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MOLECULAR BIOLOGY AND EVOLUTION OF CRYSTALLINS:
GENE RECRUITMENT AND MULTIFUNCTIONAL PROTEINS IN THE EYE LENS

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AUSTIN

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PREFACE

The eye lens is a remarkable transparent tissue with an important role in vision. Recent advances in our understanding of the lens in various species have revealed complex histories of molecular evolution and adaptation. In particular we have seen that the properties of the lens have always depended on the direct recruitment of existing proteins to new structural roles as crystallins. The lens turns out to be a particularly advantageous system for examination of evolutionary and developmental processes which may have wider significance beyond the lens itself.

Furthermore it has now been discovered that the molecular biology and development of the lens is intimately connected with ancient gene cascades which define "eye" in species from flies to mice. Thus we have seen a direct connection between essential tissue-determining genes such as *Pax-6* and *Sox-2* and the expression of crystallins in the lens.

This book describes our present view of the molecules of the lens in the context of the wider evolutionary history of the eye. Each chapter is intended to serve as review of specific areas; the evolution and development of the eye and the lens, the phenomenon of crystallin gene recruitment, the ubiquitous stress-related crystallins, the taxon-specific enzyme crystallins and the mechanisms of crystallin gene expression, followed by a closing summary.

Although this story has developed from studies of the lens it illustrates a number of important general processes in biology. It should therefore be of interest not only to those working directly on the eye but also to others involved in various fields of molecular and evolutionary biology.

CHAPTER 1

THE EVOLUTION OF EYES AND LENSES

Crystallins are the abundant, soluble structural proteins of cellular lenses in vertebrate and invertebrate eyes. The lens is a highly specialized tissue in a highly specialized organ. Its function is to control the refraction of light and image formation in the eye. Even though cellular lenses are relatively late and independently derived features of eyes, molecular studies of the lenses of vertebrates and invertebrates have revealed both a surprising diversity in composition and a surprising congruence in molecular mechanisms. These underlying similarities seem to be the result of a common evolutionary history shared by all metazoan eyes from a very early stage of organization.

Thus the evolutionary and developmental origins of crystallins, some of which are the results of quite recent recruitment events, are inextricably connected to the long evolutionary history of eyes and vision. Although the ability to sense and make sense of light and to modify behavior accordingly seems to be a complex and sophisticated behavior, one which is hard to mimic even with advanced technology, the origins of vision are surprisingly ancient in the history of life on earth.

LIGHT AND LIFE

Life is inherently opportunistic and inevitably it has made good use of the solar energy which penetrates the atmosphere as visible light. Green plants use chlorophyll-based photosynthetic systems in specialized organelles to harness the energy of light in chemical bonds[1] while some bacteria such as *Rhodopseudomonas* use the unrelated bacteriochlorophylls for a similar purpose.[1] Certain archaebacteria such as the halophile *Halobacterium halobium* (now *salinarium*) also use light as a source of energy. These prokaryotes possess an integral membrane protein called bacteriorhodopsin which consists of seven transmembrane α-helices arranged in a bundle.[2,3] A lysine residue in the seventh helix binds the chromophore retinaldehyde (retinal) through a Schiff's base linkage. When the bound retinal absorbs a photon it undergoes a

stereoisomerization from all-*trans* to 13-*cis*.[2,3] This triggers a conformational change in the protein and serves to activate a proton pump which provides an energy source for the cell.[3,4] A closely related protein, halorhodopsin, acts in a similar way as a light-powered chloride pump.[2] However archaebacteria, protists and animals go beyond using light as an energy source to exploit the broad spectrum, high frequency and directionality of light as the most information-rich of sensory media.

Remarkably it has been found that in addition to bacteriorhodopsin and halorhodopsin prokaryotic *Halobacteria* also possess two related proteins called SRI and II, where SR denotes sensory rhodopsin.[2,5,6] As their name suggests, these sensory proteins are required for a phototropic response. In eukaryotes, eyespots and eyes in species ranging from the unicellular photosynthetic protists *Chlamydomonas*[7] and *Euglena*[8] to complex multicellular animals also make use of the chromophore retinal bound to membrane proteins. In animals such as *Drosophila* and mammals, these proteins are known as opsins[9,10] (Fig. 1.1). Even in *Chlamydomonas* it seems likely that a similar retinal-binding, opsin is responsible for the response to light.[7] Like bacteriorhodopsin, opsins are integral membrane proteins with seven transmembrane α-helices in which retinal is bound via a Schiff's base to a lysine in the seventh helix. In animal opsins absorption of a photon causes a conformational change both in the bound chromophore retinal, usually from 11-*cis* to all-*trans*, and in the opsin itself. This change in structure initiates an amplifying cascade of signaling events, culminating in a release of neurotransmitters and a nerve impulse.[9-11] Depending on the organism involved this can lead to movement towards a candle flame or to understanding the written word.

The striking structural and functional similarities of the bacteriorhodopsin and opsin families could be the result of common descent from a single original "invention" of this protein motif at a very early stage in evolution. One might even speculate that this system for light absorption arrived in eukaryotic cells through a prokaryotic symbiont in a manner similar to the acquisition of organelles such as mitochondria and chloroplasts.[12] Unfortunately, there is far too little sequence similarity between bacteriorhodopsins and opsins to demonstrate homology.[13] By itself this does not eliminate the possibility of an evolutionary relationship since tertiary structure and functionality are often found to be well conserved even in the absence of obvious sequence similarity, as for example in the relationship between the 70 kDa heat shock proteins and actin.[14] However prokaryotic and eukaryotic opsins also differ in the stereoisoforms of retinal they bind. It is quite possible that in spite of their similarities the two families of proteins separately converged on the same structure since this seven-helical motif is thought to be extremely common in membrane protein receptors.[10,15,16]

While we may not be able to demonstrate common ancestry of bacteriorhodopsins and animal opsins there is still the real possibility that the visual pigment of the eukaryotic protist *Chlamydomonas* is related to animal opsins.[7] If true, this could place the root of eye evolution

