RESEARCH HIGHLIGHTS

Membrane sculptor kicks off endocytosis

Clathrin-mediated endocytosis allows cells to internalize ligands through the invagination of plasma membrane to form vesicles. For this to occur, the membrane needs to be bent into shape by proteins such as clathrin (which forms a scaffold around the vesicle) and adaptor proteins such as AP-2 (which connect the clathrin coat to transmembrane cargoes). Henne *et al.* now show that FCHo proteins (FCHo1/2), thought to function in sculpting vesicle shape, also have an essential earlier role in the initiation of vesicle formation (*Science* **328,** 1281–1284; 2010).

The authors found that RNAi knockdown of the FCHo proteins abolished vesicle formation, whereas overexpression increased vesicle budding rate and ligand uptake. FCHo1/2 were found to mark sites of vesicle formation and interact with the scaffolding proteins Eps15 and intersectin, which in turn recruit AP-2. FCHo proteins contain an F-BAR domain, which recognizes and induces curvature in membranes. Mutation of crucial residues in the F-BAR domain of FCHo2 resulted in loss of membrane binding and sculpting, and prevented the correct formation of vesicles.

The authors propose that FCHo1/2 are key proteins in the recruitment of downstream components and generation of curvature during the formation of vesicles in a range of cells. GD

Cdks target Vps34 to control autophagy

Autophagy is an important degradation pathway during nutrient-poor conditions. The class III PI3 kinase, Vps34, has an essential role in autophagy that is dependent on its interaction with Beclin 1. Cyclin dependent kinases (cdks) mainly regulate the cell cycle, but Cdk5 controls neuronal development and function. Yuan and colleagues (*Mol. Cell* **38,** 500–511; 2010) provide a new link between cdks and autophagy: Cdk1 and Cdk5 phosphorylate Vps34 to inhibit its interaction with Beclin.

The authors found that mitotic cells have fewer autophagosomes and reduced levels of the PI3K product, phosphatidylinositol-3-phosphate (PtdIns3P). They identified a putative site (Thr 159) for the mitotic kinase Cdk1 on Vps34, verified that it is a target for Cdk1/Cyclin B1, and found that phosphorylation of this site increases during mitosis.

Cdk5/p25 (p25 is a Cdk5 activator) also phosphorylates Vps34 *in vitro* on three sites including Thr 159. In mice, induced expression of p25 increases Thr 159 phosphorylation of Vps34 in the brain, demonstrating that this event occurs *in vivo*. Thr 159 is situated in the Beclin-binding region and overexpression of Cdk5/p25 inhibits the Vps34–Beclin interaction in a manner dependent on Thr 159 phosphorylation. Furthermore, under serum starvation conditions, p25 expression reduces Vps34 kinase activity, PtdIns3P-production and autophagy.

These data indicate how autophagy may be regulated during mitosis and could be important to understand how cdks act in neurodegeneration. CKR

Advancing pore assembly

In budding yeast, the process of *de novo* nuclear pore complex (NPC) assembly has remained elusive but is thought to require three integral membrane nucleoporin proteins. Doye and colleagues now identify Pom33 as a fourth integral membrane protein that has so far gone unnoticed at the pore. They find that Pom33 shows dynamic association with NPCs and is important for their normal membrane distribution (*J. Cell Biol.* **189,** 795–811; 2010).

Pom33 was previously found in a screen for yeast genes that show synthetic-lethality with the nucleoporin Nup133. Doye and colleagues now show that Pom33–GFP is dynamically associated with the pore, and this behaviour is conserved in a vertebrate homologue. Pom33 is predicted to be a multipass transmembrane protein and shows strong genetic interactions with key pore components — both members of the Nup84 complex and the Ndc1 network. It associates physically with Rtn1, a membrane protein implicated in pore assembly. Loss of Pom33 triggers clustering of pores and also affects the density of pores in the daughter bud. This, together with the observation that depletion of Nup170 in a Pom33 mutant results in pore defects, suggests that Pom33 is important for efficient pore assembly and stabilization.

From the predicted structure of Pom33, the authors speculate that it may act to stabilize the interface between the pore and the membrane during assembly. Whether its function is conserved in vertebrates remains to be seen. AS

Actin and exocytosis coordinate neurite formation

In mice cortical neurons, Ena/VASP-regulated actin dynamics are required for filopodia formation during the extension of the neurites that will become axons and dendrites. In the absence of Ena/VASP, some neurites extend through a laminin-rich extracellular matrix, although it has been unclear how. Gupton and Gertler (DOI 10.1016/j.devcel.2010.02.017) have found that laminin controls actin dynamics through the Arp2/3 complex.

To inhibit VASP-mediated neuritogenesis and follow only laminin-dependent events, the authors artificially tethered VASP to the mitochondria. They found that laminin activates neuronal integrins, which then recruit the Arp2/3 actin nucleation complex through FAK and Src kinase activation, switching filopodia formation away from Ena/VASP-dependent actin dynamics.

Rapid insertion of membrane and proteins in the growing neurites is essential to drive the increase in surface area that accompanies neuritogenesis, but a role for regulated exocytosis, which depends on the activity of specific SNARES for membrane fusion events, is unclear. The authors monitored exocytic events downstream of laminin and Ena/VASP following treatment with tetanus neurotoxin (which inhibits the SNARES VAMP1, 2 and 3) and an N-terminal fragment of VAMP7 (which functions as a dominant-negative). They find that neuritogenesis requires regulated exocytosis and that laminin–Arp2/3 acts through VAMP7-mediated fusion, whereas Ena/VASP acts through VAMP2. Thus, two routes, each using a specific machinery to control actin dynamics and regulated exocytosis, drive neurite formation. Understanding how they are both coordinated will require further studies. NLB

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