Molecular Cell, Vol. 17, February 18, 2005, Copyright ©2005 by Elsevier Inc. DOI 10.1016/j.molcel.2005.02.004

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 lysozomes, 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). Further complexity is provided by the presence of ubiquitin-related proteins that participate in similar pathways and thereby regulate additional processes. For clarity, we refer to ubiquitin and its relatives collectively as ubiquitin-like proteins (Ublps).

E1s use ATP hydrolysis to provide the chemical potential needed for the subsequent transfer steps in the Ublp pathways. This is achieved by a two-step mechanism in which the Ublp C terminus is first adenylated and then transferred from the adenyl group to an E1 cysteine. 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 Ublp NEDD8. This E1 is a heterodimer of APPBP1 and UBA3 proteins that serves as a model for the E1 of other Ublps. 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 Ublp (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 Ublps and defines the relative position of the E1 and E2 cysteine residues between which the Ublp is transferred.

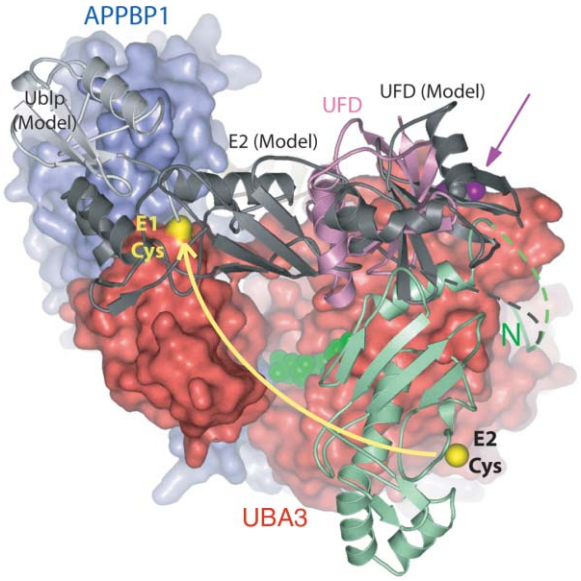

Figure 1. Possible Model for E1 Conformational Change

This composite model was generated by overlap of various crystal structures of the NEDD8 E1 enzyme (APPBP1-UBA3). Crystallographically observed conformations are shown in colors. The UBA3 UFD and bound E2 are shown in ribbon representation. E1 and E2 cysteines (yellow spheres) are separated by over 50 Å. The speculative model (gray) puts E1 and E2 cysteines next to each other by rotation about UBA3 Leu349, located in the linker between the UFD and the rest of UBA3 (purple arrow). A possible location of NEDD8 (Ublp, white) bound to the E1 cysteine has been built that would avoid steric clash and would be in position for transfer to E2. For reference, the adenylation site is indicated by the bound ATP (bright green), the site occupied by NEDD8 before adenylation is out of view, and the NEDD8 N-terminal peptide is labeled N, with the flexible residues connecting to the E2 core shown as dashed lines.

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 Ublp 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 Ublp biochemistry, because the several binding partners whose structures are known in complex all contact an overlapping surface on ubiquitin, implying that competi-

tion effects might facilitate Ublp movement between different binding partners within a pathway. A related curiosity is that E1's E2 binding domain adopts the ubiquitin fold—it is therefore called the ubiquitin-fold domain (UFD)—and despite lack of sequence similarity, the UFD surface that contacts E2 overlaps with the equivalent face of ubiquitin buried in all currently known protein complexes. One obvious possibility to consider is that E2 bound Ublp might facilitate product release by competing with the UFD interaction, although simple modeling suggests that a Ublp could not simultaneously be bound to the E2 cysteine and contact the UFD binding surface, and potential mechanistic significance for the molecular mimicry is not clear.

An important idea advanced by Huang et al. (2005) concerns the role of structural rearrangements during the E1 reaction cycle. A model for the intact E1-E2 complex can be constructed by superimposing the UFD of the E2 complex on the UFD of the full-length E1 structures. The resulting composite model places the E1 and E2 cysteine residues more than 50 Å apart. This indicates that a large conformational change will be needed to allow Ublp transfer, and this could likely be achieved by rotation about residues that link the UFD to the rest of UBA3 (Figure 1). Huang et al. support their model by constructing variant E1 proteins with altered sequences for the linker peptide and show that conformationally restricted residues prevent NEDD8 transfer to E2, whereas more flexible residues do not. Structural studies on the E1 for SUMO have also highlighted the importance of relative domain motions (Lois and Lima, 2005).

The conformational changes invoked by Huang et al. have parallels with differences observed in crystal structures of ubiquitin E3 enzymes, where large-scale domain rotations allow Ubl transfer between E2 and E3 cystsine residues (Huang et al., 1999; Verdecia et al., 2003). The need for additional conformational changes has also been invoked in the transfer of Ubl between the E1 adenylation site and catalytic Cys, because they are separated by 30 Å in the currently available structures. It has been understood, almost since determination of the first protein structures, that protein function often requires flexibility (Perutz, 1972). The emerging picture of E1 mechanism provides a particularly interesting example of this fact.

Andrew P. VanDemark and Christopher P. Hill
Department of Biochemistry
University of Utah School of Medicine
Salt Lake City, Utah 84132

Selected Reading

Hershko, A., Ciechanover, A., and Varshavsky, A. (2000). Nat. Med. *6*, 1073–1081.

Huang, L., Kinnucan, E., Wang, G., Beaudenon, S., Howley, P.M., Huibregtse, J.M., and Pavletich, N.P. (1999). Science *286*, 1321–1326.

Huang, D.T., Miller, D.W., Mathew, R., Cassell, R., Holton, J.M., Roussel, M.F., and Schulman, B.A. (2004). Nat Struct. Mol. Biol. *11*, 927–935.

Huang, D.T., Paydar, A., Zhuang, M., Waddell, M.B., Holton, J.M., and Schulman, B.A. (2005). Mol. Cell *17*, 341–350.

Lois, L.M., and Lima, C.D. (2005). EMBO J., in press. Published online: January 20, 2005. 10.1038/sj.emboj.7600552.

Perutz, M.F. (1972). Nature *237*, 495–499.

Pickart, C.M. (2001). Annu. Rev. Biochem. *70*, 503–533.

VanDemark, A.P., and Hill, C.P. (2003). Nat. Struct. Biol. *10*, 244–246.

Verdecia, M.A., Joazeiro, C.A., Wells, N.J., Ferrer, J.L., Bowman, M.E., Hunter, T., and Noel, J.P. (2003). Mol. Cell *11*, 249–259.

Walden, H., Podgorski, M.S., and Schulman, B.A. (2003). Nature *422*, 330–334.

Zheng, N., Wang, P., Jeffrey, P.D., and Pavletich, N.P. (2000). Cell *102*, 533–539.

Molecular Cell, Vol. 17, February 18, 2005, Copyright ©2005 by Elsevier Inc. DOI 10.1016/j.molcel.2005.02.006

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 phylogenic 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