RESEARCH HIGHLIGHTS

Akt-ing to control lipid metabolism

The liver responds to insulin by blocking gluconeogenesis and stimulating lipogenesis. Insulin-induced lipid synthesis requires induction of the transcription factor SREBP1c, which is mediated by the activation of mTORC1 (mammalian target of rapamycin complex 1), through the Akt-dependent inhibition of the TSC1–TSC2 (tuberous sclerosis protein 1 and 2) complex. Yecies *et al.* now identify an mTORC1-independent pathway that induces SREBP1c to promote lipogenesis in the liver (*Cell Metab.* **14,** 21–32; 2011).

To elucidate the role of mTORC1 in the regulation of hepatic lipid metabolism, Yecies *et al.* genetically ablated *Tsc1* to generate a liver-specific, insulin-independent mTORC1 gain-of-function model (*LTsc1KO*). Surprisingly, rather than increase lipid accumulation, sustained mTORC1 activity protected the mice from liver steatosis. This was due to decreased hepatic SREBP1c expression and impaired lipogenic gene expression, indicating that mTORC1-independent mechanisms were also required for SREBP1c induction and lipogenesis.

The *LTsc1KO* mouse phenotype is similar to that of liver-specific *Akt2* knockout mice and indeed *LTsc1KO* hepatocytes exhibited reduced Akt2 activity, consistent with reports that mTORC1 inhibits the insulin-Akt response through negative-feedback mechanisms. The authors established the importance of Akt in this context, by rescuing the lipogenic defect of *LTsc1KO* hepatocytes with constitutively active Akt2, and showed that in hepatocytes Akt2 represses INSIG2, an inhibitor of SREBP1c induction. These data uncover an mTORC1-independent mechanism for Akt-mediated regulation of liver lipogenesis. AIZ

This way up: Septins guide microtubules

Formation of a polarized epithelial cell requires rearrangement of the cell's microtubules. As the flat cell rises up into a column, the microtubules are formed into a complex network consisting of bundles aligned from the top to the bottom of the cell, and a meshwork of shorter filaments underneath the basal and apical membranes. Bowen *et al.* (*J. Cell Biol.* **194,** 187–197; 2011) now show that septin GTPases are needed to guide the microtubule network.

Using immunofluorescence microscopy, the authors found that in polarizing MDCK cells septin filaments overlap with perinuclear microtubule bundles and the distal ends of peripheral microtubules. Knockdown of SEPT2 (a member of the septin family) or expression of a dominant-negative SEPT2 mutant disrupted the spatial organization of the microtubules within the cell, compared with controls. To understand this, the authors performed time-lapse microscopy, which showed that microtubules moved along septin filaments as they grew. Knockdown of SEPT2 resulted in a loss of microtubule directionality and the microtubules ended up becoming entangled. Furthermore, SEPT2 knockdown increased microtubule shrinkage, suggesting septins inhibit microtubule depolymerization and thus maintain persistent microtubule growth.

Hence, septin control of microtubule growth and directionality seems to allow for correct organization of microtubules in establishing a polarized epithelial cell. GD

SNARE proteins regulate autophagosome biogenesis

Pre-autophagosomal structures mature into phagophores and autophagosomes on induction of autophagy. However, the mechanisms governing autophagosome biogenesis and maturation are incompletely defined. Two reports in *Cell* now illuminate the requirement for SNARE proteins in the formation and maturation of autophagosomes (*Cell* **146,** 290–302; 2011 and *Cell* **146,** 303–317; 2011).

Previous studies have implicated Atg8 in expansion of the phagophore membrane in yeast. Klionsky and colleagues found that Atg8 is not required for membrane hemifusion; instead, they detected an important role for Q-SNARE and R-SNARE proteins in autophagosome formation. These SNAREs were essential for recruitment of Atg9 to the phagophore assembly site and for subsequent autophagosome biogenesis.

In mammalian cells, Atg16L associates with pre-autophagosomes and phagophores, but is absent from mature autophagosomes. Rubinsztein and colleagues found that the SNARE protein VAMP7 co-localizes with Atg16L on autophagosome precursors. Depletion of VAMP7 or its partner SNAREs caused accumulation of Atg16L-positive vesicles, but blocked generation of mature autophagosomes. The authors went on to show that these SNAREs mediate homotypic fusion of vesicles that are Atg16L-positive, but LC3-negative, and as such are crucial for autophagosome maturation.

These reports provide compelling insight into distinct roles for SNARE proteins in autophagosome biogenesis. EJC

TALEs from the genetic engineering of stem cells

Owing to the peculiar properties of their DNA-binding specificity, transcription activator-like effectors (TALEs) from the plant pathogen *Xanthomonas* can be engineered to bind virtually any DNA sequence. Jaenisch and colleagues exploit this property to drive targeted modifications in both human embryonic stem cells and induced pluripotent stem cells (*Nat. Biotechnol.* **29,** 731–734; 2011), in which genetic engineering by homologous recombination is inefficient.

TALEs bind DNA through 34-amino-acid-long repeats, in which two amino acids per repeat are hypervariable to recognise a specific base. Rearrangements of TALE repeats allow the generation of novel DNA-binding specificities. When TALE domains are coupled to an endonuclease, TALENs can target endogenous genes, creating deletions or fusion proteins to monitor expression. The authors used this system to target five different genomic sites in pluripotent stem cells and generated clones expressing fusion proteins of both the protein OCT4, which is expressed in pluripotent cells, and the protein PITX3, which is only induced following differentiation.

In a separate study, Jaenisch and colleagues used zinc-finger nucleases, which have previously been developed for genetic engineering in pluripotent stem cells, to create isogenic human pluripotent stem cells differing only in the presence of specific α-synuclein variants that had been linked to early onset of Parkinson's disease (*Cell* **146,** 318–331; 2011). Using zinc-finger technology, however, can be laborious as extensive optimization is required to construct nucleases that would target unique sites of the genome. TALENs, with their defined combinatorial properties, could become the future of genetic engineering in stem cells. NLB

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