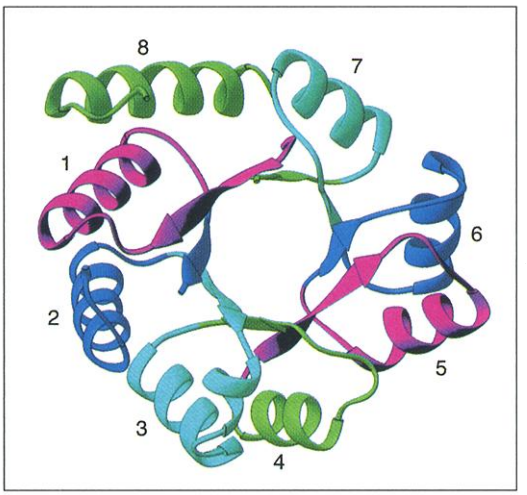
PERSPECTIVES: PROTEIN EVOLUTION

On the Ancestry of Barrels

Edith Wilson Miles and David R. Davies

The avalanche of new three-dimensional protein structure determinations, currently about 50 per week, provides an exciting resource for examining the evolutionary relationships within protein families. This is particularly important because structural resemblances can often be observed between two proteins even when the sequence homologies are quite low (*1*). Most proteins consist of multiple domains linked together in a single polypeptide chain. The similarity between domains in many multidomain proteins, such as those in the immunoglobulin domain superfamily (*2*), indicates that they have evolved through gene duplication and fusion. However, this mode of evolution need not be limited to multidomain structures. On page 1546 of this issue, Lang *et al.* (*3*) present structural data on a common single-domain protein, the eightfold β/α barrel (*4*), which indicate that this structure arose from the duplication and fusion of the gene of a common half-barrel ancestor. The study confirms previous proposals based on sequence analyses (*5*, *6*).

Lang *et al.* report the x-ray structures of two histidine biosynthetic enzymes, HisF and HisA, from a hyperthermophile, *Thermotoga maritima* (*3*). Both enzymes are β/α barrels, as previously predicted (*6*). Remarkably, in both enzymes, the loops on opposite sides of the COOH-terminal face are found to be very similar in length and structure (for example, loop 1 resembles loop 5). Furthermore, HisF contains two phosphate ions that are located on opposite sides of the COOH-terminal face, mimicking the two phosphates of the substrate, as predicted previously (*6*). The main chain atoms of the half barrels of both HisA and HisF superimpose closely with root mean square deviations from 1.4 to 2.1 Å, showing strong structural homology. Lang *et al.* use these structural results to align the sequences of the four half barrels of the structures. Although the percentage of similar residues is low (22%), five identical residues occur in each sequence, including an aspartate that may play different catalytic roles in HisA and HisF. Finally, Lang *et al.* determine the activity of each enzyme in the reaction catalyzed by the other. Remarkably, HisF is also active in the reaction catalyzed by HisA, namely the isomerization of a phosphoribosyl intermediate. HisA has no activity in the more complicated HisF reaction.



The classic β/α barrel. The structure, also called TIM barrel (named for triose phosphate isomerase), is built from eight repeated β strand/α helix units. Units 1 and 5 are shown in pink, 2 and 6 in blue, 3 and 7 in cyan, and 4 and 8 in green. Lang *et al.* provide evidence that two new β/α barrel structures, HisA and HisF, evolved from a half-barrel ancestor (*3*). This figure is based on the structure of another β/α barrel enzyme, the α subunit of tryptophan synthase from *Salmonella typhimurium* (*12*). The figure was created with the program Ribbons and the coordinates 1bks from the Protein Data Bank. The original structure has three extra α helices, which have been deleted to create a classic β/α barrel.

Have all β/α barrel proteins evolved from a common ancestral barrel? The new results show that HisA and HisF are closely related in structure not only to each other but also to three β/α barrel enzymes in the tryptophan biosynthetic pathway (TrpC, TrpF, and TrpA) (*3*). The three Trp enzymes are similar in structure and bind the phosphate of the substrate at the same site (*7*). This phosphate binding site is also found in a number of other β/α barrels, including the new HisA and HisF structures. Thus, it is highly likely that all these related β/α barrel enzymes arose by divergent evolution from a common ancestor or from one another.

The ancestral β/α barrel structure was probably a homodimer consisting of two identical half barrels, with a similar relationship to the current molecules as the human immunodeficiency virus (HIV) protease homodimer has to the pepsins, which consist of two domains formed by a single chain (*8*). It would be interesting to see whether the two half-barrel structures of HisA and HisF can assemble to form a functional barrel structure. Another β/α barrel protein, the α subunit of tryptophan synthase, can be reconstituted from separate proteolytic fragments of unequal size (*9*).

The combined results suggest that many, if not all, β/α barrels have evolved from a common ancestral half barrel, followed by diversification of catalytic function. Evolutionary change could result in loss of one or both of the phosphate binding sites and the addition of new regulatory or catalytic sites. Recent work demonstrates that the β/α barrel provides an ideal scaffold for the creation of new enzyme activities by rational design and directed evolution (*10*).

Is the half barrel the smallest ancestor? The symmetry of the β/α barrel could reflect evolution through repetitive duplication of an even smaller stable unit (*1*). Proteins such as the leucine-rich repeat ribonuclease inhibitor contain as many as 16 repeats of a simple β/α motif (*11*). The presence of repeated structural motifs in these and many other proteins suggests that these proteins also arose from gene duplication and fusion of relative small subdomains.

The authors are at NIH, Bethesda, MD 20892–0830, USA. E-mail: edithm@intra.niddk.nih.gov; david.davies@nih.gov

References and Notes

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13. We thank G. Cohen for preparing the figure.