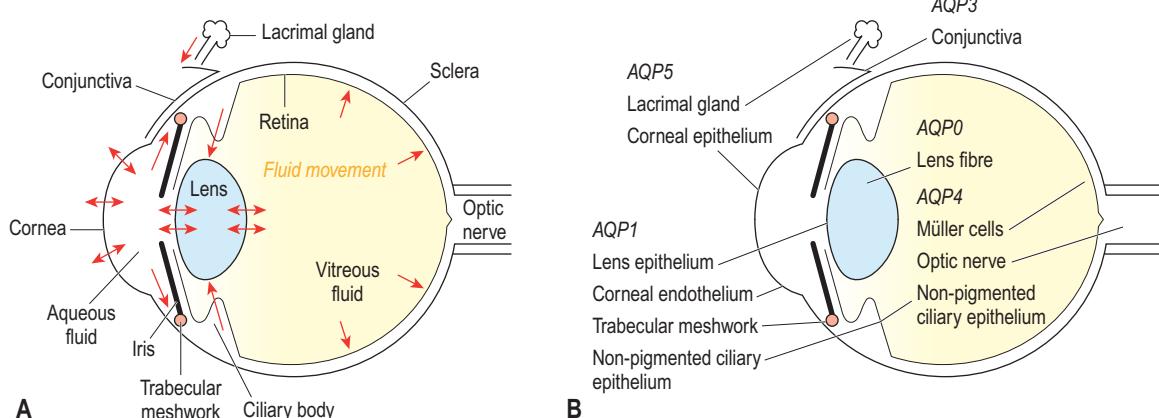


FIGURE 4-53 Fluid transport and aquaporin expression in the eye. (A) Routes of fluid movement showing secretion by lacrimal gland and ciliary body, absorption by trabecular meshwork and retinal pigment epithelium, and bidirectional movement in cornea and lens. (B) Sites of aquaporin (AQP) water channel expression in ocular tissues. (From Verkman, 2003, with permission from Elsevier.)

Extracellular matrix

The only extracellular matrix of any importance in the lens is the capsule. The capsule is constructed as for any epithelial cell basement membrane of type IV collagen and heparan sulphate proteoglycan, and acts as a diffusion barrier for the lens. Fibronectin is localized to the anterior capsule, while tenascin is present in the posterior capsule. Tenascin is one of a family of matrix-cellular proteins which includes thrombospondin and SPARC (sialo-protein associated with rods and cones, see p. 183 and Fig. 4.19), the last being required for lens transparency. The $\alpha_5\beta_1$ integrin is present in the anterior lens epithelium, while the $\alpha_6\beta_1$ integrin receptor for laminin is present in equatorial and lens fibre cells, both of which are migratory.

Semipermeable membranes and physiology of the lens

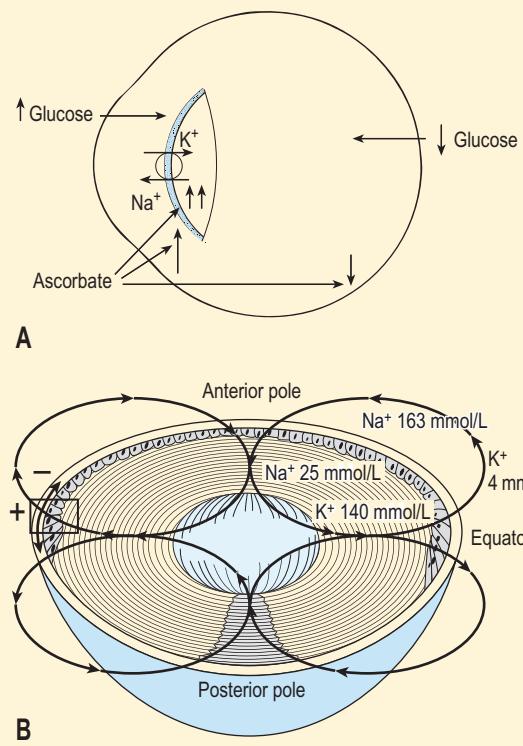
As indicated above, the lens behaves like a very large syncytium or single cell, both electrically and chemically. Active pumping mechanisms, based on Na^+/K^+ ATPases exist to pump Na^+ ions out of the lens, while chloride and water are transported into the cell (see Box 4-15). The pump is located in the epithelium with the highest concentration at the lens equator, while lens fibres do not have a pump function. As a result, electrical current density across the epithelium is highest at the equator, generating a flow of current from the equator to the lens centre, and this appears to be critical for intra-lens microcirculation (see Box 4-15).

Barriers to ion and solute transport occur at the capsule and at the plasma membranes of the epithelial and fibre cells. The capsule is permeable to small molecular weight proteins (<50 000 Da), including low molecular weight crystallins, but prevents diffusion of large molecules.

At the epithelial barrier, the cells show the typical polarization of other epithelial cells but lack tight junctions at the lateral cell surface. Instead there is an extensive system of gap junctions, which permits rapid intercellular communication, thereby allowing the cells to behave as a syncytial sheet. At the junction between the epithelium and the fibre cell, the main transport mechanism is rapid endocytosis via coated vesicles, while the very extensive system of gap junctions between each lens fibre permits rapid interfibre

BOX 4-15 TRANSPORT OF MOLECULES ACROSS THE LENS SURFACE

The lens behaves like a syncytium in which K^+ is transported into the lens and Na^+ is transported out via Na^+/K^+ ATPase present in the lens epithelium (**A**). Interestingly, the concentration of the ATPase is highest in the equatorial zone of the lens epithelium where much of the ionic transport occurs. This sets up electrical gradients with differential electrical potential differences between the lens equator and the lens poles, in part accounting for the electrical dipole that occurs in the lens (**B**). The lens also contains specific glucose transporters and transporter molecules for ascorbate and water, which ensure adequate metabolism and minimize free radical damage.



(Part B reproduced from McCaig et al., 2005.)

cell movement of metabolites, amounting in effect to a microcirculation (see Figs 4-48 and 4-49). Indeed, 50% of the fibre cell plasma membrane protein consists of AQP0, while the gap junctions in lens are to some degree specialized (more efficient) than other cell types in that AQP0 appears to assist in channel formation between the cells.

The Na^+/K^+ ATPase pump in the epithelium actively exchanges Na^+ (pumped out) for K^+ (pumped in). The Na^+ passively diffuses anteriorly down a concentration gradient present in the vitreous, across the posterior lens capsule into the lens body, where it rapidly diffuses to the anterior epithelium and is pumped out into the aqueous. K^+ ions are handled in the reverse direction, eventually diffusing passively across the posterior capsule into the vitreous. Inward currents at the anterior and posterior poles occur in the extracellular space while outward currents driven by the ATPase pump occur at the epithelium, particularly the equatorial epithelium. Critical to this process is the presence of K^+ channels in the epithelium but not in the lens fibres, which allow an overall Na^+/K^+ exchange.

While this simple pump-leak model serves to explain ion transport across the lens body there are several unanswered questions, such as the mechanism of $\text{Ca}^{2+}/\text{Mg}^{2+}$ transport, which also occurs via a specific ATPase. The $\text{Ca}^{2+}/\text{Mg}^{2+}$ ATPase is most abundant in the lens cortex. In addition, specific transporter proteins for glucose (GLUT1 in epithelium, an Na^+ -dependent SGLT transporter in lens fibres) and amino acids exist in the plasma membrane of lens fibre and epithelial cells.

Transport of water across the lens has also been shown to occur in an anteroposterior direction at a rate of about $10 \mu\text{L}/\text{h}$, probably involving AQP0, thus participating in the overall lens microcirculation, and helping to rid the lens of waste products and maintain transparency.

LENS METABOLISM

Carbohydrate

Glucose from the aqueous humour is the main source of energy for lens metabolism. Glucose enters the cell via an insulin-dependent glucose transporter located in the plasma membrane (GLUT1). Both the glycolytic and the pentose phosphate pathways are used, and under conditions of excess glucose the sorbitol pathway is entrained (Fig. 4-24). About 80% of glucose is consumed by the lens via anaerobic glycolysis. The pentose phosphate pathway uses about 10% of the remaining glucose, providing sugar residues for nucleotide synthesis. Aerobic glycolysis via the citric

acid cycle occurs only in the lens epithelium because these are the only lens cells to possess mitochondria. The epithelium also possesses most of the aldose reductase, indicating that any metabolic activity occurring via the sorbitol pathway takes place in this cell. Under normal circumstances, less than 5% of glucose is used up in the sorbitol pathway. Indeed, it is unlikely that sorbitol has a significant role in the induction of complications of high ambient glucose (diabetes). Aldose reductase is apparently induced by osmoreceptors (such as aquaporins) and interferes with NADH-binding proteins, thus disturbing regulatory free radical scavenger mechanisms (see below). In addition, aldose reductase has recently been shown to have PGF_{2α} synthase activity, is involved in the COX2 pathway, and is regulated by IL-1β. Indeed, inhibition of aldose reductase is reported to prevent allergic rhinitis by blocking the PI3 kinase/Akt/GSK intracellular signalling pathway (see Ch. 7, p. 445). Thus, it has a more direct role in inflammatory mechanisms, which are also implicated through other routes in the complications of diabetes.

Protein

Synthesis of new protein ceases with lens fibre cell formation, and all changes that occur to lens proteins after this stage are post-translational modifications. Phosphorylation of many proteins occurs, including crystallins, cytoskeletal proteins and AQP0. Several phosphorylation systems exist, including a cAMP-dependent protein kinase A and a phospholipid-dependent protein kinase C. Certain drugs enhance phosphorylation of intermediate filaments, including β-adrenergic compounds.

Numerous enzyme activities have been detected in lens protein extracts but the level of enzyme protein is very low. Some of the taxon-specific crystallins have apparent enzyme activity such as ε crystallin from duck lens (lactic dehydrogenase), ρ crystallin from frog (aldose reductase) and ε crystallin from frog (lung prostaglandin F synthase). These findings are of evolutionary rather than physiological significance but because several of these enzymes are induced in cells undergoing stress it has been suggested that stress responses may be the common denominator in these homologies (see above, heat-shock protein and α crystallin). Stress proteins and long-lived lens

crystallins may require similar properties to maintain stability and durability in anaerobic conditions. Thus, crystallins, especially the α and $\gamma\beta$ series, are among the most conserved proteins known and interestingly are not restricted to the lens ($\alpha\beta$ has been found in heart, lung, brain and retina). The promoter sequence of the αA gene has been shown to be lens-specific and has the capability of driving foreign genes selectively into its sequence. This has been proposed as an explanation for the interchangeability of function for apparently identical proteins from widely divergent sources. Similarly, the promoters for χ crystallins are lens-specific. During development and growth there is differential expression of the various crystallin genes in a highly regulated manner.

In addition to the evidence for enzymic activity in the crystallin proteins, the lens has several other proteolytic enzymes, including endo- and exopeptidase activity and membrane-associated proteases. Historically, the denudcation of the lens fibre has been described as a form of attenuated apoptosis, taking place over several days rather than hours. In addition, some of the 'death' enzymes such as caspases are activated during this process, although mice deficient in caspases 3 and 6 have normal lenses. In addition, a key component of apoptosis, namely the phosphatidylserine 'inside-out' membrane flip, does not occur. Despite these caveats, it is likely that much of the signalling machinery involved in apoptosis and lens fibre differentiation overlaps in function. In addition, autophagy does not play a part in this process since Atg5 $-/-$ (autophagy defective) mice also have normal lenses.

The neutral endopeptidases calpain I and II (cysteine Ca^{2+} -dependent enzymes) and their inhibitors, calpastins, have also been detected in lens cells. Substrates for these enzymes include cytoskeletal proteins and crystallins, and their role is probably related to protein turnover. Calpain I is present in the epithelium and lens cortex but not in the nucleus. Dysregulation of calpain genes has been suggested as a cause for age-related cataract.

Increased degradation of proteins occurs with age, particularly that of MIP26, which may have significance for coordinated intercellular functions of lens fibres and contribute to cataract. Of interest is the fact that ubiquitin conjugation, a protein degradation system in which the small 8.5 kDa ubiquitin molecule

binds to proteins before degradation, is markedly reduced in aged versus young lens nuclei.

Lipid

The unusually high concentrations of sphingomyelin, cholesterol and saturated fatty acids impart rigidity to the cell membrane (see above), which may be important in maintaining intercellular connections. In particular, the cholesterol bilipid domains ensure a high content of cholesterol in the neighbouring regions, thus maintaining stability of the membrane even if there are significant changes in the membrane phospholipids (Fig. 4-54).

In addition, high levels of phosphatidylinositol are also found in the lens, suggesting significant receptor-mediated second messenger activity in lens cells, e.g. responsiveness to hormones and catecholamines.

Redox systems in the lens microenvironment

The lens is constantly exposed to attack by oxidative agents; indeed there is a high level of hydrogen peroxide in normal aqueous and peroxidase activity is also present in the lens itself. Several enzyme systems are available to minimize or buffer the effects of oxidants, including catalase, superoxide dismutase, glutathione peroxidase and glutathione S-transferase. The lens contains high levels of glutathione (3.5–5.5 $\mu\text{mol/g}$ wet weight), with the highest concentration in the epithelium, and detoxification via the mercapturic acid pathway is an important pathway in the lens. Glutathione is produced from the interaction between glutamate and cysteine in lens cells. According to one theory glutathione diffuses towards the centre of the lens, where lens fibre cells are essentially non-metabolizing and thus unable to generate glutathione. Diffusion would occur in the opposite direction to the proposed microcirculation in the lens (see p. 229) which is dependent on ion and water transport and this difference of viewpoint has not yet been resolved. However, the current flow and the lens dipole measurements (see Box 4-15) support a pump-driven circulation rather than diffusion.

Glutathione is also important in protecting thiol groups in proteins, especially cation-transporting membrane proteins in the lens, which additionally accounts for its unusually high concentration in this tissue. More than 95% of glutathione is in the reduced state.

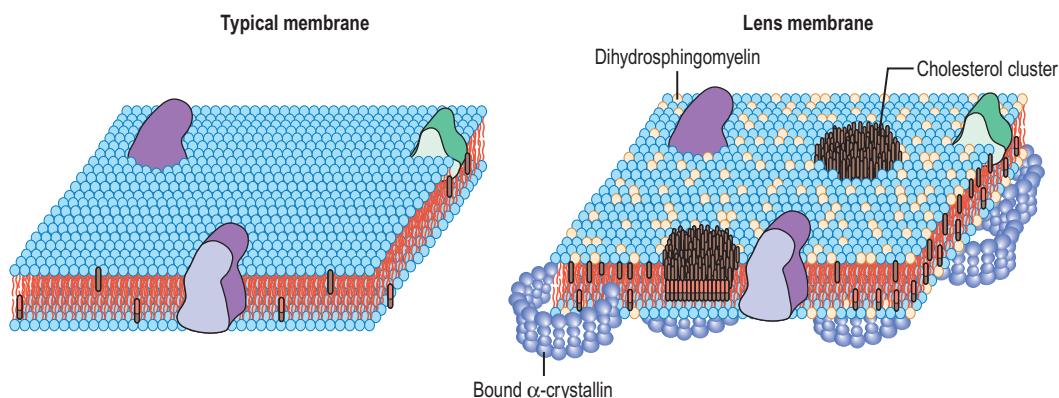


FIGURE 4-54 Figure on the right shows a ‘typical’ membrane containing few cholesterol molecules. On the left is shown a human lens membrane which contains many cholesterol molecules while most of the lipid is associated with crystallins and other lens-specific molecules such as aquaporin. The lipid-saturated membranes are highly ordered and stiff. The major lipid is dihydrosphingomyelin. (From Borchman and Yappert, 2010.)

Catalase and low levels of superoxide dismutase have also been identified in lens epithelium, indicating that these systems are also probably important.

AGEING IN THE LENS AND CATARACT FORMATION

The transmission of light decreases with age, especially for the lower wavelengths (up to a factor of 10), to the point that at low levels of illumination an apparent tritanopia can occur (see Ch. 5, p. 302). Morphologically, the cells lose cytoskeletal organization and develop vacuolation and electron-dense bodies. An increase in sodium concentration is accompanied by a decline in the membrane potential of the lens, suggesting ion channel dysfunction. Enzymatic activities decline in the lens nucleus but not in the cortex or epithelium. In addition, the appearance of water clefts in the lens as an early sign of cataract suggests decreased function of AQP0 (MIP26) and fluid transport.

Ageing of the lens and cataract formation are not synonymous. In age-related nuclear cataract there is extensive oxidation of cystine and methionine residues on lens proteins, while in aged lenses without cataract, oxidation is much less. Glutathione SH (oxidized glutathione) (see Box 4-8, p. 194) is the key. Post-translational modification of lens proteins continues throughout life. In addition to cross-linking and degradation, which occur in any stable protein system, non-enzymatic glycation is a conspicuous event. In general, $\gamma\delta$ crystallins are synthesized in young lenses, while production of $\gamma\sigma$ and β crystallins increases with

age. In addition, most of the α crystallin is lost from the water-soluble compartment to the water-insoluble compartment, as are some of the β and γ forms.

Non-enzymatic glycation of crystallins occurs at the ϵ amino groups of lysine, especially the high molecular weight aggregates of α crystallin. *In vitro*, this reaction produces a yellow fluorescent pigment similar to that seen in the ageing human lens. Interaction between various amino groups and aldehydes released from free radicals, especially through lipid, (per)oxidation produces fluorophores and ceroid/lipofuscin. In spite of the colour changes, the amount of protein that is glycated is less than 5% in an aged lens, which is considerably less than for other long-lived proteins such as haemoglobin and collagen. Lens crystallin glycation is more likely to be the result of its interaction with oxidized ascorbic acid than glucose on the basis of intra-lens concentrations, and it is possible that glutathione, by maintaining ascorbic acid in its reduced state, inhibits this process.

AQP0 also undergoes modification with age, losing a 5000 Da peptide to become MIP22 in increasing concentration. Cleavage occurs at both the C- and N-terminal ends of the molecule.

Reduced vision in cataract is caused by increased light scatter by lens proteins

The transmission of light by the lens is reduced when the ordered packing of the lens crystallins is disturbed. This can be induced in many ways, such as increased water accumulation within the lens, formation of high

molecular weight lens protein aggregates, and vacuole formation within the lens fibres with age.

Certain metabolic conditions are associated with cataract, the best known being the cataract of diabetes and a similar lens opacity in galactosaemia. In these forms of 'sugar' cataract, accumulation of water in the lens fibres was previously thought to result from the accumulation of non-degradable polyols such as sorbitol and galactitol in the lens fibre cells. High glucose/galactose concentrations in the aqueous lead to increased intracellular accumulation of glucose, which saturates the normal anaerobic glycosis pathways. Accordingly, aldose reductase is thought to fail in regulating the polyol pathway and polyols (such as sorbitol) accumulate in the cells, thereby increasing the osmotic drag of water into the cell via activation of the osmoreceptor AQP0. However, aldose reductase also affects PGF_{2α} synthesis and inflammatory processes via PGF_{2α} (see p. 197) and thus the precise role of aldose reductase in cataract formation is unclear.

Whatever the mechanism, the dysregulation in cellular metabolism, with reduction in the levels of cellular ATP and glutathione, and secondary damage to the cell, plus the increased water content causes phase separation between protein-rich and protein-poor regions of the cells and increased light scatter (cataract). As individual cells loosen their interdigitations with neighbouring cells, water clefts and vacuoles appear within the lens substance. As cells die, there is progressive increase in opacification, which in the lens cortex is seen as 'spoke-like' opacity and in the nucleus is characterized by the accumulation of insoluble protein aggregates and chromophores, causing the nucleus to change colour from yellow to red to black.

Cataract formation is caused by any insult to the lens

Since the lens is designed for the transmission of light, it responds to any insult that disturbs normal development or metabolism by opacification, even if this is only for a temporary period. Thus, certain congenital cataracts appear to affect only the fetal nucleus; radiation cataract may be limited if only a discrete area of the lens bow region is affected; sunflower cataract of trauma may be the result of shearing forces momentarily separating lens fibre cells, which then restore their interconnections; and certain forms of cataract such as

the 'feather' cataract after vitrectomy are thought to be the result of large volumes of fluid transfused through the vitreous cavity at too low a temperature or an incorrect electrolyte composition. Interestingly, such cataracts may be reversible, suggesting that the lens microcirculation is temporarily unable to cope with increased fluid load but can recover when bulk fluid flow is normalized. Similarly, 'cold' cataract is induced in young animals and is caused by the reversible precipitation of γ crystallins by phase separation in the fetal nucleus.

Many forms of cataract have been attributed to specific mutations in lens crystallins probably related to impaired molecular packing as well as several other proteins. For instance, a mutation in the Wolfram gene (*WFS1*) has been identified as the cause of congenital nuclear cataract while another gene, the *FCO1* gene, has also been implicated in a recessive form of congenital cataract.

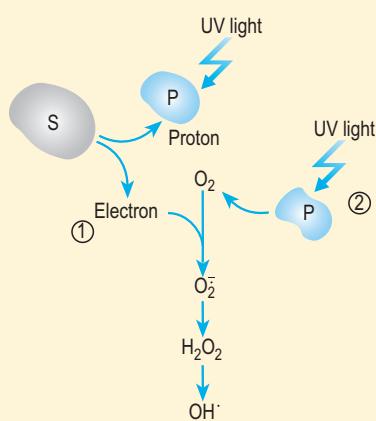
Age-related cataract formation is multifactorial

A great wealth of studies during the past 20 years has shown many biochemical changes associated with age-related cataract. In summary, these include an increase in the insoluble components of the lens, an increase in chromophores, increased protein cross-linking and aggregation, and oxidation of amino acid groups. There is a concomitant decrease in anti-oxidant enzyme systems and increased proteolytic activity. The level of glutathione is also reduced. Normal lenses contain a trypsin inhibitory activity, which may regulate age-related proteolytic activity. The major protein change in cataractous lens is the loss of α A crystallin and the selective loss of γ S crystallin. In addition there are numerous degradation peptides detectable in the water-soluble component. These mechanisms are summarized in [Figure 4-55](#).

