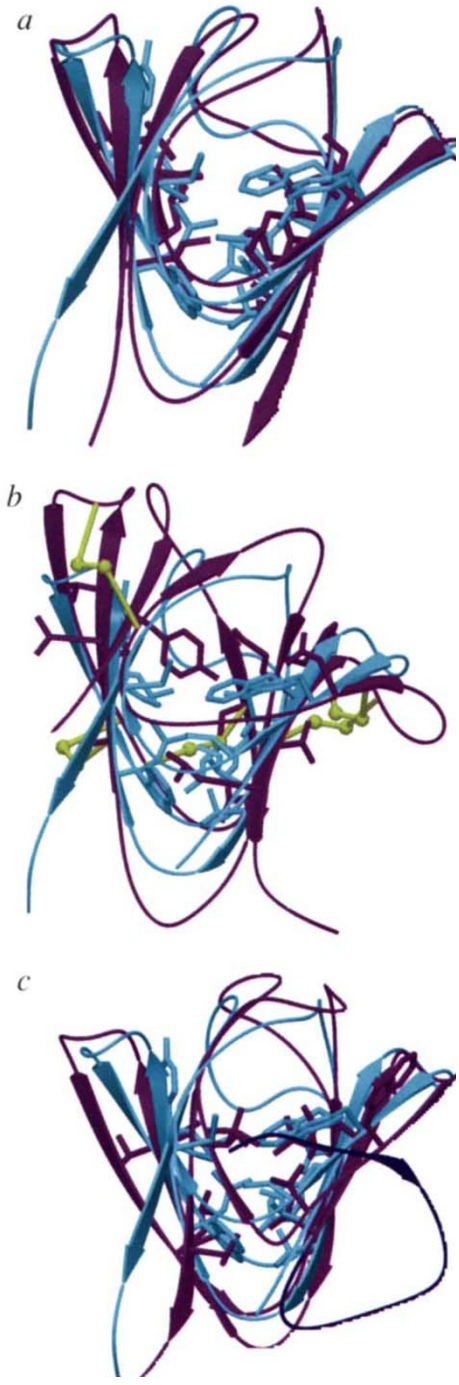
An eye on crystallins

Eye lens crystallins are evolutionarily old proteins that have acquired new functions. Some, with restricted taxonomic distribution, are the result of direct gene recruitment of enzymes, while others — the α, β and γ-crystallins — are more ancient and are ubiquitous in vertebrates. The α-crystallins belong to the HSP26 superfamily: the origins and ancestral functions of the βγ superfamily, on the other hand, are more mysterious. These proteins have four characteristic motifs with a distinct sequence signature, organized into two highly symmetrical domains[1,2] showing that they must have evolved by duplication of an ancestral one-domain protein which in turn arose by duplication of a single structural motif. The sequence signature has allowed the detection of distant members of the superfamily, beginning with the two-domain protein S (PS) of the bacterium *Myxococcus xanthus*[3], whose βγ structure (Fig. 1*a*, pink) was confirmed by NMR spectroscopy[4]. The structural similarity between these prokaryotic and vertebrate proteins is strong enough to detect motif permutation within the domains, suggesting their independent histories of duplication and fusion events.



Recently, it was suggested[5] that the killer toxin from the yeast *Willopsis mrakii* (WmKT)[6] represents the one-domain βγ ancestor (Fig. 1*b*, pink). An ancestor should exhibit basic features common to its descendants and should generally be more similar to its diverging descendant lineages than they are to each other. WmKT, which lacks the sequence signature and has several structural differences from other βγ proteins, fails this test.

A closer illustration of the one-domain ancestral structure is provided by spherulin 3a (s3a) of the slime-mould *Physarum polycephalum*, first identified by sequence signature and modelling[7]. Its calcium-loaded structure has now been solved (Fig. 1*c*, pink) by NMR[8] showing it is very similar to a single domain of a γ-crystallin. Comparisons of s3a, PS and Wmkt domains to the superfamily archetype γB-crystallin (light blue) show the clear similarities in folding (Fig. 1). However, in the case of WmKT (Fig. 1*b*), major differences are also apparent, particularly in the lengths of loops and orientation of the β-sheets. Furthermore, while hydrophobic core residues in s3a, PS and γB form equivalent clusters (Fig 1*a*,*c*), the core of WmKT is quite different and the structure is stabilized by disulphide bonds (Fig. 1*b*, yellow). WmKT is not only too divergent from prokaryotic and eukaryotic βγ structures to represent an ancestor, it may not even be part of the same evolutionary lineage, instead resulting from convergence to a stable fold.

As shown by ultracentrifugation, s3a dimerizes[9] and may thus echo the evolutionary process which led to two-domain proteins. Although the solution structure presents no evidence for intermolecular contacts, s3a has an N-terminal extension (Fig. 1*c*, dark blue) that contributes a short extra strand to the first β-sheet. Perhaps the single domain of s3a dimerizes by strand exchange using the N-terminal extension and is thus an ancient example of domain swapping?

Fig. 1 ***a***, The N-terminal domain of PS and the single domains of ***b***, WmKT and ***c***, s3a shown in pink, are each superposed on the N-terminal domain of γB, shown in light blue, drawn using the program SETOR[10]. In PS the motifs are permuted with respect to γB.

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