NEWS AND COMMENTARY

Evolutionary genetics

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Seeing the light: the role of inherited developmental cascades in the origins of vertebrate lenses and their crystallins

J Piatigorsky

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The problem of how evolution has crafted complex organs such as the animal eye continues to bamboozle biologists. The origins of lenses that allow animals to focus an image are a fulcral aspect of vision research. A recent report by Shimeld *et al* (2005) sheds light on how lenses first arose.

Vertebrate eyes are hugely complex sensory organs. Darwin himself admitted that he found it difficult to envision the sequence of events that could have led to evolution of vertebrate eyes. Throughout the animal kingdom, eyes come in many forms, consistent with the idea that visual organs developed independently in different animals (eg Land, 2005). Scientists were startled, therefore, when all the invertebrates and vertebrates investigated were shown to use a similar (although not identical) cascade of proteins regulating gene expression in eye development (eg Gehring, 2005). This suggested that eyes had a single evolutionary origin, although it remains inconclusive as to whether eyes arose once or several times independently (eg Fernald, 2004). The origin of lenses is one of the puzzles of eye evolution, as their abundant proteins (crystallins) often show marked differences among species. Recently, Shimeld *et al* (2005) provided fresh insights into the origin of the vertebrate lens by investigating the expression of a newly discovered gene related to vertebrate crystallin genes in the light-sensitive urochordate, *Ciona intestinalis* – known by the undignified name of sea squirt.

Shimeld *et al* (2005) provide evidence that vertebrates obtained lenses by tapping into regulatory circuits controlling expression of a structural gene in the sea squirt. This gene encodes a protein (Ci-βγ-crystallin) with weak sequence similarity to the vertebrate βγ-crystallins. All vertebrate lenses accumulate βγ-crystallins (among other crystallins) for transparency and refractive power (eg Bloemendal *et al*, 2004). Motile sea squirt larvae have a pair of simple eyes (called ocelli) without lenses; the eyes are lost during metamorphosis into sedentary adults. Ci-βγ-crystallin is not expressed in the ocelli of *Ciona*, but is expressed in at least two other larval structures: the otolith and the palps. The otolith is a sensory, opsin-containing structure in the brain, which shares a common developmental origin with the ocellus; the palps are structures extending from the head for adhering to a substratum during metamorphosis.

Vertebrate lens βγ-crystallins have two domains, each comprising two structural motifs with signature folds called 'Greek keys' (eg Blundell *et al*, 1981; D'Alessio, 2002). Shimeld *et al* (2005) demonstrate by crystallography that Ci-βγ-crystallin has one domain with the characteristic βγ-crystallin folds (Figure 1). Each of the two motifs in the single-domain Ci-βγ-crystallin is encoded in a separate exon, as are the motifs in the vertebrate β-crystallin genes (Inana *et al*, 1983).

Shimeld and colleagues speculate that the one-domain Ci-βγ-crystallin gave rise to the two-domain vertebrate β-crystallins by gene duplication and fusion events.

Shimeld *et al* (2005) compared the expression pattern of the endogenous *Ci-βγ-crystallin* gene with the activity of its promoter in transgenic *Ciona* and *Xenopus* larvae (see Figure 2). They found that the *Ci-βγ-crystallin* promoter was active in the otolith and palps of transgenic *Ciona* larvae, consistent with the natural expression pattern of the *Ci-βγ-crystallin* gene. Significantly, the promoter was also active in different visual structures of transgenic *Xenopus* larvae and, occasionally, the otic (ear) vesicle. An implication of these elegant results is that the *Ci-βγ-crystallin* gene had the regulatory elements in place for high lens expression in urochordates before vertebrates evolved lenses. Shimeld *et al* (2005) conclude that vertebrate lenses and their co-opted crystallins were derived together by employing pre-existing regulatory circuits used in the nervous system of a primitive chordate. The *Ci-βγ-crystallin* promoter activity in the *Xenopus* otic vesicle is also noteworthy in view of the earlier connections made between eye and ear evolution (Piatigorsky and Kozmik, 2004).

The findings of Shimeld *et al* (2005) indicate that the *Ci-βγ-crystallin* promoter was not altered secondarily to achieve lens activity in vertebrates but was incorporated into the newly evolving eye by regulatory cascades of the developing visual system. However, the authors do not consider that closely related species have distinctly different crystallins. Thus, other scenarios may have favored the emergence of small heat-shock proteins (shsp)/α-crystallins, which exist in, and are highly expressed
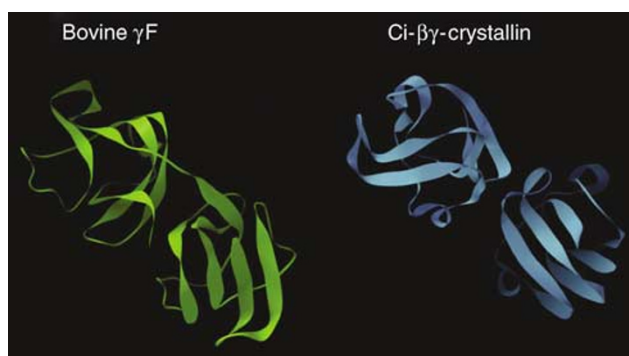