The possibility that UV light might cause or hasten some of the effects of age has been suggested by the observation that age changes appear to be more marked in the region of the visual axis than in the equatorial region of the lens. Current views are that oxidative events are the most likely mechanism of cataract formation. Near-UV light is absorbed by tryptophan, which in sunlight is converted to N-formyl-kynurenine, a fluorescent chromophore similar to 3-hydroxy-kynurenine, a second UV

BOX 4-16 FREE RADICAL DAMAGE AND THE LENS

Free radical damage in the lens may occur through oxidative metabolism but is considered mostly to be the result of UV damage by activation of endogenous photosensitizers.



Photosensitizers act by one of two mechanisms: (1) triplet sensitizer absorbs a proton from a substrate, leading to the production of free radicals (SO_2 and H_2O_2); (2) sensitizer reacts with O_2 , leading to singlet O_2 . Typical photosensitizers include riboflavin, tryptophan and kynurenine (all present in the lens).

absorbent molecule in the lens. Both these compounds can act as photosensitizers and lead to the production of the free radical singlet oxygen (see Box 4-8). Free radicals downregulate the function of critical lens enzymes such as Na^+/K^+ ATPase and lead to lens swelling and opacification, at least in the rat model. Other free radicals generated by near-UV light such as hydrogen peroxide have been implicated in the dysfunction of hexokinase, an enzyme central to glucose utilization in the lens.

Oxygen increases the rate of photo-oxidation, and vitamin E, ascorbic acid and glutathione reduce the effects of light damage (Fig. 4-55).

The role of UV light in human cataract is unclear, although exposure to UV-B is reportedly associated with an increase in cortical and posterior subcapsular cataract but not in nuclear cataract formation. Interestingly, aged human lenses appear to absorb more UV-A and even visible light than young lenses.

Certain trace metals and compounds are associated with cataract. Experimental depletion or excess of selenite leads to cataracts by a mechanism that appears to be closely interwoven with Ca^{2+} homeostasis. In contrast, cyanate induces carbamylation of lens proteins and cataract, a process that can be prevented experimentally with aspirin. Interestingly, aspirin usage may also delay the onset of cataracts in humans.

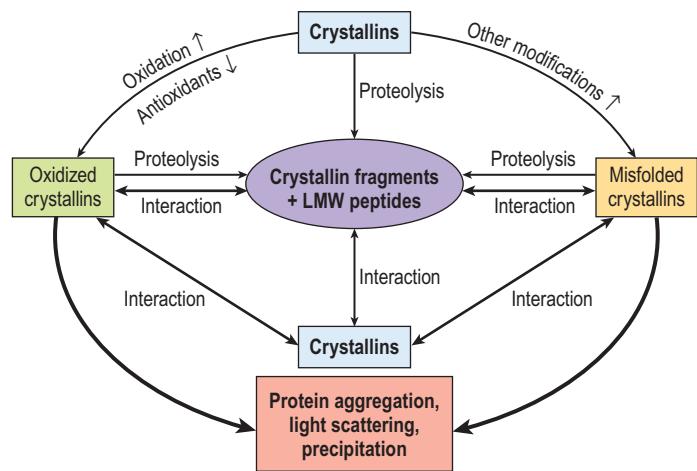


FIGURE 4-55 Schematic representation of the proposed role of crystallin fragments in ageing of the lens and development of cataract. (From Sharma and Santhoshkumar, 2009.)

Inhibition of cholesterol synthesis also leads to cataract in experimental animals. Potentially, reduced levels of magnesium may promote cataract formation.

Metabolites and chemicals such as pyruvate and caffeine have been shown to prevent effects of UV and selenium *in vitro*, respectively.

In summary, although many factors may contribute to cataract formation, probably the most important is glutathione consumption and unrestricted oxidation of intrinsic lens protein.

Mechanism of age-related cataract formation: a failure of chaperone function

As shown above, there are several processes which can lead to damaged lens proteins. Oxidation, carbamylation, deamidation and other perturbations of $\beta\gamma$ crystallins lead to their progressive inability to sustain normal intermolecular interactions. As they denature and precipitate, the α crystallins bind the unfolded proteins, but unlike true chaperones they do not have the ability to refold the $\beta\gamma$ crystallins. As a result, the chaperone capacity of the α crystallins is consumed and the complexes precipitate within the lens fibre cells, forming the insoluble protein fraction that increases with age.

The vitreous

THE VITREOUS BODY IS A TRUE CONNECTIVE TISSUE CONTAINING COLLAGEN, GAGS AND CELLS

The vitreous is 98% water, 1.0% macromolecules, and the rest solutes and low molecular weight materials.

The matrix

The vitreous transmits light by the same mechanism as the cornea, i.e. its collagen fibrils (10–20 nm) are thinner than half the wavelength of light, and the interfibrillar space is filled with GAGs (hyaluronan) at intervals that reduce the effects of diffraction in the system. Collagen imparts the gel structure to the vitreous body, is predominantly type II, similar but not identical to cartilage type II collagen (vitreous collagen has more galactosyl-glucose side chains and a higher content of alanine), and is arranged in a lattice structure in which the fibrils are suspended in a viscous hyaluronan solution. This structure is lost with age and in disease by a process known as syneresis in which the hyaluronan molecules are degraded to

smaller moieties and the collagen fibrils coagminate to form larger fibrils, becoming visible as 'floaters'. Some type VI and type IX collagens are also present and play structural roles in gel formation. In addition, a hybrid molecule composed of type V/XI α chains has been detected in vitreous in trace amounts and has been implicated in vitreous fibril formation as for cornea. In cartilage, collagen type II fibrils exist in two forms, thin (~16 nm) and thick (~40 nm), and collagen type XI is found exclusively in the thin fibrils (Fig. 4-56). Presumably collagen type XI has a similar role in the vitreous in regulating fibril diameter.

A recent member of the small leucine-rich repeat (SLR) extracellular matrix protein family, termed optin, has been identified in vitreous and also in ligament, skin and retina. It also has a role in regulating fibril thickness in the vitreous similar to collagen type IX and V/XI α proteoglycans. Importantly, optin has anti-angiogenic properties.

The normal vitreous in the young adult has a distinct architecture (see Ch. 1, p. 37). The cortex has a higher concentration of collagen and hyaluronan than the central vitreous and, in addition, the cortex contains other GAGs such as chondroitin sulphate, which may be important in vitreo/retinal apposition.

In the central vitreous gel, hyaluronan is essentially the sole GAG. Hyaluronan occurs as stiff, open coil disaccharide chains, which in solution become entangled at concentrations above 300 $\mu\text{g}/\text{mL}$ and thus add support to the gel matrix. Hyaluronan concentrations in human vitreous vary between 100 and 400 $\mu\text{g}/\text{mL}$ and the molecule binds to type IX collagen, which acts as a proteoglycan to bind the hyaluronan to the collagen fibrils. However, hyaluronan is not essential for maintaining the gel structure of the vitreous. Therefore, some long-range interactions mediated via type IX collagen are necessary. Type IX collagen is a small non-fibrillar type of collagen that contains several non-collagenous domains; these act as the proteoglycan bridges. In addition, the chondroitin sulphate proteoglycan is present in vitreous at a concentration of approximately equal ratio with type IX collagen. Traces of chondroitin sulphate also comprise part of the proteoglycan versican in the vitreous with a molecular weight of 2×10^4 to 4×10^4 Da. Hyaluronan is highly polydisperse (variable molecular sizes) and its breakdown products can activate innate immune cells

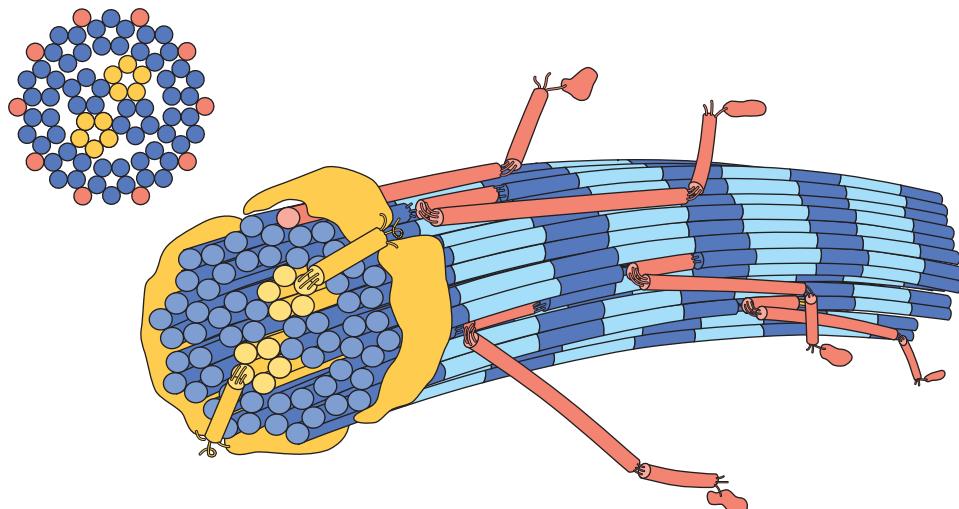


FIGURE 4-56 Schematic representation of the 10 + 4 structure of a type II collagen microfibril, two pairs of collagen type XI forming a central core surrounded by 10 type II microfibrils. Vitreous collagen has a very similar arrangement of type XI/IX/II assembly with fewer cross-links. (From Kadler et al., 2008.)

(see Ch. 7, pp. 388–389). However, as indicated above, depolymerization or even loss of hyaluronan does not of itself destroy the vitreous gel structure.

Vitreous cells

The vitreous contains a single monolayer of cells (hyalocytes), which line the adult vitreous cortex and are responsible for production of hyaluronan in the gel. However, there is no regeneration of collagen in the vitreous and thus there is no reconstitution of the gel after syneresis.

Hyalocytes are of two types: fibrocyte-like and macrophage-like. The latter are equivalent to the resident myeloid cells present in most non-CNS tissues. The role of hyalocytes in health and disease remains obscure. However, they do increase in number with age and have been implicated in macular hole formation (see Ch. 9, p. 513).

PHYSICOCHEMICAL PROPERTIES OF THE VITREOUS GEL

The viscoelasticity of the vitreous protects the retina during eye movement and deformations of the globe

The vitreous gel is non-compressible but highly viscoelastic. Thus it responds to deformations of the globe by altering its shape to comply with external forces, but permits rapid restoration of global

architecture. In this respect it behaves as a shock absorber, similar to synovial fluid, which also has a very high content of hyaluronan. These properties of the vitreous are the result of its matrix structure, particularly its content of high molecular weight hyaluronan. This molecule has a very large hydrodynamic volume and at the concentrations present in the human vitreous completely fills the interfibrillar spaces. Deformability of the vitreous has mostly been measured experimentally *in vitro*. However, recent MRI imaging has allowed *in vivo* measurements to be made which reveal that even on eye movement there is considerable deformability of the vitreous gel, which may have relevance for traction events on the retina and risk of retinal detachment (Fig. 4-57). With age, this shock-absorbing property declines as syneresis takes place (see p. 242).

Vitreous retards bulk flow of fluid and diffusion of small molecules

The flow of fluid through solutions of GAGs is variably retarded, depending on the nature and molecular weight of the GAG, and is greatest with hyaluronan. Flow of aqueous from the posterior chamber towards the retina is therefore slower in young eyes with formed vitreous gel than in older eyes where the vitreous gel has undergone liquefaction. In addition,

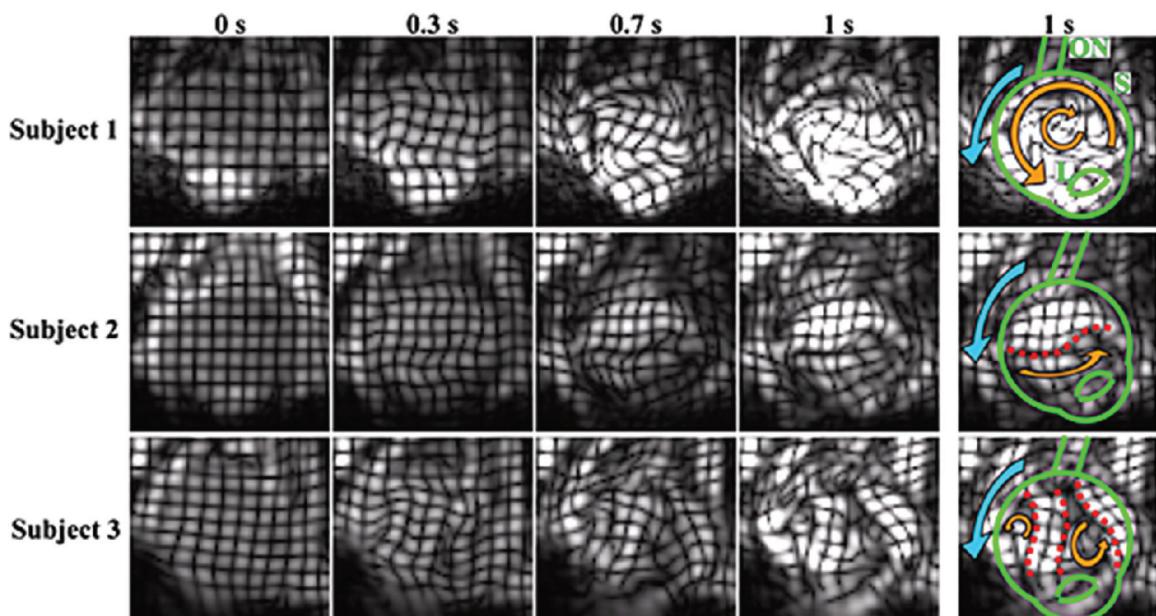


FIGURE 4-57 The technique of complementary spatial modulation of magnetization was used to show the deformation of the vitreous gel during adduction movement of the eye. These changes are shown in frames 1, 5, 10 and 15 and are overlaid with sketches demonstrating the deformation in frames 5, 10 and 15. Differences in the deformations were observed for individual subjects as shown with subject one showing homogeneous viscoelasticity while strong 'whirling' movements were observed in subject 3. (From Piccirelli et al., 2012.)

diffusion of small molecules such as glucose is retarded by the hyaluronan in the vitreous. Electrolytes are more affected in their transvitreal transport by electrostatic interactions with hyaluronan, which is a polymeric polyelectrolyte (see p. 187).

The vitreous is important for maintenance of lens clarity (sooner or later the lens develops cataract after vitrectomy) and this has been attributed to the greater access of oxygen and thus the increased risk of oxidation events due to reactive oxygen species (Fig. 4-58). Transport of fluid and electrolytes in a posterior direction across the retina is an important mechanism in the process of retinal apposition to the RPE (see next section).

The retina

THE NEURAL RETINA IS HIGHLY ORGANIZED IN LAYERS

The retina has two components, the neural retina and the RPE (see Ch. 1, p. 38).

Metabolic function in the retina

The retina's metabolism correlates with its blood supply: the outer retina, comprising the photoreceptors and the RPE, has a high metabolic activity and receives most of its blood supply from the choroid, while the metabolism of the inner retina is supplied by the retinal circulation and is much less demanding on high energy supplies.

Glucose metabolism in the retina

Despite having the highest rate of aerobic glucose consumption of any tissue, a large proportion of the glucose utilized in the retina is converted to lactate. Lactic acid production, oxygen utilization and glucose consumption are also highest in the presence of $\text{CO}_2/\text{bicarbonate}$ buffering systems, suggesting a role for carbonic anhydrases in the retina. Most of the glucose utilization in the retina is taken by the photoreceptors (>80%). Sodium-coupled monocarboxylate transporters also occur in the retina, in astrocytes and Müller cells, where amongst other duties, they function in

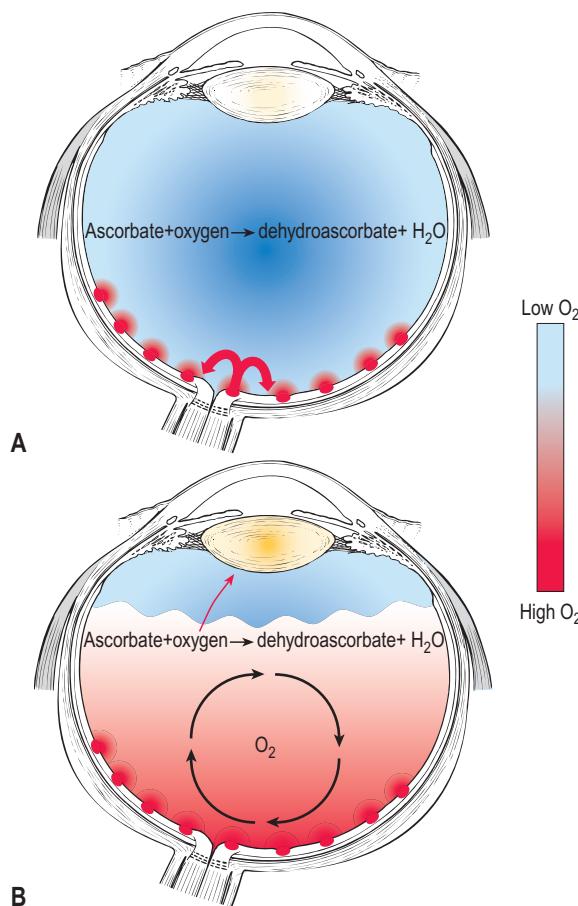


FIGURE 4-58 The distribution of oxygen (A) in the normal eye and (B) after degeneration or removal of the vitreous body. Oxygen normally diffuses from the retinal vessels into the vitreous but most of the oxygen has been consumed by retinal tissue and the concentration declines towards the central region of the gel. In the degenerate vitreous or the vitrectomized eye, more oxygen enters the fluid-filled vitreous cavity and combines with ascorbate as well as diffusing to the lens where it is relatively toxic and can promote nuclear cataract through oxidative damage. (From Beebe et al., 2011.)

'lactate shuttling' between glycolytic and oxidative pathways of glucose metabolism.

The retina can also metabolize other substrates for (ATP) energy stores such as glutamate, glutamic acid, malate and succinate. Retinal glucose is used to produce glutathione via the pentose phosphate pathway, which can be upregulated under conditions of oxidative stress. However, NADPH for glutathione stores is also produced by other non-pentose-

dependent systems involving malate and isocitrate. Glucose, glutathione and oxygen are all required for generation of electrical activity, including the ATPase-dependent 'dark currents' (see Ch. 5, Box 5-6, p. 288) in the retina.

The retina is regarded as an insulin-independent tissue, i.e. glucose enters retinal cells by transport mechanisms that are regulated directly by the extracellular concentration of glucose rather than indirectly by insulin. Glucose transport occurs by facilitated diffusion via GLUT 1 and GLUT 3 transporter proteins, similar to glucose transport in the brain. GLUT 1 and GLUT 3 are present in endothelial cells of the blood-retinal barrier, where much of the transport occurs. The insulin-linked GLUT 2 and recently GLUT 4 have also been identified in retinal tissue. Photoreceptors respond to insulin via a retina-specific insulin receptor, which is similar to brain insulin receptor in that it exists in a 'tonic' state of activity and does not change in conditions of fasting or excess glucose. The insulin receptor phosphorylates cyclic GMP-gated channels, which is central to phototransduction (see below).

Most of the retinal glucose metabolism is dealt with by retinal neurones. However, Müller cells are also likely to be involved in facilitating glucose metabolism in the retina, as energy stores, as well as 'managing' lactate production, since they contain high levels of glycogen, especially in species that lack a retinal blood supply. In addition, lactate released by Müller cells can be metabolized by photoreceptors. Photoreceptors in turn release glutamate, which is taken up and metabolized by Müller cells ('lactate shuttling').

Protein metabolism in the retina

Many of the neurotransmitters required for normal retinal cell function occur as free amino acids in the retina (see p. 265). Most of them are generated during glucose metabolism in the citric acid cycle; in addition, taurine, which is not a neurotransmitter but appears to be essential for, and is avidly taken up by, photoreceptor cells, is the most abundant amino acid in the retina although its receptor/transporter has not been identified. Taurine also regulates voltage-gated channels in ganglion cells. Taurine is not incorporated into proteins but has other functions. Interestingly, taurine may have a protective role for the retina in diabetes. Glutamate is neurotoxic and is converted by the retina to

glutamine by glutamine transferase (synthase) localized to the Müller cells. Transport of amino acids is now known to require specific amino acid transporters, for which there are several 'systems' (systems y , b , B , b^o and more). Some are linked to Na^+ transport while others are independent of Na^+ intake. Rapid uptake of amino acids is essential not only for the supply of neurotransmitters but also for arginine

uptake, which is necessary for the synthesis of nitric oxide, the major regulator of endothelial cell function.

Protein synthesis, as studied by methods such as leucine incorporation, is most active in the photoreceptors during such processes as photoreceptor renewal (see Box 4-17).

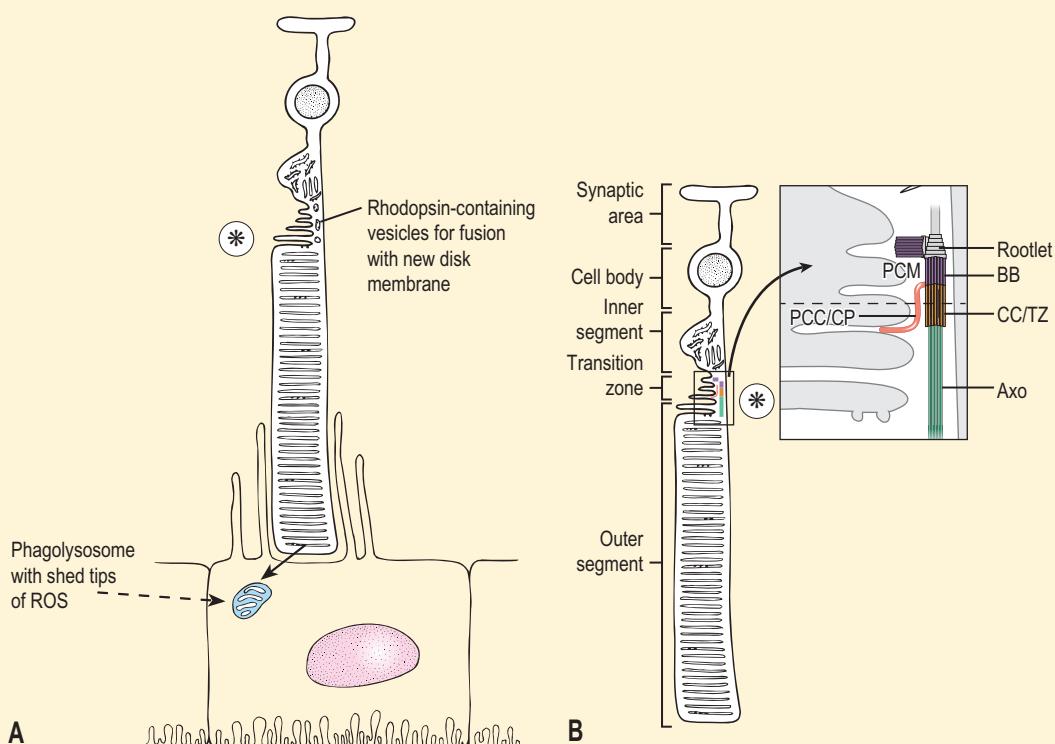
In addition to retina-specific proteins (see below), several proteins common to many tissues are present.

