the deletion mutant *RAD6Δ* and *SUG1-25* yeast had similar defects in gene silencing, and, although the *SUG1-25* mutation has no effect on H2B ubiquitylation, *RAD6Δ* yeast are unable to recruit Rpt6 to active promoters. Furthermore, ChIP analysis showed that a point mutation in the gene encoding H2B, which prevents ubiquitylation of H2B, also inhibits Rpt4 recruitment to chromatin.

So, Ezhkova and Tansey propose that H2B ubiquitylation by Rad6 recruits proteasomal ATPases to promoters. The proteasome components then move along the gene with RNA polymerase II, and use their ATP-dependent chaperone activity to reconfigure chromatin and allow access of histone methyltransferases to their target lysine residues — thereby coupling H2B ubiquitylation to transcription-dependent methylation of H3.

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References and links

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MEMBRANE TRAFFICKING

Adapting to a new role

In *Nature Cell Biology*, two papers now show that the Golgi-localized, γ-ear-containing, Arf-binding (GGA) family of adaptor proteins can interact directly with ubiquitin. Studies from Puertollano and Bonifacino, and the Piper and Boman laboratories, have found that these adaptors seem to have a new role in sorting ubiquitylated cargo at mammalian endosomes and the yeast *trans*-Golgi network (TGN), respectively.

GGAs are mainly associated with the TGN, where, in mammalian cells, they are known to sort mannose-6-phosphate receptors (MPRs) by binding to acidic-cluster–dileucine motifs in the MPR cytoplasmic tails. However, in the first study, Puertollano and Bonifacino showed that mammalian GGAs also colocalize with early endosomes. Using RNA interference, they showed that depleting GGA3 levels caused MPRs and internalized epidermal growth factor (EGF) to accumulate in enlarged early endosomes, which indicates that these proteins have entered a compartment they cannot leave.

These effects are similar to those seen after depleting proteins of the endosomal machinery that selects ubiquitylated cargo for delivery to the degradation pathway (ubiquitylated EGF receptors with bound EGF are sorted into this pathway). The authors therefore tested whether GGAs can bind ubiquitin and found that they can: GGA3 has the highest avidity of the mammalian GGAs and its VHS–GAT region is responsible for this binding.

In the final part of their study, Puertollano and Bonifacino showed that this region of GGA3 can also bind a subunit of the endosomal machinery mentioned above. They therefore propose that the effects of GGA3 depletion "…reflect a function of this protein in endosomes, which may be mediated by its interaction with the ubiquitin sorting machinery". However, they note that mammalian GGAs might also sort ubiquitylated cargo at the TGN.

The residues that mammalian GGAs use to bind acidic-cluster–dileucine motifs are not conserved in yeast Ggas. So, in the second study, Piper and co-workers used a yeast two-hybrid screen to identify sorting motifs that interact with Ggas. And, they found that Gga2 binds ubiquitin directly through its GAT domain.

Next, they investigated the role of Ggas in the trafficking of general amino-acid permease-1 (Gap1). Gap1 is expressed at the cell surface when the nitrogen source is poor. However, when the

nitrogen source improves, cell-surface Gap1 is ubiquitylated and sorted into the degradation pathway, and newly made Gap1 is ubiquitylated and sorted directly from the TGN to endosomes (and so bypasses the plasma membrane).

The authors found that, in the absence of Ggas, Gap1 is sorted from the TGN to the cell surface when it would usually be directed to endosomes. In addition, the endocytic delivery of cell-surface Gap1 to the degradation pathway seemed to be compromised. When they monitored Gap1 trafficking in the presence of Gga2 lacking its GAT domain, they again found that the TGN-to-endosome sorting of Gap1 is disrupted. However, in this case, the endocytic delivery of cell-surface Gap1 was mostly unaffected.

Piper and colleagues therefore propose that yeast Ggas bind ubiquitylated proteins at the TGN and divert them away from the secretory pathway to endosomes. And, together, these two studies have highlighted a new role for GGAs as ubiquitin-sorting receptors in membrane-trafficking events.

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WEB SITES

Juan Bonifacino's laboratory:
http://dir2.nichd.nih.gov/nichd/cbmb/Juan_Bonifacino.html

Robert Piper's laboratory:
http://www.physiology.uiowa.edu/faculty/faculty/piper.htm