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Structure/function of protein-folding and protein-transport factors

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Proteins destined to be secreted, expressed on the cell surface, or resident in intracellular membrane-bound compartments are synthesized on the rough endoplasmic reticulum (ER) and transported through the secretory pathway. A vesicle-mediated transport system carries these proteins through the compartments in which folding, acquisition of disulfide bonds, and other post-translational modifications occur. Quality-control mechanisms insure that only proteins having achieved an acceptable fold progress through the secretory pathway, and defective products are recycled. Recycling programs are further involved in turnover of cytoplasmic material, and non-canonical vesicle transport mechanisms can capture proteins synthesized in the cytoplasm and deliver them to membrane-bound compartments.

One of the most important post-translational modifications of proteins in the secretory pathway is disulfide-bond formation. Although it was originally supposed that oxidized glutathione is the primary source of disulfide bonds for ER proteins, glutathione proved to be dispensable. Instead, recent genetic studies in yeast revealed that the enzyme Ero1p (Endoplasmic reticulum oxidoreductin 1 protein) generates disulfide bonds in the ER. Ero1p is an essential flavoenzyme that interacts with protein disulfide isomerase (PDI) to shuttle oxidizing equivalents to protein substrates. Further studies suggested the existence of an alternative pathway for disulfide bond formation in the ER and identified the protein Erv2p (Essential for respiration and viability 2 protein). Erv2p, like Ero1p, is an ER membrane-associated flavoenzyme. However, unlike Ero1p, which functions anaerobically, Erv2p uses oxygen as an ultimate electron acceptor in the generation of disulfide bonds. The discovery of Ero1p and Erv2p raised the following questions. What are the structures and mechanisms of these two enzymes? Do they have unique substrates and physiological roles?

We have recently determined the structure of Erv2p using the technique of X-ray crystallography (Figure 1). The highly helical Erv2p has a novel FAD-binding fold with no resemblance to other FAD-binding proteins. Erv2p activity relies on two pairs of cysteines: a CGEC sequence in the active site, and a CGC sequence in the carboxy-terminal tail of the enzyme. By combining structural and biochemical data with in vivo observations, we suggest a model in which the carboxy-terminal tail can accept electrons from substrate proteins and shuttle them to the active-site disulfide bond. From there, the electrons are deposited on molecular oxygen via the bound FAD.

In addition to disulfide-bond formation, vesicle trafficking in the secretory pathway is also a subject of our work. One of the central proteins for secretion in yeast is Sec18p. Sec18p and its mammalian orthologue NSF (N-ethylmalemide-sensitive fusion protein) are ATPases that chaperone disassembly of a complex composed of NSF attachment proteins (SNAPs) and SNAP-receptors (SNAREs). SNAREs are a family of integral membrane proteins distributed between vesicles and target organelle membranes, and the assembly of SNAREs on opposing membranes into stable helical bundles is thought to drive membrane fusion. Although it is not fully understood why the disassembly activity of Sec18p/NSF is crucial for vesicle transport, unraveling used or erroneous SNARE complexes by Sec18p/NSF may prime membranes for fusion.

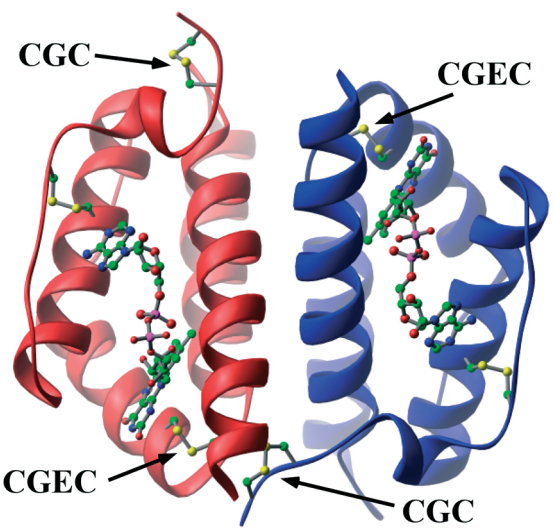

Fig. 1 *Ribbon diagram of Erv2p with di-cysteine motifs indicated and FAD shown in ball-and-stick.*

