In This Issue

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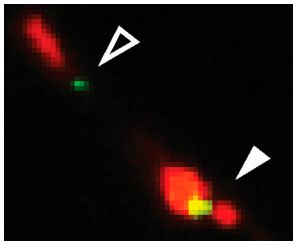
Release notes for dense-core vesicles



DCV secretion events (green) occur both inside (filled arrowhead) and outside (open arrowhead) synapses (red).

Van de Bospoort et al. describe how Munc13 proteins control when and where dense-core vesicles (DCVs) are released from neurons.

Neuronal DCVs contain neuropeptides and other factors that promote brain development and modulate synaptic transmission. Like neurotransmitter-containing synaptic vesicles (SVs), DCVs are released in response to action potentials and calcium influx, but relatively little is known about how neurons control DCV secretion. Van de Bospoort et al. designed a fluorescent probe to monitor the release of individual DCVs from hippocampal neurons in vitro.

Though DCVs weren't enriched in synaptic terminals, they were preferentially secreted at synapses upon neuronal stimulation. DCV release from other parts of the neuron was less efficient and required prolonged stimulation. To investigate why DCVs are secreted more efficiently at synapses, Van de Bospoort et al. examined the Munc13 family of presynaptic proteins, which, by helping to assemble the SV fusion machinery, are essential for SV release.

DCVs were still secreted from neurons lacking Munc13 proteins, but their release required prolonged stimulation and no longer occurred preferentially at synapses. When Munc13-1 was overexpressed, on the other hand, it localized throughout neurons and boosted the efficiency of extrasynaptic DCV release, such that brief stimuli induced secretion equally from synaptic and nonsynaptic sites.

Therefore, although Munc13 proteins aren't required for DCV exocytosis, they facilitate secretion at synaptic termini. The researchers now want to investigate how DCVs are recruited into synapses and to determine why DCV and SV secretion are regulated differently.

Van de Bospoort, R., et al. 2012. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201208024.

Opening up the ER's gatekeeper

Trueman et al. describe how the Sec61 channel decides whether to open up and let proteins pass through it into the endoplasmic reticulum (ER).

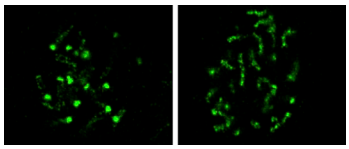
Proteins are directed to the ER by hydrophobic signal sequences and by cytosolic accessory factors that deliver them—either co- or posttranslationally—to Sec61 channels in the ER membrane. To permit protein translocation, Sec61's α subunit undergoes a conformational change that unblocks its central pore and opens up a "lateral gate" through which signal sequences can be inserted into the channel. To understand how this conformational change is triggered, Trueman et al. focused on a cluster of polar residues on either side of the lateral gate and on an adjacent patch of apolar residues in Sec61α's "plug domain," which blocks the central pore when the channel is closed.

Replacing these residues with polar amino acids boosted Sec61's activity in budding yeast, enabling the channel to translocate proteins with minimally hydrophobic signal sequences that would normally remain in the cytosol. But substituting hydrophobic amino acids for the lateral gate polar residues inhibited Sec61's activity. Many proteins were no longer transported into the ER, although proteins with highly hydrophobic signal sequences, such as the transmembrane domains of integral membrane proteins, were still translocated normally.

The lateral gate and plug domain residues therefore represent a "gating motif" that opens up the Sec61 channel to proteins with sufficiently hydrophobic signal sequences. Mutations in the motif—which is conserved in bacteria—affect channel function by altering the stability of its closed conformation. Senior author Reid Gilmore now wants to investigate how the cytosolic factors that assist protein translocation influence channel opening.

Trueman, S.F., et al. 2012. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201207163.

Segregating Bub1's mitotic functions



Aurora B (green) concentrates at centromeres in wild-type cells (left) but spreads along chromosome arms in the absence of Bub1 kinase activity (right).

Ricke et al. reveal that mice expressing an inactive version of the mitotic kinase Bub1 aren't susceptible to cancer, even though they frequently missegregate their chromosomes.

Bub1 is an essential protein that has several functions in mitosis, including activation of the mitotic checkpoint, which prevents cells from entering anaphase until every chromosome is correctly attached to the mitotic spindle. Mice expressing reduced or excessive amounts of Bub1 show chromosome segregation defects, aneuploidy, and increased rates of tumor development. The contribution of Bub1's kinase activity to these processes is unclear, however.

Ricke et al. generated mice expressing a kinase-dead version of Bub1 ($Bub1^{KD}$) in place of the wild-type protein. Aside from a slight decrease in male fertility, these animals were perfectly healthy, despite showing high levels of chromosome missegregation and aneuploidy. Mitotic checkpoint proteins were still recruited to kinetochores in the absence of Bub1 kinase activity, but the Aurora B kinase, which destabilizes incorrect kinetochore–spindle attachments, failed to accumulate at the centromeres of $Bub1^{KD}$ cells, resulting in the misalignment of mitotic chromosomes. Bub1 promoted Aurora B's recruitment to centromeres by phosphorylating histone H2A on threonine 121.

Though aneuploidy is thought to drive tumorigenesis, $Bub1^{KD}$ mice were no more prone to tumors than wild-type animals. This suggests that, although Bub1 suppresses tumors by promoting accurate chromosome segregation, its kinase activity is required for some other step in tumor progression. The authors now want to investigate whether the viability of $Bub1^{KD}$ cells is dependent on the closely related protein BubR1.

Ricke, R.M., et al. 2012. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201205115.