In This Issue

CAST into the synapse

For the nervous system to function properly, neurotransmitters must be released in a regulated fashion from an active zone in the presynaptic membrane, but relatively little is known about how synaptic vesicles are directed to this zone in the first place. On page 577, Ohtsuka et al. characterize a novel protein associated with the active zone.

The cytomatrix at the active zone (CAZ) is believed to be important in determining synaptic vesicle localization. In the new work, the authors began with traditional biochemistry, comparing crude membrane and postsynaptic density fractions from rat brain to identify proteins associated with the synapse. One novel protein was found and named CAZ-associated structural protein (CAST). Electron microscopy places CAST at the CAZ in conventional synapses. CAST associates with two known CAZ proteins, RIM1 and Munc13–1, in a ternary complex that associates with the CAZ protein bassoon, providing the first evidence that CAZ proteins form a network of protein–protein interactions in vivo.

CAST appears to help determine RIM1 localization in neurons, and the authors found that CAST is expressed in the early stages of synapse formation in primary cultured neurons. The data suggest that complexes of CAZ proteins associate with vesicles during the early stages of synapse formation, and that these protein–vesicle complexes are then transported to the newly forming synapses. Fusion of the vesicles with the plasma membrane could then determine the location of the new active zone.

A database search uncovered a putative orthologue of CAST in *C. elegans*, suggesting that the new protein is a conserved component required for CAZ formation in metazoans. If so, then targeted disruption of CAST in mice or worms should provide additional insight into the formation of this crucial structure.


green) and Bassoon (red) colocalize at the early stages of synapse formation.

Getting trapped in a rough neighborhood

The ER is differentiated into rough ER, where membrane-bound polysomes translate proteins for insertion into or translocation across the ER membrane, and smooth ER, which lacks polysomes. But how is this differentiation established? Nikonov et al. (page 497) analyzed the lateral diffusion of translocon complexes in the ER membrane. The results suggest that association of translocons with polysomes provides the basis for ER differentiation, and that translocons remain assembled even when they are not translocating nascent polypeptides.

The authors were able to measure the diffusion of translocon complexes in the ER membrane by transfecting a temperature-sensitive mutant cell line with a GFP-tagged version of the translocon-associated oligosaccharyltransferase component Dad1. In cells that are actively carrying out translation, the diffusion constant of GFP-Dad1 is about seven times less than the diffusion constant of a freely diffusing control protein. When translation initiation is inhibited or nascent polypeptide chains are terminated, the GFP-Dad1 diffusion constant increases significantly, but remains two- to threefold less than that of the control protein.

The results suggest that as a polysome associates with multiple translocon complexes, tethering them together, diffusion of the resulting array is severely restricted. The extension of multiple polypeptide chains from this array into the viscous lumen of the ER may further restrict diffusion. The diffusion rate of GFP-Dad1 in cells where translation is inhibited suggests that the translocon complex remains assembled even when it is not associated with a polysome. These free translocon complexes are able to diffuse in the ER membrane considerably faster than complexes associated with polysomes.

According to the authors’ model, polysome arrays, once formed, may remain relatively immobile and define the location of the rough ER. Translocon complexes released from these arrays after translation termination could diffuse relatively freely through the ER before reassociating with the polysomes to initiate a new round of translation.
Barrier-to-autointegration factor (BAF) was first described as a cellular activity that prevents retroviral DNA from undergoing suicidal autointegration, but its function in uninfected cells remained obscure. Segura-Totten et al. (page 475) have now performed a detailed biochemical characterization of BAF. The work defines critical functional motifs of this DNA-bridging protein, and suggests that BAF is essential for chromatin decondensation and nuclear envelope assembly and growth.

Previous work had shown that BAF binds to DNA and to proteins containing a LEM domain, a structure that defines a family of nuclear membrane proteins.

**BAF goes nuclear**

Using biochemical assays and a panel of site-directed mutants, the authors identified residues of BAF required for the protein to bind to itself, to DNA, and to emerin, a LEM-containing protein. Adding a low concentration of wild-type BAF enhances chromatin decondensation and nuclear growth in a *C. elegans* egg extract system, but a high concentration of BAF blocks both processes.

The results suggest that LEM-containing proteins bind to the middles of BAF dimers, while DNA binds to the left and right sides. During nuclear assembly, these interactions attach chromatin to the inner nuclear membrane, promoting chromatin decondensation and nuclear envelope growth. The availability of site-directed BAF mutants that produce a range of nuclear assembly phenotypes in the *Xenopus* system should provide a basis for future studies on BAF function.

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Two channels, one program

The biophysical and structural characteristics of chloride ion channels have been studied extensively, but little is known about the molecular regulation and signaling pathways associated with these highly conserved proteins. Rutledge et al. (page 435) analyzed the activation of the CLH-3 chloride ion channel in *C. elegans* during meiotic cell cycle progression and in response to oocyte swelling. The work demonstrates the utility of the worm system in studying these channels, and suggests that CLH-3 and its putative mammalian orthologue, CIC-2, respond to similar regulatory inputs to carry out similar physiological functions.

The authors found that during oocyte maturation, or in response to oocyte swelling, CLH-3 is activated by serine/threonine dephosphorylation. RNAi inhibition demonstrates that the dephosphorylation is mediated by CeGLC-7α and CeGLC-7β, phosphatases that help to regulate meiotic and mitotic cell cycles in worms. Rat CIC-2 heterologously expressed in mammalian cells is also activated by serine/threonine dephosphorylation, suggesting that the two channels share a common regulatory mechanism, despite their wide evolutionary separation. Rutledge et al. suggest that both channels may depolarize membranes to transduce signals between cell types, such as worm oocytes and the surrounding contractile sheath cells, that are coupled by gap junctions.

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Endostatin (right) prevents secondary axis induction by β-catenin (left).

**A new path for endostatin**

The ability of endostatin to inhibit angiogenesis has produced sensational headlines, but efforts to understand how this collagen fragment actually works have drawn considerably less fanfare. On page 529, Hanai et al. now demonstrate that endostatin acts through a novel pathway to inhibit Wnt signaling, suggesting that the effects of endostatin on tumors may be more complex than previously thought.

Using *Xenopus* embryonic development as a model system, the authors found that high concentrations of endostatin produced developmental abnormalities characteristic of Wnt signaling defects, a notion that was confirmed in cultured mammalian cells. Endostatin seems to act through a novel pathway to target β-catenin, a mediator of Wnt signaling, for proteasomal degradation. A downstream transcriptional activator that acts independently of β-catenin rescues cells from two of the effects of endostatin: cell cycle arrest and inhibition of endothelial cell migration.

The new work raises the possibility that endostatin may have direct antitumor effects, mediated by the inhibition of Wnt signaling, in addition to its antiangiogenic activity. The results also implicate Wnt and β-catenin signaling defects, a notion that was confirmed in cultured mammalian cells. Endostatin seems to act through a novel pathway to target β-catenin, a mediator of Wnt signaling, for proteasomal degradation. A downstream transcriptional activator that acts independently of β-catenin rescues cells from two of the effects of endostatin: cell cycle arrest and inhibition of endothelial cell migration.

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