nature Structural Biology October

One in the eye

A double insight into visual accommodation by the eye lens in birds and into the activity of a superfamily of metabolic enzymes is provided by the structure of turkey lens δ-crystallin.

THE lens of the eye presents an interesting biomechanical puzzle: not only must it be transparent to allow the passage of light, and supply a refractive index gradient to avoid aberration, but its constituent materials must be highly stable to insult by ultraviolet light, radicals and so on because lens proteins cannot be regenerated. One might imagine that such demanding requirements would have led to the evolution of a highly specialized lens material. In reality these criteria are met by a motley collection of proteins — mainly metabolic enzymes or their relatives that have been 'hijacked' into the lens during evolution. The structure of one of these constituent proteins, δ-crystallin, is reported in this month's *Nature Structural Biology*[1] and, as well as describing another new protein fold, it sheds light on both the protein's role as a crystallin and its ancient function as an enzyme of the urea cycle.

The α- and β/γ-crystallins are ubiquitous in vertebrates, indicating that these proteins were recruited to the eye at the very beginning of lens evolution. The α-crystallins are similar to the small heat-shock family of proteins and the β/γ-crystallins share homology with the spore coat protein S from *Myxococcus xanthus*.

δ-Crystallin, on the other hand, is a taxon-specific crystallin found only in birds and reptiles, suggesting that it was recruited to the lens in a common ancestor of all reptiles and birds after divergence from the line leading to mammals[2,3]. All the taxon-specific crystallins are either fully paid-up enzymes or are closely related to enzymes: δ-crystallin shares over 90% sequence identity to argininosuccinate lyase (ASL), an enzyme involved in arginine biosynthesis and, in ureotelic species, the urea cycle. ASL and δ-crystallin (which no longer has enzymic activity) form part of a superfamily of metabolic enzymes, including fumarase, aspartase and adenylosuccinase, so the new crystallin structure will provide a template for the structures of its more distant relatives.

Like all the other members of the superfamily, δ-crystallin is a tetramer. The most striking feature of the overall structure is its highly helical nature. Each of the subunits of the tetramer consists of three domains. Domains 1 and 3 have a similar topology: two helix–turn–helix motifs stacked at right angles to one another, which may have arisen by partial gene duplication. These two domains are joined through domain 2, which consists for the most part of a five-helix bundle. Two of these subunits pack tightly together in a head-to-tail fashion and the tetramer is formed from a dimer of these dimers. The subunit contacts are mainly between the helix-bundle domains, and for part of their length they form an impressive 20-helix bundle — the compact heart of the tetramer.

The lens is one of the very few tissues in vertebrates where the molecules that were laid down during embryonic development persist throughout adult life. The lens cells, as they develop, synthesize large amounts of protein, of which over 90% are crystallins. Nuclei and other organelles are then lost and the cells elongate, reaching lengths of up to a centimetre, and migrate away from the front of the lens. The lack of protein synthesis in the differentiated fibre cells means that, to maintain the transparency and refractive properties of the lens, the crystallins must remain intact for the entire lifetime of the organism. The symmetrical nature of the heart of the δ-crystallin tetramer, and of the repeated Greek key motifs of the β/γ-crystallins, is thought to be important in stabilizing proteins in the lens.

The recruitment of such a large α-helical protein to the lens of the avian eye probably serves a second function. The amount of water in the lens correlates with its refractive power and softness, and hence its ability to accommodate. The wide-ranging visual accommodation peculiar to the eyesight of birds requires the centre of the lens to have liquid-like properties. In the crystals, δ-crystallin interacts mainly with the solvent and makes few contacts with other tetramers. On the other hand, the lens in most other vertebrates (the short-sighted rat, for example) has a glass-like centre, consisting of the small β- and γ-crystallins, and in these crystals protein–protein interactions are much more extensive.

The crystallins also offered a new perspective on molecular evolution when it was discovered that the genes for ε- and τ-crystallins also encode lactate dehydrogenase-B, and α-enolase, respectively. This phenomenon of gene 'sharing'[2,3] — whereby a protein acquires a new function apparently unrelated to the original — is thought to have arisen through modification of gene expression, and could explain how the ancestral ASL gene gave rise to δ-crystallin. In the chicken, both genes have a lens-preferred enhancer, suggesting that ASL was recruited to the eye before gene duplication took place. Presumably the constraints of the new lens-specific function of ASL were not consistent with optimal enzyme activity; these two independent evolutionary pressures then favoured gene duplication and further independent selection.

Nonetheless, the high degree of similarity between δ-crystallin and ASL allow Slingsby and colleagues[1] to make deductions about the catalytic mechanism of the latter and indeed about the superfamily as a whole. The most highly conserved regions of the proteins of the superfamily cluster in the same region to form a cleft, around which are a bevy of almost invariant residues that are for the most part well placed to affect catalysis.

The variety of proteins found in the lens would suggest that recruitment is a fairly neutral process, but it is hard to believe that the crystallins are selected purely at random. Most of the crystallins are related or identical to oxoreductases that bind pyridine nucleotide cofactors, which may act as ultraviolet filters and reduce glare. A connection has also been noted with enzymes associated with carbohydrate metabolism, many of which are induced by heat shock, suggesting that the crystallins play a role in stress tolerance[4]. It will be interesting to see whether the crystallins have functions apart from acting as packing material in the lens.

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Also in this month's *Nature Structural Biology*: Max Perutz celebrates the life and achievements of Linus Pauling; catching enzymes in the act — inosine bound to adenosine deaminase and the NADPH-trichosanthin complex; parallel evolution of the control of yeast phosphorylase; heptad repeats and four-helix bundles; pectate lyase in complex with calcium; predicting new serine proteinase inhibitors; and designing receptor antagonists.

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4. deJong, W. W. *et al. Trends biochem. Sci.* **14**, 365–368 (1989).