Getting lamin B1 processed and organized

Lamins are the building blocks of the nuclear lamina, a complex polymer attached to the nuclear envelope that is thought to be important for nuclear stability, chromatin organization, and gene expression. On page 1223, Maske et al. identify the protease responsible for processing lamin B1, demonstrate the existence of a nuclear receptor specific for carboxymethylated lamin B1, and show that posttranslational processing may control the localization of this lamin into subdomains within the nuclear envelope.

After being farnesylated, the COOH-terminal CAAX domain of lamin B1 is cleaved by an endoprotease, and the new COOH terminus of the protein is then methylated by the enzyme Icmt. Using a monoclonal antibody that distinguishes proteolyzed from unproteolyzed lamin B1, the authors determined that proteolysis specifically requires the CAAX endoprotease Rce1. Separate pools of farnesylated but unproteolyzed and proteolyzed but unmethylated lamin B1 appear in the nucleus, but retention of the protein’s COOH terminus in the nuclear envelope requires carboxymethylation, indicating that a nuclear receptor specifically recognizes the fully processed form of the protein. When farnesylation is inhibited, the residual mature form occupies defined subdomains of the nuclear lamina, and the authors have preliminary evidence that these subdomains are also present in untreated cells.

The results suggest that methylation of lamin B1 is a novel mechanism controlling the higher order organization of the nucleus. Lamin B1 interacts with chromatin, so the protein’s controlled localization to subdomains of the lamin might organize interacting chromatin into similar domains. The authors are now trying to identify the nuclear receptor for carboxymethylated lamin B1.

Since the well-known oncogene ras also requires processing of its CAAX domain, CAAX endoproteases, including Rce1, are popular targets for a new generation of experimental anticancer drugs. It was initially thought that CAAX processing was unnecessary in nondividing cells, but the new findings show that interfering with lamin B1 processing disrupts the integrity of the nuclear envelope, highlighting the potential for unforeseen side effects with the new drugs.

How to block botox

From both chemical weapons inspectors and plastic surgeons, botulinum toxins are the focus of intense interest. But how do these incredibly toxic proteins get into cells? On page 1293, Dong et al. demonstrate that botulinum neurotoxin B (BoNT/B) uses the vesicle proteins synaptotagmin I and synaptotagmin II as cellular receptors, and that a fragment of synaptotagmin II can inhibit the toxin’s effects in animals.

Of the seven known neurotoxins expressed by Clostridium botulinum, BoNT/A, B, and E are the most common causes of botulism in humans and the major choices for both bioterrorist and pharmaceutical uses. In previous work, researchers identified several cellular proteins that can bind to these toxins, but there were conflicting data about which, if any, of the candidate receptors actually mediate cellular entry. Once inside neurons, the toxins act as proteases to block exocytosis, ultimately leading to paralysis and death.

Using both loss-of-function and gain-of-function approaches, Dong et al. show that synaptotagmin I or synaptotagmin II can act as a receptor to internalize BoNT/B into PC-12 cells. Fusion of synaptic vesicles with the plasma membrane, and thus display of vesicle proteins on the cell surface, occurs during excitation. Indeed, BoNT internalization is dependent on electrical activity in two different cell types. Fragments of synaptotagmin II effectively block the binding of BoNT/B to cultured cells and inhibit the activity of the toxin in mice, providing strong evidence that the interaction is biologically relevant.

In the mouse experiments, an injection of synaptotagmin II fragments partially protected the animals against a subsequent challenge with BoNT/B. This is the first demonstration that a fragment from a bacterial toxin receptor can antagonize the toxin in animals. The authors are now defining the precise requirements for BoNT/B–synaptotagmin II interactions, and are also trying to identify the receptors for other Clostridium neurotoxins.
NgCAM takes a scenic route to the axon

To establish and maintain their asymmetrical structure, neurons must sort different proteins to their somatodendritic and axonal membranes. Previous work has supported three different models to explain selective transport to axons: direct transport to the axon; selective fusion of vesicles with the axon; and transport to the somatodendritic membrane followed by transcytosis. A detailed analysis by Wisco et al. (page 1317) now identifies evidence that the adhesion molecule NgCAM can take two of these three paths.

Some recent evidence suggests that vesicles containing NgCAM are blocked from fusing with the somatodendritic membrane in the first place. But in a kinetic analysis, Wisco et al. show that in fact NgCAM does transiently appear on the somatodendritic membrane, but is then endocytosed and sent to the axonal membrane by a transcytotic pathway.

The NgCAM protein encodes all of the signals necessary to direct it to the transcytotic pathway. A mutant NgCAM protein lacking the transcytosis signal is instead sent directly to the axonal membrane from the trans-Golgi network, suggesting that two distinct pathways are available to NgCAM, but transcytosis normally supersedes direct targeting. The authors now hope to determine whether this circuitous route is unique to NgCAM or common to many axonal proteins.

A coil of actin (red) springs open in front of the Limulus sperm nucleus (blue), showing that this process relies on mechanical energy stored in a molecular spring. Springs also underlie other phenomena such as bacteriophage infection.

To penetrate the jelly coat of an egg, Limulus sperm extends a bundle of actin filaments from a coiled position in the sperm head into a sturdy 60-mM-long rod called the acrosomal process. The reaction takes only five seconds. The authors calculated the amount of mechanical energy theoretically required to drive the movement from the energy stored in the structure and expended during extension. Neither ATP hydrolysis nor calcium binding provides enough energy during the reaction, but the potential mechanical energy in the coiled actin bundle is more than sufficient to drive acrosome extension. The data suggest that calcium binding triggers, but does not power, a progressive mechanical uncoiling reaction, extending the acrosomal process like a Jack-in-the-box toy.

A coil of actin (red) gets internalized (red) on the way to the axon (blue).

NgCAM gets internalized (red) on the way to the axon (blue).

NgCAM protein: Plakin repeats (green) take Shot to adherens junctions (red).

Plakin repeats (green) take Shot to adherens junctions (red).

A long version of Short stop

The Drosophila Short stop (Shot/Kakapo) gene encodes several protein isoforms, some of which may link integrins to microtubules. In analyzing the Shot locus, Röper and Brown (page 1305) found something odd: a previously unnoticed exon encoding a series of plakin repeats. The only known function of plakin repeats though is to interact with cytoplasmic intermediate filaments, which flies lack.

Based on a biochemical analysis, the plakin repeats are incorporated into a gigantic isoform of Shot that is the third-largest protein discovered in flies. This isoform includes an actin-binding domain, the plakin repeats, a microtubule-binding domain, and spectrin repeats, and is found in adherens junctions, a localization that seems to be determined by a portion of the plakin domain. Reducing the quantity of the largest Shot isoform in early embryos weakens epithelial intercellular contacts, so it is essential for maintaining epithelial integrity.

The authors propose that the giant Shot isoform helps link the adherens junction to an associated belt of actin filaments and microtubules. This novel intermediate filament-independent activity of plakin repeats may be a conserved function of the domain, or it could be a distinct adaptation in insects, where a lack of intermediate filaments left the plakin domain free to evolve a new function.

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