In This Issue

Pilings in a lipid sea

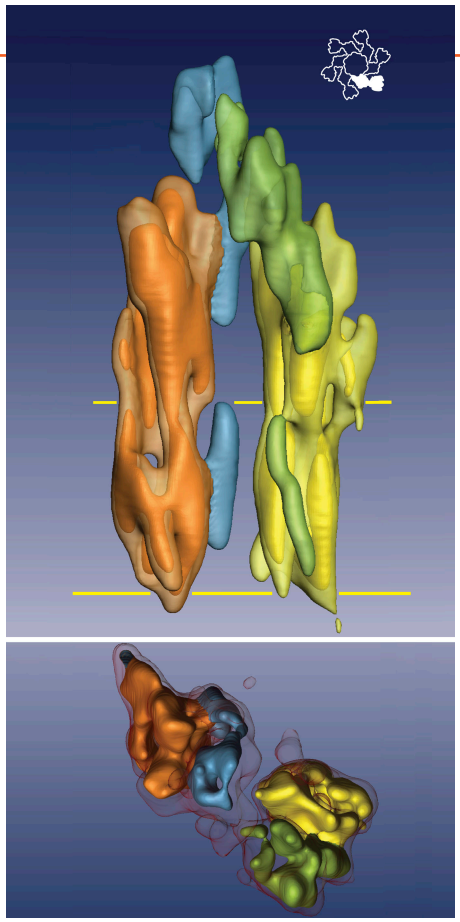
Tetraspanin contains a tightly packed quartet of transmembrane helices, according to a new, high resolution electron microscopy structure deduced by Min et al. (page 975). The rigid tetraspanin proteins may thus act as stable pilings in a lipid sea, say the authors.

Tetraspanins associate with a number of important transmembrane proteins such as integrins to form distinct signaling networks, called tetraspanin webs. Lipids trapped in the networks create microdomains with characteristic compositions and unique properties.

The web under study here was made up of uroplakins. Two uroplakin tetraspanins each pair with a single transmembrane partner forming a heterotetramer subunit, six of which then form a 16-nm wide, ring-shaped particle. A two-dimensional crystalline array of these particles contributes to a remarkable urothelial permeability barrier, which keeps urine on one side and body fluid on the other.

These arrays are particularly suitable for electron microscopic studies. At 6 Å resolution, the team could assign secondary structures to certain regions of the particle. The angle between membrane-traversing α-helices is minimal, so that the helices can pack tightly together. Each single transmembrane partner is shaped like an L that covers the tetraspanins and connects to a neighboring subunit.

The relatively rigid tetraspanin structure is ideal for docking other tall signaling transmembrane proteins. Tetraspanins can also help these proteins to pass messages into the cell, and are themselves the receptors and signaling conduits for some bacteria and viruses. Future structural studies should reveal how these signals are transduced to trigger a wide variety of cellular responses. JCB

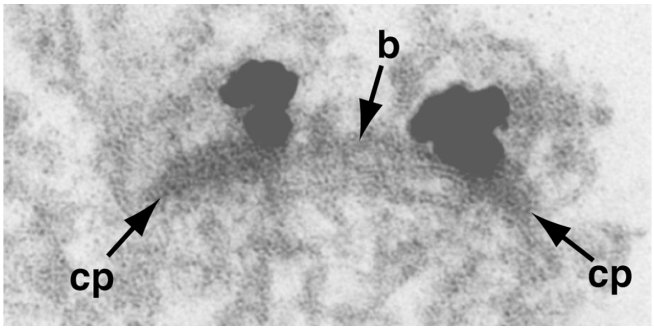


Tetraspanin forms a rigid structure.

SPB building blocks

A straight rod of Sfi1p appears to act as a simple building block that aids the doubling of the budding yeast spindle pole body (SPB), say Li et al. (page 867).

For many years, John Kilmartin and colleagues have painstakingly tracked, using electron microscopy (EM), the duplication of the SPB, which is budding yeast's version of the centrosome. The painstaking part comes in because only a few sections happen to catch the tiny SPB in the act. The SPB has a protrusion called the half bridge. At some point this half bridge doubles in size. Another SPB accumulates on the far end of this structure, then the two SPBs split apart. Before the splitting occurs, the far ends of the two half bridges are too close to be resolved by regular light microscopy.



Sfi1p reaches from its NH_2 terminus (black) at each central plaque (cp) across the bridge (b).

The group now takes a look at two half bridge proteins: Sfi1p, and the Cdc31p centrin. Sfi1p has ~20 repeats that are thought to bind centrin. Crystal structures of 2–3 Sfi1p repeats with bound centrin now reveal that Sfi1p forms a straight rod of α-helix with one centrin bound to each repeat. The centrins line up along the long axis of the Sfi1p but also coil around it and interact with each other end-on-end. The coiling means that side-to-side interactions between filaments are infrequent, perhaps providing space for bridge-nucleated microtubules to sneak through the structure and access the rest of the cell.

An Sfi1p-centrin complex with 15 repeats, examined by EM, is also a long rod. Extrapolating its length to the in vivo situation is consistent with the rod determining the length of the half bridge. Finally, the group shows that Sfi1p's NH_2 terminus is at the SPB whereas the COOH terminus is at the far end of the half bridge (or the center of the structure once the half bridge duplicates).