BOX 4-17 PHOTORECEPTOR RENEWAL

Photoreceptor renewal occurs by synthesis of new protein-rich membrane at the outer limiting membrane, with shedding and phagocytosis of the outer segment tips by the RPE (A). Disks are formed by the evagination of the plasma membrane \circledast at the junction between the inner and outer segments, while rhodopsin and other proteins synthesized in the ER and Golgi apparatus are transported in vesicles for fusion to the newly formed plasma membrane \circledast . Photoreceptor renewal is similar in rods and cones; phagocytosis of the receptor tips occurs in a diurnal manner. The mechanism of phagocytosis is unclear but involves a membrane glycoprotein CD36, which is also involved in the uptake of apoptotic neutrophils and oxidized low-density lipoprotein by haematopoietic cells such as macrophages. In

addition, rod outer segment (ROS) phagocytosis is associated with induction of cyclo-oxygenase-2 (COX-2), an enzyme involved in prostaglandin synthesis. Recent studies have also shown the importance of the cilia in the functioning of the photoreceptor, since genetic mutations in a number of genes controlling cilia proteins produce forms of retinal degeneration now known as ciliopathies (B).

There are four distinct compartments in photoreceptor primary cilia, indicating known proteins that define their respective extent, are (1) distal cilium or axoneme (Axo; green); (2) connecting cilium/transition zone (CC/TZ; orange); (3) basal body (BB; purple); and (4) periciliary complex or ciliary pocket (PCC/CP; red). These compartments serve discrete functions in the cilium (see inset in Figure).



For instance, laminin is present in vessel structures, fibronectin in the interphotoreceptor space, and matrix proteoglycans are widely distributed throughout the retina. Tenascin-C is present in the extracellular matrix and is thought to play a role in preventing myelination of retinal neurones. However, tenascin-C knockout mice show no myelination of their retina. Several growth factors are present in the retina, such as insulin-like growth factor 1 (IGF-1) and acidic and basic fibroblast growth factor (bFGF). Basic FGF is not only present in basement membranes of vessels but is also distributed in such regions as the photoreceptor layer, where it may play a trophic role in outer segment renewal.

The retina contains a high content of lipid (20%)

The predominant lipids in the retina are the phospholipids, phosphatidylcholine and phosphatidylethanolamine (in total around 80%). There is also a high content of polyunsaturated fatty acids in the retina, especially in the outer segments, some containing more than six double bonds (known as 'supraenes'). This renders the retina particularly susceptible to oxidative damage (see p. 193). Disk membranes of photoreceptors are rich in phosphatidylethanolamine while the plasma membrane has higher levels of cholesterol. This is relevant to the activity of rhodopsin because it is inhibited in the presence of the sterol. Thus the older disk membranes at the tip of the photoreceptor (see Box 4-17) have lower levels of cholesterol than the fresh, newly formed disks, allowing easier activation of rhodopsin at the photoreceptor tip.

Lipid metabolism is varied and complex in the retina; thus, in addition to synthetic activities in microsomes, exchange of bases between different lipid species occurs, while frequent acylation–deacylation reactions also occur. Lipids are continually undergoing degradation via phospholipases and modifications including decarboxylation and methylation by the appropriate enzymes.

BLOOD FLOW IN THE RETINA

Blood flow is determined by a balance between the perfusion pressure and the resistance in the blood vessels. Blood flow in the retina is autoregulated. In general, this means that the retinal vessel calibre varies with the cardiac output to ensure that blood

flow is kept constant, although it has also been interpreted as meaning that the blood flow is varied according to the nutritional demands of the tissue. In the adult, the blood flow in the retina is maintained constant over a range of ocular perfusion pressures from 45 to 145 mmHg and this is mostly achieved by changes in blood vessel diameter, as shown in mice deficient in the endothelial nitrous oxide synthase.

Retinal blood flow comprises about 5% of the total flow to the eye, the majority passing through the choroid.

The blood–retinal barrier regulates the passage of molecules into the retina

The blood–retinal barrier is maintained by tight junctions that exist between the endothelial cells of the retinal vessels and similar tight junctions in the RPE (see pp. 254–258). Thus, the retinal vessels and the RPE are impermeable to the passage of molecules greater than 20 000–30 000 Da, and small molecules such as glucose, amino acids and ascorbate are transported by facilitated diffusion (mostly GLUT 1 at both sites, but also GLUT 3 in the retinal vessels). Similar transporters are present for amino acids including arginine, and both classes of transporters are present in smooth muscle cells and pericytes ensuring rapid transport and uptake of these moieties into the retinal tissue. Arginine for instance is central to the production of NO by endothelial nitric oxide synthase (eNOS), a critical regulatory element in retinal blood flow (see above). Endothelial cells principally metabolize glucose by anaerobic glycolysis.

Although the retina is considered an insulin-independent tissue, the endothelial cells and pericytes possess high-affinity receptors for insulin, IGF-1 and IGF-2. The role of these receptors in the regulation of glucose transport in the retina is not clear because their effect is delayed for some hours, suggesting that they stimulate protein synthesis and the production of new transporters rather than recruit existing transporters, as occurs in insulin-dependent tissues such as muscle.

Retinal blood flow may be partly under autonomic control

Retinal vessels possess all four types of high-affinity adrenergic receptors, although in low numbers. In

addition there is indirect evidence that, despite well-recognized mechanisms of autoregulation, some degree of autonomic control exists in humans. How this might occur in the absence of nerve fibres is unclear, but it is possible that autonomic nerves in the choroid supply vessels at the optic nerve head. The retinal vascular bed may be one of the few systems to lack perivascular mast cells, an important response element for catecholamines.

Retinal blood flow is responsive to hyperoxia (vasoconstriction) and hypercapnia (vasodilatation), the latter via the prostaglandins PGD₂ and PGE₂. Other mediators of changes in vessel diameter include the eicosanoid, PGI₂, endothelin and nitric oxide. PGI₂ and endothelin have been detected in retinal vessels and presumably are released under appropriate conditions. Nitric oxide is released via endothelial nitric oxide synthase (see Ch. 7, p. 385) and provides basal levels of vascular dilatation. Retinal illumination induces release of nitric oxide but autoregulation in retinal vessels is not significantly affected by nitric oxide. Contractile activity in the retinal vessels is attributed to the pericytes, whose role therefore may be to regulate blood flow. Their early loss in diabetes may account for the increase in blood flow that occurs in the retina in diabetes, and may contribute to the development of retinopathy.

PHOTORECEPTORS

Photoreceptors are specialized for reception of visual stimuli and have unique characteristics (see Ch. 1).

Metabolism and turnover

Photoreceptors are some of the most highly metabolic cells in the body, utilizing glucose both aerobically and anaerobically. Photoreceptor outer segments lie in apposition to the RPE in the interphotoreceptor matrix between the apical microvilli of the RPE cell. Extensive protein and lipid synthesis ensures a continuous turnover of new outer segment membrane at the junction with the inner segment; the tips of the outer segment containing the 'oldest' disks are phagocytosed as small packets of about 200 disks by the RPE cell, a process that occurs in a diurnal manner just after light onset (Fig. 4-59). Three RPE cell surface receptors have been identified which are involved in the phagocytosis of

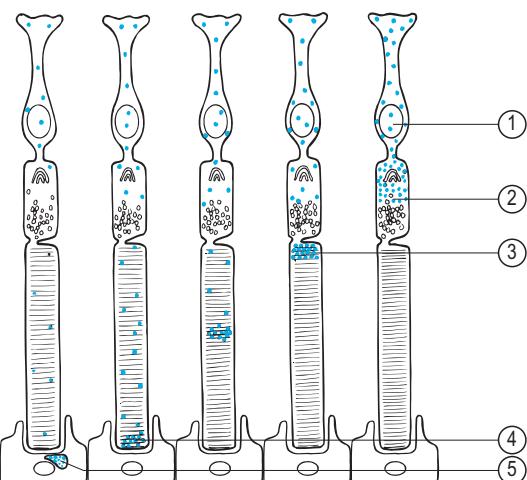


FIGURE 4-59 Photoreceptor renewal and points where damage may occur: during (1) transcription; (2) post-translational modification; (3) incorporation into disk membrane; (4) disk shedding; (5) phagocytosis by RPE.

outer segments, namely CD36, the tyrosine kinase MerTK and the integrin $\alpha_5\beta_1$. Loss of these proteins may lead to retinal degenerations (MerTK: human retinitis pigmentosa and rat RCS disease; $\alpha_5\beta_1$:RPE lipofuscin accumulation). Diurnal regulation of phagocytosis is under control of the secreted glycoprotein milk fat globulin-EGF8. Activation of MerTK may be under autocrine regulation of its natural ligand, Gas6, which is also expressed in RPE cells or via 'tubby' proteins which are expressed in photoreceptors and generate an 'eat me' signal similar to that which occurs in apoptotic cells.

Complete renewal of the rod outer segment takes about 9–10 days. In contrast, although cone outer segments are phagocytosed in a similar manner, the process appears to be more random and occurs when darkness occurs; cone membranes and their integral proteins are much more stable and long-lasting.

Insertion of rhodopsin into the disk plasma membrane follows a well-defined pathway from the inner segment RER to the outer segment plasma membrane infolding (see Box 4-18). Glycosylation of rhodopsin takes place through combined co-translational and post-translational events in a classic lipid-carrier mechanism using Dol-P-P-GlcNAc (Fig. 4-60), which can be inhibited by tunicamycin. Acylation of rhodopsin also occurs in the membrane via palmitic acid.

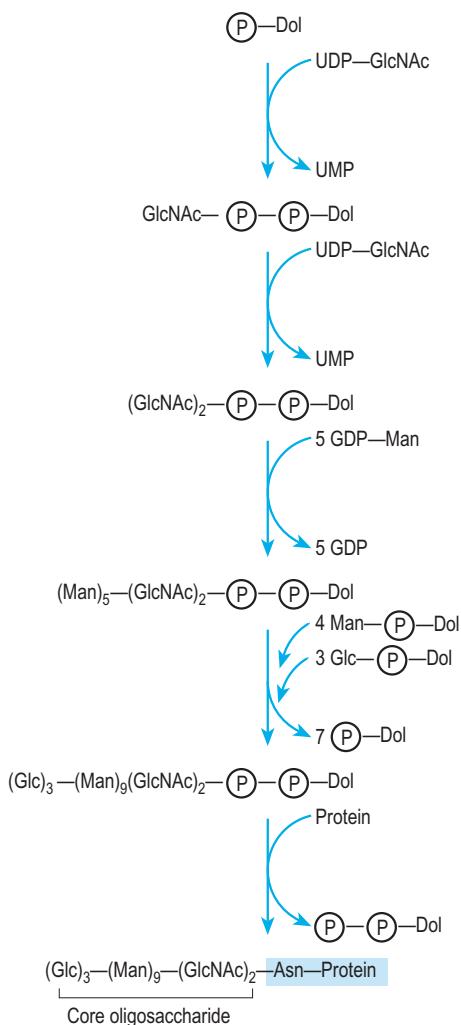


FIGURE 4-60 Glycosylation and acetylation of rhodopsin.

Lipids in photoreceptors are replaced both by membrane turnover and by molecular replacement. The abundant stores of phosphatidylcholine are synthesized from large intracellular pools of free choline and phosphorylation by ATP; the activated choline then reacts with 1,2-diacylglycerol to form phosphatidylcholine. Similar mechanisms operate for the synthesis of phosphatidylserine and phosphatidylethanolamine; all three phospholipids are synthesized in the RER but are transported to the newly forming outer segment membrane by different mechanisms. The role of the high concentrations of docosahexanate

phospholipids in photoreceptors has recently been identified as enhancing the function of many of the proteins involved in the visual transduction cascade, such as GDP-bound transducin (see below).

Several chemical reactions are associated with disk shedding, although the specific stimulus and its site of origin (i.e. the photoreceptor or the RPE cell) are not known. The circadian light–dark rhythm of shedding is under the control of melatonin- and/or 5-methoxytryptophol-synthesizing enzymes that are predominantly active in photoreceptors (Fig. 4-61). Melatonin synthesized and released in the retina does not enter the circulation but acts locally. The retina has widely distributed melatonin receptors, both the MT1 and MT2 (Mela and Melb) receptors being found in the photoreceptors and in inner retinal amacrine cells. A third receptor (Melc) has been detected in non-human species. In contrast to melatonin, dopamine appears to counter-regulate retinal circadian rhythms by suppressing melatonin synthesis through the D2/4 receptor, while melatonin works on the MT2 receptor in the reverse direction. Dopamine may also influence a second circadian regulated system involving melanopsin, a photopigment present in inner retinal ganglion cells, and possibly does so through activating the MT2 receptor (see p. 267).

The mammalian retina also appears to possess an autonomous melatonin-responsive circadian oscillator independent of central (suprachiasmatic nucleus) control and rhodopsin and cone opsin synthesis are in phase with this rhythmic oscillation. However, several other compounds have an effect on disk shedding, such as excitatory amino acids, glutamine and aspartate, while certain divalent ions are also essential (Ca^{2+} , Mn^{2+}). Recent studies in mice lacking the D4 dopamine receptor have shown that this membrane protein may regulate several of the controlling mediators of disk shedding apart from melatonin, such as a light-sensitive pool of cAMP.

Phosphoinositide metabolism is considerably greater than phosphatidylcholine or phosphatidylethanolamine metabolism in the photoreceptor but its precise role in phototransduction is unclear (see p. 258). Cytidine triphosphate is also a product of light transduction and is linked to phosphatidylinositol formation.

BOX 4-18 RHODOPSIN SYNTHESIS

Insertion of rhodopsin into the outer segment plasma membrane is facilitated by the lack of a signal peptide, which permits integration of opsin into the lipid bilayer by

co-translational coupling of glycosylation and asymmetric insertion via specific insertion sequences in the protein.

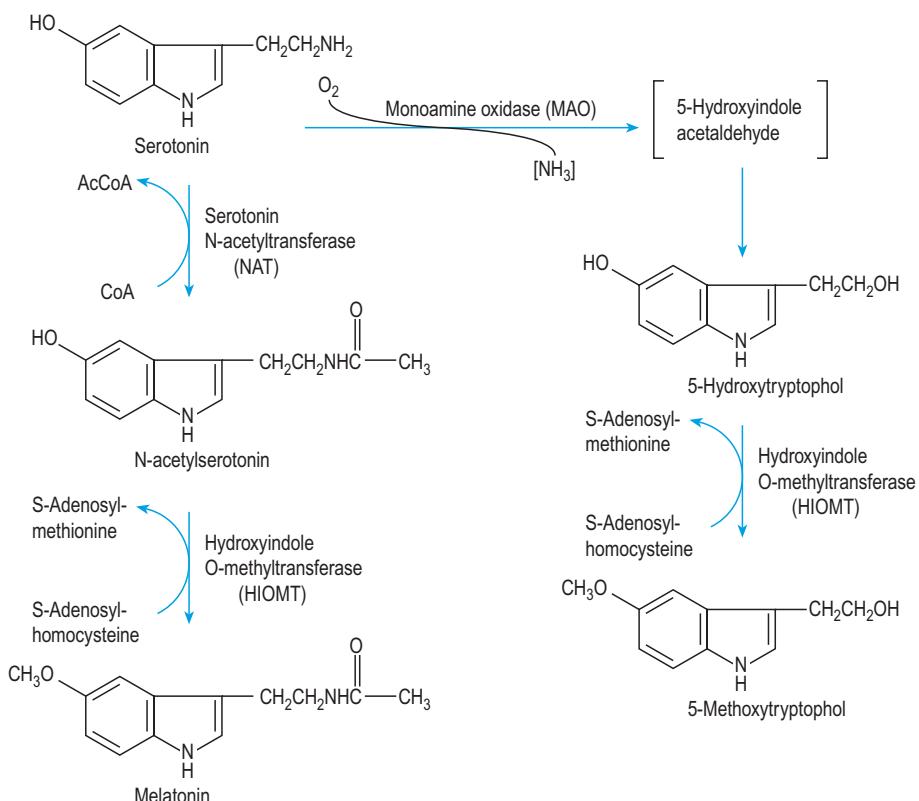
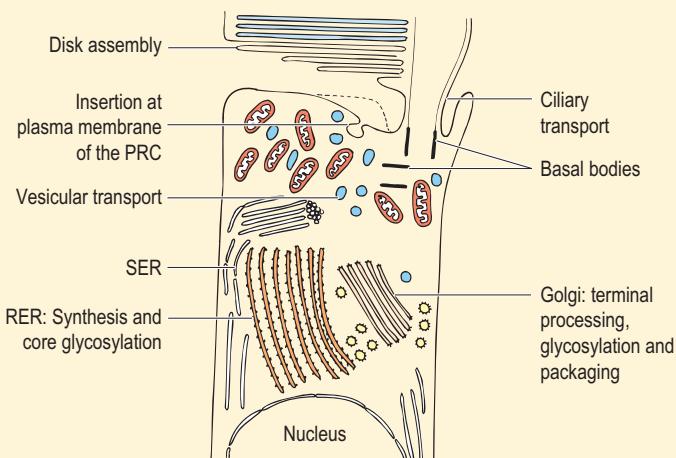


FIGURE 4-61 Regulation of disk shedding. Disk shedding may be under the control of melatonin-synthesizing enzymes such as serotonin N-acetyltransferase (NAT), which is present in the pineal gland and retina.

Photoreceptor cell-specific proteins

The highly differentiated visual cell contains many unique proteins, including integral membrane proteins, membrane-associated proteins and cytosolic proteins, of which more than 30 alone are involved in the phototransduction cascade. Some of these are shown in Table 4-8. However, there are many more proteins, and many of them have been evolutionarily

conserved. For instance, 57 genes and their respective proteins were identified and selected in a recent study for analysis and categorized in three sets: photoreceptor cell type-specific, process-specific (e.g. phototransduction itself, the retinoid cycle and developmental regulation) and a specific functional role (e.g. opsin, G protein, etc.).

The protein composition of the disk membrane is predominantly rhodopsin (90%), whereas the plasma membrane has a wider range of cell-specific proteins and less rhodopsin (50%). Rhodopsin is the visual receptor protein; proteins such as peripherin/RDS (Fig. 4-62) and the spectrin-like protein Rom-1 have structural functions in the maintenance of the photoreceptor shape, similar to those of spectrin/ankyrin-like proteins in red cells. Both are members of the tetraspanin family of proteins, and may also have a role in membrane fusion important for rod disk generation.

Remarkably different mutations in the RDS protein (Fig. 4-63) produce different clinical types of retinal degeneration such as autosomal dominant retinitis

Integral membrane proteins	Peripheral/cytosolic proteins
Rhodopsin	Arrestin (48 kDa protein, S antigen)
cGMP channel	Transducin
Na ⁺ /Ca ²⁺ -K ⁺ exchanger	Phosphodiesterase
Glucose transporter	Phosducin
Guanylate cyclase	Rhodopsin kinase
Peripherin/RDS	Guanylate cyclase
Rom-1	
ABCR/rim protein	
Retinal dehydrogenase	

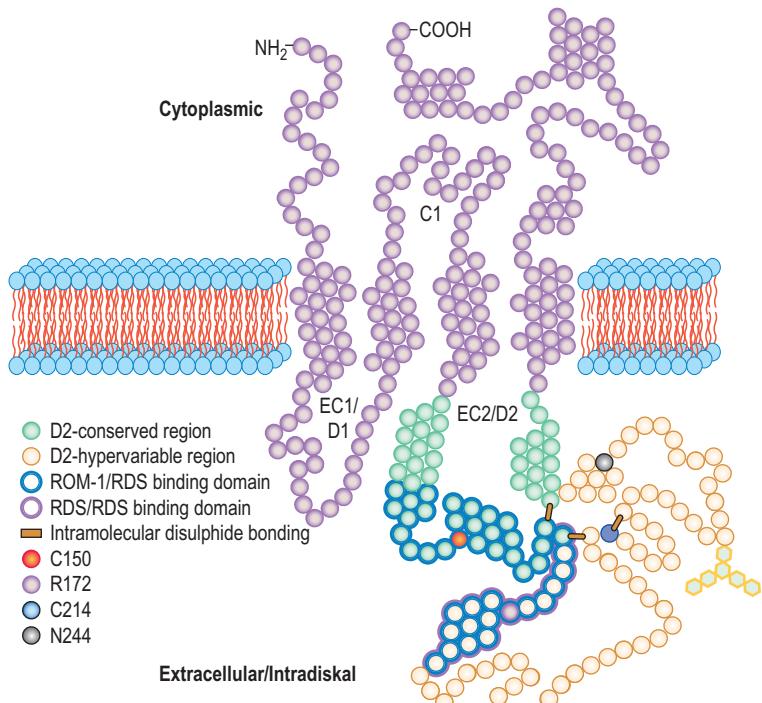


FIGURE 4-62 Structure of the RDS/peripherin protein. Structural features of the RDS protein. RDS contains four transmembrane domains, two intradiskal loops (D1 and D2), cytoplasmic N- and C-termini, and a small cytoplasmic loop (C1). (From Chakraborty et al., 2013.)

