E1 on the Move

E1 enzymes activate ubiquitin or related proteins and pass them to E2 enzymes. A recent structure and associated biochemical studies (Huang et al., 2005) show how an E1 binds its cognate E2 and indicates that large, conformational changes will be an integral component of the E1 reaction cycle.

A dramatic form of posttranslational modification occurs when lysine side chains or N termini of target proteins are covalently bound to the C terminus of another protein called ubiquitin (Hershko et al., 2000). This modification is applied to many protein substrates and is interpreted by a varied array of binding proteins that function in a remarkable diversity of biological pathways, including targeting to proteasomes, targeting to lysosomes, signaling pathways, transcriptional processes, and viral budding. The large number of ubiquitin substrates results from a biochemical pathway that starts with a single E1-activating enzyme, which transfers the ubiquitin to E2 enzymes, which in turn collaborate with a plethora of E3 enzymes to transfer ubiquitin to substrates (Pickart, 2001).

Our understanding of this process owes much to a series of structural and biochemical studies from the Schulman lab working on the E1 for the Ubip NEDD8. This E1 is a heterodimer of APPBP1 and UBA3 proteins that serves as a model for the E1 of other Ubip. One earlier insight concerned the requirement for the second step, i.e., transfer to the E1 cysteine, which is not, in principle, needed to increase the chemical potential for subsequent transfer reactions but may be required to avoid a topological trap (VanDemark and Hill, 2003; Walden et al., 2003). The binding site for NEDD8 has been determined in detail for the adenylation step (Walden et al., 2003), and a similar arrangement was demonstrated recently for the E1 of the SUMO Ubip (Lois and Lima, 2005). It has also been shown that the NEDD8 E1 specifically recognizes its cognate E2 enzyme, in part, through interactions of an inherently flexible sequence extended from the N terminus of the folded E2 core domain, although other E2s lack sequences equivalent to NEDD8 E2’s N-terminal extension and cannot utilize this mechanism (Huang et al., 2004). A previously missing piece of the puzzle is now provided by the latest Schulman lab publication (Huang et al., 2005), which describes the structure of a complex between an E2 core domain and the binding domain at the C terminus of UBA3. This interaction is presumably equivalent for the E1-E2 complexes of all Ubips and defines the relative position of the E1 and E2 cysteine residues between which the Ubip is transferred.

The new structure reveals details of how the NEDD8 E1 recognizes the core domain of its cognate E2, and analysis of sequence differences suggests that the equivalent interface can specify appropriate E1-E2 partnerships in the other Ubip pathways. The structure also shows that the E2 surface buried against E1 overlaps with the surface buried in the two currently known E2-E3 complex structures (Huang et al., 1999; Zheng et al., 2000). One has to be careful in these comparisons, because the E1 and E3 complexes are with different E2 molecules and there are many types of E3, but the obvious implication is that E2s cannot simultaneously bind E1 and (at least some) E3 enzymes. Huang et al. suggest that the inferred competitive binding might contribute to efficient transfer of E2 from E1 to an E3 complex. This idea may have parallels with other aspects of Ubip biochemistry, because the several binding partners whose structures are known in complex all contact an overlapping surface on ubiquitin, implying that competi-
Spiraled Origins

Recent studies have established that the eukaryotic actin-based cytoskeleton has prokaryotic origins. In addition to regulating cell shape and polarity, Gitai et al. (2005) provide convincing evidence that the Caulobacter actin homolog MreB also mediates the early segregation of the chromosomal origin, a typical functional role of the eukaryotic tubulin-based cytoskeleton.

The evolutionary history of all living things is often illustrated as a tree; the roots of life’s origin split into branches of genetic connections that symbolize the divergence of independent species. Often the tree is fine scaled to include leaves of biological pathways or protein families. This representation of phylogenetic analysis serves as a guide to understanding both the underlying mechanisms of ancient functional changes and whether these changes were selectively advantageous or merely happenstance. Although the components of the two basic eukaryotic and prokaryotic cytoskeletal systems share low sequence conservation, the atomic structures of actin- and tubulin-like proteins have been highly conserved (Amos et al., 2004). Emerging evidence suggests that these structural similarities are not strong assurances of functional conservation, and indeed the connections of divergence are not figurative branches of linearity but seem to be characterized by somewhat spiraled role reversals.

The cytoskeletal machinery regulates a medley of diverse cellular processes necessary for cell differentiation and growth. In eukaryotes, the tubulin-based cytoskeletal system mediates mitosis and chromosome...