CHAPTER 6

A BRIEF HISTORY OF LENS AND CRYSTALLIN RECRUITMENT

Equipped with photosensitive eyespots whose development was under the control of a gene cascade originating with *Pax-6,* simple metazoans began to thrive. Even a primitive level of vision conferred great advantages. Improvements in optics and in processing the sensory data which was gathered conferred even greater advantages. In the ancestors of vertebrates part of the enhancement of the eye occurred through the evolution of a cellular lens. A single change in the differentiation program of a layer of cells covering the retina caused swelling and cell elongation to form a simple concentrating lens. Associated with the stress of this event, certain gene families were induced in the elongating cells. These included genes involved with synthesis and protection of cytoskeleton and with other aspects of osmotic stress. In the vertebrate lineage, these genes included a small heat shock protein homologous to αB-crystallin and at least one member of the βγ-crystallin superfamily, perhaps homologous to βB2-crystallin. These were highly stable proteins able to interact closely with other components of the cytomatrix.

The stress responses in these elongating cells would initially have been no different from those inducible by similar stresses in other cells and the induced genes would not yet have become crystallins. However the function of the new lens would have been greatly improved by increasing its protein concentration and refractive index. The stress proteins which were already necessary for the primitive lens would have been among the easiest targets for further induction. This could have occurred in a tissue-preferred way if these genes acquired binding sites for transcription factors expressed in the eye as part of the "eye cascade." Several different evolutionary experiments must have occurred and most would have failed. For example, high expression of stress genes outside the lens in the retina might have had a deleterious effect on vision while some combinations of eye-related transcription factor binding sites might have caused overexpression in parts of the central

nervous system and elsewhere. However selection of successful experiments produced a fine-tuned, lens-specific system.

These modifications in gene expression would have involved not only the proto-crystallins but also the transcription factors themselves. Expression patterns of members of the eye cascade must also have been modified to produce a new tissue, the lens. Indeed, it appears that *Pax-6* itself was co-opted for this new program. While continuing its ancient role in defining eye as a whole it acquired an additional role in specifying lens.

Once the path for the development of the lens was established it continued to be refined, setting up differential expression patterns within the tissue and throughout its development. Terminally differentiated fiber cells evolved to provide the core of the lens. These cells acquired a different complement of transcription factors to allow for different patterns of gene expression within the lens. The original complement of recruited proteins began as dual function stress proteins and crystallins. With time and with new demands they experienced adaptive conflicts which were resolved by gene duplication and specialization. A lens-specialized αA-crystallin evolved to play a particular role in association with lens cytoskeleton. The β-crystallins multiplied allowing for more complex patterns of protein expression during development. A very highly specialized protein family specific to the fiber cells also arose, perhaps by duplication from the ancestral βγ-crystallin of the earliest lens. The members of this family were the γ-crystallins which acquired specializations in structure allowing the lens to achieve even higher protein concentrations and higher refractive index.

At this stage the lens had become the sophisticated optical device of the aquatic vertebrate eye. However its evolution was not over. As the vertebrate eye moved from an interface with water to one with air the lens and other parts of the eye adapted to the new environment. Lens-hardening γ-crystallins were replaced or diluted by newly recruited crystallins. This time the recruitment process, occurring in an established tissue, made use of a different class of protein. There was no benefit to be gained from further introductions of stress proteins. Instead metabolic enzymes were recruited. Just as before, the genes for the new crystallins acquired binding sites for transcription factors involved in lens specification and their expression in lens was enhanced while their non-lens expression continued. Unlike the crystallins, these proteins were not specifically adapted for a high protein concentration environment and they contributed to a general softening and hydration of the lens. However it is likely that they were still required to be able to form stable interaction with the cytoskeleton which formed the essential scaffolding of the lens cells. Indeed, even outside the lens many metabolic enzymes are found anchored to cytoskeleton.

While the primary impetus to the recruitment of enzyme crystallins was modification of the optical properties of the lens, other factors were also involved. Some reptiles adopted a habit in which UV glare became a problem. The δ-crystallin in their soft lenses did not provide any opportunity for resolving this problem. However a second enzyme crystallin recruitment proved beneficial. When LDHB was recruited as ε-crystallin, archosaur lenses acquired a mechanism for increased UV filtering. Similar patterns occurred in other lineages. In mammals there was a complex history involving lens-softening, a reversion to hard spherical lenses and then a series of re-modifications with reductions in γ-crystallin expression and various enzyme crystallin recruitments.

Although the lens is a highly specialized tissue in a highly specialized organ, the interplay of development and tissue specification with gene expression, molecular evolution and the environment which has shaped it must have occurred over and over again in different living systems during the history of life on this planet.

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