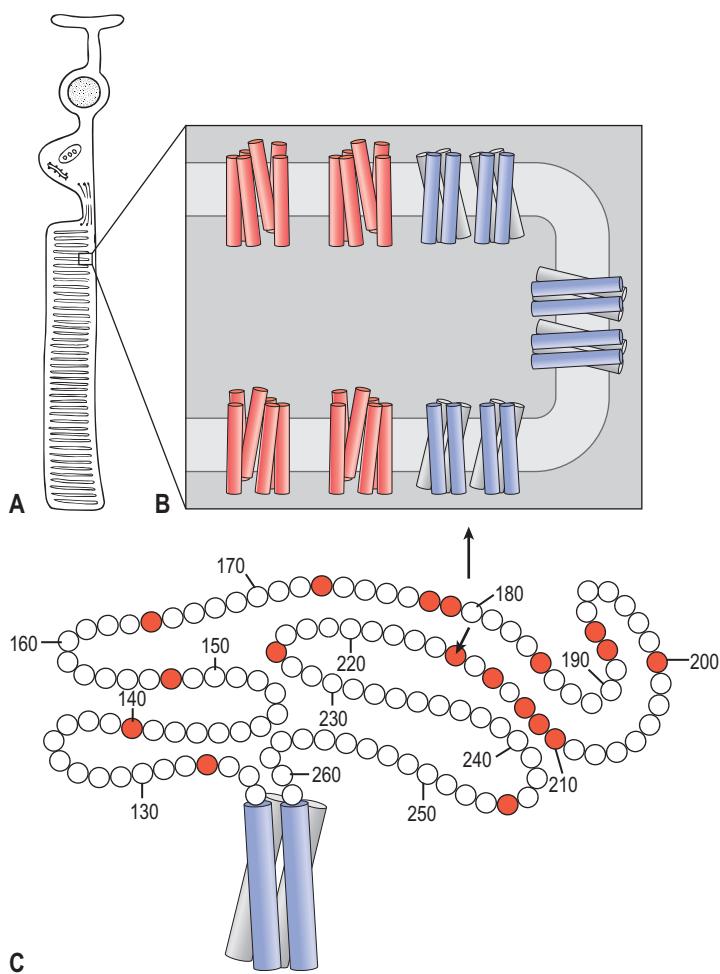


FIGURE 4-63 The rim region of photoreceptor disk shown in **A** are expanded in **B** to reveal the 4-span (tetraspanin) arrangement of the RDS/peripherin molecule with the intradiscal portion of the molecule shown in **C**. Specific amino acids locating mutations responsible for forms of retinitis pigmentosa are identified in red. (From Vos et al., 2010.)

pigmentosa and macular dystrophy (see Ch. 9, p. 514). The $\text{Na}^+/\text{Ca}^{2+}$ exchanger facilitates ionic transport during phototransduction and maintains Ca^{2+} homeostasis. In addition, the spectrin-like protein Rom-1 appears to be linked to a second ion-channel protein of 67 kDa. Stacking of rod and cone disks is a central organizational requirement for ordered photoreceptor function and a set of glutamic acid-rich proteins (GARPs) interact with RDS/Rom to both structure stacking of disks and participate in ion-channel functions.

In contrast to the integral membrane proteins, many of the peripheral membrane and cytosolic proteins are not exclusive to the photoreceptor and are intimately involved in the light amplification cascade

itself (see below). Transducin is a member of the family of G proteins and is composed of three chains (α , β , γ), which dissociate during the light response.

Certain other proteins are located in the photoreceptors as well as in other areas of the retina; these include the IGF-1 receptor, IGF-1-binding protein, and FGF, both of which have been implicated in the induction of proliferative diabetic retinopathy (see Ch. 9, p. 504). In addition, there is a glucose transporter (GLUT 1) for control of intracellular glucose levels.

Melanopsin comes of age

Because it was recognized that certain light-sensitive processes, such as those entrained in a circadian fashion, were likely to reside at a different site from

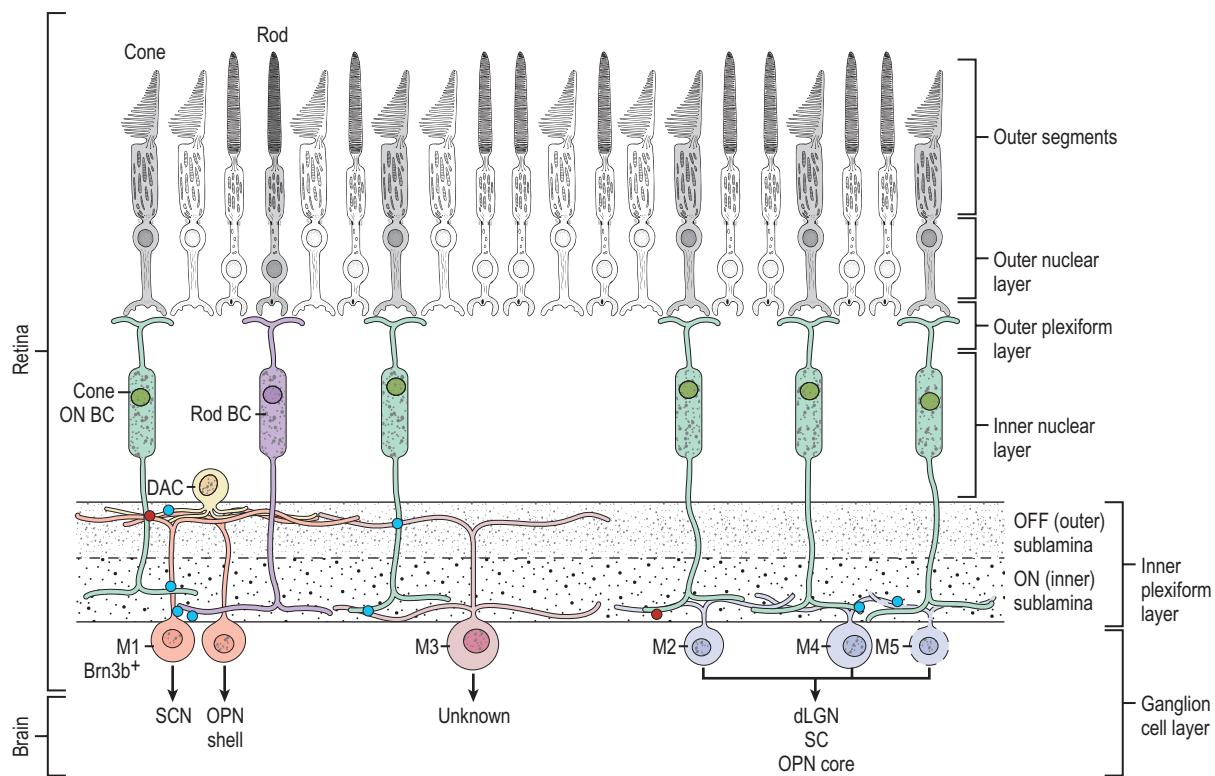


FIGURE 4-64 Five distinct morphological types of melanopsin-expressing ipRGC exist, as shown here, and project to different parts of the brain such as the lateral geniculate nucleus (LGN), the suprachiasmatic nucleus (SCN) and the olfactory pretectal nucleus (OPN) (see Chs 1 and 5). (From Schmidt et al., 2011.)

visual photoreception, a search for other photoreceptors was initiated. Early studies indicated that the likely site was intraocular and probably retinal and in due course a photopigment, melanopsin, was detected in specialized retinal ganglion cells with large receptive fields (see Ch. 5, p. 306). These are now known as intrinsically photosensitive retinal ganglion cells (ipRGCs) of which there are five types with different functions, including entrainment of the circadian clock, sleep and pupillary responses to light (Fig. 4-64).

Photoreceptors are easily damaged but readily regenerate

The extensive metabolism and rapid turnover of photoreceptor outer segments render them highly susceptible to damage. Damage may occur at any level from synthesis of new membrane to phagocytosis of the outer segment tips (see Fig. 4-59).

Photoreceptors also degenerate when they are separated from the RPE, as in retinal detachment, or when there is a subretinal collection of fluid; photoreceptors are lost in inflammatory and metabolic retinal diseases and are probably highly susceptible to free radical damage. Specific photoreceptor loss occurs in various forms of retinal degeneration: inherited forms of these disorders are collectively known as retinitis pigmentosa and for many of them mutations in retinal specific proteins have been described (see p. 261 and Ch. 3, p. 151). To date, more than 150 gene mutations have been associated with various retinal degenerations.

Damage to the retina also occurs as part of normal physiology via both light- and oxygen-induced mechanisms. Around one-third of the mutations associated with retinal degenerations occur in proteins associated with the photoreceptor cilium, the connecting structure between the inner and outer segments (see Ch. 1, p. 41). These are known as ciliopathies, in

which the Wnt signalling pathway is implicated (see eFig. 4-5). The *Wnt/Drosophila wingless* family of genes express highly conserved secreted glycoproteins which spread across tissues to reach their targets where they interact with the products of two sets of genes (*Frizzled* and genes for low-density lipoprotein-related proteins, LRPs). In the fruit fly, *Drosophila*, a species that is frequently used to study molecular genetics, Wnt signalling has been shown to be central to normal photoreceptor development as well as to central nervous system development, probably through regulation of the expression of pro-survival factors such as *Dickkopf* (*Dkk3*) in cells. In fact this mechanism highlights the fact that photoreceptor damage in retinal degenerations and other conditions is mediated via apoptosis, which may occur by both caspase-dependent and caspase-independent mechanisms. Light-induced retinal damage varies with the intensity, wavelength, duration, cyclical nature and previous antioxidant status.

For instance, it has been shown that cyclical light is less injurious to the retina than constant

illumination of equivalent power, an effect associated with higher levels of ascorbic acid, vitamin E and glutathione, and lower levels of 22:6(n-3) fatty acids in the tissue. RPE65, the retinoid cycle enzyme and an intrinsic retinal pigment epithelium protein with a critical amino acid residue at position 450, regulates the rate of rhodopsin synthesis through the action of two proteins, elongation of very long fatty acids-like 1 (ELOVL1) and fatty acid transport protein 4 (FTAP4), both of which have very long fatty acid synthase activity. This demonstrates the complex biochemical machinery which is in place to protect photoreceptors from light damage. The death signal for photoreceptors involves induction of the pro-apoptotic transcription factor AP-1 such that inhibition of regeneration of rhodopsin or suppression of AP-1 can prevent light-induced damage to photoreceptors (Fig. 4-65).

Extensive antioxidant enzyme systems are present in the retina and, under conditions of stress such as light injury, levels of glutathione peroxidase and glutathione S-transferase are markedly raised. In

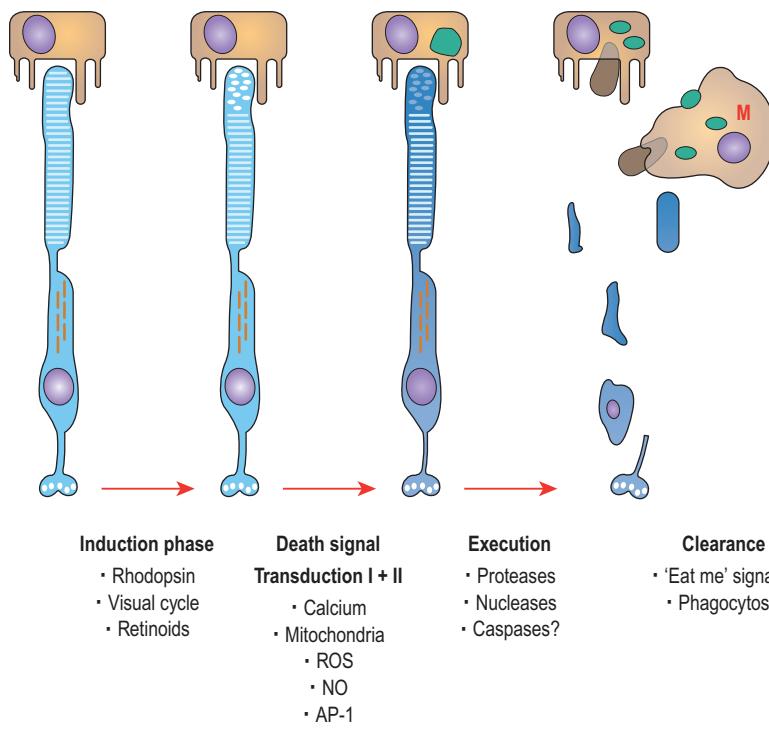


FIGURE 4-65 Schematic drawing of major components which contribute to light-induced photoreceptor apoptosis. Induction phase: rhodopsin is essential in this process. Death signal transduction: requires Ca^{2+} and transcription factor AP-1. Execution: mechanism is not clear, and may require caspases. Clearance: signals inducing phagocytosis by RPE and, in acute light damage, macrophages, are not known. (From Wenzel et al., 2005, with permission from Elsevier.)

addition, photoreceptor expression of glutathione peroxidase4 (Gpx4) is absolutely required for normal photoreceptor development. Superoxide dismutase (the Cu²⁺/Zn²⁺ form) is also present in significant amounts in several layers of the retina, but levels of catalase are low. Despite this, treatment with either of these protective agents as well as other antioxidant nutrients, N-acetylcysteine and thioredoxin can prevent retinal degeneration in experimental models. Melatonin also has important antioxidant activity. In addition, vitamin E is of major importance in reducing photoreceptor damage, mainly by its role in inhibiting lipid peroxidation at different stages in the process of free radical damage (see Box 4-16).

Iron in the form of Fe²⁺ ions can interact with hydrogen peroxide to produce high levels of hydroxyl radicals (see Box 4-8, p. 194) and cause retinal injury. Local release of iron may occur in diseases involving retinal haemorrhage and when intraocular foreign bodies are present. Ceruloplasmin, which contains 95% of the copper in the serum, mediates iron metabolism via ferroxidase, which converts ferrous to ferric iron before it is delivered to serum transferrin after efflux from the cell. Patients with the recessive disorder leading to altered ceruloplasmin blood levels have severe progressive retinal degeneration. Iron homeostasis is essential to normal retinal function and the five main regulatory proteins are present in the RPE cell (Fig. 4-66).

The macular region of the retina is particularly susceptible to light damage. Interestingly, this region contains additional yellow pigments lutein and zeaxanthin and a further pigment mesozeaxanthin, which may be transported to this region from the blood and whose function is thought to be related to reducing glare from short-wavelength blue light. These pigments, which belong to the family of carotenoids (tetra-terpenoids with 40 carbon isoprene backbone) (Fig. 4-67), also have an antioxidant effect, particularly at low levels of oxygenation. They are referred to as xanthophyll macular pigments and can be measured non-invasively in the retina using Raman spectroscopy. Sustained levels of these pigments are considered protective against macular degeneration.

Finally, melanin in the RPE layer is a very effective free radical scavenger, particularly in the reduced

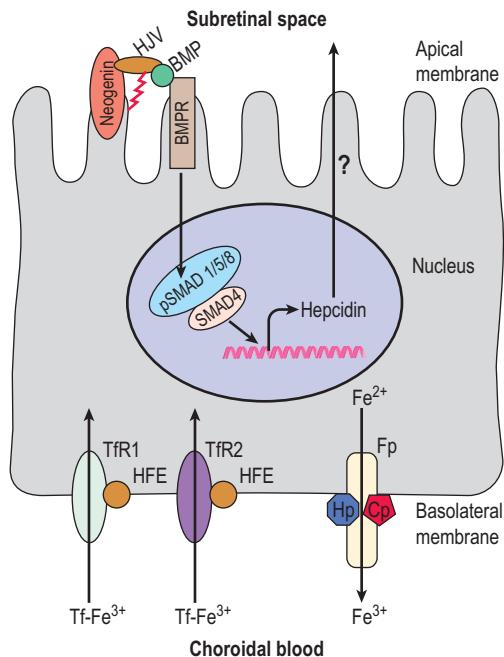


FIGURE 4-66 Expression pattern of haemochromatosis genes in retinal pigment epithelium (RPE). TfR, transferrin receptor; HFE, HLA-like protein involved in iron homeostasis; Fp, ferroprotein; Hp, hephastin; Cp, ceruloplasmin. (From Gnana-Prakasam et al., 2010.)

form, where it may act as a trap for stray light-induced free radicals emanating from the photoreceptors, but the role of melanin is more likely to be related to protection of the RPE cells themselves because these are terminally differentiated cells. Melanin may act as an antioxidant due to its ability to bind metal ions such as Fe²⁺, a process well known to occur in fungi. Melanin granules in RPE cells appear to be connected to the lysosomal enzyme system in RPE cells and loss of melanin is associated with age-related macular degeneration.

The interphotoreceptor matrix is the biological glue for retinal adhesion

The interphotoreceptor matrix extends from the outer limiting membrane of the retina (see Ch. 1, p. 41) to the surface of the RPE cell. It is an extremely narrow space, almost a potential space, but contains some unique and physiologically important molecules. These include interphotoreceptor retinol-binding protein (IRBP; accounts for at least 70% of the

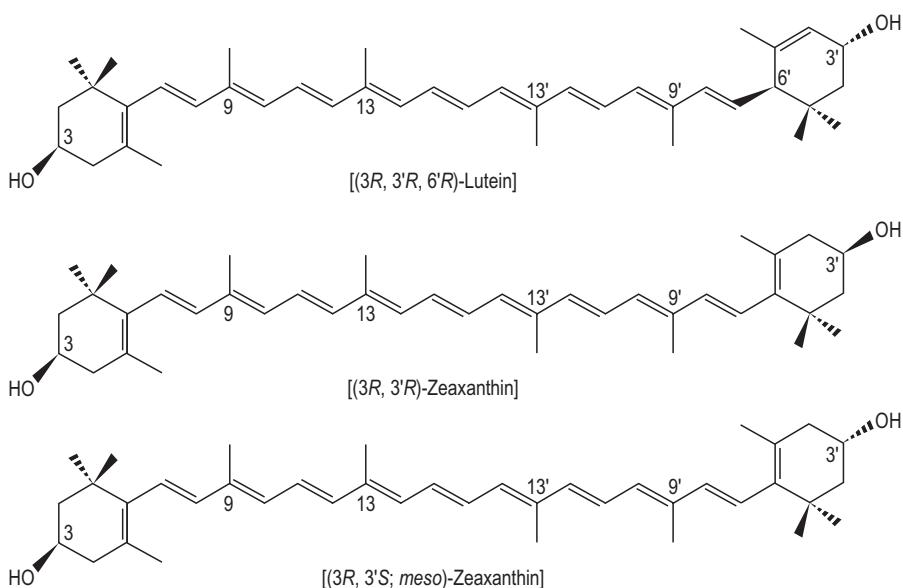


FIGURE 4-67 The chemical structure of macular pigments. (From Abdel-Aal et al., 2013.)

interphotoreceptor matrix protein), which transports retinoids between the RPE cell and the photoreceptor, and several species of proteoglycan, which provide a coating for the photoreceptor outer segment. Interestingly, the 6-sulphated (DeltaDi6S) chondroitin species appears to be more prominent around cones than around rods. IRBP appears to be essential for photoreceptor survival but is not required for the visual cycle. In addition, there are several cell surface proteins that form part of the glycocalyx of the cell but may also play a part in maintaining retinal apposition, which is essentially maintained by the bulk flow of fluid across the retina. These include fibronectin, intercellular adhesion molecule 1 (ICAM-1) (see Ch. 7) and the CD44 antigen, also known as the hyaluronan receptor. There is also a second hyaluronan-binding protein, SPARC (sialo-protein associated with rods and cones), which may also be important in providing a scaffold to the matrix.

The interphotoreceptor matrix of the cone is compartmentally separated from that of the rod by an insoluble matrix sheath containing its own specific proteoglycans, which presumably play a role in the regulation of their different forms of visual excitation. Chondroitin-6-sulphate appears to be the major proteoglycan–GAG in the cone matrix sheath, while a

similar but less well-defined sheath around rod outer segments appears to be composed of sialyl conjugates. It has been suggested that these proteoglycan sheaths around both rods and cones are important in retinal–RPE adhesion, for instance via hyaluronan–CD44 interactions on the apical microvilli as well as via SPARC and SPARCAN through hyaluronan-mediated motility receptor (RHAMM) domains.

The interphotoreceptor matrix contains other GAGs, including non-sulphated chondroitin and hyaluronan (about 14% of total). These are present as proteoglycans and are probably mostly synthesized by the RPE cell. There are also a number of proteolytic enzymes and their inhibitors, including matrix metalloproteinases and tissue inhibitor of matrix metalloproteinases (TIMPs) mostly as RPE cell surface bound molecules to mannose-6-phosphate receptors. In addition, $\alpha\beta$ crystallins have been identified in the IPM derived from the RPE in an exosome-mediated mechanism.

THE RETINAL PIGMENT EPITHELIUM

The RPE is a pluripotent cell

The retinal pigment epithelium (RPE) is a multifunctional pluripotent cell, which befits its embryological

origins. It expresses many of the proteins considered characteristic of other cell types. Thus it possesses several cytokeratins characteristic of epithelial cells, yet also contains vimentin, which is a mesenchymal cell protein. One group of microfilaments, namely actin, myosin, α actinin and vimentin, is organized as a ring (or belt) around the cell and inserts into typical zonulae occludens; a second type of microfilament bundle, containing actin, myosin, fodrin and vimentin, occurs in the apical processes and may be involved in photoreceptor renewal.

Under conditions of stress, RPE cells may express proteins more typical of macrophages and other myeloid cells. These include receptors for the Fc portion of immunoglobulin, the CD68 molecule and inducible nitric oxide synthase (iNOS) (see Ch. 7, p. 385). RPE cells also express the leucocyte marker CD36, which is involved in ROS phagocytosis (see p. 254) as well as $\alpha_5\beta_1$ integrin and the tyrosine kinase MerTK. In addition, RPE cells contain high quantities of phosphatidylcholine and phosphatidylinositol with high levels of saturated fatty acids and a high content of arachidonic acid. This may explain to some extent their ready ability to generate prostaglandins with immunosuppressive properties. The high content of cholesterol in RPE membranes indicates a low plasma membrane fluidity compared, for instance, with rod outer segment membranes, whose cholesterol content is low.

