Perspective

The Recruitment of Crystallins: New Functions Precede Gene Duplication

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GENE DUPLICATION, COMPLETE (*1*) OR PARTIAL (*2*), HAS been recognized as a major force in molecular evolution. The crystallins, abundant proteins of the eye lens with structural roles in the refractive properties of the tissue (*3*), have taken a different pathway in evolution, in which the acquisition of new functions precedes gene duplication (*4*). Crystallins are diverse, with distinctive patterns of taxon-specific expression (*3*). While some crystallins are specialized for lens, the taxon-specific crystallins are often identical to enzymes found in lesser amounts in other tissues (Table 1).

The α-, β-, and γ-crystallins are represented in all vertebrate lenses. Of these three multigene families, only the gene for αB-crystallin is expressed outside the lens and is overexpressed in some neurological diseases (*5*). Both of the α-crystallins (αA and αB) are related to the stress-inducible small heat-shock proteins (*6*). The β- and γ-crystallins are structurally related to each other and are distantly related to microorganismal dormancy proteins that may also be induced by certain stresses, including osmotic shock (*6*).

In contrast, the taxon-specific crystallins are identical or very closely related to enzymes. These enzyme crystallins were first noticed in birds, where they substitute for the γ-crystallins (*3*). Enzyme crystallins are also found among mammals (Table 1). The most abundant mammalian taxon-specific crystallin identified at present is cytoplasmic aldehyde dehydrogenase/η-crystallin, which may account for up to one-quarter of the soluble protein in the lenses of macroscelids (elephant shrews) (*7*). Even among invertebrates, the cephalopods have abundant crystallins closely related to enzymes (Table 1).

The recruitment of enzymes as crystallins illustrates a model of molecular evolution in which changes in expression occur before, or instead of, gene duplication (Fig. 1). Two enzyme crystallins, lactate dehydrogenase–B (LDH-B)/ε-crystallin (*4*) and α-enolase/τ-crystallin (*8*), have been shown to be encoded in single genes in the duck (*9, 10*). About one-tenth of the protein of the duck lens (*4*) and about one-third in the hummingbird lens (*11*) is ε-crystallin. In duck and swift lenses, ε-crystallin has retained high LDH (*4, 11*) activity, and τ-crystallin in turtle lenses has some enolase activity (*10*). These two taxon-specific crystallins are examples of the recruitment of an enzyme to a new structural role. At the same time, the enzyme preserves its catalytic function and correct expression outside the lens. The bifunctional enzyme crystallin has thus acquired a new role by modification of gene expression, either transcriptionally or posttranscriptionally. Because two distinct protein phenotypes are produced by the same transcriptional unit, this phenomenon has been called gene sharing (*12*). The recruitment of crystallins from preexisting proteins by gene sharing differs from the model in which gene duplication is required for the evolution of new functions (*1*).

Once an enzyme has been recruited to serve as a structural protein in lens, in addition to its conserved role in metabolism, it is subject to at least two independent sets of evolutionary pressure. This may lead to sequence modifications that enhance its function as a crystallin but that are not of benefit to the enzymatic role. This seems to have occurred in LDH-B/ε-crystallin in some species (*4, 11*). In some cases this could lead to an adaptive conflict that might be resolved either by reversion, with loss of crystallin expression, as illustrated by the loss of δ-crystallin in swifts (*11*) and the absence of ε-crystallin in chickens and other birds (*4*), or by gene duplication and separation of function (Fig. 1).

An example of how crystallin recruitment can be followed by gene duplication and subsequent partial separation of function is provided by the argininosuccinate lyase (ASL)/δ-crystallin family. Of the two tandemly arranged chicken δ-crystallin genes, δ1 is specialized for lens expression and produces >95% of the lens δ-crystallin messenger RNA (mRNA); by contrast, the δ2 gene, which appears to encode the enzymatically active ASL, produces most of the ASL/δ-crystallin mRNA in nonlens tissues, although it is still much more abundant in the lens than in other tissues (*13*). In ducks, however, both δ-crystallins, including the enzymatically active ASL/δ2-crystallin, are abundant in lens (*14*). Consequently, ASL activity in the duck lens is 1500-fold higher than that in the chicken lens (*12*). It is likely that the gene for ASL developed high expression in the lens before duplication because a lens-preferred enhancer is present in the third intron of both chicken δ-crystallin genes (*13, 15*). A side effect of this lens-driven duplication is that novel isoforms of ASL could arise in various tissues of those birds and reptiles that retain nonlens expression of both ASL/δ-crystallin genes.

In a more ancient example of gene duplication after crystallin recruitment, one of two genes for the α-crystallins has apparently become completely specialized for lens (Fig. 1). This family probably arose when a gene coding for a stress protein was recruited as a crystallin in an early ancestor of vertebrates (*3, 5*). This gene duplicated, generating both the gene for αB-crystallin, which presumably retained the original function while also serving as a crystallin, and the gene for αA-crystallin, which specialized for lens. The separation in function of these two genes may have been assisted by their segregation onto different chromosomes (*3*). The genes for the less differentiated δ-crystallins of the chicken are still

Table 1. A listing of the major families of crystallins, their approximate distribution, and their relationships. References for most can be found in (*3*); for η and μ see (*7*); and for **J** see (*24*). ζ is a functional quinone reductase, although it is not related in sequence to enzymes of similar function (*25*). (NADPH, reduced form of nicotinamide adenine dinucleotide phosphate.)