Kilmartin suggests that the half bridge duplicates when an unknown signal causes two copies of Sfi1p to link to each other via their COOH termini. Then the NH_2 terminus of the new Sfi1p nucleates the growth of the new SPB. The signals controlling these events are not known. The test of this model is to see whether an Sfi1p with fewer repeats will make SPBs with shorter half bridges. JCB

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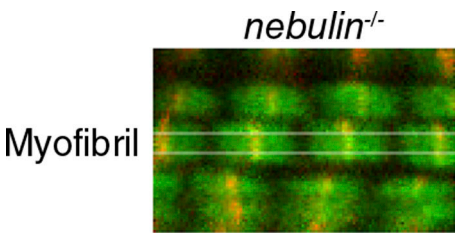
Muscular measuring stick

Nebulin acts as a measuring stick for the thin actin filaments in muscle, say Bang et al. (page 905). Longer thin filaments result in greater overlap with myosin thick filaments and thus the ability of a muscle to bear greater loads.

Distinct muscles have different biophysical properties and requirements, and the lengths of the many nebulin isoforms have been suggested to correlate with thin filament lengths in different muscles. Some attempts have been made to reduce nebulin function in culture, but what was really needed was a study in a living animal.



In mice lacking nebulin, thin filaments are shorter and more uniform among muscles.

The team therefore used gene targeting to delete the nebulin gene in mice. Mutants were born in the expected numbers but ate little, thrived less, and died within days. Their muscles looked fine at birth but became disorganized with use, eventually forming messy aggregates that are also seen in humans with nebulin mutations.

Thin filaments in four muscles had almost identical lengths of ~1 μm in the mutants. In wild type mice, by contrast, these same muscles had various different lengths—all longer than 1 μm. The researchers suggest that a nebulin-independent mechanism ensures that actin filaments in muscle reach the 1 μm mark. But nebulin must be present to guide further actin growth, perhaps by protecting the growing filament from capping proteins. They now plan to test this model by expressing specific nebulin isoforms in specific locations, to see if this is sufficient to determine different thin filament lengths. JCB

Transcriptional glue

Up to 60% of all cellular transcription in budding yeast occurs in the tandem arrays of rDNA. That transcription can prevent separation of sister chromatids during mitosis, according to Machín et al. (page 893).

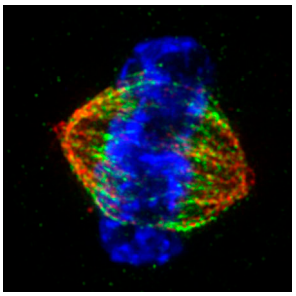
The researchers started with Cdc14 phosphatase, which is needed to get cells out of mitosis. Cells mutant for Cdc14 could be forced out of mitosis by expressing a mitotic kinase inhibitor, but the majority of these cells died because they did not resolve their rDNA. But either reducing the amount of rDNA or inhibiting rDNA transcription was sufficient to allow these cells to survive and segregate rDNA correctly. Angelika Amon (MIT, Cambridge, MA) is reporting similar findingings in a paper in press.

There are two main theories to explain this surprising result. A combination of DNA replication and transcription may increase supercoiling and thus promote catenation. Or the proteins that do the cotranscriptional processing of rRNA may form a sticky mess that holds the sister chromatids together.

The stickiness may be disrupted by the chromatin-condensation complex condensin, which normally loads onto rDNA but fails to do so when Cdc14 is defective. Alternatively, condensin may contribute by reducing the amount of rDNA transcription. Old results suggested that rDNA transcription continued unabated through mitosis, but Machín et al. are retesting that assertion. Meanwhile, the relevant target for Cdc14's activity remains a mystery. JCB

Biased spindle formation

A microtubule-stabilizing protein called HURP forms a gradient around chromosomes that stabilizes spindles, say Wong and Fang (page 879).



HURP (green) is localized close to chromosomes (blue).

HURP was previously associated with hepatocellular carcinoma, but Wong and Fang picked it out as being induced during mitosis and showing covariation with known mitotic regulators. Almost half of all HURP-depleted cells had one or more unaligned chromosome during metaphase. The kinetochores of these chromosomes were not attached to microtubules, and even attached kinetochores were under less tension than in a normal spindle.

HURP has also shown up recently as part of a complex from frog egg extracts that is required for the conversion of aster-like to spindle-like structures (Koffa, M.D., et al. 2006. *Curr. Biol.* 16:743-754). The complex consists of two characterized microtubule-associated proteins (TPX2 and XMAP215), a plus end-directed microtubule motor (Eg5), a mitotic kinase (Aurora A), and HURP. This complex is dependent on the activity of chromatin-localized Ran-GTP, thus helping to focus the spindle around chromosomes.

This is consistent with Wong and Fang's finding that HURP is found on microtubules and in a gradient that peaks around chromosomes. They also found that HURP binds to and stabilizes microtubules. HURP's activity may be needed as, based on mathematical modeling, unbiased microtubule growth is too inefficient for kinetochore capture given the time constraints of mitosis.

The HeLa cells depleted of HURP eventually escaped from their cell cycle arrest induced by unattached chromosomes. The same was true if the cells were treated with low levels of microtubule depolymerizing drugs that induced some chromosome detachment. This suggests that the mitotic checkpoint is weak in certain tumor-derived cells, which would promote genomic instability. JCB