Turnover in normal RPE is similar to that in endothelial cells (i.e. very slow or nil) since it is a post-mitotic cell, but under certain circumstances RPE cells can proliferate and contribute to pathological processes as in retinal detachment (see Ch. 9, p. 492). When appropriately stimulated, RPE cells can synthesize and secrete growth factors such as FGF, IGF-1 and interleukin-1, which most likely have a role in the normal physiology of the retina. In addition, RPE cells are an important inducible source of vascular endothelial growth factor (VEGF). The production of growth factors by the RPE is not fully understood teleologically and it may play a part in regulating other tissues, such as the choroid and sclera, and thus indirectly have a role in the development of myopia (see eFig.

4-5). However, in pathological situations this may contribute to conditions such as diabetic retinopathy and subretinal neovascularization. To offset this risk,

RPE cells constitutionally secrete a specific anti-angiogenic protein, pigment epithelium-derived factor (PEDF), as well as secreting thrombospondin-1 into the pericellular matrix.

The role of the RPE as the second site of the blood-retinal barrier (see Ch. 1, p. 40) is based on normal tight junctions containing the tight junction-specific proteins claudin-3 and -19 and is completed with a ring of cytoskeletal actin-myosin (see above); since the RPE also has a major role in pumping fluid across the retina from the subretinal space to the choroid, the tight junctions are integrated with a series of membrane transporters including Cl^- and HCO_3^- channels, under control of ATPases and carbonic anhydrase, as well as GLUTs 1 and 3. Thus bidirectional transport of various metabolites occurs, but the main bulk flow of fluid from the retinal side to the choriocapillaris is mostly by CFTR, the Ca^{2+} -dependent Cl^- channel, which is one of the family of ABC transporters (mutations in which are responsible for certain forms of retinal degeneration) rather than by aquaporin 1. Bestrophin 1, the gene responsible for Best's disease, may also be involved in either Cl^- or HCO_3^- transport in the RPE. The RPE cell is therefore a highly polarized epithelium, organized for transport, with its apical microvilli in apposition to the photoreceptor cell and its basal infoldings towards the choroid (Fig. 4-68).

RPE cells also express proteins on their apical surface that would be basolaterally expressed in other epithelia. These include N-CAM (a cell adhesion molecule) and EMMPRIN (extracellular matrix metalloproteinase inducer), which are likely to be involved in photoreceptor adhesion and phagocytosis, respectively. In addition, the phagocytosis receptor $\alpha_5\beta_1$ integrin is expressed on the apical surface with the Na^+-K^+ ATPases involved in transport. Other apical membrane-associated proteins include ezrin, which is associated with long apical microvilli, radixin and moesin/yurt, some of which are essential to apical basal polarization and are dependent on a range of signalling molecules such as a lipid phosphatase (PTEN), a serine-threonine kinase, and a transmembrane protein (Crumbs). Reversed apical polarization occurs postnatally and is the result of suppressed decoding of specific basolateral signals.

The RPE sits on a prominent basement membrane, Bruch's membrane, composed of five discrete layers,

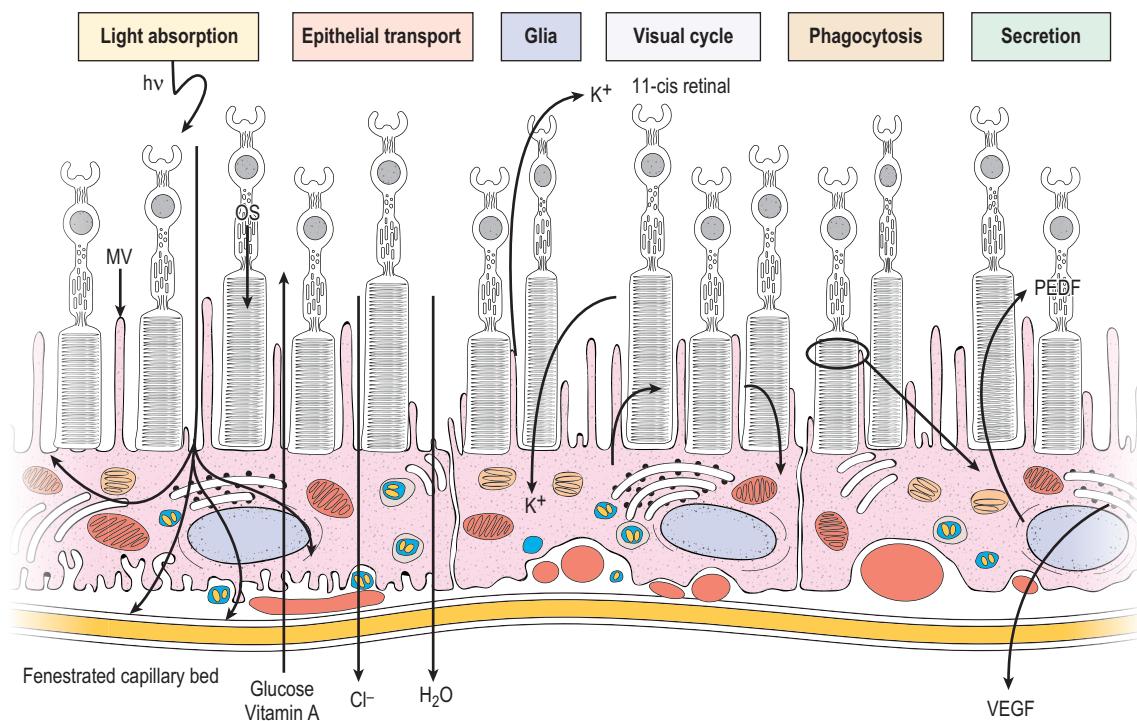


FIGURE 4-68 Summary of retinal pigment epithelium (RPE) functions. PEDF, pigment epithelium-derived growth factor; VEGF, vascular epithelium growth factor. (From Strauss, 2005.)

part of which incorporates the basement membranes of the RPE and the choriocapillaris, respectively. In addition to proteoglycans and matrix proteins typical of any basement membrane, Bruch's membrane contains hyaluronan and chondroitin sulphate plus types I, III, VI and VII collagen, and elastin.

Photoreceptor function is critically dependent on a healthy RPE layer

The RPE has multiple functions (Table 4-9) but its essential role is to maintain the physiology of the photoreceptors. Thus it removes 'spent' photoreceptor tips in the diurnal process of receptor renewal and participates in 11-cis retinol recycling. Indeed, it has been known for over 100 years that contact with the RPE was necessary for the bleached retina to regain its 'visual purple'.

As stated above, during the process of photoreceptor disk renewal the outer segment tips are shed in a diurnal manner and removed by the RPE cells in a short burst of phagocytic activity. Cone outer

TABLE 4-9 Functions of the RPE

Photoreceptor renewal
Retinal attachment
Interphotoreceptor matrix production
Transport of water and metabolites
Retinoid metabolism
Blood-retinal barrier
Immunoregulation
Free radical scavenging

segments are similarly removed by the RPE cells but the process is considerably slower. Phagocytosed outer segment tips are digested in the extensive RPE phagolysosomal system, a process that continues throughout life. Solubilized waste material is then transported across the extensive basal infoldings of the cell into the choriocapillaris. It is not surprising therefore that, with age, there is accumulation of lysosomal bodies and lipofuscin pigment, which may reflect the declining ability of the RPE cell to handle large amounts of relatively indigestible material. This may

be linked to reduced melanin production with age. This partly digested rod outer segment 'retinoid' material, some of which is known as A2E (see below), has been implicated in the development of age-related macular degeneration.

Although some retinoid material will be incorporated in the phagocytosed outer segment tip, this material is not used for regeneration of bleached rhodopsin. Instead this occurs in the cytosol of the RPE cell. The conversion of 11-cis retinal to the all-trans retinal during phototransduction is accompanied by release of the chromophore into the interphotoreceptor matrix (Fig. 4-69; and see below). Regeneration of the 11-cis retinal from the all-trans retinal takes place only in the RPE, since retinol isomerase is unique to this cell. Therefore, the all-trans retinal must somehow

enter the RPE cell by a means other than outer segment tip phagocytosis. A slightly different process occurs for cone chromophore regeneration. In cones, after phototransduction, 11-cis retinal is reduced to all-trans retinal, which is then transported to Müller cells, where it is converted to 11-cis retinol and transported back to cones, where it is reconverted back to 11-cis retinal (Fig. 4-69). This last step can only occur in cones.

How the transport of the chromophores in the interphotoreceptor matrix is achieved is not yet clear, but it is probably more subtle than the proposed mechanism of retinoid shuttling in which IRBP 'transports' the retinoids between the two cells; rather, it is dependent upon the 'buffering' effect of IRBP, in which low-affinity binding of retinoid to IRBP permits its release at the appropriate site depending on the local concentration and amount of chromophore bleach. This notion is indirectly supported by evidence from the IRBP knockout mouse; this mouse has a fully functional neurochemical visual cycle but an accelerated dark adaptation response. In contrast, the exclusive localization of the high-affinity binding protein cellular retinol-binding protein (CRBP) to the RPE cell will help to drive the 'flow' of all-trans retinol in this direction and assist in the development of concentration gradients. Docosahexanoic acid, an important photoreceptor fatty acid, in contradistinction to palmitic acid, induces a rapid and specific release of 11-cis retinal from one of the two retinoid binding sites on IRBP.

For rods most of this activity takes place in the dark. However, IRBP also functions in transporting 11-cis retinol to cones which can take place in light conditions. There are two binding sites for the chromophore on the IRBP molecules which may also have functional significance in terms of vitamin A transport.

The RPE is polarized with tight junctions, but transport is bidirectional

The transport function of the RPE is bidirectional in that, as shown above, retinoid transport, as well as glucose transport, is achieved through polarized distribution of specific receptors (Fig. 4-68). In contrast, the bulk of transport occurs from the retina to the choriocapillaris, particularly for digested outer

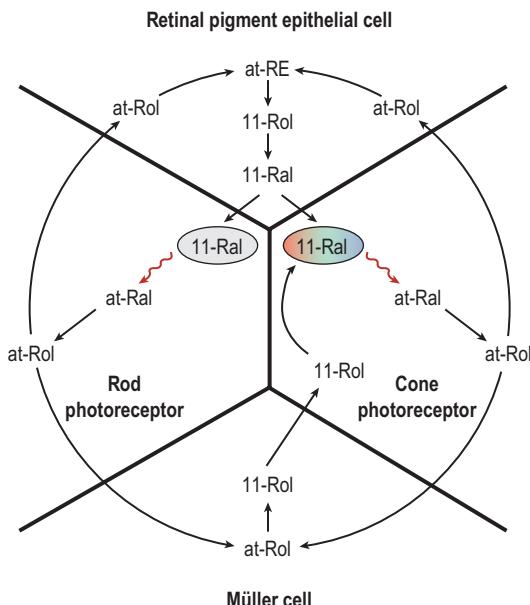


FIGURE 4-69 Reactions of rod and cone visual cycles. The areas separated by solid lines represent cellular compartments of a retinal pigment epithelial cell, rod and cone photoreceptor cells, and Müller cells. The ovals surrounding 11-Ral represent rod (grey fill) and cone (tricolor fill) visual pigments. Photoisomerization reactions are shown in red. All other chemical reactions are catalysed by enzymes. Retinoids are chaperoned by retinoid-binding proteins (not shown) during intercellular and intracellular diffusion. Diffusion of at-Rol from rod photoreceptor cells to Müller cells has not been demonstrated. at-RE, all-trans retinyl esters; at-Ral, all-trans retinal; at-Rol, all-trans retinol; 11-Ral, 11-cis retinal; 11-Rol, 11-cis retinol. (From Saari, 2012.)

segment material. There is also a significant bulk flow of fluid from the retina to the choroid, probably via 'solute drag' through mechanisms such as an Na^+/K^+ ATPase pump located in the apical plasma membrane of the RPE and by transport of non-ionic solutes such as amino acids and glucose. Interestingly, RPE cells do not appear to express the water transporters aquaporin 3–5, unlike Müller cells and astrocytes. However, active HCO_3^- transport appears to be the major ion linked to fluid transport and is mediated by a carbonic anhydrase-regulated system (see section on ciliary body, p. 217). There are six isoforms of carbonic anhydrase in the RPE cell. The exchange of ions (and bulk water) establishes an electrical potential across the RPE, positive on the retinal side, of 5–15 mV, and also maintains steady pH regulation in the face of high lactic acid production by the outer segments of the photoreceptor cell. However, as indicated above, the Ca^{2+} -activated Cl^- channel, CFTR, and bestrophin membrane proteins are currently considered strong players in trans-RPE fluid flow.

The net rate of fluid transport across the RPE is about 4–6 $\mu\text{L}/\text{cm}^2$ per hour. Transretinal fluid flow has been proposed as a major mechanism for maintaining retinal apposition; indeed, it has been suggested that clinical RPE detachments may result from a breakdown of the transport mechanisms for fluid across the RPE as a result of focal damage.

Thus any process that impairs retinol transport into the RPE from central stores in the liver will affect vision; because vitamin A requirements are supplied through the diet, any condition affecting this, such as protein–calorie malnutrition or one of the malabsorption syndromes, may produce visual symptoms. One of the earliest symptoms of severe malnutrition, endemic in developing countries, is night blindness caused by lack of vitamin A.

The RPE also contains α and β_2 adrenergic receptors, plus enzymes of the cytochrome P₄₅₀ drug-metabolizing system. In addition, melanin, a major constituent of RPE cells is effective in drug detoxification. The melanin content of RPE cells decreases with age.

The chemistry of the visual response

The main function of the retina is to convert light energy into an interpretable signal for cortical cells in

the brain. This process begins with photochemical events in the photoreceptor.

PHOTOCHEMICAL REACTIONS IN THE RETINA

Rhodopsin, vitamin A and photoreceptor turnover

Rhodopsin, a 348 amino acid long major integral cell membrane protein in rod photoreceptor outer segments, is synthesized in the ER and Golgi apparatus of the inner segments and transported in protein-rich vesicles to the outer segment, where fusion occurs with the newly formed disk membranes in the periciliary ridge complex (see ciliopathies). It has three glycosylation sites containing various branched combinations of N-acetylgalactosamine and mannose residues. Opsin is a seven-turn (α helix) membrane-spanning protein containing a serine- and threonine-rich cytosol-exposed C-terminus, which is variably phosphorylated, and an intradisk N-terminus (Fig. 4-10). This structure follows the general pattern for G protein types of membrane receptor such as the adrenergic and muscarinic receptors which induce cell signalling on binding of their specific ligand by activating adenyl cyclase to raise intracellular concentrations of the second messenger cAMP (see pp. 243–245 and Ch. 6, pp. 172 and 258). The ligand for rhodopsin, 11-cis retinal, is already bound in the dark and dissociates when activated by a photon of light.

Rhodopsin behaves like a genuine receptor, but with differences: in the resting state (i.e. in the dark), Na^+ channels in the rod outer segment plasma membrane are held 'open' by cGMP, synthesized by guanylate cyclase. This provides the electrochemical basis for the relative depolarization of the photoreceptor outer segment compared with other cells (−57 versus −78 mV). On stimulation of rhodopsin with light, transdisk membrane signalling occurs via sequential activation of other membrane-bound proteins, transducin and phosphodiesterase, to lower the cytosolic concentration of cGMP, i.e. it acts like a second messenger in reverse (see below). This has the effect of closing the leaky Na^+ channels in the plasma membrane and causing a relative hyperpolarization (to −87 mV), thereby generating the electrical response (see below).

Activation of rhodopsin is achieved via isomerization of retinol, a vitamin A compound that lies 'nested' between the first and last transmembrane loops of the

rhodopsin molecule, with its long axis in the plane of the membrane (Fig. 4-10). Modelling studies show the relationship between the chromophore pocket and the opsin molecule (Fig. 4-70). In this reaction, the tail of 11-cis retinal on conversion to all-trans retinal becomes elongated and more perpendicularly disposed to the isoprene retinal ring structure; this has the effect of generating greater interaction between the retinal and its binding sites to the side-chains of amino acids Lys206 and Lys296, thereby heightening the energy state of the rhodopsin molecule. All-trans retinal becomes converted to all-trans retinol, which does not fit within the rhodopsin transmembrane loops and it is during this stage that rhodopsin converts through its various intermediaries to opsin, the changes being demonstrable by colour bleaching (Fig. 4-70). The chromophore thus detaches from the bleached opsin and diffuses away into the interphotoreceptor matrix to be taken up by the RPE cell; in the dark it undergoes isomerization within the RPE cell (see Figs 4-69 and 4-70) to 11-cis retinol, which binds to cellular retinal binding protein (CRALBP) and becomes converted to 11-cis retinal-CRALBP, which is transported to the cell membrane and is transferred to interphotoreceptor retinal binding protein (IRBP) on which it is shuttled back to the photoreceptor to reattach to rhodopsin and recommence the cycle.

The process of 11-cis retinal binding to opsin occurs by the formation of a Schiff base to Lys296, releasing a single molecule of water. Interestingly, 'ordered' water molecules are also included in the overall conformation of rhodopsin as tightly bound 'prosthetic' structural moieties giving cohesion to the overall structure of the molecule (see Fig. 4-10). It is possible that these water molecules are held to the structure through electrochemical bonds that become disrupted when light converts 11-cis retinal to all-trans retinal, thus creating space for the chromophore to detach.

There are three cone opsins in humans which have around 50% homology to rhodopsin and to each other apart from the red and green opsins which are 95% homologous to each other (Fig. 4-71). The release of all-trans retinal from cone pigments is much faster than from rhodopsin and even binding of *cis*-retinal is less stable, possibly related to the amount of water binding.

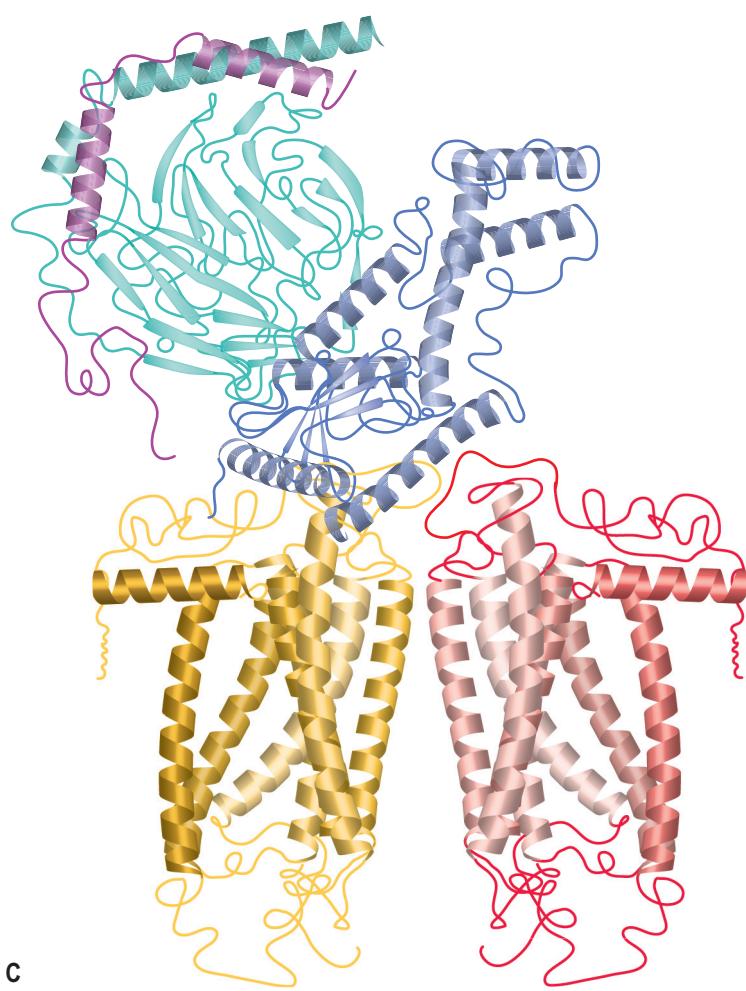
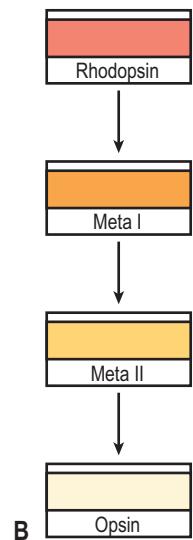
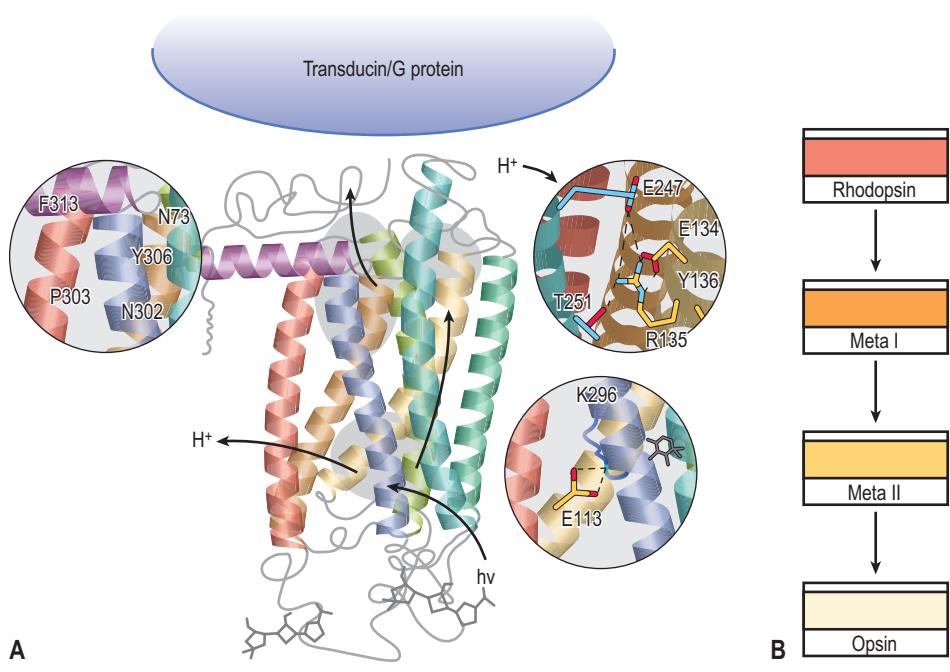
The conversion of 11-cis retinal to all-trans retinal and then to all-trans retinol is the fundamental chemical reaction to take place during the visual impulse, and the chemical reaction has been known for many years. Laser flash photolysis studies have chemically identified the intermediates that occur in the breakdown of vitamin A (see Figs 4-69 and 4-70). Normal vision depends on a plentiful supply of vitamin A, which must be provided exogenously because humans cannot synthesize it. Dietary vitamin A comes from β -carotene in plants, while animal sources provide the chromophore as a retinyl ester linked to fatty acids. Thus, visual deterioration, especially at low illumination (night blindness), is an early sign of malnutrition and malabsorption syndromes, but sensitive electrophysiological tests may be required to reveal its full extent. Retinoids are stored in the RPE as non-toxic retinyl esters; retinoic acid, which can also be synthesized during retinoid metabolism, has many functions in cell biology and has recently been shown to have a role in immune tolerance, but free retinoic acid is toxic to cells and thus has to be generated from retinyl esters, similar to visual retinoids. In the retina, all-trans retinol to 11-cis retinol isomerization is regulated by palmitoylation under the control of the RPE-specific protein RPE-65 (see Fig. 4-69) whose role serves to switch off the visual cycle in the dark.

