Playing FtsZ before sporulation

*Bacillus subtilis* puts its division plane in the right place with the help of cytoskeletal spirals, according to new results from Sigal Ben-Yehuda and Richard Losick (Harvard University, Cambridge, MA).

In a growing *B. subtilis* cell, the tubulin-like protein FtsZ assembles into a ring structure at the division plane in the middle of the cell. Upon sporulation, cell division is asymmetric, and FtsZ rings form at the poles. Previous models suggested that the shift occurred when FtsZ assembly was blocked at the midcell and activated at the poles.

Ben-Yehuda and Losick examined a GFP fusion of FtsZ during sporulation and discovered that this model was inadequate. The fusions revealed a spiral-like intermediate of FtsZ, which over time extended from the midcell toward both poles. The spirals eventually gave way to polar rings, one of which became the division plane. Thus, generation of polarity requires that the ring still form at the midcell, with the spirals causing its relocation. The spirals were also seen moving from the poles to the midcell when bacteria entering sporulation were returned to growth medium.

The spirals may result from elevated levels of FtsZ present during sporulation, as overexpression of *ftsZ* in *Escherichia coli* has also been shown to cause the formation of spirals. “Possibly, it is an intrinsic property of FtsZ that high concentrations cause it to form spirals,” says Losick. “Or maybe the ring structure is actually a tight spiral, and only the periodicity changes.”


Sorting it out, without clathrin

Clathrin-independent endocytosis is revealing itself at last. Intermediary organelles in this pathway have been difficult to identify, due in part to a dearth of markers and, until recently, difficulties in blocking the clathrin-dependent process. But now, Benjamin Nichols (MRC Laboratory of Molecular Biology, Cambridge, UK) has identified a set of endosomes that are uniquely involved in clathrin-independent trafficking.

Nichols’ results demonstrate that vesicles containing caveolin-1 define a set of early endosomes that are distinct from those that form from clathrin-coated pits. Proteins that were endocytosed independently of clathrin, including GPI-anchored proteins and the cholera toxin B subunit, were found within the caveolin-1–positive endosomes. Even in the absence of clathrin-mediated endocytosis, these proteins were delivered from the plasma membrane to the Golgi.

Although caveolin-1 provides a useful marker for the pathway, the protein was not important for endocytosis. Nichols found that caveolin-1 was sorted away from Golgi-bound vesicles, and diminished caveolin-1 levels did not inhibit clathrin-independent endocytosis. Caveolin-1–containing endosomes have previously been shown to transport SV40 virus to the ER. Nichols believes these may be the same organelles, although this is not yet proven.

The function of the clathrin-independent pathway will be better understood when specific inhibitors can be identified. For now, Nichols hypothesizes that clathrin-independent endocytosis is important for delivery of certain plasma membrane lipids to the trans face of the Golgi, the site of lipid raft formation and Golgi cargo sorting.


Getting a GRIP on kinesin movement

Building a highly polarized cell requires the specific transport of proteins to their final destinations. Kinesin and its binding proteins can direct this differential transport in neuronal cells, according to new results from Mitsutoshi Setou, Nobutaka Hirokawa (University of Tokyo, Tokyo, Japan), and colleagues.

Kinesin can transport vesicles along microtubules to the axon by binding to the scaffolding protein JSAP1. But now it appears that the motor is not always partial to the axon. Using the glutamate receptor subunit GluR2 as cargo, Hirokawa’s group demonstrated that kinesin could also transport its cargo to dendrites, where the receptors are required.

Upon this discovery, Hirokawa says, “we were then interested in understanding how the same motor could determine the direction of transport.” They found their answer in a screen for kinesin binding partners, which identified a glutamate receptor–interacting protein, GRIP-1. Whereas kinesin bound to GRIP-1 was recruited to dendrites, kinesin bound to JSAP1 moved to axons. It is thus the scaffolding proteins that direct kinesin and its cargo toward their destination.

Previously identified scaffolding proteins, such as JSAP1, have shown a preference for binding to kinesin light chain. Sure enough, GRIP-1 binds the heavy chain. Thus, the choice of subunit bound may be important in determining the direction kinesin travels.

New dyes developed by Guido Gaietta, Roger Tsien, Mark Ellisman (University of California at San Diego, La Jolla, CA), and colleagues allow efficient fluorescent and electron microscopy for studying the location, trafficking, assembly, and turnover of proteins and protein complexes. “We were interested in being able to go from light microscopy to the EM level,” says Ellisman. “In particular, we wanted better resolution than with immunogold labeling.” To do so, the group examined FLAsH, a biarsenical derivative of fluorescein. FLAsH binds to small amino acid extensions containing the sequence C-C-X-X-C-C, which can be added to recombinant target proteins. Binding of FLAsH causes the ligand to emit a strong green fluorescence, and binding of ReAsH, a red variant, leads to red fluorescence.

ReAsH is the variant that is useful for EM, because it can generate singlet oxygen upon illumination. Singlet oxygen drives localized polymerization of the substrate diaminobenzidene (DAB) into an insoluble form that can be viewed by EM. “Because the fluorescent label binds directly to the protein you are trying to localize, and the DAB polymer deposits directly nearby the fluorophore, the resolution is better [than immunogold labeling],” says Ellisman. Additionally, this technique does not require the diffusion of large antibodies into the fixed specimens.

The group put their new label to work by examining the protein connexin43, which multimerizes to form gap junctions. Pulse-chase with first FLAsH and then ReAsH revealed newer (i.e., red) molecules of connexin at the outer edge of large clusters of gap junctions known as plaques. Green molecules were concentrated in the center, indicating that older connexins are endocytosed from the middle of the plaques. EM demonstrated that newly synthesized connexins were first sent to nonplaque sites on the cell surface before flowing to the edge of a plaque.

The team expects there will be many uses for the FLAsH-ReAsH system, especially in revealing how large complexes, such as receptor patches at synapses, are formed. “Anyone who has a question about how a cell manages the assembly of large macromolecular structures of a protein should benefit from the use of this system,” says Ellisman.


Widening the target for cancer treatment

Doctors treating cancer patients should aggressively target the area surrounding a tumor during surgery and radiation therapy. That suggestion comes as a result of new studies from Timothy Padera, Rakesh K. Jain, and colleagues (Harvard Medical School, Boston, MA), who report that metastasis occurs through lymphatic vessels in the tumor margin and nearby normal tissue.

Cancer cells can metastasize by escaping a tumor through either blood or lymphatic vessels. Jain’s group questioned whether cancerous cells escape through lymphatic vessels within the tumor itself. In various transplanted and spontaneous tumors in mice, and in human tumors, markers of lymphatic vessels were found within the tumors, but functional analysis revealed that these vessels were not draining fluid.

Jain suggests that lymphatic vessels in tumors may collapse from the high pressure exerted by the rapidly proliferating cancer cells. The findings also act as a warning against the use of markers alone to identify functioning lymphatic vessels in tumors. “A combination of markers as well as functional studies will be necessary,” Jain says.

Although lymphatic vessels in the tumor were not functional, those in the margin of the mouse tumors were either normal in appearance or, in tumors overexpressing VEGF-C, larger in diameter than normal vessels. With increased size came increased lymphatic metastasis, suggesting that marginal lymphatics are sufficient for metastasis. “The larger size of these vessels increases the opportunity for cancer cells to escape,” says Jain. Thus, radiation therapy should be directed at tumor margins in addition to their centers, and VEGF-C may provide a good target for drug treatments.