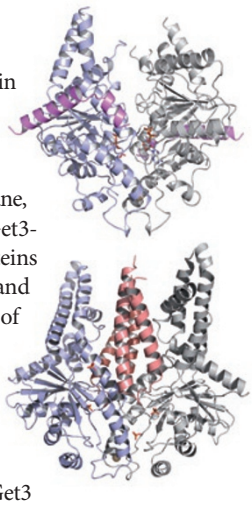
Anchored tails



Tail-anchored (TA) membrane proteins contain a C-terminal membrane anchor and are post-translationally delivered to the ER by the Get3 ATPase. Get3 interacts with the two receptor proteins Get1 and Get2 at the membrane, but the molecular mechanism underlying Get3-dependent membrane insertion of TA proteins has been unknown. Denic, Dötsch, Sinning and colleagues now report the crystal structures of Get3 in complex with the cytosolic domains of Get1 and Get2 in different functional states. Both receptor proteins are required for TA proteins to insert in a nucleotide-dependent manner. The cytosolic domain of Get1, which is necessary and sufficient for Get3 binding, was crystallized in complex with Get3. The Get3–Get1 complex is a symmetric heterotetramer, with two Get3 molecules in the open state and two Get1 molecules binding at the interface of the Get3 dimer. Get1 contacts both Get3 monomers, with one interaction surface providing high affinity while the second interferes with nucleotide binding and forms only with the open state of Get3. The authors also determined the structures of the semi-open Get3–Get1 complex and the closed Get3–Get2 complex. Comparing the two Get3–Get1 structures suggests that Get1 stays bound to Get3 through the high-affinity interface during the transition from the fully closed to the open state. In contrast to Get1, two Get2 molecules bind away from the Get3 dimer interface such that each Get2 molecule contacts only one Get3 subunit. Get1 and Get2, which can bind simultaneously to Get3, share a partially overlapping binding site. The authors propose that TA protein binding locks Get3 in the closed state and that Get2 tethers Get3–TA to the ER membrane. Get1 associates with Get3–TA–Get2, partially displacing Get2 and resulting in an insertion-competent complex. Although the precise timing of ATP hydrolysis is still unknown, the authors favor a scenario in which TA protein binding induces ATP hydrolysis in Get3. Upon interaction with the Get1-Get2 receptor, the stored energy from hydrolysis drives Get3–Get1 through the semi-open to the open state and facilitates TA protein insertion. (*Science* doi:10.1126/science.1207125, published online 30 June 2011) *AH*

Small but mighty

Small molecules have been used to target specific proteins and regulate their activity *in vivo*. Two recent papers describe systems in which small molecules control the levels of target proteins by triggering their degradation. The first report is from Wandless and colleagues, who previously developed systems fusing the protein of interest to destabilizing domains that cause its rapid degradation. One such system used FK506- and rapamycin-binding protein (FKBP) as the destabilizing domain; the small molecule Shield-1 binds and stabilizes FKBP. Now these authors turn the concept around and engineer a version of FKBP with a C-terminal 19-residue tail, which binds to FKBP's active site and stabilizes it. When Shield-1 is added, the C-terminal tail is displaced and acts as a degron to promote rapid degradation of the fusion; a four-residue motif (RRRK) was shown to be sufficient to cause rapid degradation of yellow fluorescent protein (YFP). In the second paper, Crews and colleagues use the commercial HaloTag system, in which the protein of interest is fused to a modified bacterial dehalogenase that can form a covalent bond with synthetic bifunctional ligands. The authors screened HaloTag ligands with different hydrophobic moieties for their ability to destabilize fusion proteins. The rationale was that the fusion protein containing such an exposed hydrophobic tag would be recognized as an unfolded protein and eliminated through the ubiquitin-proteasome system. The selected hydrophobic tag was shown to work well in cell culture and in animal models (zebrafish embryos and mice). Both systems are a welcome addition to the arsenal of tools to manipulate the levels of proteins of interest in cells, in a rapid and efficient way. (*Nat. Chem. Biol.* doi:10.1038/nchembio.598 and doi:10.1038/nchembio.597, published online 3 July 2011) *IC*

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Heavy metal

P1B-type transmembrane ATPases transport copper from the cytoplasm into different organelles or out of the cell, but no atomic structure of a complete P1B-type transporter has previously been available. In humans, both Menkes and Wilson's diseases are linked to defects in copper transport by the P1B-type transporters ATP7A and ATP7B, respectively, underscoring the importance of understanding this process. Now, Møller, Nissen and colleagues report the crystal structure of the P1B-type copper transporter from *Legionella pneumophila*, LpCopA, at 3.2-Å resolution. Their final model presents almost the entire protein, although the heavy-metal binding domain (HMBD) could not be completely resolved. However, there was continuous low-resolution electron density in the region of the HMBD, allowing the authors to approximate its location and mechanism of action. The transport process appears to be quite similar to that used by the calcium transporter SERCA1a. A platform, lined by an amphiphilic MB′ helix and a straight M1 transmembrane helix, encompasses three highly conserved amino acids. These residues can provide a docking site for the HMBD (or a copper chaperone) and an entry site for copper ions, allowing transport through internal membranous ion-binding sites. The ions then exit on the periplasmic side of the transporter. Because of the similarity between LpCopA and ATP7A, the authors were able to map known mutations associated with Menkes disease to their structure. The majority of mutations are found in regions that affect various aspects of transporter function, providing insight into the mechanisms by which these mutations contribute to disease. (*Nature* **475**, 59–64, 2011) *SM*

Mediating transcription

It has become increasingly clear that transcriptional regulation occurs at the elongation step as well as at initiation. It is now known that RNA polymerase II (RNA pol II) pauses near promoters and that the regulation of movement into elongation is complex and involves multiple factors, including those found in a so-called super elongation complex (SEC). Using a proteomics approach followed by an array of functional analyses, Conaway and colleagues have now found that the mediator complex component MED26 plays a crucial role in this regulation. They found that MED26 associates with components of the SECs through its conserved N terminus, and mutational analysis identified residues required for recruitment of these complexes. Further analyses indicated that during steady-state transcription, these interactions are required for RNA pol II to be found in the bodies of genes and for transcription of many genes including *myc*. However, under heat shock, it was found that MED26 is required for appearance of RNA pol II at the promoter. Therefore, these studies identify MED26 as playing a critical role at multiple regulatory steps in transcription, particularly those instrumental to recruitment of SECs and movement into the elongation phase of transcription. (*Cell* **146**, 92–104, 2011) *SL*