Agrin at CNS synapses

At neuromuscular junctions, the extracellular matrix protein agrin directs the formation of postsynaptic structures. Agrin is also found in neurons of the central nervous system (CNS), so does it also direct synaptic development there? On page 923, Hoover et al. provide the first evidence of a specific agrin receptor involved in the development of synapses between two neurons.

The authors previously identified an agrin-induced signaling pathway in CNS neurons. The new work shows that the domain of agrin responsible for inducing this signal is within 20 kD of the carboxy terminus of the protein. A fragment from within this functional domain binds to the putative agrin receptor, but does not activate the signaling pathway, suggesting that receptor activation requires binding to two sites. Using the fragment as a probe reveals that agrin and its putative receptor colocalize at neuron–neuron synapses. The agrin receptor in the CNS is distinct from receptors that mediate agrin function at neuromuscular junctions.

The data suggest that agrin directs synaptic development or function in all neurons, regardless of their locations or neurotransmitter phenotypes. Hoover et al. are now trying to identify the agrin receptor in the CNS through affinity purification and expression library screening.

Catching a kinase in the act

Using a cleverly designed set of molecular probes, Violin et al., reporting on page 899, provide the first real-time analysis of phosphorylation and dephosphorylation downstream of protein kinase C (PKC). The results provide a striking demonstration of the importance of phosphatases in PKC signaling. When second messenger concentrations decrease, PKC interaction with the membrane loosens, PKC activity is rapidly lost, and the activity probe is quickly dephosphorylated.

The authors designed a reporter protein that immediately indicates changes in PKC activity. Phosphorylation or dephosphorylation of the reporter causes a change in fluorescence resonance energy transfer. When tethered to the membranes of appropriately stimulated cells, the reporter is phosphorylated and dephosphorylated in sustained oscillations, in step with waves of calcium release. Similar reporter proteins, designed to monitor other signaling parameters, demonstrate that the oscillations can be driven by calcium waves alone, or by concurrent waves of calcium and diacylglycerol.

The rapid oscillations suggest that phosphatases act like brakes that are always on; when PKC activity decreases, dephosphorylation immediately predominates. By directing the reporter to specific intracellular sites, the authors can now ask whether PKC is active when anchored to nonmembrane sites by scaffold proteins, and determine the dynamics of phosphorylation and dephosphorylation at these sites.

Fusion promoter or fusion inhibitor?

Mononuclear phagocytes can fuse to form osteoclasts or multinuclear giant cells. The latter are hallmarks of Crohn’s disease, granulomas, tumors, and fungal and HIV infections. Though the precise function of these syncytia remains unclear, Takeda et al. (page 945) now provide important new insights into phagocyte fusion, suggesting that the process may differ considerably from other types of cell fusion.

Earlier work identified several cell surface proteins required for phagocyte fusion, but the authors focused on CD9 and CD81, transmembrane proteins in the tetraspanin family that promote the fusion of gametes, myoblasts, and virus-infected cells. Surprisingly, the expression of both proteins is elevated in mononuclear phagocytes cultured under normal conditions, but under fusogenic conditions CD9 and CD81 expression is reduced.