**THE EYE IS** a fascinating model of the complexity that can be produced by evolution. While the most primitive eyes may have been simple light-sensitive patches, subsequent elaboration produced eyes capable of image formation. In vertebrates and some invertebrates this is achieved by cellular lenses. To focus light, a lens must have a higher refractive index than the surrounding medium. This is produced by very high concentrations of soluble structural proteins called crystallins. Since lens proteins may be the longest-lived in vertebrates, crystallins must be able resist large-scale aggregation and maintain their structure for extremely long periods. However, they are no more inherently transparent than many other proteins. Transparency depends only on maintenance of short-range order between scattering centres that are not large on the order of the wavelength of light.

**Crystallins are multifunctional proteins**

It was a great surprise to discover that, in spite of their unusual role, many of the most abundant crystallins are not specialized structural proteins and are not even lens specific. In particular, there is a remarkable class of taxon-specific crystallins, restricted to specific evolutionary lineages (Table I), which turn out to be metabolic enzymes directly recruited to a new role by modification of gene expression without prior gene duplication (Fig. 1). This kind of gene recruitment reverses the conventional view that gene duplication must precede the acquisition of new protein functions. Thus, in birds and crocodiles, the glycolytic enzyme lactate dehydrogenase B (LDHB) serves two distinct functions as both an enzyme and a crystallin, a major constituent of the lens. In a hummingbird it accounts for almost half of total lens protein. Similar stories apply to other independently recruited enzymes scattered throughout vertebrate evolution.

Their abundance, occasional lack of activity, restricted distribution and variability argue that taxon-specific crystallins were not recruited because of their enzyme activity. They are bulk structural proteins contributing to refractive index. However, evolution is pragmatic and subsidiary roles could be very useful. For example, some enzyme crystallins are able to sequester NAD(P)H in the lens, something which could protect against oxidation or even filter UV radiation. This idea is particularly attractive in the case of LDHB/γ-crystallin whose distribution among birds correlates well with exposure to light.

Since a single protein becomes subject to different, perhaps competing, sets of selective pressure, the multifunctionality of enzyme crystallins is not without consequence. For example, most species that use LDHB as γ-crystallin exhibit unusual changes in LDHB sequence, including replacement of an otherwise conserved surface phenylalanine residue by glycine. This removal of an exposed hydrophobic side chain evidently has no beneficial effect on enzyme activity and must be driven by the second role as a lens protein. In some cases adaptive conflicts may arise when changes favourable for one role

![Table I. Eye lens crystallins and their relationships with enzymes and stress proteins](image-url)

**Table I. Eye lens crystallins and their relationships with enzymes and stress proteins**

<table>
<thead>
<tr>
<th>Crystallin Distribution</th>
<th>[Related or Identical]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ubiquitous stress crystallins</td>
<td></td>
</tr>
<tr>
<td>α</td>
<td>All vertebrates</td>
</tr>
<tr>
<td>γ</td>
<td>All vertebrates (embryonic γ not in birds)</td>
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<tr>
<td>Taxon-specific enzyme crystallins</td>
<td></td>
</tr>
<tr>
<td>δ</td>
<td>Most birds, reptiles</td>
</tr>
<tr>
<td>ε</td>
<td>Crocodiles, some birds</td>
</tr>
<tr>
<td>ζ</td>
<td>Guenon-pig, degu rock cavy, camel, llama</td>
</tr>
<tr>
<td>η</td>
<td>Elephant shrews</td>
</tr>
<tr>
<td>λ</td>
<td>Rabbits, hares</td>
</tr>
<tr>
<td>μ</td>
<td>Kangaroo, quoll</td>
</tr>
<tr>
<td>ρ</td>
<td>Frogs</td>
</tr>
<tr>
<td>τ</td>
<td>Lamprey, turtle, moderately abundant in most vertebrates</td>
</tr>
<tr>
<td>S</td>
<td>Cephalopods</td>
</tr>
<tr>
<td>Ω</td>
<td>Octopus</td>
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<tr>
<td>J</td>
<td>Cubomedusan jellyfish</td>
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**Lens crystallins: gene recruitment and evolutionary dynamism**

Graeme Wistow

In a novel evolutionary process, enzymes and stress proteins have undergone direct gene recruitment as eye lens crystallins in a number of independent events. This may have allowed a dynamic response to changing visual environments during evolution. In spite of their diversity, many crystallins may share an origin in essential developmental processes such as cell elongation.

