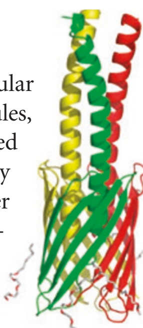
Transporting passengers



For pathogenic Gram-negative bacteria, extracellular virulence factors, which can include adhesion molecules, cytotoxins and invasive proteins, must be transported across both the inner and outer membranes. Many of these proteins are members of the autotransporter family and are comprised of an N-terminal signal peptide adjacent to a passenger (effector) domain and a C-terminal translocator domain. After Sec-dependent secretion across the inner membrane, the translocator domain inserts into the outer membrane, facilitating extracellular translocation of the passenger domain. The autotransporter family can be divided into the monomeric conventional autotransporters and the trimeric autotransporters, which have a short translocator domain that forms SDS-resistant trimers in the outer membrane. With only the structure of a conventional autotransporter, the NalP protease, known, there is debate about how translocation of the passenger domain occurs. The Waksman and St. Geme labs now present the crystal structure of the *Haemophilus influenzae* Hia adhesin trimeric autotransporter translocation domain, providing additional insight into passenger domain translocation. The translocator domain forms a trimeric β-barrel, with four β-strands contributed from each monomer. This channel is spanned by three N-terminal α-helices, one from each monomer, that are crucial for trimer formation. The structure is strikingly similar to that of NalP, which forms a 12-strand β-barrel spanned by a single N-terminal helix, suggesting a common translocation mechanism. Functional analyses support a 'hairpin' translocation model, where the C-terminal region of the passenger domain, which forms a long helix in Hia and NalP, initially adopts a hairpin structure in the β-barrel that allows the rest of the domain to pass from the periplasm to the extracellular surface. (*EMBO J.*, advance online publication 11 May 2006, doi:10.10138/sj.emboj.7601132) *MM*

Relocate as needed

Silent information regulator (Sir) proteins are a family of NAD^+-dependent protein deacetylases. The best-characterized member of this family, Sir2, is a histone deacetylase that is required for the maintenance of silenced chromatin at several locations in *Saccharomyces cerevisiae*. One of the yeast Sir2 homologs, Hst2, is found in the cytoplasm. Although its exact biological function is not clear, Hst2 has been reported to affect nuclear silencing. The mammalian counterpart of yeast Hst2 is SirT2. SirT2 is also localized in the cytoplasm, and the conservation of localization for these homologs from evolutionarily distant organisms suggests a common function. Reinberg and colleagues now show that SirT2 and Hst2 preferentially deacetylate modified Lys16 of histone H4 (H4K16Ac) both *in vitro* and in the cell. Both Hst2 and SirT2 seem to affect H4K16Ac levels globally, in contrast to some other Sir members, which act in a gene-specific manner. H4K16Ac levels change with cell-cycle progression and are highest at S phase but drop substantially at the G_2/M transition. The authors show that SirT2 is localized in the cytoplasm during most of the cell cycle but moves into the nucleus and becomes associated with the chromatin in the G_2/M transition and during mitosis. This observation indicates that SirT2 is responsible for lowering the level of H4K16Ac at these stages of the cell cycle. Accordingly, H4K16Ac levels in $SirT2^{-/-}$ cells remain high during mitosis. Together, these results describe a new and evolutionarily conserved function for SirT2 and Hst2. (*Genes Dev.*, published online 28 April 2006, doi:10.1101/gad.1413706) *HPF*

Research highlights written by Hwa-ping Feng, Boyana Konforti, Sabbi Lall and Michelle Montoya.

AID and the single strand

Antibody gene diversity occurs by two distinct processes: somatic hypermutation and class switch recombination. Somatic hypermutation is initiated by antigen binding and results in the generation of point mutations in the variable regions of immunoglobulin (Ig) genes, allowing the selection of high-affinity antibody variants. Activation-induced cytidine deaminase (AID) is a single-stranded DNA deaminase required for somatic hypermutation. Genetic evidence supports a model in which AID directly introduces base changes in the Ig locus, which are then processed into mutations. Other processes that generate single-stranded DNA would also be predicted to stimulate AID-dependent deamination. For example, transcription has been shown to be strongly correlated with somatic hypermutation both *in vivo* and *in vitro*. Now, Papavasiliou and co-workers recapitulate this mutagenesis *in vitro* using plasmid DNA, recombinant AID, *Escherichia coli* RNA polymerase (RNAP) and nucleoside triphosphates. They find that deamination is not strand specific—cytidines on both the template and nontemplate strand are deaminated, and the frequency of the mutations decreases as the distance from the promoter increases. Both these properties of the *in vitro* system are similar to what is observed at the Ig locus *in vivo*. Finally, the authors show that AID directly interacts with the elongating polymerase *in vitro*. Although the data strongly suggest that AID and transcription are necessary and sufficient for hypermutation *in vitro* and *in vivo*, not all transcribed genes are hypermutated *in vivo*, so the mechanism by which AID is specifically targeted to the Ig locus still remains to be determined. (*Mol. Cell. Biol.* **26**, 4378–4385, 2006) *BK*

This is your PI3-kinase on drugs

The phosphoinositide 3-kinases (PI3-Ks) regulate a range of cellular processes and are potential targets for drug-based cancer therapy. However, this family encompasses 15 members with distinct substrate specificities and complex biological roles, rendering specific PI3-K inhibition difficult. Shokat and colleagues have examined the specificity of an array of small chemical PI3-K inhibitors, expanding on the two pan-specific inhibitors previously in use. They started by examining inhibitors targeting PI3-K subsets *in vitro*, finding that drugs target specific isoforms despite high sequence similarity. They then determined structures of p110γ (a PI3-K) complexed with inhibitors and found, for example, that the inhibitor PIK-39 induces formation of a novel hydrophobic pocket that mediates inhibitor interaction. On the basis of the structures of more potent inhibitors, a phenol moiety predicted to project into a deep affinity pocket was added to PIK-39, creating a compound 20- to 60-fold more active than its parent. Using these new PI3-K inhibitors, the authors were able to dissect the roles of isoforms *in vivo*, a goal previously thwarted by the complex lethal phenotypes of PI3-K mutants. Their analysis indicates that p110α is the main mediator of insulin signaling in adipocytes and myotubes. Finally, the authors examined the effect of their inhibitors on glucose uptake in mice, finding that a p110α inhibitor prevents an insulin-stimulated decline in blood glucose. The study demonstrates the power of using systematic *in vitro* and structural analyses of small chemical inhibitors to drive drug design, while also assessing specific PI3-K functions. Further analysis is required to elucidate the structural basis of inhibition and the biological roles of other PI3-Ks. (*Cell* **125**, 733–747, 2006) *SL*

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