Figure 1 Ribbon models of a βγ-crystallin 'Greek key' fold in γF-crystallin from the bovine lens and from Ci-βγ-crystallin from *Ciona*. The models were derived from the RCSB Protein Data Bank.
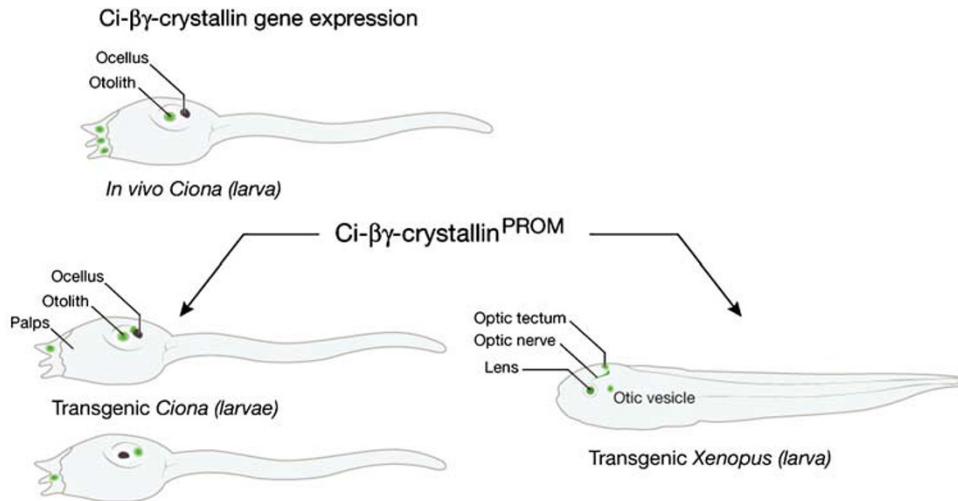
Figure 2 Expression of Ci-$\beta\gamma$-crystallin in *Ciona* and of Ci-$\beta\gamma$-crystallin^{PROM} in transgenic larvae of *Ciona* and *Xenopus*. Expression of the endogenous gene in *Ciona* (upper) was identified by immunofluorescence using a rabbit polyclonal anti-Ci-$\beta\gamma$-crystallin antibody. Ci-$\beta\gamma$-crystallin^{PROM} has 1 kb of 5′ flanking region of the *Ci-$\beta\gamma$-crystallin* gene as a promoter fragment fused to the green fluorescent protein reporter gene. The green color represents expression of the gene or transgene. See Shimeld *et al* (2005) for further technical details.
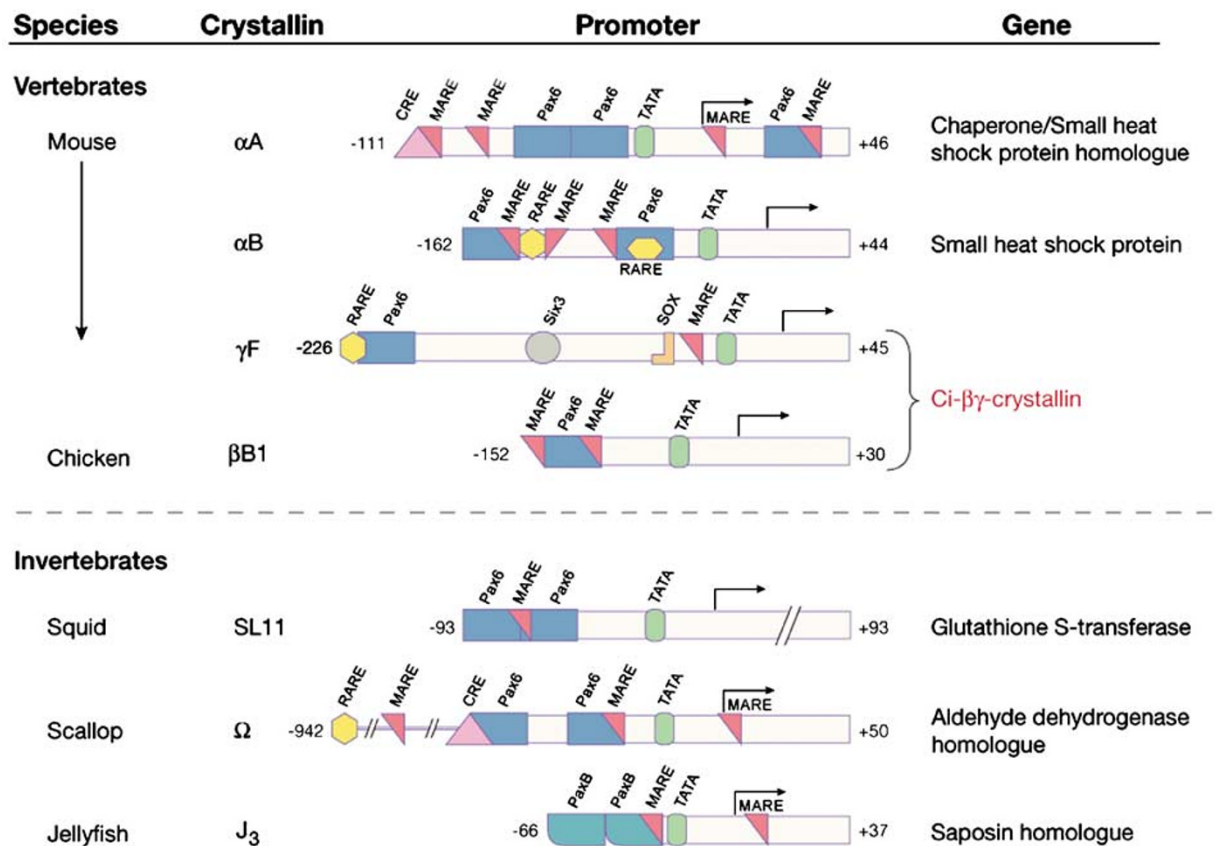

