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βγ-crystallin redux

Evidence for a particular evolutionary pathway is virtually always indirect. Many shy away from defending any particular pathway because they understandably distrust conclusions based on inference rather than experimentation. But even a die hard empiricist would have to admit that the evidence for the evolution of the βγ-crystallins, built up from comparisons of sequence and structure and function, presents a compelling set of observations from which to infer particular steps in the evolution of this superfamily of molecules.

The physiological roles of members of the βγ-crystallin superfamily can be easily rationalized in terms of their most obvious functions—with consequences for understanding the evolutionary pressures that gave rise to their present forms. In the eye lenses of vertebrates, βγ-crystallins must provide a gradient of refractive index, and because the cells in the centre of the lens undergo almost no turnover, or even new protein synthesis, they must be unusually stable proteins. Initial appearances can, however, be deceiving: the crystallins in general are notorious for having other functions that have nothing to do with sight[1] (for example, ε-crystallin is identical to lactate dehydrogenase B). Although the βγ-crystallins have no apparent identity or close similarity with enzymes of distinct function, given the multifunctional nature of the other crystallins it pays to distrust our initial impressions about the importance of acute vision in selecting their structure. Indeed, a primary clue to the evolutionary origin of the βγ-crystallins comes not from a lens protein at all, but is instead found in the NMR structure of a yeast killer toxin presented on page 662 of this issue[2].

The killer toxin from *Williopsis mrakii* (WmKT) is not involved in vision, but rather in suppressing the growth of rival yeasts. Yeast killer toxins take a variety of forms—some are thought to form pores in the competitor, some inhibit proton pumping, some inhibit adenylate cyclase. The ~10,000 M_r secreted toxin from *W. mrakii* inhibits β-(1,3)-glucan synthetase, and thus the production of β–glucan, an important polysaccharide constituent of the cell wall[3].

The structure of this toxin is interesting in its own right, but is made more so because it is an excellent candidate to be the structural precursor for the entire βγ-crystallin superfamily. The basic secondary structural unit making up the βγ-crystallins is the ~40-residue Greek-key motif[4,5]. Both γB-crystallin[4] and βB2-crystallin[5] (see Fig.) are two-domain proteins, each domain composed of two Greek keys. It is natural to hypothesize that these crystallins arose from a monomeric or perhaps dimeric single-domain protein. A scenario of gene duplications and fusions, interspersed with a few insertions and deletions, suffices to connect a hypothetical single-domain precursor with both sides (β and γ) of the

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A β2B-crystallin dimer, a complex member of the superfamily. The two domains (red and blue) each contain two Greek key motifs. Figure taken from ref. 6.

superfamily[4–6]. A genetic duplication of a single-domain protein, then some divergence, and then fusion would lead to a two domain, four Greek key protein. That a duplication of the single, two-Greek key protein occurred is supported by the observation that the Greek keys bear a closer resemblance when compared between domains than within a single domain[4]. The scenario for the evolution of the β-crystallin side is more problematic[6]: it may have arisen from the monomeric γ-crystallins through a relatively simple 'domain-swap'; however, the loss of an intron between Greek key motifs at an early, single-domain stage argues in favour of a less straightforward explanation[6]. Whatever the scenario for evolution of both branches of this superfamily, it has been generally accepted that there should have existed a single domain, two-Greek key precursor—something very much like the 88 amino-acid long WmKT[2].

The structural similarity that the one-domain WmKT bears to the γB-crystallin domains is not accompanied by any appreciable sequence homology (another candidate—based on sequence data—for a single-domain precursor is spherulin 3a from *Physarum*; its structure is not yet available). In fact, nothing apparent in the WmKT sequence alone would have led one to believe that it contains Greek key motifs[2]. But its 3D structural resemblance to a single βγ-crystallin domain argues that it represents the stable fold that gave rise to the members of the superfamily, and is diverged from some common one-domain ancestor.

WmKT at first glance has nothing functionally in common with a protein used in the lens, but it is perhaps too soon to conclude that they share no common functions at all. As mentioned above, the crystallins in general appear to have an unusual capacity to lead a double life (or at least to be closely related by sequence and thus evolution to a protein with another role)[1]; any appeal to understanding their evolution in terms of function must account not only for clear vision, but for possible past roles as well. βγ-crystallins in particular must be stable; WmKT is also a very stable protein, although stability in WmKT is accomplished in a different way[2]. Perhaps a functional commonality can be found in considering that βγ-crystallins must be properly ordered with respect to each other within the lens or that they are thought to order water molecules in a way important for maintaining proper refractive index in a water-poor environment[4]. In this regard, it may be worth noting that some relatives of β- and γ-crystallins, Protein S from *Myxococcus* and spherulin 3a are involved in the formation of dehydrated spores[1]. Whether any of these functional properties can be ascribed to WmKT seems tenuous, but it will be informative in any case to understand other functions of this toxin, such as how it inhibits its enzyme target.

As with any evolutionary scenario, the evidence that the WmKT fold is the precursor of the two-domain βγ-crystallins rests on observation and not experimentation. The closeness of resemblance, however, and the fact that such a precursor had long been hypothesized[4], puts a minimum of strain on our ability to infer an evolutionary pathway. If these were facts surrounding a political scandal, they would support a whole industry for conspiracy theorists. And why not? Evidence for the steps in evolution at a molecular level is rarely more clear-cut than this. It seems that iterations of gene duplication and fusion have built on the simpler Romanesque of a single-domain protein resembling WmKT to create the Gothic of the βγ-crystallins; in the finest tradition of evolution, little has gone to waste, nothing was torn down, only replicated, modified, and buttressed.

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