Phototransduction: the conversion of light energy to an electrochemical response

The conversion of the energy stored in a single photon of light to an electrical response is possible because of the extensive amplification of the molecular cascade involved in closure of the Na^+ channels (see eBox 4-5).

These channels are kept open by cGMP (termed cyclic nucleotide gated channels, CNGP), which acts as a second messenger in this system.

In the dark, open Na^+ channels allow Na^+ to exit and Ca^{2+} to enter and maintain a relative depolarization. This is linked to a $\text{Na}^+/\text{Ca}^{2+}$ exchanger, which maintains a steady intracellular concentration of Ca^{2+} . In the light, the Na^+ channels are closed, leading to a relative hyperpolarization and a decline in the intracellular Ca^{2+} concentration. Closure of the Na^+ channels is achieved by hydrolysis of cGMP by phosphodiesterase (PDE), one of the responses that is greatly amplified in the cascade. Signal amplification is achieved by activation of many molecules of



transducin by one molecule of metarhodopsin (R^*), probably in the region of 300x (Fig. 4-72 and eBox 4-5).

In this sense, activated rhodopsin acts like an enzyme since it is available to activate further molecules of transducin until the light is switched off by the combined action of recoverin (rhodopsin kinase) and arrestin (also known as S antigen). Similarly, around 600 molecules of cGMP are hydrolysed by a single molecule of PDE and, indeed, the limitation of its activity is determined by the availability of cGMP. There is a further three-fold amplification of channel opening by one cGMP molecule.

Phototransduction is a biological cascade

The mechanism of cGMP hydrolysis in phototransduction has been extensively investigated; the sequence of events in this response is outlined in Figure 4-72.

Certain enzymes play important roles in this process, including guanylate cyclase and cGMP phosphodiesterase. Guanylate cyclase activation restores cGMP levels with the help of recoverin (see Fig. 4-72), while the enzyme R^* is deactivated to the phosphorylated form R-P and bound by arrestin (S antigen). During these changes there is considerable redistribution of photoreceptor molecules in the shift from light to dark (Fig. 4-73). Amplification is therefore a function of the time interval at each of the stages of processing.

Does phosphoinositide metabolism have a role in phototransduction?

From the above it would appear that much of the transduction mechanism has been deduced; however, the role and mechanism of Ca^{2+} influx are unclear. Ca^{2+} mobilization is the product of a major second messenger system in many cells, and is activated via receptor-mediated activation of phospholipase C (PLC) and G (PLG) proteins. This results in the

hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP_2) to form inositol triphosphate (IP_3) and diacylglycerol (DAG). IP_3 induces mobilization of calcium stores, while DAG activates protein kinase C. Two major calcium-binding proteins, calmodulin and GCAP1 and 2 (calcium-dependent modulator of guanylate cyclase), are involved in phototransduction. Both PKC and PLC regulate the translocation of arrestin from the inner to the outer segments in the dark.

In the invertebrate retina, light induces hydrolysis of PIP_2 , with production of IP_3 and DAG, but its role in the transduction cascade is unclear. However, there is evidence that an integral transmembrane phosphatidylinositol transfer protein prevents retinal degeneration in *Drosophila* (the rdg B protein). The evidence in the vertebrate retina is even less convincing. It is possible that this mechanism may have some other function related to membrane conductance and mobilization of ion stores, but unrelated to transmission of an electric impulse. However, there is little doubt that phosphoinositide metabolism and turnover is a major pathway in the photoreceptor cell.

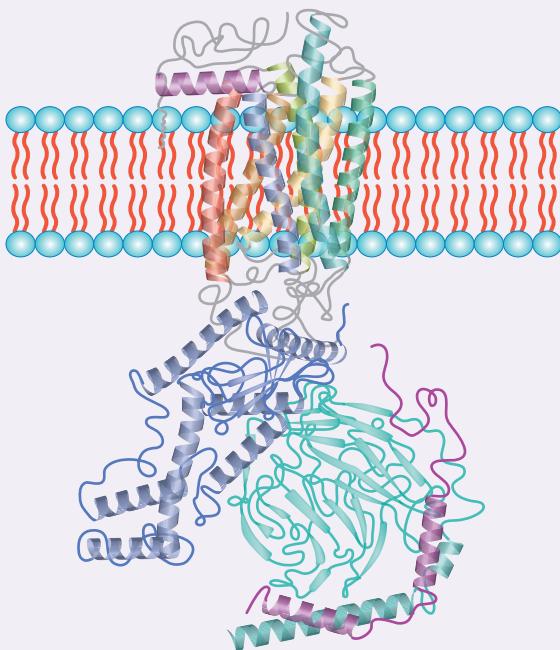
Renewal of photoreceptors is associated with accumulation of lipofuscin pigment in the RPE cells

Estimates suggest that RPE cells phagocytose about 2000–4000 disks per day. To cope with this phagocytic load, the RPE cell has an extensive lysosomal enzyme system that can digest about 50% of its load within 1–2 hours (important in terms of cyclic rod outer segment tip shedding). Uptake of shed rod outer segment tips is receptor mediated, through the $\alpha_v\beta_5$ integrin receptor signalling, the receptor tyrosine kinase Mer (Mertk) and CD36. CD36 recognizes oxidized phosphatidylcholine, a product of aged outer segment tips, and may be involved in the engulfment process rather than initial binding. Interestingly, the $\alpha_v\beta_5$ receptor is also involved in retinal adhesion to

FIGURE 4-70 Activation of rhodopsin by light. (A) Rhodopsin absorbs a photon of light, which leads to isomerization of 11-cis retinal to all-trans retinal. Transmission of this signal to the cytoplasmic surface triggers nucleotide exchange on the heterotrimeric G protein transducin. Transducin dissociates and activates the downstream signalling events. (B) Spectral changes in rhodopsin upon activation as detected by photography. Once rhodopsin in its dark 11-cis retinal-bound state (A) is exposed to light, it immediately goes through a series of photointermediate states, including metarhodopsin I (Meta I) (B), and eventually progressing to the Rho^* (metarhodopsin II (Meta II)) activated state (C). Upon treatment with hydroxylamine, the chromophore is hydrolysed, resulting in a largely colorless solution. (C) Shows a model of the G protein rhodopsin complex. (From Palczewski, 2012.)

eBox 4-5**Phototransduction cascade and channel opening**

Activation of a single molecule of rhodopsin generates an amplification cascade that leads to the opening of many channels, and the induction of a change in the resting potential of the photoreceptor.



Type 2 rhodopsin (Rh) (rainbow coloured) embedded in a lipid bilayer (heads red and tails blue) with transducin (GDP) below. G_α is coloured red, G_β blue and G_γ yellow. There is a bound GDP molecule in the G_α-subunit and a bound retinal (black) in the rhodopsin. The N-terminus of rhodopsin is red and the C-terminus blue. Anchoring of transducin to the membrane has been drawn in black. PDE, phosphodiesterase.

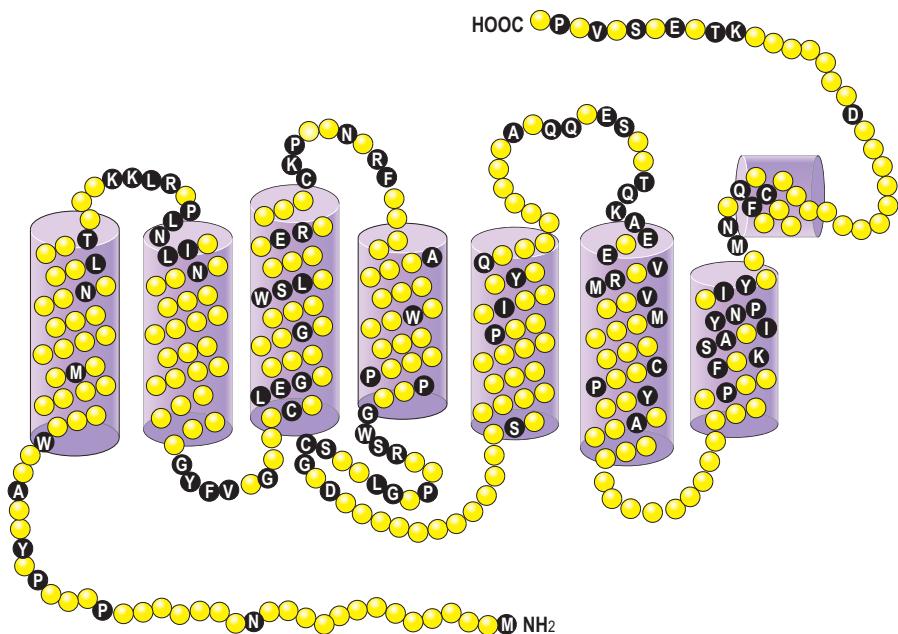


FIGURE 4-71 Sequence identity among the human rod and cone opsins. Opsin secondary structure is represented in two dimensions based on alignment with the known structure of bovine rhodopsin. Residues that are identical between the human rod and human red, green and blue cone opsins are denoted as black circles with the conserved amino acid residues identified. (From Tang et al., 2013.)

the RPE layer, functioning in a different diurnal cycle for phagocytosis.

With age, accumulation of retinoid material and lipofuscin occurs in the RPE. Free all-*trans* retinal, produced after the photoactivation of rhodopsin by light, is considered to bind to phosphatidylethanolamine (PE), forming N-retinylidene-PE (NRPE) from which N-retinylidene-N-retinylphosphatidylethanolamine (A2PE) is generated by the binding of a second molecule of all-*trans* retinal. Phospholipase D then cleaves PE from A2PE to generate A2E, which has been identified as a potential culprit in the lipofuscin responsible for age-related macular degeneration (AMD). This process is light dependent and thus retinal light damage is thought to be a major factor in development of AMD. However, recently, 11-*cis* retinal has also been shown to generate A2E, which raises a question mark concerning this model of light damage to the retina.

Photoreceptors are easily damaged, particularly by excess light radiation, and several free radical scavenging mechanisms are in place to minimize the damage. In addition, vitamin E has a significant role as a free radical scavenger (see Box 4-19).

RPE lysosomes contain a battery of enzymes capable of degrading complex lipid–glycoprotein aggregates

Phagocytosis of rod outer segment tips takes place in stages. First, there is formation of the primary phagolysosome by fusion of the endosome with the lysosome. This may then fuse with lipofuscin granules or with melanosomes to form melanolipofuscin bodies. With age, and experimentally in vitamin E deficiency, there is a greater shift towards fusion with lipofuscin granules. Eventually these bodies are degraded and their products are transported out of the cell. However, the accumulation of insoluble material between the basement membrane and the RPE cell has been attributed to a breakdown in the capacity of the cell to deal with this load. In addition, accumulation of lipofuscin is associated with reduced phagocytic capacity of RPE cells.

SYNAPTIC EVENTS BETWEEN PHOTORECEPTORS AND CELLS OF THE INNER NUCLEAR LAYER

Bipolar cells of the inner nuclear layer (INL) synapse either with rod or with cone photoreceptors but not with both. In addition, they synapse with other cells

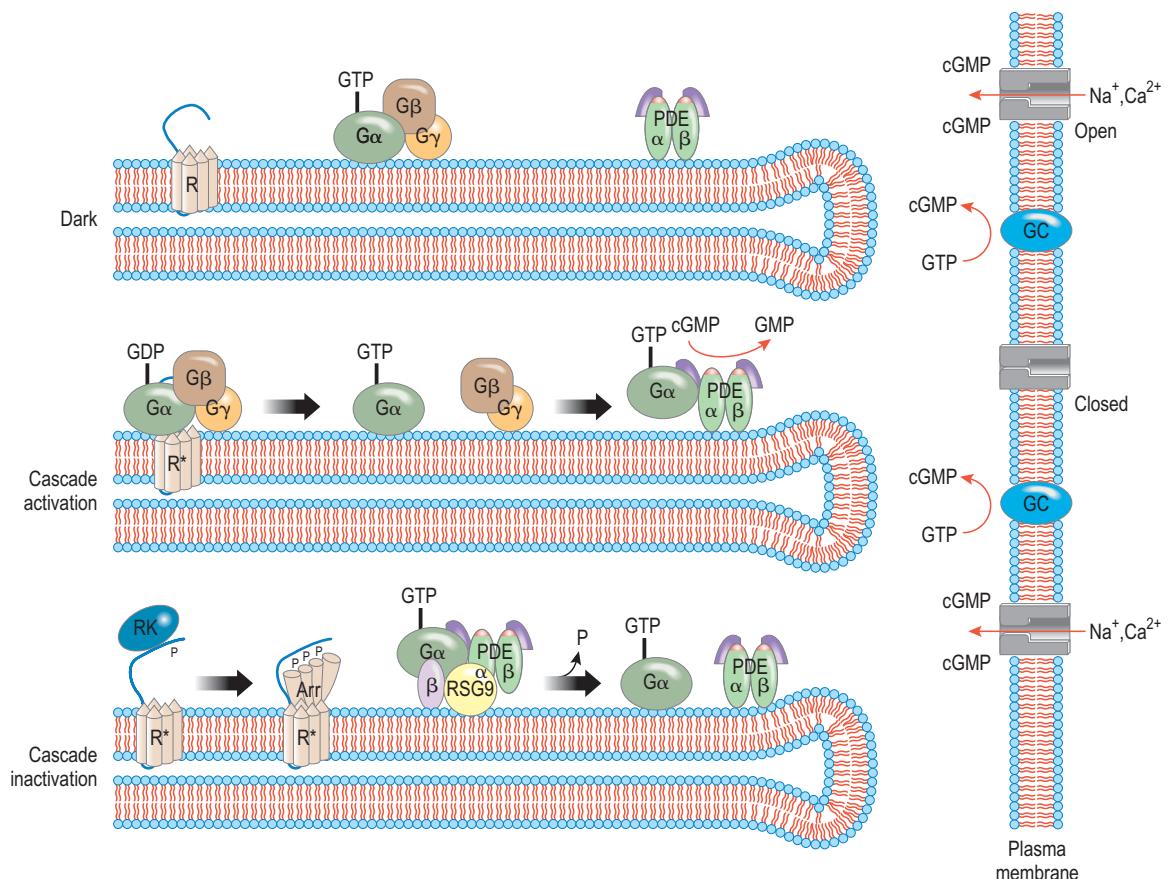


FIGURE 4-72 Schematic of phototransduction cascade activation and inactivation. The upper disk illustrates inactive rhodopsin (R), transducin ($G\alpha$, $G\beta$ and $G\gamma$ subunits), and PDE (α , β and γ subunits), in the dark. The reactions in the middle disk illustrate light-induced transducin and PDE activation. The reactions in the lower disk represent R^* inactivation via phosphorylation by rhodopsin kinase (RK) followed by arrestin (Arr) binding and transducin/PDE inactivation by RGS9– $G\alpha\beta$ -PDE $\alpha\beta$ complex. (From Burns et al., 2005, with permission from Elsevier.)

in the INL such as horizontal cells. There are around 10 different types of cone bipolar cells but only one type of rod bipolar. Several types of neurotransmitter may be involved (Table 4-10). In the resting state, the dark currents produced by the open cation channels in the outer segment (see Ch. 5, Box 5-6, p. 288) are accompanied by high levels of neurotransmitter release at the synaptic junction with the bipolar cells. In the light, there is a decrease in transmitter release, which alters the transmission of electric potentials in the bipolar cells. However, the situation is more complex than this.

Two types of bipolar cell

Synaptic transmission at neuromuscular junctions is mediated by acetylcholine, released from the nerve

ending when the action potential wave arrives. Binding of acetylcholine to its receptor on the muscle initiates a second message, which causes a depolarization in the cell owing to opening of the voltage-gated Na⁺ channels.

At the synaptic junction between the photoreceptor cell and the bipolar cell, a different type of reaction occurs. First, the photoreceptor does not transmit an action potential wave, but presents a graded hyperpolarization response, which depends on the intensity of the light stimulus. In the dark, while the photoreceptor is in a relatively depolarized state, the neurotransmitter glutamate (see Table 4-10) is released from the presynaptic photoreceptor terminal and binds to a retina-specific metabotropic receptor (see next section for definitions) on one type of bipolar cell (the

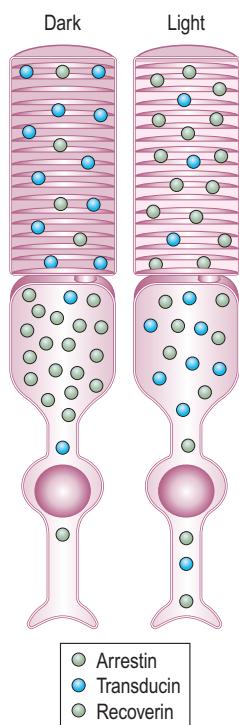


FIGURE 4-73 Schematic representation of transducin, arrestin and recoverin subcellular distribution in the dark-adapted and light-adapted rod. (From Burns et al., 2005, with permission from Elsevier.)

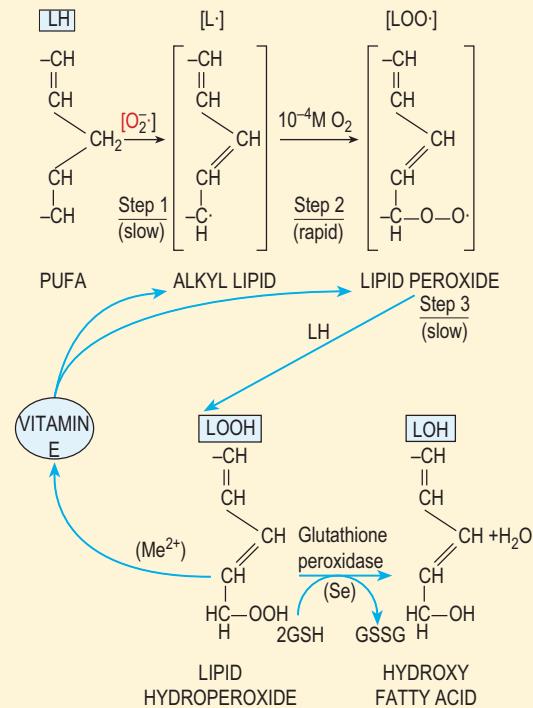
ON-bipolar), which produces hyperpolarization and keeps the bipolar Na^+ channel closed. When light activates the photoreceptor, glutamate release ceases and the intracellular concentration of cGMP rises, thus opening the Na^+ channels and causing depolarization of the cell membrane, allowing the ON-bipolar cell to transmit a signal.

In contrast, glutamate binding to the ionotropic receptor on a second type of bipolar cell (the OFF-bipolar; see Ch. 5) has the reverse effect of inducing hyperpolarization. To summarize, therefore, when light activates the photoreceptor and transmits the message to the bipolar cell, ON-bipolar cells are depolarized by reduction in glutamate release and, OFF-bipolar cells are hyperpolarized by an increase in glutamate release. Additionally, glutamate may bind to horizontal cells, where it also produces hyperpolarization.

This type of graded response is achieved by specialized photoreceptor structures termed ribbon synapses which are exquisitely organized to transmit

BOX 4-19 VITAMIN E AS A FREE RADICAL SCAVENGER

Vitamin E limits free radical damage at several stages in lipid peroxidation.



Regeneration of vitamin E under light stress requires ascorbic acid. In experimental vitamin E deficiency there is a marked accumulation of lipofuscin in the RPE, which may be relevant to a similar accumulation of lipofuscin in age-related macular degeneration. PUFA, polyunsaturated fatty acid.

information. Several membrane proteins are involved in the cytoskeletal structure and function of the synapse, such as dystrophin and retinoschisin, which have clinical relevance (mutations in retinoschisin cause retinoschisis, a retinal degeneration involving splitting of the retinal layers) as well as in the inter-synaptic cleft, a space containing other essential matrix components (Fig. 4-74). In both ON- and OFF-bipolar cells, the action of glutamate is considered excitatory, since there is a defined response.