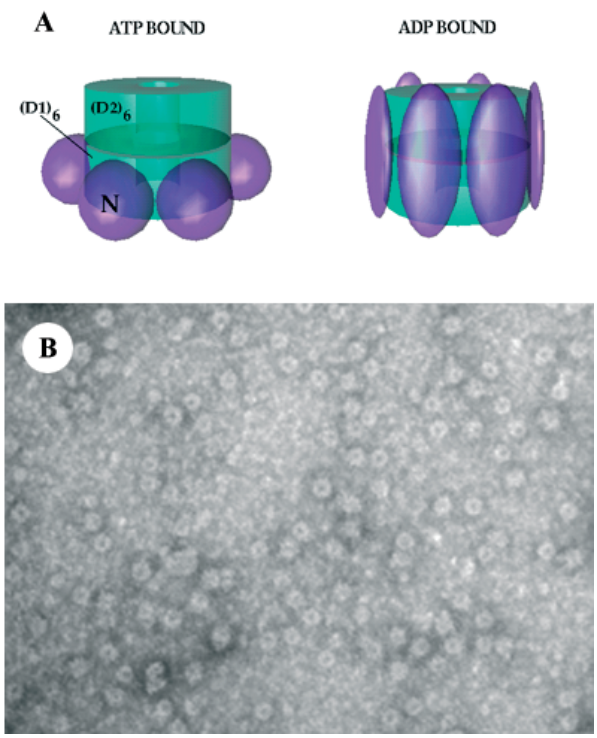

Fig. 2 *(A) Schematic model of the Sec18p/NSF structure, illustrating the structural changes that occur in the enzyme during the nucleotide hydrolysis cycle. Purple spheres represent N domains, and green rings represent the D1 and D2 domains. This figure is derived from Hanson, et al. (1997) Cell 90, 523-535. (B) Electron micrograph (x 33,000) of a field of Sec18p particles. The sample was stained with 2% uranyl acetate.*

Sec18p/NSF is a large and complex protein. Each subunit of Sec18p/NSF is roughly 85 kD and is composed of three domains: N, D1, and D2. The D1 and D2 domains have ATPase activity; D1 activity is required for the catalysis of SNARE disassembly, whereas D2 mediates Sec18p/NSF hexamerization in the presence of nucleotide. Electron microscopy (EM) studies show that the Sec18p/NSF complex is a ring-shaped particle (Figure 2). It was possible to see, at low resolution, two states of the N domain representing different points in the nucleotide hydrolysis cycle.

Attempts have been made in multiple laboratories to determine the structure of the Sec18/NSF hexameric complex by X-ray crystallography with no success so far. However, the structures of individual domains have been determined in our lab and others. Our current goal is to determine the structure of the whole Sec18p complex and to understand the conformational changes involving the N domain to provide insight into the mechanism of membrane fusion in the secretory pathway. We are using cryo-EM 3D reconstruction techniques to work toward intermediate-resolution models of Sec18p conformations into which we can dock the known high-resolution domain structures.

Sec18p/NSF may be involved in special vesicle-trafficking events, in addition to the standard secretory pathway. When a cell is starved, it can undergo a process called autophagocytosis in which large sections of the cytoplasm are 'swallowed' by vesicles with double membranes and transported to the lysosome for digestion. A protein called GATE-16 is essential for autophagocytosis and becomes covalently attached to membranes in a conjugation reaction resembling ubiquitination. In collaboration with the laboratory of Zvulun Elazar in the Department of Biological Chemistry, we have determined the structure of GATE-16 by X-ray crystallography, and we are currently exploring the possibility that attachment of this protein to membranes enhances the interaction with Sec18p/NSF.

The structural studies described above have provided snapshots of key players in fundamental cell-biological processes. The combination of high-resolution structural studies with biochemical or genetic research conducted in our own laboratory or in those of our close collaborators has contributed to the development of structure-based models for the mechanisms of protein modification and trafficking in the secretory pathway.

Selected Publications

Gross, E., Sevier, C.S., Vala, A., Kaiser, C.A., and Fass, D. (2002) A new FAD-binding fold and intersubunit disulfide shuttle in the thiol oxidase Erv2p, Nature Struct. Biol. (in press).

Paz, Y., Elazar, Z., and Fass, D. (2000) Structure of GATE-16, membrane transport modulator and mammalian ortholog of autophagocytosis factor Aut7p, J. Biol. Chem. 275, 25445-25450.

Babor, S.M., and Fass, D. (1999) Crystal structure of the Sec18p N-terminal domain, Proc. Natl. Acad. Sci. USA 96, 14759-14764.

Acknowledgments

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