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are deleterious for another. Such conflicts could be resolved by reversal of recruitment (reversion) or by gene duplication and separation of functions (Fig. 1). Both of these avenues have been explored by crystallins. Since the recruitment of LDHB/ε-crystallin occurred in a common ancestor of birds and crocodiles, reversion must have occurred in species that do not now use ε-crystallin and whose LDHB has a “normal” sequence. Interestingly, two related species that have ε-crystallin, swifts and hummingbirds, have maintained the LDHB sequence. Instead they have eliminated or reduced their content of another taxon-specific protein, δ-crystallin. Conceivably, an unfavourable interaction between these two proteins was solved by a reciprocal reversion of δ-crystallin recruitment.

δ-Crystallin, found in birds and reptiles, also illustrates the other solution to adaptive conflict, gene duplication. Birds have two active δ-crystallin genes. δ2-Crystallin is the enzyme arginino- succinate lyase (ASL) while δ1-crystallin, although highly similar in sequence, has no enzyme activity. After the recruitment of ASL as δ-crystallin in an early ancestor of birds and reptiles, adaptive conflict may have provided the selective pressure for gene duplication, allowing one gene to make the subtle sequence modifications necessary to enhance lens function while the other maintained enzymatic function. In duck both proteins are still crystallins while in chicken specialization has resulted in decreased ASL/δ2-crystallin expression in lens (see Ref. 10 for references) (Fig. 1). Gene duplication is an important process in molecular evolution. Perhaps δ-crystallin points to a more general phenomenon where proteins first acquire new functions by sequence drift, for example gaining new binding capability, then become subject to adaptive conflict. This would provide selective pressure for duplication and separation of functions instead of relying on two unselected steps in duplication and neutral drift.

In vertebrates there is a second and more ancient class of crystallins represented in all species. Just as the taxon-specific crystallins are enzymes, the ubiquitous α-, β- and γ-crystallins are related to stress proteins and have non-lens expression and can act as a kind of molecular chaperone, solubilizing heat-stressed proteins. The β- and γ-crystallins are related to each other and to two unusual proteins, spherin 3a of the eukaryote Physarum polycephalum and protein S of the
Lenses and cell elongation

If one process in both ontogeny and evolution characterizes all cellular lenses, it is the elongation of cells (Fig. 2). In some lizards and other vertebrates the tiny parietal or median eye, equivalent to the mammalian pineal, has a lens consisting simply of a monolayer of elongated cells. Although of quite different ontogeny, these lenses resemble the earliest developmental stages of lenses in the familiar lateral eyes in which a monolayer over the presumptive retina thickens into columnar cells, the lens placode (see Ref. 20). Conceivably this recapitulates how the first lenses arose in evolution. In lateral eyes the development of the lens goes further. The epithelial layer containing the lens placode invaginates to form the lens vesicle. The placode cells become the primary fibres and extend to fill the lens. The epithelial layer serves as a stem cell population, allowing the lens to grow throughout life and to match the size of a growing eye as new fibre cells overlay the old. The cellular elongation during this process in both ontogeny and evolution is a common feature of all stages and may influence lens gene expression.

Remarkably, enzyme crystallin recruitment has also occurred in the independently evolving lenses of cephalopods. Octopus and squid make use of S-crystallins, members of the same superfamily as glutathione S-transferases (GST), stress-inducible, detoxification and anti-oxidation enzymes (see Refs 1 and 17). Another abundant protein in the octopus lens, Ω-crystallin, is closely related to a different detoxification enzyme family, the aldehyde dehydrogenases (ALDH). Coincidentally, the enzyme ALDH I serves as η-crystallin in elephant shrews, ALDH III is the major component of mammalian corneal endothelia, another transparent eye tissue (see Ref. 5 for references), while a distantly related member of the same superfamily, λ-crystallin, is found in the muscle-derived, diffusing 'lens' of squid light organs, another remarkable example of convergent evolution. Odd convergences like this probably say more about the underlying recruitment process than about any unusual transparency of these proteins.

bacterium *Myxococcus xanthus*, both of which play important roles in the formation of dehydrated spores. While no existing organism represents the ancestor from which vertebrates evolved, pre-vertebrate lenses probably contained an α-crystallin/shsp and a βγ-crytatnin superfamily member.

Figure 2

Cell elongation in ontogeny and evolution of lenses. Primitive lenses could have arisen as simple elongated monolayers, a possibility illustrated in the reptile parietal eye. Such a structure could have been ancestral to multilayered structures capable of continuous growth. Conceivably, the ontogeny of the vertebrate lens recapitulates this evolutionary process. Cell elongation is a common feature of all stages and may influence lens gene expression.
lenses of some invertebrates are less well understood but they have obvious similarities in their arrangement of concentric layers of elongated cells (see Ref. 21).