TABLE 4-10 Retinal neurotransmitters and neuromodulatory peptides

Agent	Site of action
Neurotransmitters	
Glutamate	Photoreceptor
γ -aminobutyric acid	Horizontal amacrine cells
Glycine	Amacrine cell
Taurine	
Tyrosine derivatives	
Dopamine	Horizontal amacrine cells
Noradrenaline	
Adrenaline	
Tryptophan derivatives	
Serotonin	Photoreceptor
Aspartate	Photoreceptor
β -alanine	
Histidine derivative	
Histamine	
Neuromodulators	
Peptides	
Vasoactive intestinal peptide	Amacrine, ganglion cells
Angiotensin I and II	Amacrine, ganglion cells
Substance P	Amacrine, ganglion cells
Luteinizing hormone releasing hormone and thyrotrophin releasing hormone	Amacrine, ganglion cells
Leu- and metenkephalin	Amacrine cell
β -endorphin	Amacrine, ganglion cells
Somatostatin	Amacrine, ganglion cells
Neurotensin	Amacrine cell
Glucagon	Amacrine cell

Glutamate is unlike acetylcholine in that there is no enzyme that rapidly degrades the neurotransmitter and shuts down the response; instead, the glutamate merely diffuses away or is taken up by nearby glial cells and inactivated.

The differential binding of glutamate to ON- and OFF-bipolar cells via specific receptors (see below) is a direct chemical correlate of the electrophysiological and psychophysical responses that can be obtained from these cells, and is a good demonstration of nature's use of a binary (ON-OFF) system to process complex sensory phenomena. Apparently, the segregation of responses at the bipolar cell level is retained to the level of the visual cortex.

Neurotransmitters and neuromodulators

In contrast to glutamate, other neurotransmitters such as glycine and GABA have a lateral inhibitory action at the synaptic junction and are considered to be neuromodulators, i.e. they modulate the effect of glutamate; neuromodulators have a much longer action, often mediated through second messenger systems in the cell. There are many different types of neurotransmitter, most of which are amino acids or amino acid derivatives, and the majority are active in transmission of electric impulses within the mammalian retina. As indicated above, typical excitatory transmitters include acetylcholine and glutamate, while γ -aminobutyric acid (GABA) and glycine are inhibitory to their function. Several other neuromodulatory peptides exist which have direct or indirect effects on neural responses. Dopamine is probably the best known of these (see below). In addition, recent studies indicate that neuronal nitric oxide synthase may modulate retinal signalling via release of nitric oxide.

Glutamate is the major neurotransmitter in the retina

As indicated above, glutamate (Fig. 4-75) is the major mediator of synaptic transmission between the photoreceptor and the bipolar and horizontal cells. The functional organization of the retina is centred around the specificity of glutamate for four discrete types of glutamate receptor on different types of cells (ON- and OFF-bipolar cells, and two types of horizontal cell). Glutamate receptors are classified as ionotropic (iGluR) and metabotropic (mGluR). iGluR are by far the more ubiquitous and are further grouped as:

- N-methyl-D-aspartate (NMDA) selective receptors
- quisqualate receptors
- AMPA/kainate receptors on hyperpolarizing bipolar cells
- APB (sign-inverting glutamate receptor agonist) receptors.

Each of these receptors has specific characteristics related to whether it is ionotropic (directly affecting ion channels, e.g. NMDA) or metabotropic (primarily affecting aspects of neuronal cell metabolism, such as cGMP levels, and secondarily altering ion-channel permeability, e.g. APB receptors) (see Fig. 5-12, p. 295). Synaptic transmission in ganglion cells may also utilize glutamate. However, for some time it has

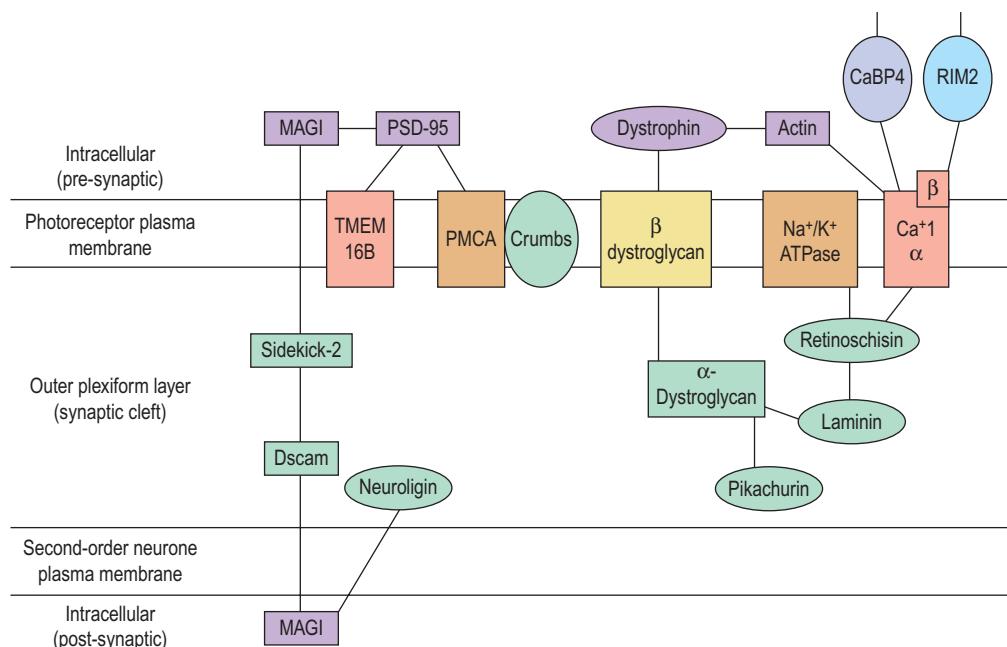


FIGURE 4-74 Proteins central to the organization of the outer plexiform layer (OPL). Two major nodes of protein–protein interactions occur in the extracellular matrix (ECM) of the OPL ECM within the OPL. The Sidekick-2 trans-synaptic scaffold attaches MAGI proteins in apposed cells, with putative lateral presynaptic interactions to PSD-95. PSD-95 also appears to anchor the Cl(Ca) channel. TMEM16B and PMCA extrusion pump at non-ribbon sites in the terminal. Scaffolding interactions between a transmembrane dystroglycan complex and the retina-specific ECM proteins pikachurin and retinoschisin, linked through synaptic laminin and cytoplasmic actin, while also attaching to the extracellular face of transmembrane CaV channels and the Na⁺/K⁺-ATPase. (From Mercer and Thoreson, 2011.)

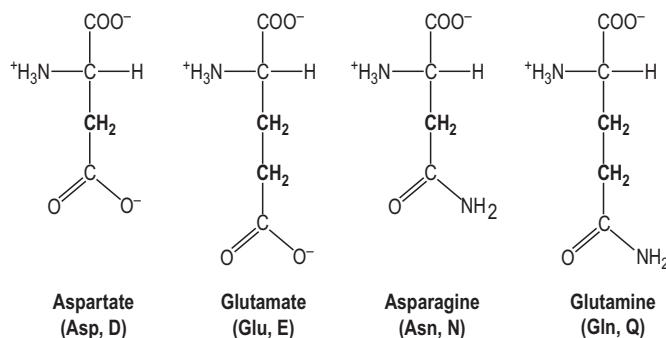


FIGURE 4-75 Chemical structure of the acidic amino acids aspartate, glutamate, asparagine and glutamine.

been proposed that small cyclic nucleotides may confer gating activity, and recent studies have confirmed this (Fig. 4-76). The cAMP and cGMP appear to have opposing gating effects in their interactions with horizontal/amacrine cells and ganglion cells in the regulation of Ca²⁺ influx and in addition appear to be modulated themselves by neuronal nitric oxide

synthase (nNOS) derived from neighbouring amacrine cells. The precise role of NO in the retina is unclear but may be important in visual adaptation.

Horizontal and amacrine cells

Horizontal and amacrine cells directly modify the rate of electrical firing in bipolar cells by release of

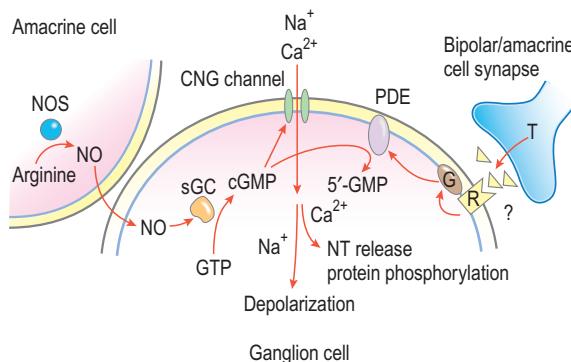


FIGURE 4-76 Schematic representation of pathways that might regulate the activity of the cGMP-gated channel in retinal ganglion cells. The cGMP synthesis could be stimulated by nitric oxide adjacent amacrine cells. Activation of cGMP-gated channels will increase Ca^{2+} influx and enhance Ca^{2+} -driven processes. At the same time, G protein-coupled receptors activated by neurotransmitters from bipolar and amacrine cell terminals may regulate the activity of one or more PDEs which control hydrolysis of cGMP and thus the activity of the cGMP-gated channels. (From Barnstable et al., 2004; modified from Ahmad et al., 1994.)

excitatory (horizontal cells) and inhibitory (horizontal and amacrine cells) neurotransmitters. Glutamate is the major excitatory transmitter of horizontal cells, while GABA, a derivative of glutamate distributed widely throughout the retina, functions particularly in the horizontal and amacrine cells as an inhibitory mediator. Recently, it has been shown that some horizontal cell negative feedback to cones can be mediated via glutamate-gated Ca^{2+} channels. Glycine is also an inhibitory neurotransmitter in amacrine cells, which have a significant role in determining the size of receptive fields of individual ganglion cells (see Ch. 5).

Acetylcholine is a major excitatory neurotransmitter of amacrine cells and often co-localizes in GABAergic starburst cells, suggesting that these cells can act in a complex excitatory/inhibitory manner in relation to ganglion cell receptive fields. Ganglion cells are thought to utilize glutamate as a major transmitter.

Amacrine cells also receive input from the third class of photoreceptor in the retina. Intrinsically photosensitive retinal ganglion cells (ipRGCs, see pp. 249–250) excite a subset of amacrine cells via dopamine receptors and are coupled to a further subset of GABA-responsive amacrine cells via gap junctions. In this way information regarding

irradiance, circadian rhythms and pupillary responses is integrated with primary photoreceptor responses.

Dopamine exerts a neuromodulatory effect on retinal function

Dopamine occurs in amacrine cells and inner plexiform layer cells in the inner retinal layers and clearly has a role to play in visual function because the reduced and delayed b-wave electroretinogram responses (see Ch. 5) seen in patients with Parkinson's disease are normalized on treatment with l-DOPA. Dopamine receptors (there are two broad classes and several subclasses) are present on all retinal neurones and therefore dopamine probably has many as-yet undetermined effects, one of which is to uncouple photoreceptor-driven horizontal cell gap junctions. Horizontal cells are probably the source of activity in the surround region in bipolar cell centre-surround receptive fields, suggesting that regulation of spatial contrast sensitivity may be under dopaminergic control.

Dopamine may have many other effects including regulation of AII-type amacrine cells, which couple rod-associated bipolar cells and ganglion cells, and it may even have a paracrine role in altering the level of photoreceptor cytoplasmic cAMP. Dopamine has also been implicated in development in retinal neurones because it has been shown that apomorphine inhibits form-deprivation myopia in experimental animals by a dopamine D₂-receptor mechanism acting within either the retina or the RPE.

Dopamine is inactivated by cellular re-uptake, a process mediated by specific Na^+ -dependent membrane transporter proteins, which have been cloned. Dopamine may also be antagonized by melatonin, which is synthesized and released by photoreceptors, accounting for the photopic to scotopic transition in rod responsiveness. It is also counteracted by NMDA, which may induce impaired dopamine synthesis. Interestingly, melatonin has neuromodulatory effects on dopamine-sensitive cells as well as on photoreceptors (see p. 261).

It can be seen therefore that, while much remains to be determined concerning the mediators of neural function in the retina and visual cortex, a view is already emerging of differential stimulation and inhibition of function induced by a variety of interacting

neuromodulators and transmitters, which correlates with observed psychophysical events.

Conclusion

While the eye has many 'eye-specific' biochemical and cell biological functions and roles, many of these are founded on general principles that apply to other systems and, indeed, comparison of ocular and

non-ocular processes offers much insight into basic fundamental principles. Where differences exist, they assist in highlighting the properties and functions of molecules and reactions, while introducing us to the specific mechanisms in the eye. In this way, a greater understanding of the general principles is achieved.

FURTHER READING

A list of further reading can be found online.



FURTHER READING

- Abdel-Aal el, S.M., Akhtar, H., Zaheer, K., Ali, R., 2013. Dietary sources of lutein and zeaxanthin carotenoids and their role in eye health. *Nutrients* 5, 1169–1185.
- Ambache, N., 1957. Properties of irin, a physiological constituent of the rabbit's iris. *J. Physiol.* 135, 114.
- Andersen, J.L., Kornbluth, S., 2013. The tangled circuitry of metabolism and apoptosis. *Mol. Cell* 49, 399–410.
- Appenzeller-Herzog, C., Hall, M.N., 2012. Bidirectional crosstalk between endoplasmic reticulum stress and mTOR signaling. *Trends Cell Biol.* 22, 274–282.
- Asnacios, A., Hamant, O., 2012. The mechanics behind cell polarity. *Trends Cell Biol.* 22, 584–591.
- Barabino, S., Chen, Y., Chauhan, S., Dana, R., 2012. Ocular surface immunity: homeostatic mechanisms and their disruption in dry eye disease. *Prog. Retin. Eye Res.* 31, 271–285.
- Beebe, D.C., Holekamp, N.M., Siegfried, C., Shui, Y.B., 2011. Vitreoretinal influences on lens function and cataract. *Phil. Trans. R. Soc. London B. Biol. Sci.* 366, 1293–1300.
- Bishop, P.N., 2000. Structural macromolecules and supramolecular organisation of the vitreous gel. *Prog. Retin. Eye Res.* 19, 323–344.
- Bloomfield, S.A., Volgyi, B., 2009. The diverse functional roles and regulation of neuronal gap junctions in the retina. *Nat. Rev. Neurosci.* 10, 495–506.
- Bonanno, J.A., 2012. Molecular mechanisms underlying the corneal endothelial pump. *Exp. Eye Res.* 95, 2–7.
- Borchman, D., Yappert, M.C., 2010. Lipids and the ocular lens. *J. Lipid Res.* 51, 2473–2488.
- Bovolenta, P., Esteve, P., Ruiz, J.M., Cisneros, E., Lopez-Rios, J., 2008. Beyond Wnt inhibition: new functions of secreted Frizzled-related proteins in development and disease. *J. Cell Sci.* 121, 737–746.
- Bowne-Anderson, H., Zanic, M., Kauer, M., Howard, J., 2013. Microtubule dynamic instability: a new model with coupled GTP hydrolysis and multistep catastrophe. *Bioessays* 35, 452–461.
- Boyer, N.P., Higbee, D., Currin, M.B., Blakeley, L.R., Chen, C., Ablonczy, Z., et al., 2012. Lipofuscin and N-retinylidene-N-retinylethanolamine (A2E) accumulate in retinal pigment epithelium in absence of light exposure: their origin is 11-cis-retinal. *J. Biol. Chem.* 287, 22276–22286.
- Bresson, E., Lacroix-Pepin, N., Boucher-Kovalik, S., Chapdelaine, P., Fortier, M.A., 2012. The prostaglandin F synthase activity of the human aldose reductase AKR1B1 brings new lenses to look at pathologic conditions. *Front. Pharmacol.* 3, 98.
- Briher, W.M., Yap, A.S., 2013. Cadherin junctions and their cytoskeleton(s). *Curr. Opin. Cell Biol.* 25, 39–46.
- Brizzi, M.F., Tarone, G., Defilippi, P., 2012. Extracellular matrix, integrins, and growth factors as tailors of the stem cell niche. *Curr. Opin. Cell Biol.* 24, 645–651.
- Brokerhoff, S.E., 2011. Phosphoinositides and photoreceptors. *Mol. Neurobiol.* 44, 420–425.
- Bron, A.J., Yokoi, N., Gaffney, E.A., Tiffany, J.M., 2011. A solute gradient in the tear meniscus. I. A hypothesis to explain Marx's line. *Ocul. Surf.* 9, 70–91.
- Brooke, M.A., Nitou, D., Kelsell, D.P., 2012. Cell-cell connectivity: desmosomes and disease. *J. Pathol.* 226, 158–171.
- Bulow, H.E., Hobert, O., 2006. The molecular diversity of glycosaminoglycans shapes animal development. *Annu. Rev. Cell Dev. Biol.* 22, 375–407.
- Burke, J.M., 2008. Epithelial phenotype and the RPE: is the answer blowing in the Wnt? *Prog. Retin. Eye Res.* 27, 579–595.
- Burridge, K., Wittchen, E.S., 2013. The tension mounts: stress fibers as force-generating mechanotransducers. *J. Cell Biol.* 200, 9–19.
- Butovich, I.A., 2011. Lipidomics of human meibomian gland secretions: chemistry, biophysics, and physiological role of meibomian lipids. *Prog. Lipid Res.* 50, 278–301.
- Candia, O.A., Alvarez, L.J., 2008. Fluid transport phenomena in ocular epithelia. *Prog. Retin. Eye Res.* 27, 197–212.
- Chakraborty, D., Rodgers, K.K., Conley, S.M., Naash, M.I., 2013. Structural characterization of the second intra-discal loop of the photoreceptor tetraspanin RDS. *FEBS J.* 280, 127–138.
- Cheema, A.S., Mozayan, A., Channa, P., 2012. Corneal collagen crosslinking in refractive surgery. *Curr. Opin. Ophthalmol.* 23, 251–256.
- Cipriani, V., Leung, H.T., Plagnol, V., Bunce, C., Khan, J.C., Shahid, H., et al., 2012. Genome-wide association study of age-related macular degeneration identifies associated variants in the TNXB-FKBP1-NOTCH4 region of chromosome 6p21.3. *Human Mol. Gen.* 21, 4138–4150.
- Claessen, J.H., Kundrat, L., Ploegh, H.L., 2012. Protein quality control in the ER: balancing the ubiquitin checkbook. *Trends Cell Biol.* 22, 22–32.
- Couchman, J.R., 2010. Transmembrane signaling proteoglycans. *Annu. Rev. Cell Dev. Biol.* 26, 89–114.
- Dagenais, M., Skeldon, A., Saleh, M., 2012. The inflammasome: in memory of Dr. Jurg Tschopp. *Cell Death Differ.* 19, 5–12.
- Dartt, D.A., 2009. Neural regulation of lacrimal gland secretory processes: relevance in dry eye diseases. *Prog. Retin. Eye Res.* 28, 155.
- Denu, J.M., Gottesfeld, J.M., 2012. Minireview series on sirtuins: from biochemistry to health and disease. *J. Biol. Chem.* 287, 42417.
- Desouza, M., Gunning, P.W., Stehn, J.R., 2012. The actin cytoskeleton as a sensor and mediator of apoptosis. *Bioarchitecture* 2, 75.
- Diamond, J.S., 2011. Calcium-permeable AMPA receptors in the retina. *Front. Mol. Neurosci.* 4, 27.
- Domogatskaya, A., Rodin, S., Tryggvason, K., 2012. Functional diversity of laminins. *Annu. Rev. Cell Dev. Biol.* 28, 523.
- Doughty, M.J., 2012. Goblet cells of the normal human bulbar conjunctiva and their assessment by impression cytology sampling. *Ocu. Surf.* 10, 149.
- Edwards, M.M., Lefebvre, O., 2013. Laminins and retinal vascular development. *Cell Adh. Migr.* 7, 82.
- Efron, N., Hollingsworth, J.G., 2008. New perspectives on keratoconus as revealed by corneal confocal microscopy. *Clin. Exp. Optom.* 91, 34.
- Eichmann, A., Simons, M., 2012. VEGF signaling inside vascular endothelial cells and beyond. *Curr. Opin. Cell Biol.* 24, 188.
- Elagouz, M., Stanescu-Segall, D., Jackson, T.L., 2010. Uveal effusion syndrome. *Surv. Ophthalmol.* 55, 134.
- Elhawy, E., Kamthan, G., Dong, C.Q., Danias, J., 2012. Pseudoexfoliation syndrome, a systemic disorder with ocular manifestations. *Hum. Genom.* 6, 22.
- Fanaei, M., Monk, P.N., Partridge, L.J., 2011. The role of tetraspanins in fusion. *Biochem. Soc. Trans.* 39, 524.
- Fischbarg, J., 2012. Water channels and their roles in some ocular tissues. *Mol. Aspects Med.* 33, 638–641.
- Florey, O., Overholtzer, M., 2012. Autophagy proteins in macroendothelial engulfment. *Trends Cell Biol.* 22, 374.
- Freddo, T.F., 2013. A contemporary concept of the blood-aqueous barrier. *Prog. Retin. Eye Res.* 32, 181.