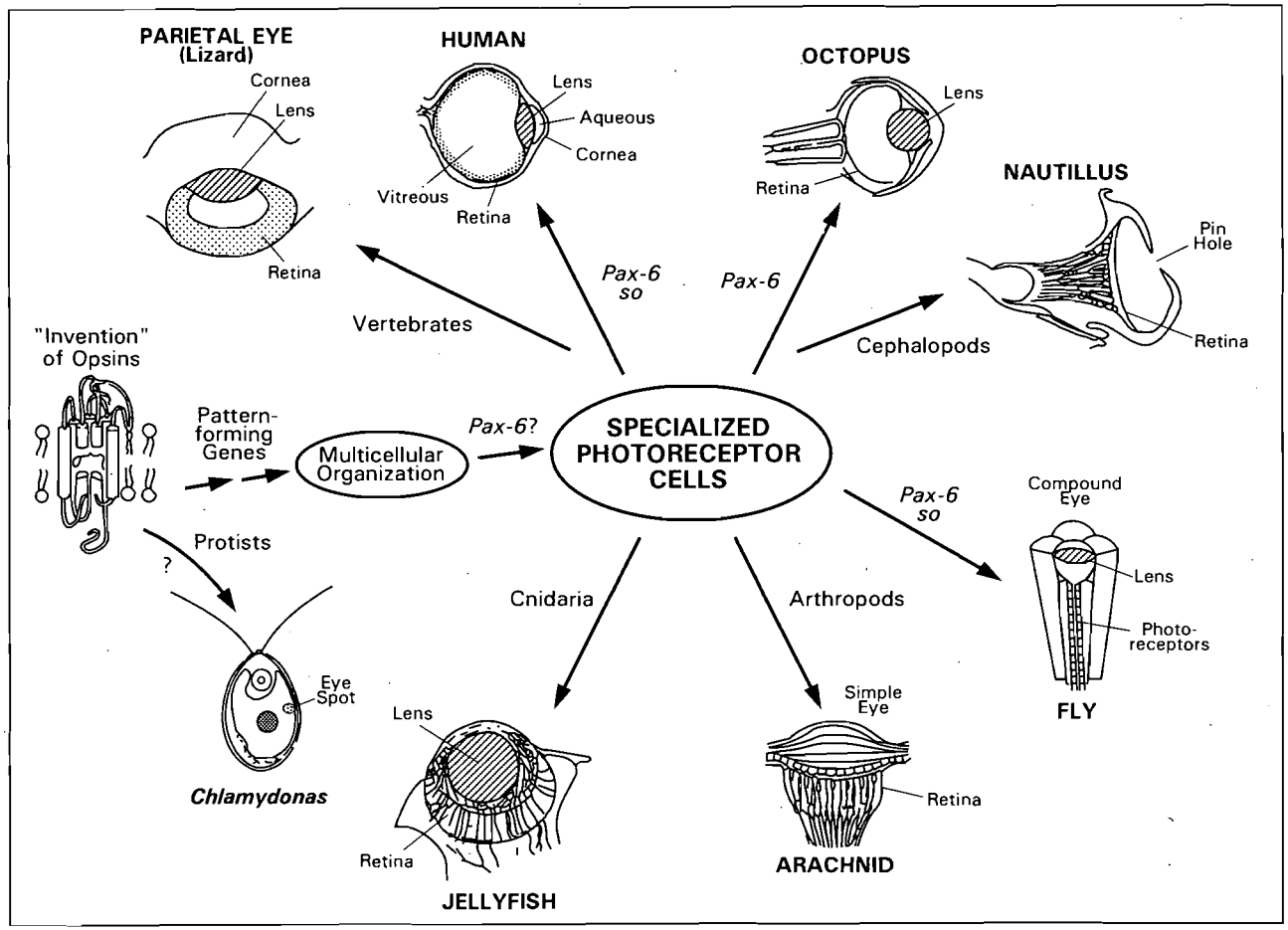

Fig. 1.1. The evolution of eyes. Diverse metazoan eyes may share a common origin and common molecular mechanisms of development involving Pax-6 *and other genes such as* sine oculis *(so).*[109] *All eyes and eyespots may share an even more ancient common origin in the evolutionary innovation of the opsin gene family. Figure is adapted from several sources including references 19, 27, 32, 110, 111.*

at least as far back as unicellular eukaryotes. Sequence data for protist opsins are eagerly awaited.

After the "invention" of opsin itself and of the mechanisms to couple its light receptor function to cellular responses the next big step in the evolution of eyes came with the arrival of multicellular organisms (Fig. 1.1). In metazoans it became possible to produce differentiated cells which could specialize in the production of opsins. These were the ancestors of the photoreceptor cells of animal eyes in which opsins are concentrated in arrays in plasma membranes. Photoreceptor cells occur in two major classes, the rhabdomeric photoreceptors composed of microvilli which are found in insect compound eyes and elsewhere or the ciliary photoreceptor cells typical of mammalian retinas.[9,11,17] These cells contain all the machinery of the visual cascade together with neural connections to transmit information to the rest of the organism. It now appears that the earliest achievement of specialized photoreceptor cells during multicellular organization may be ancestral to both rhabdomeric and ciliary photoreceptors since eyes from both lineages share the same fundamental molecular mechanisms of development and differentiation (Fig. 1.1).

THE EVOLUTION OF EYES: DIVERSITY AND SUCCESS

Even the most simple visual systems are a remarkable testimony to the power of natural selection and molecular evolution. Eyes are so useful that they are widespread in animals to the point that over 90% of living animal species have some kind of vision.[17-19] Some eyes, such as those in certain species of nematode, may be no more than light sensitive patches which may serve simply for up/down or light/dark orientation.[18,20] However, in many cases more of the information content of light is exploited by some kind of imaging system.

