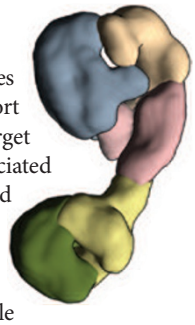
HOPS from head to tail



In eukaryotes, membrane fusion generally involves Rab-GTPase–dependent tethering of the transport vesicle to the SNARE fusion apparatus at the target membrane. GTP-activated Rab GTPases are associated with vesicle or organelle membranes and bind tethering factors to promote SNARE-mediated vesicle fusion. In the endosomal membrane system, some multisubunit tethering complexes (MTCs), such as the conserved homotypic fusion and vacuole protein sorting (HOPS) complex bind to both Rab GTPases and SNAREs. HOPS comprises six subunits: Vps11, Vps16, Vps18, the SNARE-binding Vps33, and Vps41 and Vps39, which both bind the Rab GTPase Ypt7. Raunser, Ungermann and colleagues have determined the structure of the HOPS complex, using EM, giving the first structural view of a Rab-binding MTC. HOPS has a seahorse-like shape with flexible head and tail regions. 3D reconstructions of HOPS subcomplexes and antibody labeling were used to orient the subunits within the HOPS structure. Vps39-Vps11 occupy the bulky tail, and Vps18 forms the rigid backbone connecting head and tail regions. The side of the head is formed by Vps16-Vps33. Surprisingly, Vps41 was localized to the remainder of the head region, far from Vps39. Nanogold labeling confirmed the presence of two Ypt7 binding sites at opposite ends of the HOPS complex. The authors propose that HOPS is initially recruited to late endosomes through Ypt7 binding to Vps39 and Vps41. A reorientation step may occur so that HOPS can associate with phosphoinositides and the highly curved endosomal surface. This would require Vps39 to release endosomal Ypt7, allowing it to interact with Ypt7 on the vacuole membrane and bringing the membranes closer together to allow Vps33 to promote SNARE assembly. (*Proc. Natl. Acad. Sci. USA* **109**, 1991–1996, 2012) *MM*

Inhibiting E2s

UBC13 and UbcH5 are E2 ubiquitin-conjugating enzymes important in DNA damage response that are inhibited by the deubiquitinase OTUB1. This inhibition is independent of OTUB1's catalytic activity, but requires OTUB1's N-terminal region, which enables OTUB1 to bind preferentially to E2~ubiquitin conjugates. Two complementary studies now explore the structural mechanism for OTUB1's inhibitory activity. Unexpectedly, both reveal a requirement for an additional unconjugated ubiquitin molecule bound to OTUB1's distal ubiquitin binding site, promoting tighter binding of OTUB1 to E2~ubiquitin. Wolberger and colleagues present two crystal structures: the *C. elegans* OTUB1 bound to human UBC13 and the ubiquitin aldehyde (Ubal)-conjugated chimeric *C. elegans* OTUB1 (with a human N-terminal region) in complex with ubiquitin-conjugated UBC13. The latter reveals that Ubal binding to OTUB1's distal site causes marked structural rearrangements in OTUB1. Modeling shows that the OTUB1 N-terminal helix would clash with UEV1, an ubiquitin E2 variant required by UBC13 to synthesize Lys63-linked chains, thus inhibiting the UEV1-UBC13 interaction and Lys63-linked polyubiquitination. Durocher, Sicheri and colleagues report the crystal structure of human OTUB1 bound to ubiquitin-conjugated UBCH5B, showing OTUB1 engaged with UBCH5B and free ubiquitin. The structure highlights how coupling between OTUB1's proximal and distal ubiquitin-binding sites, critical for deubiquitinase activity, is co-opted for inhibiting E2 enzymes. OTUB1 binding occludes the E3 binding site on UBCH5B and UBC13, providing a general mechanism for the inhibition of E2s. Mutations that abrogate OTUB1's inhibition of ubiquitination in the DNA damage response are located at the interface of OTUB1 with either UBCH5B or ubiquitin; such mutants cannot suppress UBC13 activity *in vitro*. (*Nature* doi:10.1038/nature10911, published online 22 February 2012; *Mol. Cell* **45**, 384–397, 2012) *SM*

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Twist in the RNAPII tail

Silencing mediated by Polycomb repressor complexes (PRCs) in embryonic stem cells (ESCs) can be accompanied by histone marks typical of active genes and RNA polymerase II (RNAPII) presence. A study by Pombo and colleagues analyzes this correlation between seemingly antagonistic Polycomb and RNAPII complexes, referred to as chromatin bivalency, at the genome-wide level. ChIP-seq analyses of PRC repression markers and RNAPII states in ESCs revealed that PRC targets exhibit a range of RNAPII variants. Silent, developmental PRC target genes were generally only associated with RNAPII with phosphorylated Ser5 in the C-terminal tail of RNAPII's largest subunit (RNAPII-S5p) at promoters and throughout coding regions. RNAPII-S5p produced transcripts that did not mature into mRNA and were not translated. Derepression of PRC target genes correlated with RNAPII-S5p levels, suggesting both physical and functional synergy between PRCs and RNAPII-S5p. A group of active genes was also associated with PRC binding, and many of these genes have roles in metabolism. These active PRC targets switch between PRC-repressed (S5p only) and PRC-active (S5p, S2p, S7p) states within the ESC population. It is thought that fluctuation from the PRC-repressed state to the active state occurs to variable extents across different PRC targets, resulting in different expression levels. (*Cell Stem Cell* **10**, 157–170, 2012) *AH*

Restoring order

The molecular chaperone Hsp33 undergoes unfolding and activation in response to oxidative stress conditions in the cytoplasm. These changes primarily involve Hsp33's C-terminal redox switch domain, which contains a flexible linker and a redox-sensitive center. In its unfolded state, Hsp33 interacts with client proteins, preventing them from misfolding and aggregating. Upon restoration of the normal, reducing environment in the cytoplasm, Hsp33 refolds and releases its clients to the DnaK chaperone system. Now Jakob and colleagues reveal how Hsp33 exerts its protective effect on protein substrates. The authors use peptide arrays with sequences from known client proteins to determine binding specificity. Together with solution studies using peptides of known structures, they find that Hsp33 prefers peptides with secondary structure over unfolded ones. This suggests that Hsp33 interacts with early unfolding intermediates and explains how it avoids binding its own unfolded redox switch domain. By limited proteolysis or hydrogen-deuterium exchange, coupled to MS analyses, the authors establish that it is the linker region within the unfolded redox switch domain that binds substrates, and notably, this interaction leads to stabilization of the linker itself. Finally, the authors follow a model substrate protein: upon refolding of Hsp33, the interacting regions within the substrate become destabilized, indicating that they undergo further unfolding, to be refolded by DnaK. Altogether, these results provide comprehensive insight into how this ATP-independent chaperone uses reversible order-to-disorder transitions to protect substrates from misfolding and to foster productive refolding. (*Cell* doi:10.1016/j.cell.2012.01.045, published online 2 March 2012) *IC*