- Giorgio, M., Trinei, M., Migliaccio, E., Pelicci, P.G., 2007. Hydrogen peroxide: a metabolic by-product or a common mediator of ageing signals? *Nat. Rev. Mol. Cell Biol.* 8, 722.
- Gnana-Prakasam, J.P., Martin, P.M., Smith, S.B., Ganapathy, V., 2010. Expression and function of iron-regulatory proteins in retina. *IUBMB Life* 62, 363.
- Gonzalez-Fernandez, F., Bevilacqua, T., Lee, K.I., Chandrashekhar, R., Hsu, L., Garlipp, M.A., et al., 2009. Retinol-binding site in interphotoreceptor retinoid-binding protein (IRBP): a novel hydrophobic cavity. *Invest. Ophthalmol. Vis. Sci.* 50, 5577.
- Gouyer, V., Gottrand, F., Desseyn, J.L., 2011. The extraordinarily complex but highly structured organization of intestinal mucus-gel unveiled in multicolor images. *PLoS ONE* 6, e18761.
- Govindarajan, B., Gipson, I.K., 2010. Membrane-tethered mucins have multiple functions on the ocular surface. *Exper Eye Res* 90, 655.
- Hafezi, F., Kanellopoulos, J., Wiltfang, R., Seiler, T., 2007. Corneal collagen crosslinking with riboflavin and ultraviolet A to treat induced keratectasia after laser in situ keratomileusis. *J. Cat. Refract. Surg.* 33, 2035.
- Hardarson, S.H., 2013. Retinal oximetry. *Acta Ophthalmol* 91, 1. Thesis 2.
- Houtkooper, R.H., Pirinen, E., Auwerx, J., 2012. Sirtuins as regulators of metabolism and healthspan. *Nat. Rev. Mol. Cell Biol.* 13, 225.
- Inder, K.L., Davis, M., Hill, M.M., 2013. Ripples in the pond – using a systems approach to decipher the cellular functions of membrane microdomains. *Mol. Biosystems* 9, 330.
- Invergo, B.M., Montanucci, L., Laayouni, H., Bertranpetti, J., 2013. A system-level, molecular evolutionary analysis of mammalian phototransduction. *BMC Evol. Biol.* 13, 52.
- Iozzo, R.V., 1998. Matrix proteoglycans: from molecular design to cellular function. *Annu. Rev. Biochem.* 67, 609.
- Ito, S., Pilat, A., Gerwat, W., Skumatz, C.M., Ito, M., Kiyono, A., et al., 2013. Photoaging of human retinal pigment epithelium is accompanied by oxidative modifications of its eumelanin. *Pigment Cell Mel Res* 26, 357.
- Jaqaman, K., Grinstein, S., 2012. Regulation from within: the cytoskeleton in transmembrane signaling. *Trends Cell Biol.* 22, 515.
- Ji, F., Jung, J., Koharudin, L.M., Gronenborn, A.M., 2013. The human W42R gammaD-crystallin mutant structure provides a link between congenital and age-related cataracts. *J. Biol. Chem.* 288, 99.
- Kadler, K.E., Hill, A., Carty-Laird, E.G., 2008. Collagen fibrillogenesis: fibronectin, integrins, and minor collagens as organizers and nucleators. *Curr. Opin. Cell Biol.* 20, 495.
- Kannan, R., Sreekumar, P.G., Hinton, D.R., 2012. Novel roles for alpha-crystallins in retinal function and disease. *Prog. Retin. Eye Res.* 31, 576.
- Kaushik, S., Cuervo, A.M., 2012. Chaperone-mediated autophagy: a unique way to enter the lysosome world. *Trends Cell Biol.* 22, 407.
- Keenan, T.D., Clark, S.J., Unwin, R.D., Ridge, L.A., Day, A.J., Bishop, P.N., 2012. Mapping the differential distribution of proteoglycan core proteins in the adult human retina, choroid, and sclera. *Invest. Ophthalmol. Vis. Sci.* 53, 7528.
- Kevany, B.M., Palczewski, K., 2010. Phagocytosis of retinal rod and cone photoreceptors. *Physiol. (Bethesda)* 25, 8.
- Kim, C., Ye, F., Ginsberg, M.H., 2011. Regulation of integrin activation. *Annu. Rev. Cell Dev. Biol.* 27, 321.
- Kitagishi, Y., Matsuda, S., 2013. RUFY, Rab and Rap family proteins involved in a regulation of cell polarity and membrane trafficking. *Int. J. Mol. Sci.* 14, 6487.
- Klingberg, F., Hinz, B., White, E.S., 2013. The myofibroblast matrix: implications for tissue repair and fibrosis. *J. Pathol.* 229, 298.
- Koch, K.W., Dell'orco, D., 2013. A calcium-relay mechanism in vertebrate phototransduction. *ACS Chem Neurosci* 4, 909–917.
- Koob, S., Reichert, A.S., 2014. Novel intracellular functions of apolipoproteins: the ApoO protein family as constituents of the Mitoflin/MINOS complex determines cristae morphology in mitochondria. *Biol. Chem.* 39, 285–296.
- Last, J.A., Liliensiek, S.J., Nealey, P.F., Murphy, C.J., 2009. Determining the mechanical properties of human corneal basement membranes with atomic force microscopy. *J. Struct. Biol.* 167, 19.
- Lee, Y.S., Tresguerres, M., Hess, K., Marmorstein, L.Y., Levin, L.R., Buck, J., et al., 2011. Regulation of anterior chamber drainage by bicarbonate-sensitive soluble adenylyl cyclase in the ciliary body. *J. Biol. Chem.* 286, 41353.
- Lee, J.G., Ko, M.K., Kay, E.P., 2012. Endothelial mesenchymal transformation mediated by IL-1beta-induced FGF-2 in corneal endothelial cells. *Exp. Eye Res.* 95, 35.
- Leitinger, B., 2011. Transmembrane collagen receptors. *Annu. Rev. Cell Dev. Biol.* 27, 265.
- Levin, M.H., Verkman, A.S., 2006. Aquaporins and CFTR in ocular epithelial fluid transport. *J. Mem. Biol.* 210, 105.
- Liton, P.B., Gonzalez, P., Epstein, D.L., 2009. The role of proteolytic cellular systems in trabecular meshwork homeostasis. *Exp. Eye Res.* 88, 724.
- Liu, W., Chun, E., Thompson, A.A., Chubukov, P., Xu, F., Katritch, V., et al., 2012. Structural basis for allosteric regulation of GPCRs by sodium ions. *Science* 337, 232.
- Logue, S.E., Cleary, P., Saveljeva, S., Samali, A., 2013. New directions in ER stress-induced cell death. *Apoptosis* 18, 537.
- Lorenzi, M., 2007. The polyol pathway as a mechanism for diabetic retinopathy: attractive, elusive, and resilient. *Exper. Diab. Res.* 2007, 61038.
- Luensmann, D., Jones, L., 2012. Protein deposition on contact lenses: the past, the present, and the future. *Cont. Lens Ant. Eye* 35, 53.
- McBrien, N.A., 2013. Regulation of scleral metabolism in myopia and the role of transforming growth factor-beta. *Exper. Eye Res.* 114, 128–140.
- McCaig, C.D., Rajnicek, A.M., Song, B., Zhao, M., 2005. Controlling cell behavior electrically: current views and future potential. *Physiol. Rev.* 85, 943.
- Malhotra, A., Minja, F.J., Crum, A., Burrowes, D., 2011. Ocular anatomy and cross-sectional imaging of the eye. *Semin. Ultrasound CT MR* 32, 2.
- Mann, G.E., Yudilevich, D.L., Sobrevia, L., 2003. Regulation of amino acid and glucose transporters in endothelial and smooth muscle cells. *Physiol. Rev.* 83, 183.
- Mao, Y., Schwarzbauer, J.E., 2005. Fibronectin fibrillogenesis, a cell-mediated matrix assembly process. *Matrix Biol.* 24, 389.
- Massague, J., 2012. TGFbeta signalling in context. *Nat. Rev. Mol. Cell Biol.* 13, 616.
- Mathias, R.T., Rae, J.L., Baldo, G.J., 1997. Physiological properties of the normal lens. *Physiol. Rev.* 77, 21.
- Mathias, R.T., White, T.W., Gong, X., 2010. Lens gap junctions in growth, differentiation, and homeostasis. *Physiol. Rev.* 90, 179.
- Maurice, D.M., 1957. The structure and transparency of the cornea. *J. Physiol.* 136, 263.
- Maxfield, F.R., van Meer, G., 2010. Cholesterol, the central lipid of mammalian cells. *Curr. Opin. Cell Biol.* 22, 422.
- Meek, K.M., Boote, C., 2009. The use of X-ray scattering techniques to quantify the orientation and distribution of collagen in the corneal stroma. *Prog. Retin. Eye Res.* 28, 369.

- Meek, K.M., Hayes, S., 2013. Corneal cross-linking – a review. *Ophthalmic Physiol. Opt.* 33, 78.
- Mercer, A.J., Thoreson, W.B., 2011. The dynamic architecture of photoreceptor ribbon synapses: cytoskeletal, extracellular matrix, and intramembrane proteins. *Vis. Neurosci.* 28, 453.
- Mimura, T., Yamagami, S., Amano, S., 2013. Corneal endothelial regeneration and tissue engineering. *Prog Retinal Eye Res* 35, 1–17.
- Moreau, K.L., King, J.A., 2012. Protein misfolding and aggregation in cataract disease and prospects for prevention. *Trends Mol. Med.* 18, 273.
- Mosher, D.F., Adams, J.C., 2012. Adhesion-modulating/matrix-cellular ECM protein families: a structural, functional and evolutionary appraisal. *Matrix Biol.* 31, 155.
- Mostowy, S., Cossart, P., 2012. Septins: the fourth component of the cytoskeleton. *Nat. Rev. Mol. Cell Biol.* 13, 183.
- Mueckler, M., Thorens, B., 2013. The SLC2 (GLUT) family of membrane transporters. *Mol. Aspects Med.* 34, 121.
- Muijnzeeks, L.D., Keeley, F.W., 2012. Molecular assembly and mechanical properties of the extracellular matrix: a fibrous protein perspective. *Biochim Biophys Acta* 1832, 866–875.
- Muller-McNicoll, M., Neugebauer, K.M., 2013. How cells get the message: dynamic assembly and function of mRNA-protein complexes. *Nat. Rev. Gen.* 14, 275.
- Nagaraj, R.H., Linetsky, M., Stitt, A.W., 2012. The pathogenic role of Maillard reaction in the aging eye. *Amino Acids* 42, 1205.
- Neisch, A.L., Fehon, R.G., 2011. Ezrin, Radixin and Moesin: key regulators of membrane-cortex interactions and signaling. *Curr. Opin. Cell Biol.* 23, 377.
- Neuhuber, W., Schrodil, F., 2011. Autonomic control of the eye and the iris. *Autonom Neurosci* 165, 67.
- Nickla, D.L., Wallman, J., 2010. The multifunctional choroid. *Prog. Retin. Eye Res.* 29, 144.
- Niehrs, C., 2012. The complex world of WNT receptor signalling. *Nat. Rev. Mol. Cell Biol.* 13, 767.
- Nightingale, T.D., Cutler, D.F., Cramer, L.P., 2012. Actin coats and rings promote regulated exocytosis. *Trends Cell Biol.* 22, 329.
- Nikitovic, D., et al., 2012. The biology of small leucine-rich proteoglycans in bone pathophysiology. *J. Biol. Chem.* 287, 33926.
- Okada, Y., et al., 2010. Neurotrophic keratopathy; its pathophysiology and treatment. *Histol. Histopathol.* 25, 771.
- Orbán, T., Gupta, S., Palczewski, K., Chance, M.R., 2010. Visualizing water molecules in transmembrane proteins using radiolytic labeling methods. *Biochemistry* 49, 827.
- Palczewski, K., 2012. Chemistry and biology of vision. *J. Biol. Chem.* 287, 1612.
- Palfy, M., Remenyi, A., Korcsmaros, T., 2012. Endosomal crosstalk: meeting points for signaling pathways. *Trends Cell Biol.* 22, 447.
- Palkovits, S., et al., 2013. Measurement of retinal oxygen saturation in patients with chronic obstructive pulmonary disease. *Invest. Ophthalmol. Vis. Sci.* 54, 1008.
- Palty, R., Hershinkel, M., Sekler, I., 2012. Molecular identity and functional properties of the mitochondrial $\text{Na}^+/\text{Ca}^{2+}$ exchanger. *J. Biol. Chem.* 287, 31650.
- Panaser, A., Tighe, B.J., 2012. Function of lipids – their fate in contact lens wear: an interpretive review. *Cont. Lens Ant. Eye* 35, 100.
- Parker, R., Wang, J.S., Kefalov, V.J., Crouch, R.K., 2011. Interphotoreceptor retinoid-binding protein as the physiologically relevant carrier of 11-cis-retinol in the cone visual cycle. *J. Neurosci.* 31, 4714.
- Peti, W., Nairn, A.C., Page, R., 2013. Structural basis for protein phosphatase 1 regulation and specificity. *FEBS J.* 280, 596.
- Piccirelli, M., Bergamin, O., Landau, K., Boesiger, P., Luechinger, R., 2012. Vitreous deformation during eye movement. *NMR Biomed.* 25, 59.
- Pickard, G.E., Sollars, P.J., 2012. Intrinsically photosensitive retinal ganglion cells. *Rev. Physiol. Biochem. Pharmacol.* 162, 59.
- Prince, J., Chuck, R.S., 2012. Refractive surgery after Descemet's stripping endothelial keratoplasty. *Curr. Opin. Ophthalmol.* 23, 242.
- Quinlan, R.A., et al., 2013. Changes in the quaternary structure and function of MJHSP16.5 attributable to deletion of the IXI motif and introduction of the substitution, R107G, in the alpha-crystallin domain. *Phil. Trans. R. Soc. Lond B. Biol. Sci.* 368, 0120327.
- Rabbani, N., Thornalley, P.J., 2012. Glycation research in amino acids: a place to call home. *Amino Acids* 42, 1087.
- Rachel, R.A., Li, T., Swaroop, A., 2012. Photoreceptor sensory cilia and ciliopathies: focus on CEP290, RPGR and their interacting proteins. *Cilia* 1, 22.
- Racioppi, L., Means, A.R., 2012. Calcium/calmodulin-dependent protein kinase kinase 2: roles in signaling and pathophysiology. *J. Biol. Chem.* 287, 31658.
- Rask-Madsen, C., Kahn, C.R., 2012. Tissue-specific insulin signaling, metabolic syndrome, and cardiovascular disease. *Arterioscl Thromb Vasc Biol* 32, 2052.
- Reinosa, R., et al., 2012. Topographical distribution and characterization of epithelial cells and intraepithelial lymphocytes in the human ocular mucosa. *Mucosal. Immunol.* 5, 455.
- Rhee, I., Davidson, D., Souza, C.M., Vacher, J., Veillette, A., 2013. Macrophage fusion is controlled by the cytoplasmic protein tyrosine phosphatase PTP-PEST/PTPN12. *Mol. Cell. Biol.* 33, 2458–2469.
- Richdale, K., et al., 2013. Quantification of age-related and per diopter accommodative changes of the lens and ciliary muscle in the emmetropic human eye. *Invest. Ophthalmol. Vis. Sci.* 54, 1095.
- Ritch, R., 2008. Exfoliation syndrome: beyond glaucoma. *Arch. Ophthalmol.* 126, 859.
- Rizzuto, R., De Stefani, D., Raffaello, A., Mammucari, C., 2012. Mitochondria as sensors and regulators of calcium signalling. *Nat. Rev. Mol. Cell Biol.* 13, 566.
- Rousselle, P., Beck, K., 2013. Laminin 332 processing impacts cellular behavior. *Cell Adh. Migr.* 7, 122.
- Saari, J.C., 2012. Vitamin A metabolism in rod and cone visual cycles. *Annu. Rev. Nutr.* 32, 125–145.
- Sahin, E., DePinho, R.A., 2012. Axis of ageing: telomeres, p53 and mitochondria. *Nat. Rev. Mol. Cell Biol.* 13, 397.
- Sasai, Y., 2013. Cytosystems dynamics in self-organization of tissue architecture. *Nature* 493, 318.
- Schaffler, A., Buechler, C., 2012. CTRP family: linking immunity to metabolism. *Trends Endocrinol. Metab.* 23, 194.
- Schlotter-Schrehardt, U., Kruse, F.E., 2005. Identification and characterization of limbal stem cells. *Exp. Eye Res.* 81, 247.
- Schmidt, T.M., Chen, S.K., Hattar, S., 2011. Intrinsically photosensitive retinal ganglion cells: many subtypes, diverse functions. *Trends Neurosci.* 34, 572.
- Sebastian, C., Satterstrom, F.K., Haigis, M.C., Mostoslavsky, R., 2012. From sirtuin biology to human diseases: an update. *J. Biol. Chem.* 287, 42444.
- Sharma, K.K., Santhoshkumar, P., 2009. Lens aging: effects of crystallins. *Biochim. Biophys. Acta* 1790, 1095.
- Shin, K., Fogg, V.C., Margolis, B., 2006. Tight junctions and cell polarity. *Annu. Rev. Cell Dev. Biol.* 22, 207.
- Shoulders, M.D., Raines, R.T., 2009. Collagen structure and stability. *Annu. Rev. Biochem.* 78, 929.

- Singh, P., Carraher, C., Schwarzbauer, J.E., 2010. Assembly of fibronectin extracellular matrix. *Annu. Rev. Cell Dev. Biol.* 26, 397.
- Sit, A.J., McLaren, J.W., 2011. Measurement of episcleral venous pressure. *Exp. Eye Res.* 93, 291.
- Soboloff, J., Rothberg, B.S., Madesh, M., Gill, D.L., 2012. STIM proteins: dynamic calcium signal transducers. *Nat. Rev. Mol. Cell Biol.* 13, 549.
- Song, B., Zhao, M., Forrester, J., McCaig, C., 2004. Nerve regeneration and wound healing are stimulated and directed by an endogenous electrical field in vivo. *J. Cell Sci.* 117, 4681.
- Song, S., et al., 2009. Functions of the intermediate filament cytoskeleton in the eye lens. *J. Clin. Invest.* 119, 1837.
- Song, M.S., Salmena, L., Pandolfi, P.P., 2012. The functions and regulation of the PTEN tumour suppressor. *Nat. Rev. Mol. Cell Biol.* 13, 283.
- Sterling, P., 2013. Some principles of retinal design: the Proctor lecture. *Invest. Ophthalmol. Vis. Sci.* 54, 2267.
- Strauss, O., 2005. The retinal pigment epithelium in visual function. *Physiol. Rev.* 85, 845.
- Stringer, J.M., Barrand, S., Western, P., 2013. Fine-tuning evolution: germ-line epigenetics and inheritance. *Reproduction* 146, R37.
- Su, X., Ohi, R., Pellman, D., 2012. Move in for the kill: motile microtubule regulators. *Trends Cell Biol.* 22, 567.
- Subczynski, W.K., Raguz, M., Widomska, J., Mainali, L., Konovalov, A., 2012. Functions of cholesterol and the cholesterol bilayer domain specific to the fiber-cell plasma membrane of the eye lens. *J. Memb. Biol.* 245, 51.
- Summers, J.A., 2013. The choroid as a sclera growth regulator. *Exp. Eye Res.* 114, 120–127.
- Suresh, S., 2007. Biomechanics and biophysics of cancer cells. *Acta Biomat.* 3, 413.
- Swamynathan, S.K., 2013. Ocular surface development and gene expression. *J. Ophthalmol.* 103947.
- Swaroop, A., Kim, D., Forrest, D., 2010. Transcriptional regulation of photoreceptor development and homeostasis in the mammalian retina. *Nat. Rev. Neurosci.* 11, 563.
- Tang, P.H., Kono, M., Koutalos, Y., Ablonczy, Z., Crouch, R.K., 2013. New insights into retinoid metabolism and cycling within the retina. *Prog. Retin. Eye Res.* 32, 48.
- Teng, P.Y., Wanek, J., Blair, N.P., Shahidi, M., 2013. Inner retinal oxygen extraction fraction in rat. *Invest. Ophthalmol. Vis. Sci.* 54, 647.
- Thevenin, A.F., et al., 2013. Proteins and mechanisms regulating gap-junction assembly, internalization, and degradation. *Physiology (Bethesda)* 28, 93.
- Thoreson, W.B., Mercer, A.J., Cork, K.M., Szalewski, R.J., 2013. Lateral mobility of L-type calcium channels in synaptic terminals of retinal bipolar cells. *Mol. Vis.* 19, 16.
- Toivola, D.M., Strnad, P., Habtezion, A., Omary, M.B., 2010. Intermediate filaments take the heat as stress proteins. *Trends Cell Biol.* 20, 79.
- Ujie, H., Shibaki, A., Nishie, W., Shimizu, H., 2010. What's new in bullous pemphigoid. *J. Dermatol.* 37, 194.
- van Reeuwijk, J., Arts, H.H., Roepman, R., 2011. Scrutinizing ciliopathies by unraveling ciliary interaction networks. *Hum. Mol. Gen.* 20, R149.
- Varma, S.D., Kovtun, S., Hegde, K.R., 2011. Role of ultraviolet irradiation and oxidative stress in cataract formation—medical prevention by nutritional antioxidants and metabolic agonists. *Eye Cont. Lens* 37, 233.
- Vos, W.L., et al., 2010. Expression and structural characterization of peripherin/RDS, a membrane protein implicated in photoreceptor outer segment morphology. *Eur. Biophys. J.* 39, 679.
- Wang, J., et al., 2011. Ultra-high resolution optical coherence tomography for imaging the anterior segment of the eye. *Ophthalmol. Surg. Lasers Imag.* 42, S15.
- Wenzel, A., Grimm, C., Samardzija, M., Reme, C.E., 2005. Molecular mechanisms of light-induced photoreceptor apoptosis and neuroprotection for retinal degeneration. *Prog. Retin. Eye Res.* 24, 275.
- Wright, A.F., Chakarova, C.K., Abd El-Aziz, M.M., Bhattacharya, S.S., 2010. Photoreceptor degeneration: genetic and mechanistic dissection of a complex trait. *Nat. Rev. Genet.* 11, 273.
- Yamada, K.M., Miyamoto, S., 1995. Integrin transmembrane signaling and cytoskeletal control. *Curr. Opin. Cell Biol.* 7, 681.
- Yamashiro, S., Gokhin, D.S., Kimura, S., Nowak, R.B., Fowler, V.M., 2012. Tropomodulins: pointed-end capping proteins that regulate actin filament architecture in diverse cell types. *Cytoskeleton (Hoboken)* 69, 337.
- Young, T.L., Metlapally, R., Shay, A.E., 2007. Complex trait genetics of refractive error. *Arch. Ophthalmol.* 125, 38.
- Yu, F.X., Guan, K.L., 2013. The Hippo pathway: regulators and regulations. *Genes Dev.* 27, 355.
- Yucel, Y.H., et al., 2009. Identification of lymphatics in the ciliary body of the human eye: a novel 'uveolymphatic' outflow pathway. *Exp. Eye Res.* 89, 810.
- Zheng, Z., et al., 2012. Sirtuin 1-mediated cellular metabolic memory of high glucose via the LKB1/AMPK/ROS pathway and therapeutic effects of metformin. *Diabetes* 61, 217.
- Zhou, L., Beuerman, R.W., 2012. Tear analysis in ocular surface diseases. *Prog. Retin. Eye Res.* 31, 527–550.
- Zhou, L., Zhao, S.Z., Koh, S.K., Chen, L., Vaz, C., Tanavde, V., et al., 2012. In-depth analysis of the human tear proteome. *J. Proteomics* 75, 3877–3885.