RESEARCH HIGHLIGHTS

Secretion hijacks autophagosomes

Canonical secretion relies on an endoplasmic reticulum (ER)-targeting signal sequence that directs proteins through the Golgi to the plasma membrane. However, many secreted proteins do not carry this signal and reach the surface through alternative routes. In two papers, Malhotra and colleagues and Subramani and colleagues describe the unconventional path taken by Acb1 (acetyl-coenzyme A binding protein) in *Saccharomyces cerevisiae* and *Pichia pastoris*, respectively (*J. Cell Biol.*, **188**, 527–536 and 537–546; 2010). Both papers show that starvation stimulates Acb1 secretion, which is unperturbed by mutations in several genes involved in conventional secretion. Secretion does require the Golgi-associated protein GRASP and is blocked by mutations that impair the formation of autophagosomes – compartments that deliver their contents to the vacuole for degradation. However, mutations blocking autophagosomal fusion with vacuoles did not stop Acb1 secretion. Autophagosomes have been shown to sometimes fuse with recycling endosomes and multivesicular bodies (MVB) before being targeted to lysosomes. Acb1 secretion was impaired by mutations in recycling endosomes and MVB ESCRT (endosomal sorting complex required for transport) components. It was also found that Acb1 secretion in *S. cerevisiae* requires a plasma membrane specific t-SNARE fusion factor and *P. pastoris* Acb1 secretion depends on a phospholipase D, a known regulator of vesicle fusion with the plasma membrane. Taken together, these data make a strong case for Acb1 being secreted through a route involving autophagosomes, endosomal compartments and plasma membrane-specific fusion factors. NLB

The rules of kinase engagement

The systematic identification of kinase–substrate relationships has been beset by difficulties, as relationships defined in high-throughput screens are frequently not recapitulated *in vitro* or *in vivo*. Benjamin Turk, Michael Snyder and colleagues have now developed an approach in *Saccharomyces cerevisiae* that both predicts how kinases recognize their targets and identifies novel substrates (*Sci Signal.* **3**, ra12; 2010). By screening a library of peptides containing a central Ser or Thr phosphoacceptor site, the authors defined phosphorylation motifs for 61 of the 122 known yeast protein kinases. For each kinase, these analyses revealed those residues flanking the phosphoacceptor site that were most sensitive to substitution, and showed that related kinases tended to phosphorylate similar target motifs. The authors also developed a "kinase code" for determining which amino acids on the kinase are important for substrate recognition.

The MOTIPS motif analysis pipeline was next employed to predict potential protein substrates from the results of the peptide screen. *In vitro* assays confirmed that most of the predicted proteins were indeed *bona fide* substrates. Intriguingly, these analyses uncovered several previously unappreciated targets for well-studied kinases, and also identified substrates for kinases about which little is known. These results provide insight into the substrate selectivity of eukaryotic protein kinases, and suggest that robust high-throughput proteomics analyses can be used to infer the function of poorly characterized kinases. EJC

Replacing histones during spermatogenesis

During the postmeiotic stages of spermatogenesis, nucleosomal histones are initially replaced by transition proteins and subsequently by protamines, which promote DNA condensation and packaging of DNA into sperm heads. The mechanisms underlying meiotic sex chromosome inactivation (MSCI) and nucleosome removal are unclear but histone ubiquitylation is thought to be important. Yu and colleagues now show that histone H2A and H2B ubiquitylation by the E3 ligase RNF8 promotes acetylation of Lys16 of histone H4 (H4K16ac) — a crucial modification for the replacement of histones by protamines — by recruiting the acetyltransferase MOF to chromatin. (*Dev. Cell* **18**, 371–384; 2010).

RNF8-deficient male mice are infertile, but don't have defects in MSCI, suggesting that the spermatogenic defects occur after meiosis. Indeed, late spermatid development and DNA condensation are compromised in RNF8-deficient mice. Chromatin-associated transition proteins are also reduced in mutant testes suggesting that histones fail to be replaced in sperm lacking RNF8. Consistent with this, very little protamine is detected in the mutant testes. Quantitative assessment of histone modifications shows that not only ubiquitylation of H2A and H2B, but also H4K16 acetylation, are decreased in RNF8-deficient elongating spermatids. The authors demonstrate that mutant testes also have less chromatin-bound MOF, and cell culture experiments place MOF-mediated H4K16 acetylation downstream of H2A and H2B ubiquitylation. This study reveals that histone ubiquitylation and histone H4K16 acetylation have important roles in global histone replacement in spermatids. FC

Fast-tracking transdifferentiation

Fibroblasts can be driven into a pluripotent state through the expression of just a few transcription factors, raising the question of how irreversible cell differentiation is. Vierbuchen *et al.* (*Nature* **463**, 1035–1041; 2010) now show that expression of three transcription factors can also directly trigger the transformation of fibroblasts into neurons.

Vierbuchen *et al.* initially found that a pool of 19 factors previously linked to neural development could drive reprogramming of mouse embryonic fibroblasts (MEFs) into neuronal cells, marked by TauEGFP. They then narrowed this down to a set of critical factors: although Ascl1 alone could produce cells with some neuronal characteristics, expression of Ascl1 in combination with Brn2 and Myt1l produced *bona fide* neurons from MEFs or postnatal fibroblasts. These 'induced neuronal' cells expressed key neuronal markers, produced action potentials, contained ligand-gated ion channels and formed excitatory synapses in culture.

Previous examples of transdifferentiation have been restricted to switches in cell types within a given lineage. This new work shows that cells can be driven to switch fate directly to an unrelated lineage, and the need to first enter an undifferentiated state can be bypassed. The therapeutic potential of the induced neuronal cells will become clear when experiments are extended to human cells and to *in vivo* models. AS

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