One of the simplest systems is found in the eye of the sea-going cephalopod mollusk *Nautilus*. The photoreceptor cells are arrayed in a curved retina much like that of the vertebrate eye.[17,20,21] Image formation is provided simply by a small hole in the front of the eye giving the form of a simple pinhole camera (Fig. 1.1). Light refracted through the pinhole can form a clear but dim image on the retina. Many other species, both invertebrates such as other mollusks, arachnids and jellyfish, and vertebrates (Fig. 1.1) have eyes in which light is concentrated and directed by a lens to give brighter images.[17,20,21] In some species, lenses may be used only as light concentrators, but elsewhere they are used for image formation, both gathering and focusing light, often correcting for the spherical and chromatic aberration which may afflict inorganic lenses as they do so.[17,22-24]

STRUCTURE OF THE VERTEBRATE EYE

The vertebrate eye (Fig. 1.2) is a spherical organ consisting of several transparent layers overlaying a photosensitive retina all contained

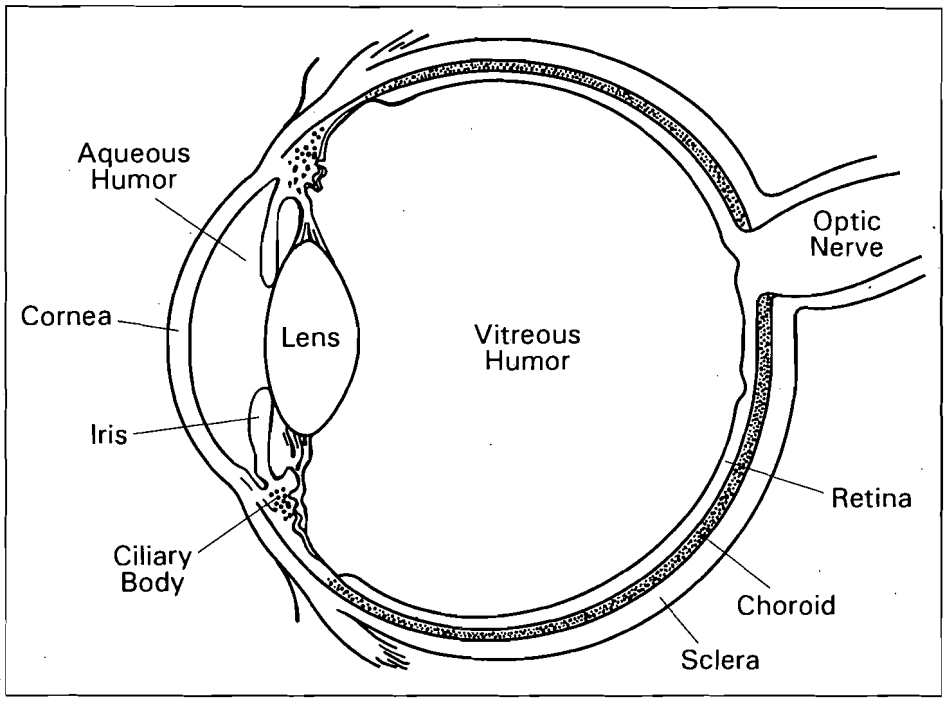

Fig. 1.2. The structure of the human eye.

in an opaque eyeball with a single anterior opening and a posterior connection to the brain via the optic nerve.[20,25,26] The first transparent layer, covering the anterior opening, is the cornea which is primarily an extracellular matrix of collagen overlaid by thin layers of endothelial and epithelial cells.[25,26] In many species the cornea provides a major part of the focusing power.[20] It also protects the interior of the eye from the external environment and absorbs much of the short wavelength ultra-violet radiation which could harm the sensitive interior. Both the physical curvature of the cornea and much of its nutrient supply derive from the aqueous humor, a clear fluid which fills the anterior chamber of the eye.[25,26] In the avascular anterior chamber the aqueous plays an essential role in transport of nutrients, growth factors and waste products for both the cornea and the anterior part of the lens.

The aperture of the eye, the pupil, is defined by a pigmented contractile tissue, the iris, which extends from the ciliary body.[25,26] Just behind the iris, suspended from the ciliary body by a system of ligaments, is the lens, a highly specialized cellular structure with various roles in producing a sharp visual image.[25,26] This image is projected onto the retina through the gelatinous vitreous body which fills the rest of the eyeball. Like the aqueous, the denser vitreous has nutritive

and other transport roles to play.[25,26] The retina consists of layers of nerve cells and the opsin-containing photoreceptors themselves.[25,26] Curiously, in the vertebrate eye these cells are arranged at the back of the retina so that light must first pass through the neural layers. Photoreceptor cells function without turnover throughout life. They continually produce membranous discs containing the opsins and other proteins of the visual cascade.[25,26] Particularly in the rod cells these form stacks of discs which, as they age, are shed and scavenged by the retinal pigment epithelium.[25,26] This light absorbing layer also serves to eliminate dazzle from internal reflection of unabsorbed light.

Other architectures for eyes, such as the use of directed bundles of photoreceptor cells perhaps coupled with wave guides rather than lenses have been exploited widely, most notably in the compound eyes of insects.[18,21] The eye of the scallop *Pecten* even makes use of a mirror instead of a lens for light gathering.[20] However this is unusual. Unlike astronomical telescopes in which the dominant form of optics is reflective, eyes are predominantly refractive.

Although we are most familiar with the idea of paired, symmetrically equivalent eyes many species have several sets of eyes, sometimes of different types. Many arthropods, such as *Drosophila*, have both compound eyes and small simple eyes with concentrating lenses.[18,21] Even vertebrates may have additional eyes. In lampreys, amphibians and some reptiles a small third (and sometimes fourth) eye, the parietal or median eye (Fig. 1.1), forms from a vesicle of neural ectoderm.[27] The posterior part of this vesicle develops photoreceptor cells to form a "retina" while the anterior part consists of a single layer of elongated, transparent cells, a "lens." The function of this eye is unknown although it seems likely that it has a role in setting diurnal rhythms. In birds and mammals the parietal eye has evolved into the pineal, the main source of the hormone melatonin.[28]

STRUCTURE AND DEVELOPMENT OF CELLULAR LENSES

A lens is basically a curved interface between regions of differing refractive indices. This kind of structure can be achieved in various ways. For a cellular lens, all that is required is for a monolayer of cells to elongate while constrained around the edges by contact with other cells, and for these cells to increase their protein concentration and hence their refractive index. Indeed, cellular lenses in both vertebrates and invertebrates are mainly composed of extremely elongated cells or, in the case of cephalopods, cell processes.[29-32] Discontinuities between adjacent cells are minimized and a uniform tissue consisting primarily of cytoplasm is formed.

