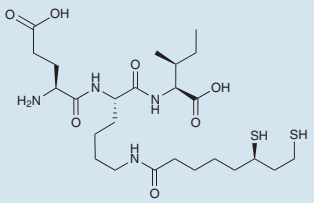
Two small steps for LipA



Lipoyl groups (disubstituted octanoyl chains covalently attached to certain proteins) have important roles in promoting enzyme activity. Lipoyl synthase (LipA) performs the final step in lipoyl-group synthesis by converting an octanoyl substrate to a 6,8-sulfur–modified chain, but the precise mechanism of this reaction was unknown. Now Douglas *et al.* have used a small-molecule substrate surrogate to elucidate the reaction pathway. During the reaction, radicals generated by the reductive cleavage of *S*-adenosyl-L-methionine catalyze the transfer of sulfur atoms from a [4Fe-4S] cluster to the unactivated octanoyl chain. By stopping the reaction prematurely via acidification, the authors could detect the substrate, the product and a monothiolated intermediate. Under nondenaturing conditions, this intermediate remained bound to LipA, a result in agreement with previous observations that LipA provides both of the sulfur atoms to a single substrate. Further analysis of the intermediate showed sulfur incorporation at only the C6 position. The complete absence of C8-monothiolated material confirms that the enzyme proceeds in a specific, stepwise manner. A substantial deuterium isotope effect further suggests that the incorporation of the second sulfur is the rate-limiting step. The dual function of LipA highlights its importance in controlling one type of post-translational modification in the cell. (*Angew. Chem. Int. Edn. Engl.* **45**, 5197–5199, 2006) *CG*

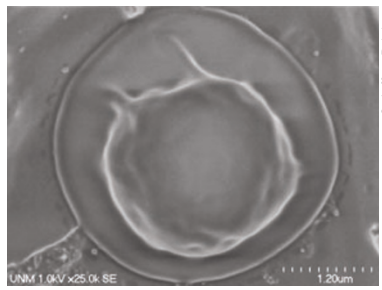
Misfolded glycoprotein ERADication

Proteins destined for secretion fold in the endoplasmic reticulum (ER). Incorrectly folded proteins fail to accumulate in the ER as a result of being degraded by a process called ER-associated degradation (ERAD). As many ER proteins span the membrane with domains on either side, a misfolded segment of a protein may be identified by separate ERAD machinery in the lumen (ERAD-L), in the cytosol (ERAD-C) or within the ER membrane (ERAD-M). From these parallel pathways, substrates are marked for proteosomal degradation. Many questions remain about the ways in which ERAD substrates are initially identified. In a recent issue of *Cell*, Denic *et al.* define early steps involved in the ERAD-L pathway. Yos9p, an ER lectin, is known to have a critical role in recognizing both the sugar and the unfolded peptide segment of misfolded glycoproteins. By affinity purification of proteins associated with Yos9p, the authors identified a protein complex consisting of the lumenal chaperone Kar2p and the transmembrane protein Hrd3p, as well as other transmembrane and cytosolic components of ERAD. Mutation of the Yos9p sugar-binding pocket did not affect the composition of the complex. Using protein mutations and truncations, the authors found that Yos9p binds directly to Kar2p and to the lumenal domain of Hrd3p. Both the Yos9p–Kar2p complex and Hrd3p were able to independently recognize misfolded proteins. As in complex assembly, initial substrate recognition was independent of glycosylation status. These results define an initial sugar-independent recognition step that must precede a sugar-dependent step involving Yos9p. The precise role of this sugar-dependent step in the ERAD pathway remains to be uncovered. (*Cell* **126**, 349–359, 2006) *JK*

tRNA flips out for sulfur

Most cellular RNA sequences start their existence as transcripts composed of the four canonical ribonucleotides, but some become chemically modified to enhance their biological functions. For example, a uridine at the wobble position of the anticodon loop for glutamate, glutamine and lysine tRNAs (U34) is converted to 2-thiouridine (s^2U34). MnmA, the *E. coli* enzyme that catalyzes the thiouridylation of U34, receives the necessary sulfide group (derived from L-cysteine) from an elaborate system of sulfur transferases. Numata *et al.* have recently solved the three-dimensional structure of MnmA and provided a molecular-level glimpse into the mechanism by which this sulfur transfer reaction may occur. The authors reported X-ray crystal structures of three forms of MnmA in complex with $tRNA^{Glu}$. In the open complex, the enzyme cradles the tRNA and specifically recognizes U34 by hydrogen bonding with a glutamine residue. Transformation to the closed complex reorganizes a surface helix to form a lid over the cavity, which induces the U34 nucleotide to flip into the active site. In the presence of ATP, U34 becomes adenylated at the C2 position, and two critical cysteine residues, C102 and C199, that were disulfide linked in the open complex become reduced. C199 accepts a sulfide directly from the sulfur transfer protein TusE; this C199 persulfide intermediate may cooperate with C102, and with Asp99 as a general acid-base, to deliver a sulfide ion to the adenylated tRNA, producing s^2U34. Although additional biochemical studies will be required to clarify this mechanism further, this structural work provides new insights into enzymatic tRNA modification. (*Nature* **442**, 419–424, 2006) *TLS*

Evaporated biosensors



C. Jeffrey Brinker

Scientists have long sought to fabricate biosensors by integrating planar silicon technology with living cells to take advantage of cellular capacity for sensitive detection of biomolecules. But cells cannot survive the harsh conditions of conventional manufacturing, nor can they retain viability without aqueous buffer. Brinker *et al.* now report that lipid surfactants can be used to stably incorporate live cells into silica-based nanostructures. Previously, the authors developed a technique in which dissolved silica and surfactant assemble into mesophases as solvent evaporates. In an effort to introduce cells into this system, the authors experimented with phospholipids as surfactants. Using X-ray scattering, they found that when *Saccharomyces cerevisiae* cells are added to their lipid-silica system, the cells act as "active colloids" to alter the mesophasic behavior of the system from lamellar to hexagonal/cubic. Fluorescence microscopy revealed that the cells surround themselves with a fluid lipid shell that seals in water and enables the cells to survive high-vacuum conditions and remain viable in air for several weeks. Cells incorporated in such planar nanostructures responded rapidly to environmental stimuli, such as a switch from glucose to galactose, even after exposure to dry conditions for 24 hours. Although this technology is at an early stage, biosensors based on this prototype could find wide application in industry and national security. (*Science* **313**, 337–340, 2006) *KM*

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