In This Issue

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Tat substrates open the portal

Aldridge et al. suggest how chloroplast proteins could induce assembly of the pore complex that transports them across the thylakoid membrane.

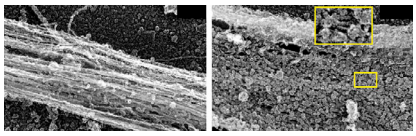
The twin-arginine translocase (Tat) complex transports folded proteins across the thylakoid membranes of chloroplasts and the plasma membrane of bacteria. The chloroplast Tat complex consists of the multipass transmembrane scaffold cpTatC and the single-pass proteins Hcf106 and Tha4, the latter of which is thought to oligomerize and form the translocase pore. To avoid excessive leakage across the thylakoid membrane, however, the complex only forms a functional pore in the presence of a substrate protein bearing a twin-arginine–containing signal peptide. Aldridge et al. examined how the Tat components interact with each other and the substrate protein to initiate pore assembly.

The signal peptide twin-arginine motif binds to a region of cpTatC exposed to the chloroplast stroma, but Aldridge et al. found that a hydrophobic portion of the peptide contacts a different site on cpTatC, located near the thylakoid lumen. The signal peptide isn't long enough to contact both sites on a single cpTatC molecule but could, the researchers suggest, insert between two cpTatC subunits and contact one site on each.

The cpTatC proteins would usually cup together like two halves of a coconut shell but could open up after the signal peptide's insertion, changing cpTatC's interactions with the other Tat components. Indeed, in the presence of a substrate protein, the pore-forming subunit Tha4 contacted regions on the concave face of cpTatC that it couldn't contact in the absence of a signal peptide. This could facilitate Tha4's oligomerization and pore formation, a proposition that now needs to be studied in more detail.

Aldridge, C., et al. 2014. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201311057.

Protein coat keeps axons buttoned up



After 4 days in vitro (left), the AIS of a hippocampal neuron contains a microtubule bundle. 17 days later (right), the microtubules are coated by a dense, fibrillar–globular coat.

Jones et al. reveal how a submembranous network of cytoskeletal proteins helps maintain neuronal polarity.

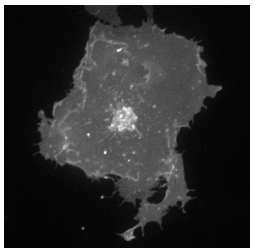
The axon initial segment (AIS) is a specialized structure that, in addition to firing off action potentials, maintains neuronal polarity by preventing axonal proteins from mixing with components of the cell body and dendrites. This latter function is thought to rely on dynamic actin filaments, which could either form a dense meshwork to restrict protein diffusion or assemble into oriented tracks for myosin motors to move specific cargo in and out of the axon. Jones et al. used platinum replica electron microscopy to examine the organization of actin filaments and other AIS components.

The researchers found that actin filaments were neither dense nor oriented within the AIS structures of cultured hippocampal neurons. Instead, the segments mainly consisted of microtubule bundles surrounded by a dense coat of known AIS components such as ankyrin G and spectrin βIV. This coat looked somewhat similar to the cytoskeletal network that ankyrin and spectrin proteins form beneath the membrane of red blood cells and could represent a barrier to the diffusion of axonal membrane proteins.

The AIS did contain sparse populations of both short, stable actin filaments—which may be part of the ankyrin G–spectrin βIV coat—and slightly longer dynamic actin fibers. The latter population was dispensable for coat formation but might be required to remodel the ankyrin–spectrin network: in the presence of the actin-depolymerizing drug latrunculin B, gaps appeared in the AIS coat at sites of axon branching. Senior author Tatyana Svitkina now wants to investigate how the structure of the AIS is affected by the loss of individual coat components.

Jones, S.L., et al. 2014. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201401045.

Probing PtdIns4*P*'s localization



A maximum intensity projection shows GFP-P4M's localization to the Golgi, plasma membrane, and late endosomes/lysosomes.

Hammond et al. develop a new probe to detect the phospholipid PtdIns4*P* in living cells.

Different cellular membranes have distinct protein and lipid compositions. The phospholipid phosphatidylinositol 4-phosphate (PtdIns4*P*) is best known for its role at the Golgi, where it helps recruit proteins involved in vesicular transport. PtdIns4*P* is also found at the plasma membrane, and some evidence suggests it could be synthesized on late endosomes and lysosomes as well. But the fluorescent probes used to detect PtdIns4*P* in living cells use the PtdIns4*P*-binding domains of Golgi proteins that also bind to other components of Golgi membranes. Accordingly, these probes preferentially label the Golgi-localized pool of PtdIns4*P* in vivo.

Hammond et al. constructed a new PtdIns4*P* biosensor using the PtdIns4*P*-binding (P4M) domain of a bacterial protein called SidM. GFP-P4M localized to the Golgi, plasma membrane, and late endosomes/lysosomes of living mammalian cells. This wide-ranging distribution was dependent on PtdIns4*P* because P4M labeling was lost when the PtdIns4*P*-hydrolyzing phosphatase Sac1 was targeted to any of these organelles. On the other hand, mistargeting the PtdIns4-synthesizing enzyme PI4KA was sufficient to recruit P4M to additional cell membranes.

PtdIns4*P*'s widespread distribution means that it can't be a simple marker of organelle identity. Instead, lead author Gerry Hammond speculates that the phospholipid may determine the distribution of other membrane components by recruiting proteins involved in vesicular transport and through a potential function in nonvesicular lipid transport between organelles. The P4M probe should help researchers investigate both this and the many other functions of PtdIns4*P*.

Hammond, G.R.V., et al. 2014. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201312072.