The division into different cells allows the formation of a gradient of refractive index as protein content varies between layers of cells.[30,33] Generally this is used to increase the apparent convexity of the lens

and to increase its focusing power. Thus the center of the lens has a higher protein concentration and hence a higher refractive index than the cortical regions.[23,30,33] There may be a two fold difference in protein concentration between these two regions. The recent discovery that the lens in a chameleon acts as if it were concave rather than convex[34] suggests that this pattern may have been reversed to fit the peculiar optical requirements of this species.

Cell elongation, which is probably a largely osmotic process, is a key feature of lens development.[35-37] In vertebrate lenses it is the first recognizable stage in lens differentiation during embryogenesis (Fig. 1.3). A patch of epidermal ectoderm overlying neural ectoderm undergoes cell elongation to form the lens placode.[25,26,31,38] As development proceeds, there is a coordinate invagination of the lens placode and the underlying neural ectoderm. The lens placode pinches off from the surrounding ectoderm to form the lens vesicle. Even at this early stage, expression of the characteristic lens proteins, the crystallins can be detected.[39,40] The neural ectoderm layer goes on to form the eye cup which gives rise to the retina, ciliary body and other structures.

The lens vesicle consists of the original elongated cells of the placode, now at the posterior of the vesicle, and an anterior layer of undifferentiated, cuboidal cells. This arrangement allows for continual growth in the lens. The posterior, elongated cells undergo further elongation and differentiation becoming the primary fiber cells. They extend until they fill the lens vesicle and come into contact with the anterior layer. The anterior cells comprise the progenitors of the lens epithelium, a stem cell-like population which persists throughout life. While the central anterior epithelial cells remain rather quiescent more posterior cells migrate towards the lens equator where they enter a proliferative zone and go through mitosis (Fig. 1.4). At the lens equator cells undergo a dramatic terminal differentiation into enormously elongated new fiber cells. These secondary fiber cells overlay the primary fibers in concentric layers in a process which continues throughout life. The original embryonic primary fibers form the so-called lens nucleus. Later, fully mature secondary fibers may also constitute part of the nucleus, the densest region of the lens and a frequent locus for cataract formation in humans. Younger secondary fibers, particularly those which are still metabolically and synthetically active form the lens cortex.

Although cell elongation is essential for lens development, it is not the only determinant of the shape of the lens. During development vertebrate lenses acquire the lens capsule, essentially a basement membrane surrounding the lens, which helps to constrain its shape. While the epithelial cells are attached to the capsule the fiber cells detach during differentiation and eventually form contacts only with other fiber cells. This contact is mediated through numerous gap junctions[41] and probably also through an abundant lens fiber cell membrane protein called MIP26,[42] a member of a large family of water channel proteins.[43]