Cell elongation makes use of fundamental cellular processes such as osmoregulation and the elaboration or reorganization of cytoskeleton. Indeed, osmoregulation, the balance of protein and water, must be critical for all transparent tissues. Several crystallins have plausible connections to such processes14. For example, ß-crystallin of Rana is related to rat aldose reductase1, an enzyme implicated in osmoregulation, while other enzyme crystallins may bind substrates such as lactate, arginine, proline and other small molecules which can also be osmolytes (see Refs 1 and 22). The theme extends to the ubiquitous crystallins since ß-crystallin and the microorganism relatives of ß-crystallins are induced by related kinds of osmotic stress12,21. As for involvement with cytoskeleton, various glycolytic enzymes have been shown to associate with actin filaments24, while shsps like ßB-crystallin can also interact with actin and have roles in cytoskeleton assembly (see Ref. 13). As the lens evolved, the necessary refractive power must have been achieved by recruiting genes that are active under the prevailing conditions of cell elongation and whose protein products fit the broad requirements of their new role. Osmotic stress proteins, cytoskeleton chaperones and easily inducible detoxification enzymes would have been good candidates. Such an origin could have engendered enduring similarities in gene expression for groups of crystallins.

Figure 3

Acquisition of TATA-box promoters in enzyme crystallin genes. Three examples of taxon-specific crystallin genes in which lens expression depends on TATA-box promoters. In contrast, non-lens expression in ß-crystallin and expression of non-recruited ASL and ã-enolase in mammals depends on GC-rich 'housekeeping' promoters. Intriguingly, the first intron of chicken ß-crystallin genes contains (in complement) sequences similar to a human ASL gene promoter element (see Ref. 31).

Gene expression

Most gene expression studies have concentrated on ubiquitous crystallins26. As yet no unifying principle of lens preferred expression has emerged, although some common promoter sequences have been proposed25. Indeed, it would not be surprising if different mechanisms governed the activation of genes in lens cells at different developmental stages. For example, induction of embryonic, fibre-cell specific ß-crystallins can be mediated by retinoic acid26. Even so, heterologous crystallin genes have lens-preferred expression even between mice and chickens (see Refs 1 and 20), suggesting that some common mechanisms do exist.

ã-Enolase/ß-crystallin, which is expressed abundantly in lens epithelia and is a crystallin in several species (see Refs 1, 8 and 27 for references), illustrates the way in which a gene could be recruited through its response to transcriptional programs running in the lens for wider purposes. The promoter for duck ã-enolase/ß-crystallin is upregulated by the proto-oncogene c-myc (Ref. 28; R. Warwar, G. Wistow and P. S. Zelenka, submitted), which has preferred expression in lens epithelia probably as part of a general developmental mechanism29. ã-Enolase, which is a marker for various stem cells29, responds in the context of this general program with the result that it
is expressed at fairly high levels in lens epithelia, probably in all species. Stepped enhancements of expression by various (as yet unknown) mechanisms could have led to the recruitment of this enzyme as γ-crystallin in some species. A transgenic model of such a recruitment process shows that the mouse lens is refractory to a sevenfold increase in expression of this enzyme.

The duck γ-crystallin gene shares an interesting feature with other taxon-specific crystallins, the δ-crystallins. The promoters of these genes, like those of ubiquitous crystallins (see Ref. 20 for references), contain TATA boxes while their unrecruited human homologues (Ref. 31 and see Ref. 27 for references) have GC-rich 'housekeeping' promoters (Fig. 3). Furthermore, both chicken δ-crystallin genes have extra 5' untranslated exons relative to their human counterpart and even contain 'promoter-like' sequences in the first intron (Fig. 3), suggesting that they might at some time have acquired alternative TATA-box promoters. This is exactly what has occurred in the more recent recruitment of a quinone oxidoreductase gene as ζ-crystallin in the guinea-pig. This gene has an upstream, GC-rich promoter from which non-lens transcripts derive and a downstream, TATA-box promoter which gives rise to lens transcripts (Fig. 3). Functional analysis of the ζ-crystallin gene (D. Lee, P. Gonzalez and G. Wistow, submitted) has shown that the lens promoter by itself is sufficient for lens-specific expression. The recruitment of ζ-crystallin may have occurred by the insertion of the lens promoter into an intron of an existing, functional gene without disturbing the normal expression of that gene.