Figure 3 Diverse crystallin genes and their promoters in vertebrates and invertebrates. Note the similarity in regulatory elements in the promoters of the distinct crystallin genes. CRE, cyclic AMP-responsive element; MARE, Maf regulatory element; RARE, retinoic acid receptor regulatory element. αA- and αB-crystallins were derived by gene duplication. αA-crystallin has lost stress inducibility; αB-crystallin is stress inducible. SL11-crystallin was derived by gene duplication of an authentic glutathione *S*-transferase gene and specialized for lens expression; it still has weak enzymatic activity. Ci-$\beta\gamma$-crystallin is the presumed ancestor to vertebrate $\beta\gamma$-crystallins (Shimeld *et al*, 2005). (I am grateful to Ales Cvekl (Albert Einstein College of Medicine) for help in identifying the regulatory elements.)

in, all vertebrate lenses, and for the diverse taxon-specific enzyme-crystallins that are present in a scattered fashion in selected groups of animals (eg Wistow and Piatigorsky, 1988; de Jong *et al*, 1989). The enzyme-crystallins comprise individual metabolic enzymes (or extremely similar proteins derived from duplicated genes) present at

high concentrations specifically in the lenses of certain species where they act as crystallins; these and other taxon-specific crystallins are not expressed highly in lenses of species that did not recruit them as crystallins. The promoters of the diverse vertebrate crystallin genes have many structural similarities, as might be expected if they had converged independently to produce high activity in response to the factors responsible for lens development (Cvekl and Piatigorsky, 1996) (Figure 3).

Shimeld *et al* (2005) consider it unlikely that lenses evolved earlier and degenerated in urochordates. Be that as it may, many invertebrates, including cephalopods, scallops and jellyfish, do have well-developed cellular lenses that have recruited diverse proteins as crystallins (Tomarev and Piatigorsky, 1996). Examples include the saposin-related/ J3-crystallin of jellyfish (Piatigorsky and Kozmik, 2004) and the aldehyde dehydrogenase-related/Ω-crystallin of scallops (Carosa *et al*, 2002). The taxon-specific crystallin promoters of these invertebrates show structural convergence similar to the vertebrate crystallin promoters (see Figure 3). Moreover, a similar cast of transcription factors are employed for eye development in vertebrates and invertebrates (Gehring, 2005). Apparently, lenses have evolved both by assimilating cells, transcription factors and potential crystallins already integrated into developmental pathways connected to eye lineages, and also by convergent acquisition of new crystallins by promoter modification. It remains a challenge to determine the evolutionary relationship between lenses of ancient invertebrates and of vertebrates.

Finally, Ci-$\beta\gamma$-crystallin, in common with other lens crystallins, has been recruited from a widely expressed gene; clearly, it does not perform a refractive function in *Ciona*, which lacks a lens. Members of the $\beta\gamma$-crystallin superfamily of proteins appear connected to stress protection and growth control (eg Clout *et al*, 2001; Wistow *et al*, 2005). Thus, another challenge posed by the study of Shimeld *et al* (2005) is to identify the sensory and non-sensory functions of Ci-$\beta\gamma$-crystallin in *Ciona*. This strategy could provide valuable insights into the non-refractive functions of the vertebrate $\beta\gamma$-crystallins, which are expressed outside of the lens in a number of tissues, including the retina, heart and testis.

J Piatigorsky is at Laboratory of Molecular and Developmental Biology, National Eye Institute, National Institutes of Health, Bethesda, MD 20892, USA.
E-mail: joramp@nei.nih.gov

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