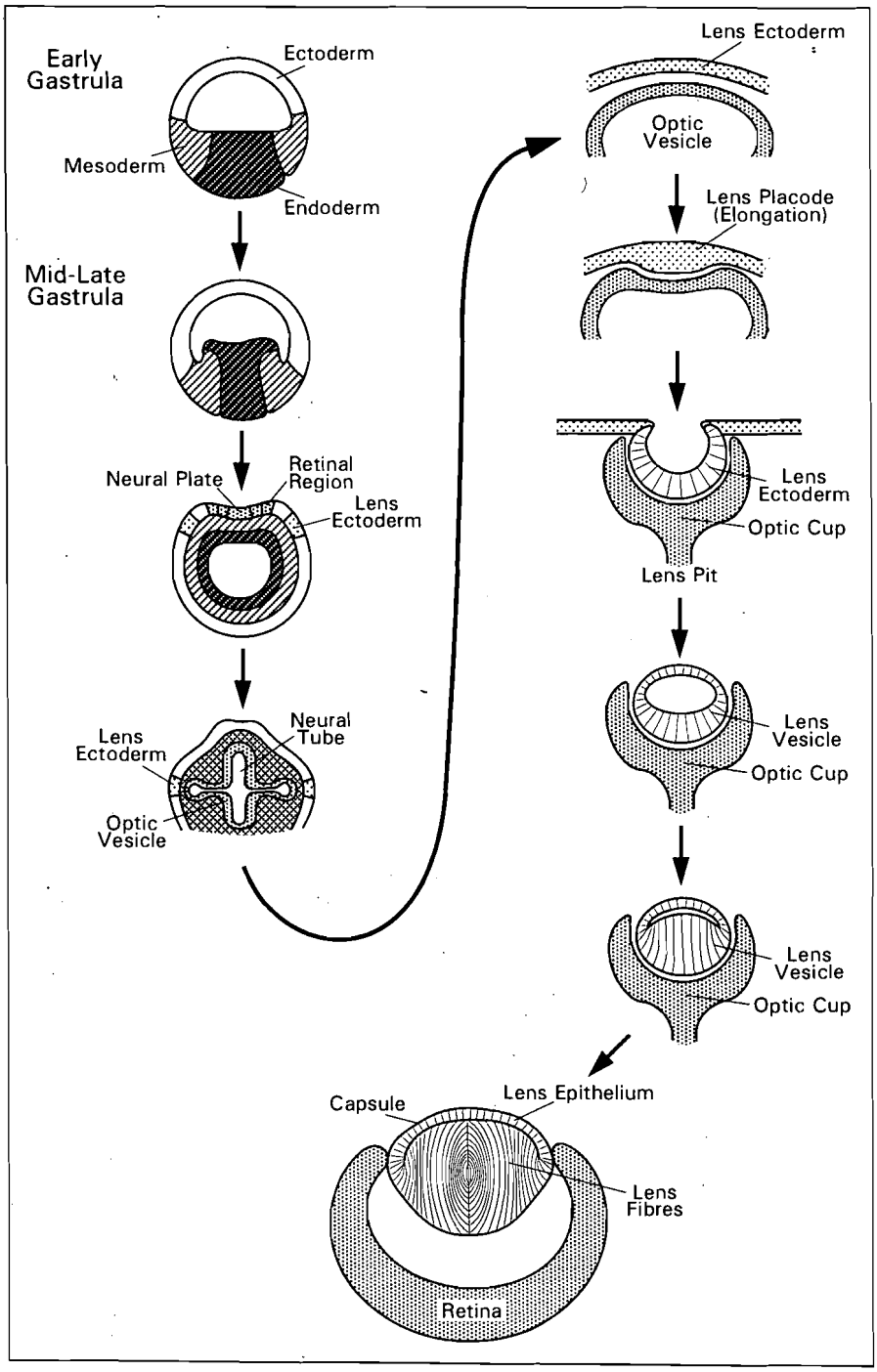

Fig. 1.3. The ontogeny of the vertebrate eye. This figure is a composite derived from a model of lens induction in Xenopus laevis [38] *shown on the left side, and later stages of eye and lens differentiation in the rat*[29] *shown on the right.*

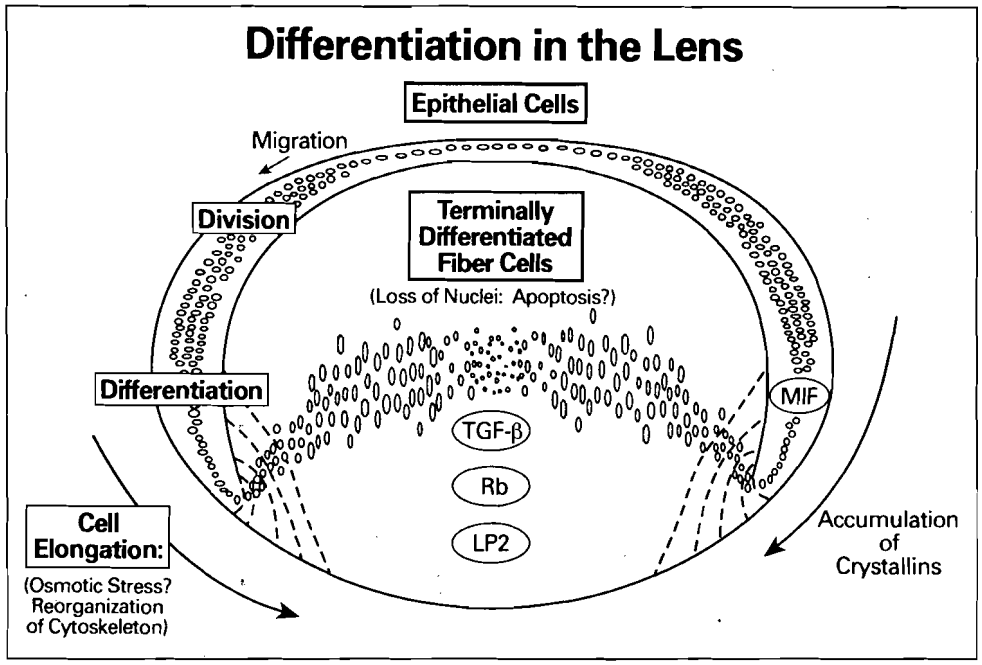

Fig. 1.4. Differentiation in a generalized vertebrate lens. Some non-crystallin molecular markers identified in various species are indicated; MIF,[59] TGFβ1,[58] Retinoblastoma protein (Rb)[112] and LP2, a lipid binding protein in lens.[113]

As fiber cells mature and are overlaid by younger layers their terminal differentiation continues and cellular organelles are lost. Nuclei, mitochondria and the structures of the endoplasmic reticulum are eliminated at a sharp division in the lens between one cell layer and the next.[44] From an optical standpoint the loss of organelles is usually interpreted as a loss of potential sites for light scattering along the optical axis of the lens. The loss of nuclei also eliminates any possibility of the fiber cells resuming proliferation, something which would certainly disrupt lens structure and transparency. However it is possible that the loss of nuclei is not an end in itself but is simply an inevitable part of the program of differentiation in this tissue. Lens cell differentiation has some intriguing parallels with programmed cell death mechanisms. Most notably the cell nuclei condense and the chromosomal DNA breaks down in a characteristic nucleosomal ladder.[45] Eventually the nuclei disappear into the cytoplasm. It has been pointed out that this also has some similarity to an abortive mitotic phase and the loss of nuclei may be the result of a failure of this phase to complete.[46]

Whatever the reason for nuclear breakdown, it is clear that mature fiber cells lose their ability to express genes or to synthesize new

protein and even decline in general metabolic capability.[47] Proteins in the mature fiber cells must survive without turnover throughout life. Lens proteins which are synthesized in the embryo and persist throughout life may thus be the oldest in the organism.

Growth Factors and Lens Differentiation

An unusual feature of the vertebrate lens is its relative isolation from other tissues. Although the lens may be vascularized during mammalian (but not avian) embryonic development the mature lens in all vertebrates is a completely avascular system.[47] This is necessary for transparency, but it means that nutrients and waste products must make their way to and from the lens from surrounding structures by diffusion. The closest structures are the ciliary body, to which the lens is attached through equatorial connections to the capsule, and the iris. Anteriorly lies the aqueous humor and the cornea. Posteriorly lies the vitreous and the retina. These surrounding structures communicate with the lens by means of diffusible growth factors and hormones and it is likely that the lens communicates with the rest of the eye in the same way. Growth of different tissues in the eye needs to be well coordinated and a deficiency in growth of one part, such as the lens, will lead to a general disruption of growth in the eye and microphthalmia.[48-50]

