In This Issue

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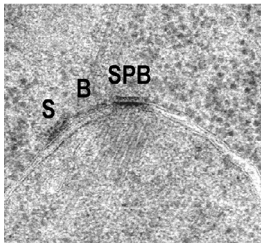
Building a bridge to a new spindle pole body



In a wild-type cell, a satellite SPB (S) begins to assemble at the end of a bridge (B) extending from a mother SPB.

Seybold et al. describe how three proteins interact to form a structure that initiates the duplication of spindle pole bodies (SPBs), the yeast equivalent of centrosomes.

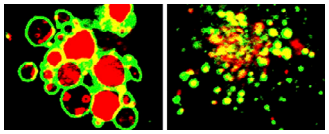
Budding yeast SPBs are embedded in the nuclear envelope and must be duplicated once per cell cycle. A structure called the half bridge juts out from the SPB on both the nuclear and cytoplasmic sides of the nuclear envelope. The cytoplasmic part of this structure contains ~60 Sfi1 molecules, aligned in parallel with their N termini embedded in the SPB's central plaque. In G1, the distal C-terminal domains of these Sfi1 molecules bind to the C termini of additional Sfi1 molecules, extending the half bridge into a bridge. A new SPB then assembles around the Sfi1 N-terminal domains exposed at the far end of this structure. Two other proteins, Kar1 and Cdc31, localize to the cytoplasmic region of the half bridge/bridge, but their roles in SPB duplication remain unclear.

Seybold et al. used super-resolution microscopy to show that Kar1, an integral nuclear membrane protein, localizes to the center of the bridge. Accordingly, Kar1 bound to the C-terminal region of Sfi1 that forms this part of the structure. In the absence of Kar1, the bridge arched away from the nuclear envelope as if it was no longer anchored to the outer nuclear membrane. Artificially cross-linking Sfi1 to Kar1's transmembrane domain flattened out the bridge and restored cell viability.

Cdc31, a homologue of the centrosomal protein centrin, binds to both Kar1 and Sfi1. Seybold et al. found that the protein stabilizes the half bridge/bridge by cross-linking neighboring Sfi1 molecules. Indeed, says senior author Elmar Schiebel, all three proteins stabilize each other's presence in the structure in order to promote SPB duplication.

Seybold, C., et al. 2015. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201412050

P2X4 promotes lysosome fusion



Lysosomes (red) are enlarged in a cell (left) expressing an active version of P2X4 (green). This effect is blocked when the channel's activity is inhibited (right).

The calcium channel P2X4 stimulates lysosome fusion by activating calmodulin, Cao et al. reveal.

The fusion of lysosomes with each other and with other organelles requires calcium release from the lysosome, but what triggers this release, how calcium crosses the lysosomal membrane, and how it stimulates fusion, remains unknown. Cao et al. focused on the role of lysosomal P2X4, an ATP-activated calcium channel regulated by luminal pH.

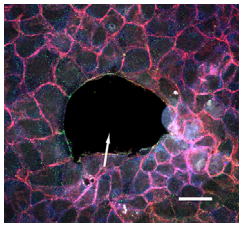
Because ATP is always present in the lysosomal lumen, Cao et al. think that luminal pH may be an important regulator of P2X4 activity and lysosome fusion. Overexpressing the channel in cells led to the formation of abnormally large lysosomes. Increasing the pH of the lysosomal lumen also caused enlarged lysosomes and stimulated lysosome fusion. These effects were blocked by knocking down P2X4, inhibiting the channel, or chelating cytoplasmic calcium ions.

The researchers then turned their attention to how calcium induces organelle fusion after its release into the cytosol. Calcium stimulated P2X4's association with calmodulin, thereby recruiting this calcium-binding protein to lysosomal membranes. Inhibiting calmodulin blocked P2X4's ability to promote lysosome enlargement, indicating that calmodulin acts downstream of the calcium channel, perhaps by binding to the SNARE proteins that drive lysosome fusion.

Senior author Xian-Ping Dong now wants to investigate how P2X4's activity is coordinated with the function of another lysosomal calcium release channel, TRPML1, which he believes has a distinct role in promoting lysosome fission.

Cao, Q., et al. 2015. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201409071

Kinases KISS and tell



Rho-kinase (blue), Scrib (red), and Shroom2 (green) colocalize at the contractile edge of an epithelial wound.

Amano et al. describe a new method to identify the substrates of protein kinases.

There are ~500 protein kinases in the human genome and, collectively, they regulate a wide variety of cellular processes by phosphorylating numerous substrate proteins. Most of these substrates remain unknown, however, so Amano et al. devised a new technique, called kinase-interacting substrate screening (KISS), to identify kinase targets.

The researchers first used the KISS approach to look for substrates of Rho-kinase. The catalytic domain of this enzyme was loaded onto affinity beads, which were then incubated with rat brain lysates in the presence or absence of ATP. Sensitive mass spectrometry techniques were then used to identify the bound substrate proteins and determine which of their residues were phosphorylated. In total, the researchers catalogued 356 phosphorylation sites on 140 different proteins, most of which had not previously been identified as Rho-kinase substrates.

Amano et al. verified several of these new substrates, including the cell polarity protein Scrib. Phosphorylation of a serine residue in Scrib's C-terminal domain enhanced the protein's assembly into a ternary complex with Rho-kinase and the actin-binding protein Shroom2. This complex preferentially localized to the free edges of epithelial cells, where it promoted myosin light chain phosphorylation and actomyosin contractility.

The researchers successfully applied KISS to eight other kinases, each of which phosphorylated a distinct set of proteins. Senior author Kozo Kaibuchi plans to deposit his team's results in a public database.

Amano, M., et al. 2015. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201412008