However, alternative lens-specific promoters are not general features of taxon-specific crystallin recruitment. PCR analyses of 61, 82 and 5-crystallins in the duck show that the same promoters function in lens and non-lens tissues (Fig. 3). Indeed, lens expression of the specialized chicken δ1-crystallin gene is conferred by an intron-located enhancer which binds both general and lens-specific factors15. The duck LDHB/ε-crystallin gene even violates the 'requirement' for a TATA box: it has a housekeeping promoter responsible for expression in both lens and heart17. There may be as much variety in mechanisms of gene recruitment as there is in the genes recruited.

**Taxon specificity and evolutionary dynamism**

Although there are clearly neutral elements in the selection of enzyme crystallins, there could also be selective mechanisms at work. The dramatic changes in lens protein composition during evolution may have been strongly influenced by changing visual environments (Fig. 4). The vertebrate eye evolved in the sea, an optically dense medium requiring lenses with high refractive indices. In fish lenses, in the most hydrated and highest refractive index regions of mammalian lenses, and in the hard, myopic lenses of rodents the major crystallins are γ-crystallins1. Of all crystallins, these highly symmetrical, cysteine-rich proteins are perhaps the most specialized and most specific1,18. Their three-dimensional structures and biological distribution suggest that these proteins have a special role in low-water environments and in high-refractive-index lenses (Fig. 4). They may bind very little water since an unusually high proportion of their polar residues are involved in intramolecular hydrogen bonds and ion pairs19.

As vertebrates emerged into the air there would have been great advantages in softening and reducing the refractive index of their aquatic lenses, allowing accommodation and focusing at a distance. There must have been strong selective pressure to eliminate γ-crystallins or dilute them with suitable proteins of more normal hydration properties. Indeed, birds, with superb diurnal visual acuity, have completely replaced embryonic γ-crystallins with taxon-specific enzyme crystallins (ASL/δ, LDHB/ε, enolase/τ, see Ref. 1). The same selective forces driving this process should have acted on the ancestors of mammals, leading to enzyme recruitment and loss of γ-crystallins (Fig. 4).
fact, this has occurred but with surprising variability. Some marsupials have α-crystallin\[^9\] and elephant shrews (primitive placental) have ALDH1/γ-crystallin\[^{10}\]; rabbits and hares have λ-crystallin (see Refs 1 and 4), and some rodents and camels have NADPH:quinone-oxidoreductase/ç-crystallin (see Ref. 38 for references). As an equally valid strategy, humans have reduced γ-crystallin levels by eliminating or reducing expression of four out of six γ genes\[^{39,40}\]. All this suggests that the modification of mammalian lenses occurred more recently than in other vertebrates. This is consistent with another odd feature. In mammals, the six γ-crystallins, encoded by closely linked genes, are 80–98% identical while the γ-crystallins of frogs, like vertebrate β-crystallins, are much less well conserved and generally share only 50% identity (see Refs 1 and 36 for references). Mammalian γ-crystallins thus look considerably younger than β-crystallins or the γ-crystallins of frogs. Some of this must be due to gene conversion\[^{26}\] but both clustering and similarity could also be explained if the mammalian γ-crystallin locus arose from recent gene multiplication.

Mammals were small and few from the Triassic to the Cretaceous periods and some, the ancestors of modern placental mammals, seem to have survived by adapting to a nocturnal niche (Fig. 4), an idea suggested by the relatively poor colour vision of modern placental mammals (see Ref. 19). Colour vision requires daylight and has no selective value in the dark. Similarly, nocturnal, burrowing mammals have no use for soft accommodating lenses and could even benefit from myopic lenses like those of mice and rats. Perhaps during a nocturnal episode in mammalian evolution, γ-crystallin genes underwent a renaissance, amplifying an active gene to form the modern cluster. At the same time and for similar reasons, any pre-existing lens-saltentizing enzyme crystallins could have been lost. Later, the explosive radiation of diurnal mammals in the Tertiary would have led to a reprise of this whole scenario as lenses softened again, with the loss of γ-crystallins and the independent recruitment of several enzyme crystallins. The occurrence of Ç-crystallin in guinea-pigs and camels may be a rare example of independent choice of the same suitable enzyme under similar conditions.

As an interface with the outside world, the eye is peculiarly sensitive to environmental changes. Eyes can be lost altogether in subterranean animals\[^9\] while other species that move between night and day, air and water have to adjust their vision as necessary with all the expediency the evolutionary process can muster.

References

It is TIBS policy to restrict the number of references. I regret, therefore, that several original papers have not been directly acknowledged and apologize to those concerned.

30 Zaske, J. D. et al. (1992) Dev. Biol. 151, 18–26
32 Gonzalez, P. et al. (1992) Exp. Eye Res. 55, 159