The compartmentalization of the eye allows the formation of gradients of growth factors across the lens. This may be a principal mechanism for control of lens differentiation. In the absence of growth factors, explanted rat lens epithelial cells remain quiescent but at increasing concentrations of acidic and basic fibroblast growth factors (aFGF, bFGF), the same cells in culture mimic the differentiation of the lens, they migrate, divide and finally increase in volume and synthesize differentiation-specific crystallins.[51-53] In this model high concentrations of aFGF and bFGF in the vitreous, lower concentrations in the posterior chamber (the space between the ciliary body and the lens), and low concentrations in the anterior chamber would be enough to regulate lens cell differentiation. This idea fits very well with some classic experiments in which lenses were reversed anterior to posterior in the embryonic chicken eye.[54] When this was done, epithelial cells which were now positioned posteriorly elongated, mimicking the differentiation of fiber cells. More recently, when a secretable form of aFGF was targeted to lens in transgenic mice, lens epithelial cells elongated and began to express differentiation-specific crystallins.[55]

Undoubtedly other growth factors are also involved in lens differentiation. These include IGF-1 which is important for lens cell growth in chicken,[56] platelet derived growth factor (PDGF) which when delivered in pulses maintains the transparency of rat lenses in organ culture[57] and TGFβ1 (transforming growth factor) which is localized in the fiber cells of mouse lens[58] (Fig. 1.4).

These growth factors may operate through a common path. A small protein expressed with moderate abundance in embryonic chick, mouse and human lens was found to be identical to a protein previously identified as macrophage migration inhibitory factor (MIF).[59] In lens the expression of this protein is associated with the equatorial region (Fig. 1.4). MIF is expressed in a delayed early response to mitogenic growth factors including bFGF, PDGF and TGFβ1 (which is mitogenic in NIH 3T3 fibroblast).[60,61] Antisense suppression of MIF in cultured cells blocks cell proliferation while constitutive expression allows cells to grow in the absence of serum.[61] MIF may be an essential part of the growth factor response in lens and other cells.

Evolution of a Cellular Lens

From Darwin onwards an explanation of the development of the multilayered vertebrate eye by step-wise selective processes has often been cited as one of the biggest challenges to classical evolutionary theory.[62] Recently a computer modeling exercise has demonstrated a possible selective path for the evolution of an eye in which each stage is a functional improvement over its predecessors.[63,64] This shows that the evolution of an eye superficially similar to those of vertebrates could occur rapidly, although since this treatment seems to consider zones of refractive index rather than discrete cells and tissues its ontogenic and phylogenetic implications for real eyes are not clear.

The evolution of a lens requires reasonable changes in structures of the eye with some benefit or lack of deleterious effect at each stage. A primitive ancestral stage in the evolution of the lens might have consisted of a single layer of elongated cells. This would resemble in some ways the so-called lens of the parietal or median eye present in many reptiles and amphibians[27] (although to what extent this structure acts as a lens is unknown). Such a structure could have served to protect the retina physically or from harmful radiation. It could also have begun to act as a concentrator of light.[20]

The size of a lens consisting of a single layer of cells is limited by the extent to which individual cells can elongate. Through the topological trick of forming the lens vesicle, the vertebrate eye lens is freed from these constraints and can grow throughout life adding new concentric layers of cells. It is by no means obvious how this trick was performed. It presumably occurred in one step perhaps through a single mutation in a gene controlling tissue-pattern formation or cell-cell recognition leading to the separation of lens cells from their surroundings. However convergent evolution has produced superficially very similar lenses in cephalopods, jellyfish and some other invertebrates although different developmental tricks have been used in different lineages. For example, in cephalopod lenses the concentric layers of fibers are not complete cells, instead they are cellular processes extending from a lentigenic region outside the lens proper.[65,66] Yet a very similar looking lens is the result.

Crystallins: Refraction and Transparency

The refractive power of the lens derives both from its curvature and its refractive index. The refractive index of the lens is largely a property of the crystallins, the soluble proteins of the "crystalline," or clear, lens which provide its bulk refractive structure.[47,67,68] Large amounts of crystallins accumulate in lens cells, particularly in the differentiated fiber cells (Fig. 1.4). Indeed, these proteins may account for as much as 80-90% of soluble protein in a highly proteinaceous tissue and, as such, are clearly structural proteins. Some dense, high refractive index lenses such as those of fish, rodents or squid, may have a protein content of 60% wet weight or more, although in many diurnal terrestrial species, particularly birds, the content is less than half this value.

Lens transparency is maintained by short-range order between crystallins.[69,70] Phase changes in the supramolecular structure of the lens, such as those which occur in cold cataract,[71] can lead to opacity by the creation of light scattering interfaces between zones of different refractive index. The intermolecular interaction of crystallins which define their supramolecular organization depend on the sequences and structures of the individual crystallins, as described in subsequent chapters.

Crystallins were originally characterized in a few domestic vertebrate species.[47,72] Size fractionation of native proteins revealed a few conspicuous size species which were named using the Greek alphabet, a convention which has been continued for vertebrate crystallins. In mammals, three classes were recognized, the α-, β- and γ-crystallins in descending order of native size, the α-crystallins being large aggregates, the β-crystallins dimers to octamers and the γ-crystallins monomers. In chickens the γ-crystallins were absent, apparently replaced by a different, multimeric crystallin which was named δ-crystallin. Subsequently many more species were examined and a new appreciation of crystallin diversity has emerged.

Crystallins may now be classified in two broad groups; the ubiquitous and the taxon-specific[37] (Table 1.1). The ubiquitous crystallins are represented in every vertebrate species examined suggesting that they reflect the composition of the ancestral vertebrate lens. They are the α-crystallins, consisting of two gene products, αA-crystallin and αB-crystallin; the β-crystallins which in mammals and birds are a family of six genes falling into two subgroups, βA and βB; and the γ-crystallins which in mammals form one family of highly similar genes expressed embryonically and neonatally and one more distantly related gene expressed in the cortical fibers of adult lenses. β- and γ-crystallins are related and may therefore share a common origin in the lens. A hypothetical ancestral vertebrate lens might have contained one α-crystallin and one ancestral β-crystallin.