Distribution	Crystallin	(Related) or Identical
Represented in all vertebrates	α	(Small heat-shock proteins) (*Schistosoma mansoni* antigen p40) αB has nonlens expression
	β, γ	(*Myxococcus xanthus* Protein S) (*Physarum polycephalum* spherulin 3a)
Some birds and reptiles	δ	ASL
	ε	LDH-B
Some mammals	ζ	(Alcohol dehydrogenases)
	η	Cytoplasmic aldehyde dehydrogenase
	λ	(Hydroxyacyl CoA dehydrogenases)
	μ	(Dehydrogenases?)
Frogs	ρ	(NADPH-dependent reductases)
Many species	τ	α-enolase
Cephalopods	**S**	(Glutathione S-transferases)
Jellyfish	**J**	??

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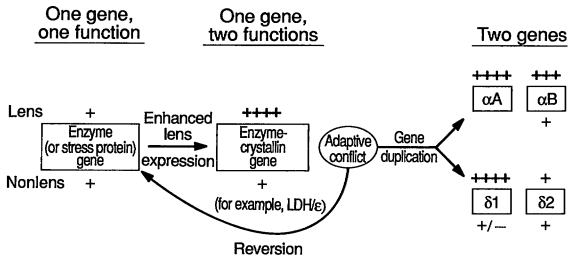

Fig. 1. A scheme for the recruitment of crystallins. During crystallin recruitment, a single gene with a single function acquires high expression in lens. If this creates an adaptive conflict, crystallin expression may be lost or gene duplication and specialization may occur. Blocks indicate genes. Plus signs above each gene give a general indication of expression in lens whereas those below indicate nonlens expression. Specific genes that provide examples of particular stages in the evolutionary process are indicated. Two different models of specialization after duplication are illustrated.

closely linked on the same chromosome and must have arisen from a more recent duplication. Thus, recruitment of crystallins probably occurred initially by elevation of gene expression without duplication (LDH-B/ε-crystallin and α-enolase/τ-crystallin), but, when necessary, this was followed by duplication with more (α-crystallin) or less (ASL/δ-crystallin) complete specialization for lens or nonlens expression (Fig. 1). For the β- and γ-crystallins, multiple gene duplications have led to gene families with six or more members that seem to be specialized for lens (*3*).

The enzyme crystallins are present in lens at amounts that greatly exceed any likely catalytic requirements. In some cases, the recruited enzymes do not maintain catalytic activity in lens, either because of posttranslational modification or, in the case of some duplicated genes, because of sequence modifications. Furthermore, similar species frequently express different enzyme crystallins, suggesting that it is not large amounts of a particular enzyme activity that are required for lens function. It is noteworthy, however, that most of these enzymes are oxido-reductases that bind pyridine nucleotide cofactors (*3, 16*). Once recruited as crystallins, these proteins may acquire useful roles related to their original functions, for example, in sequestering reduced cofactors as ultraviolet filters or as reducing potential (*4, 16*). Even so, it appears that there is a large element of neutrality in the selection of enzymes as crystallins, with many acceptable solutions.

The genetic mechanisms of crystallin recruitment are not well understood. Different crystallin genes maintain lens-preferred expression even in heterologous systems: preferential expression is observed for the chicken δ1-crystallin gene in transgenic mouse lenses (*15*) and the mouse γF-crystallin gene promoter in transfected chicken lens epithelial cells (*17*), though neither gene has a direct homolog in the heterologous host. No simple consensus sequence that directs lens-preferred expression has been identified, however. Furthermore, the expression of αA-crystallin in chicken and mouse requires different combinations of regulatory sequences, although promoter regions of both genes are able to confer lens-specific expression in transgenic mice (*18*).

Promoter elements and enhancers required for high expression in lens have been described for a number of crystallin genes (*19*). These contain regulatory motifs common to many nonlens genes, including AP-1–, AP-2–, Sp1–, and NF-KB–like and octamer-like sites (*19*). Some crystallins may have originally been recruited serendipitously for high expression in lens simply because they share transcription factors with other genes for which expression in lens is important for developmental or other reasons.

The multifunctionality of crystallins and their recruitment by altered gene expression may have parallels in other systems. In mammalian cornea, tumor-associated aldehyde dehydrogenase constitutes up to 40% of the soluble protein (*20*). As for some crystallins, this presumably involves modification of the expression of a gene with a different role elsewhere. Other examples include the neurotrophic factor neuroleukin, which is also the enzyme phosphohexose isomerase (*21*), and another protein that has four functions (protein disulfide isomerase, thyroid hormone binding protein, the β-subunit of prolyl hydroxylase, and the glycosylation site binding component of oligosaccharyl transferase) (*22*). Even some of the enzymes recruited as crystallins have been identified with other, unexpected functions (*8, 23*). Given the pragmatism of molecular evolution, multifunctionality of biological macromolecules may be more common than has been realized, and may affect rates of evolution and even medical therapies targeted at particular molecules with unanticipated secret identities.

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