Chaperones and Catalysts of Protein Folding, Modification, and Sorting

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Translation of a polynucleic acid into a polypeptide is only the first step in expression of genetic information. After translation, the polypeptide undergoes folding, post-translational modifications, assembly, sorting, trafficking, and ultimately degradation. The complex and integrated events that govern the fates of proteins in the cell are a source of fascination, and the breakdown of these mechanisms leads to disease.

Driven to explore at atomic level the macromolecules involved in these fundamental cell biological processes, the laboratory uses the technique of X-ray crystallography as its foundation. However, we combine crystallography with biochemistry and other structural techniques to answer, as thoroughly as possible, the questions that arise from our structures.

Catalysis of disulfide bond formation

A primary focus of our attention is the set of enzymes, known as sulfhydryl oxidases, that catalyze formation of disulfide bond cross-links in other proteins. We have determined or are in the process of determining the structures of enzymes that perform this reaction in a number of contexts. One enzyme, Ero1, catalyzes disulfide bonding in the endoplasmic reticulum (ER) during protein folding (Figure 1). Another, QSOX, functions later in the secretory pathway (*i.e.*, the Golgi and extracellular environment) and is thought to mediate cross-linking of fibrous extracellular matrix proteins, and assembly of proteins into covalently bonded oligomers. Expression patterns suggest that QSOX plays an important role in neural development. In a third context, sulfhydryl oxidases are found in cells infected by poxviruses and related double-stranded DNA viruses. Disulfide bonds are required for folding and assembly of poxvirus coat proteins in the generally reducing environment of the cell cytosol; since there is no cellular cytosolic sulfhydryl oxidase, these viruses encode their own.

In addition to studying the structures and catalytic mechanisms of sulfhydryl oxidases, we are discovering how some of them are regulated, an important feature to prevent the build-up of reactive oxygen species and oxidative damage to the cell as a side effect of cysteine oxidation. For example, Ero1 can be modulated by oxidation or reduction of a series of regulatory disulfide bonds that tune the activity of the enzyme to the amount of cysteine containing protein substrates in the ER (Figure 1).

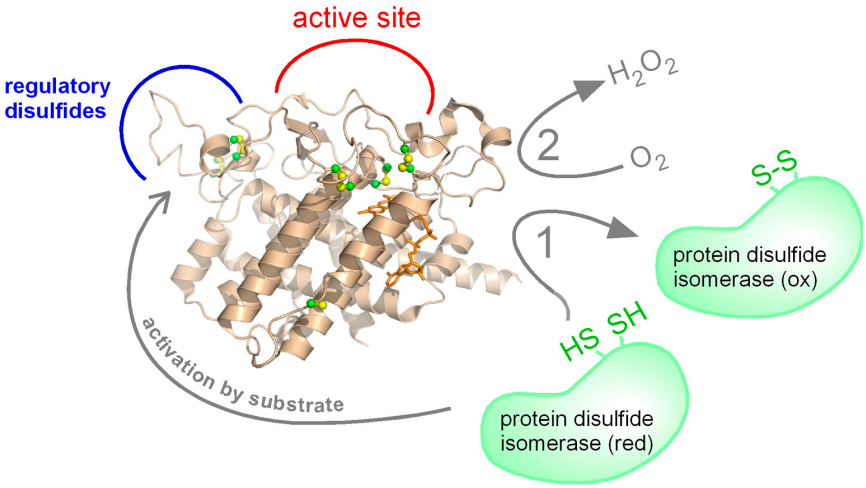

Fig. 1 *Structure of Ero1 determined by X-ray crystallography. Disulfide bonds are shown as balls-and-sticks. The FAD cofactor is orange. The two steps of the "ping-pong" mechanism of the enzyme are 1) oxidation of a substrate such as protein disulfide isomerase and 2) reduction of oxygen to hydrogen peroxide. The resting enzyme is activated by reduction of regulatory disulfide bonds, which occurs when sufficient reduced substrate is present.*

Protein trafficking

A second subject we address is how proteins are sent to various destinations and fates according to the needs of the cell. We have a new project focusing on the translocation of an amino acid permease to the plasma membrane when amino acids in the cell are depleted. We are characterizing a multi-protein complex responsible for recognizing the permease in the "transit hall" of the endosome and shuttling it to the plasma membrane under amino acid starvation conditions. We are exploring the possibility that this protein complex is a novel membrane coat complex that binds cargo and drives vesicle budding.

Furthermore, it is now well known that protein degradation is as important as protein production and localization. We have determined structures of proteins involved in both the protein-specific and the bulk recycling programs in the cell, namely the ubiquitin/proteasome system and autophagy, respectively. Autophagy has been shown to be important for elimination of aggregation-prone intracellular proteins that otherwise lead to "proteinopathies" including various neurodegenerative diseases. By studying complexes between autophagy factors and fragments of scaffolding proteins that surround polyubiquitinated protein aggregates in the cytosol, we are contributing to an understanding of the mechanism by which these protein aggregates are collected and cleared from the cell.

Retroviruses

In addition to our studies of the systems that facilitate protein folding and transport in the cell, we have a

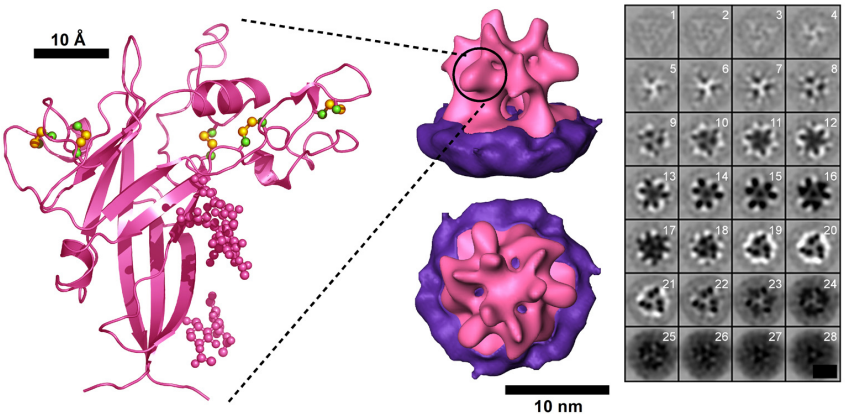

Fig. 2 *Structure of the envelope protein complex of a murine leukemia retrovirus analyzed by a combination of cryo-electron tomography and X-ray crystallography. The glycoprotein trimer is shown in pink in the central panel (side and top views) with a section of virus membrane in purple. On the right are slices through the averaged tomogram. On the left is the high-resolution structure of the receptor-binding domain of the envelope complex, with disulfides and carbohydrates shown as balls-and-sticks.*

long-standing interest in how viruses subvert these systems for their own purposes. The surface or envelope proteins of retroviruses are a case in point. These proteins utilize the disulfide-bond formation and chaperone machinery in the ER to acquire their intricate, "spring-loaded" structures, necessary for the viruses to infect new cells and propagate. We have used a combination of X-ray crystallography and cryo-electron tomography to study the structures and assembly states of these retrovirus envelope proteins (Figure 2).

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