In contrast the taxon-specific crystallins are major constituents of lenses only in defined evolutionary lineages (Table 1.1). They arose later than the ubiquitous crystallins as a result of discrete, indepen-

Table 1.1. Vertebrate crystallins

Ubiquitous: Stress-related	Taxon-specific: Enzymes	
α, β, γ	δ, ε, π	reptiles, birds
	ζ, η, λ, μ	mammals
	ρ	frogs
	τ	scattered distribution

dent recruitment events and were retained in descendant species.[37] The recruitment of taxon-specific crystallins provides an unusual opportunity to study events in the molecular evolution of species still at a stage of great diversity. Other systems may have enjoyed similar diversity in the distant past but this may have been obscured by subsequent extinctions and "rationalizations" of the pool of diversity.

Differential expression of the multiplicity of crystallins in each lens allows for establishment of smooth gradients of refractive index which enhance the optical properties of the lens, eliminating chromatic and spherical aberration.[22]

Crystallins and Stress

Although several different proteins serve as crystallins, many share the unifying of a connection to stress responses. There is a direct role for mammalian αB-crystallins in heat and osmotic shock[73-77] and other crystallins have more or less direct links with heat, osmotic, or various oxidative stresses[68,78-81] (Table 1.2). This may reflect the stressed condition of the lens itself. A tissue which undergoes enormous cell elongation, must continually maintain its balance of protein and water and be bathed in light for years on end. Crystallins seem to have been selected from proteins which are already expressed in the lens and which at high levels may have beneficial effects for lens stability. In particular several crystallins, including enzyme crystallins, may have a protective association with the cytoskeleton upon which the elongated fiber cells depend.[37,76] These properties of crystallins are discussed in later chapters.

PAX-6 AND COMMON ORIGINS

In spite of the enormous variety and evolutionary inventiveness of metazoan eyes and the high specialization of their component tissues, there is remarkable evidence that all these eyes and even derived tissues such as the vertebrate lens share a common origin. In vertebrates, flies, mollusks and worms the same molecular mechanisms are responsible for initiating the development of what otherwise appear to be such widely different eyes. Thus a gene responsible for normal eye development in man (*aniridia*)[82,83] and mouse (*small eye*)[84] turns out to be directly homologous to *eyeless* a gene essential for compound eye

Table 1.2. Crystallin connections

Heat Shock-
- αB is a sHSP in mammals
- Enolase (τ) is a HSP in yeast

Osmotic Stress-
- αB is induced by osmotic stress in mammals
- β and γ are related to proteins induced by dehydration
- ρ is related to aldose reductase, an osmotic stress protein
- Substrates of other enzyme crystallins may be osmolytes

Interaction with Cytoskeleton-
- α, β associate with cytoskeleton, plasma membrane
- LDH (ε), Enolase (τ), GAPDH (π) interact with "cytomatrix" in other cells

UV or oxidative stress-
- Several enzyme crystallins, ε, ζ, μ, ρ, π, bind NAD(P)H and sequester reduced and oxidized co-factors in lens where they could act as UV filters or as redox reagents

Chemical detoxification-
- Some enzymes used as crystallins are involved in detoxification: ζ is a quinone reductase; η is an aldehyde dehydrogenase; ρ is probably an aldo-keto reductase; squid SL1 is a glutathione reductase

development in *Drosophila*.[85] The product of these genes is a transcription factor, known in vertebrates as Pax-6, which belongs to a family of master-control factors whose expression determines the development of complex tissues.[86-89]

Indeed it seems that *Pax-6* may be the "master gene" for eye development in both mammals and flies (Fig. 1.1). When either *Drosophila* or mouse *Pax-6* is expressed ectopically in antenna, wing or leg of the fruit fly complete compound eyes develop in the targeted tissues.[90] This is a stunning demonstration of the existence of an ancient developmental control system which may be common to all eyes.

While ectopic expression of *Pax-6* could also be attempted in transgenic mouse embryos, mimicking the experiments in flies, the situation is likely to be more complex in vertebrates. After all, *Pax-6* is already expressed in other parts of the CNS[87] and even in pancreas.[91] Clearly the interplay of factors in different tissues can modulate the outcome of such developmental tinkering in less experimentally pliant organisms than *Drosophila*. There is also the interesting case of *Pax-6* in the eyeless nematode *C. elegans*. A homologue of *Pax-6*, vab-3/mab-18, has been found in this organism (A. Chisholm, personal communication).[92] It is expressed in sensory neurons and has an important role in correct formation of the "head."[93] *C. elegans* has no eyes but some other nematodes, such as *Mermis nigrescens*,[94] do have eyespots and phototaxis. It would not be surprising to find that *Pax-6* plays a key role in development of these structures. It seems likely that the

ancestors of *C. elegans* had eyes which were lost much like those of blind cave fish. What prevents eye formation in *C. elegans*? Has it lost target genes, such as those for opsins, or has it lost other factors downstream of *Pax-6* in the eye cascade?

Pax-6 operates at such a high level in the developmental cascade that it is upstream of all genes necessary to form an eye. Many of the genes which lie downstream of *Pax-6* must be different in flies and mice but the original trigger is the same for both and has been conserved for hundreds of millions of years. Other members of the cascade are also becoming known. *Sine oculis* is another homeodomain-encoding gene which is essential for eye development in *Drosophila* and it apparently has homologues in mammals which are also expressed in eye.[95] Other pattern-forming genes are also known to be expressed regionally in developing eyes of various species[96] including *Notch* of *Drosophila*[97] and numerous homeodomain-encoding genes such as *Msx-1* and *Msx-2* (formerly *Hox-7* and *Hox-8*)[98] and several *Hox* genes.[99]

This "eye cascade" must have evolved once early in metazoan evolution conferring the ability to respond to light. This gave the organisms which possessed it such advantages that their descendants came to dominate the animal world. As those descendant species radiated over hundreds of millions of years they continued to use the same ancestral molecular machinery even as the gross structure of their eyes diversified and adapted. Thus, although the common ancestor of octopus and human eyes is unlikely to have had a lens, its distant descendants both evolved superficially similar structures making use of some of the same common, inherited mechanisms.

This "master gene" role for *Pax-6* also helps explain the overlap in developmental origins and gene expression between eye and brain. In addition to eye, *Pax-6* is also expressed in the central nervous system,[87] particularly in the diencephalon[100] which is so closely related to eye in development. Its expression in various parts of the eye and brain is probably also responsible for the phenomenon of transdifferentiation among these tissues.

Eye and Brain

The eye has often been thought of as an offshoot of the brain (see ref. 67 for review). This seems logical enough since the optic cup which gives rise to the retina and ciliary body is derived from the neural ectoderm. However it has also been suggested that the eye came first.[101] Indeed it is striking to see that complex eyes are present in organisms, such as jellyfish, in which it is much harder to identify anything which could pass for a brain. This "eye-first" idea can be taken to its extreme if we entertain the possibility that the eyespots of protists share an evolutionary lineage with eyes of more complex organisms. If eyes came first, the brain could have developed as a center to process the information from the eye and to integrate it into behavioral responses.

As the image-forming and color-discriminating potential of the eye developed, so the brain developed further to make use of the information available. In this view the brain becomes a developmental extension of the retina rather than vice versa.

Whichever came first, the developmental unity of eye and brain are illustrated by the parietal eye of reptiles and amphibians[27] and its evolutionary descendent in birds and mammals, the pineal. In the parietal or median eye both a retina and a "lens" derive from the same neural ectoderm which gives rise to only the retina in the lateral eyes and which also gives rise to brain. Immunohistochemistry has suggested that the parietal eye lens shares molecular components with the lenses of the lateral eyes[102] but this has not yet been examined in detail at the molecular level. Thus the pineal which is regarded as part of the brain is descended from an eye similar in many ways to those with which we are familiar. Indeed, the chicken pineal expresses some of the same opsins as the retina of the lateral eyes.[103]

Transdifferentiation, Lens Regeneration and the Pax-6 Paradox

The close connection between differently derived parts of the eye and between eye and brain tissues is apparent in the remarkable phenomena of transdifferentiation and lens regeneration. In culture, cells from embryonic chicken adenohypophysis, iris and pigmented and neural retina can transdifferentiate to give rise to cell types resembling several differentiated tissue of the eye.[47,104-108] In particular all these systems can give rise to lens-like cells or lentoid bodies which express characteristic lens proteins. In many ways this is reminiscent of the derivation of the retina and lens of the parietal eye from neural ectoderm tissue. Lens can also be derived from other differentiated eye tissues in vivo. After lens removal in some species of newt, the dorsal and ventral iris, tissues of neural ectodermal origin, can regenerate a lens which expresses crystallins while in *Xenopus laevis* lens can regenerate from cornea.[47,65] Thus these differently derived tissues have the potential to follow the path of lens development even in the adult. However lens cells themselves are not capable of transdifferentiation into any other tissue. In this sense they are the lowest common denominator of differentiation potential in their developmental lineage.

The important role of *Pax-6* in all these tissues may be the basis for these phenomena. Recent work has shown that lens competence is a very early stage in development of the animal cap ectoderm and that earlier work which implied an inductive role for the optic cup was in error.[38] Furthermore, the earliest detection of Pax-6 expression in the chicken embryo is in a layer of cells which includes the presumptive lens.[100] Later, *Pax-6* is expressed in both lens and in neural ectoderm, including the developing retina and diencephalon.[100] Thus in vertebrates the lens appears to represent the minimal state of eye differen-

tiation. As other tissues lose expression of downstream components of the eye cascade they "revert" to the simpler level of the lens controlled by *Pax-6* and perhaps a few other high-level factors. Lens may thus be the minimal state of differentiation under control of *Pax-6* in vertebrates. It would be interesting to see whether pancreas, another site of *Pax-6* expression, could also transdifferentiate into "lens". The idea of lens being in some way the most fundamental outcome of *Pax-6* expression appears to be extremely paradoxical since the lens is by no means the fundamental tissue of the eye in any evolutionary or developmental sense. Yet, as will be described below, it turns out that expression of some crystallins, lens-specific or lens-preferred proteins, depends on binding of Pax-6 to the promoters or enhancers of their genes.

Crystallins would seem to be the final product of the cascade of gene expression necessary to form the lens. Similarly the vertebrate lens is probably the most recently evolved of eye structures. Why then are at least some crystallin genes under the control of the highest level eye "master gene"? The simplest explanation for the *Pax-6* paradox seems to be that the evolution of the lens necessitated use of transcription factors already expressed in the eye. As such Pax-6 fits the bill as much as any other factor in the cascade. At an earlier stage in evolution Pax-6 may have been principally involved in controlling other regulatory genes, such as those for other transcription factors, and may not have had a direct role in expression of eye-specific genes such as those encoding opsins. However, when the lens evolved, Pax-6 was co-opted or recruited to a new role in direct control of structural gene expression in the eye lens. Thus the gene recruitment of crystallins, described below, depended on the acquisition of Pax-6 binding sites and the consequent recruitment of *Pax-6* itself.

EVOLUTION DYNAMISM AND THE VERTEBRATE LENS: ENVIRONMENTAL PRESSURES

The eye provides a direct interface between the outside world and the internal world of perception and response. As an optical system, the properties of the eye are severely constrained by aspects of the external environment and the way of life of the organism. Thus the structure and light sensitivity of the retina differ according to whether the animal is diurnal, in which case it makes use of low-sensitivity, color-discriminating cone cell photoreceptors often with associated colored oil-drops,[20] or nocturnal, in which case it makes use of monochromatic vision through highly sensitive rod cell photoreceptors.[20] Similarly the refractive properties of the cornea and lens adapt to suit the needs of a fish, which requires a high refractive index to focus under water and has little use for vision at a distance, or of a hawk which needs a lower refractive index, accommodating lens to focus both at great distances in the air or close up in the nest.[20] The properties of

the cornea and lens may also evolve to filter harmful or dazzling radiation as species move from dim to bright light environments.

During the course of evolution vertebrate species have moved from water to land or from activity by day to activity by night. Perhaps more than any other organ this has placed unusual requirements on the eye to adapt and readapt its properties at both anatomical and molecular levels. Subsequent chapters will concentrate on describing the ubiquitous and taxon-specific crystallins, their structures and functions and the molecular biology of their gene recruitment and expression in the lens.

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