Research Roundup

Weakness as a strength

Jinun Qin (Lerner Research Institute, Cleveland, OH) is the defender of the weak, or at least of weak protein–protein interactions. His work with Julia Vaynberg, Tomohiko Fukuda, Cary Wu (University of Pittsburgh, Pittsburgh, PA), and colleagues shows that an extremely weak affinity between two focal adhesion proteins is needed for cell migration.

The focal adhesion proteins are the adaptors PINCH-1 and Nck-2, whose weak interaction ($K_\text{D} = 3 \text{ mM}$) was noted previously in yeast two-hybrid assays but not with less sensitive techniques. Using NMR, which unlike crystallography provides structural information even for very weak interactions, the group has now determined the structure of the tiny interface between the adaptors. To prove its biological relevance, they then used genetic rescue experiments to show that the disruption of the PINCH-1/Nck-2 interface in vivo impairs cell spreading and migration.

The results show that the Nck-2/PINCH-1 interaction is necessary for processes that depend on focal adhesions. Focal adhesions, in which many other components are tightly bound, may rely on this weak interaction for dynamic assembly and disassembly.

For Qin, the results extend far beyond this one pair. “In humans,” he says, “there are hundreds of thousands of protein–protein interactions. They can’t all be strong, or our cells would be glued together all the time.”

As NMR-based studies on purified proteins is relatively quick and easy, the significance of other very weak interactions (e.g., cell–cell contacts and enzyme–substrate pairs) may be determined soon.

Reference: Vaynberg, J., et al. 2005. Mol. Cell. 17:513–523.

Acyl cycles move Ras

Acyl groups cycle endlessly on and off Ras to confine the protein to the Golgi and plasma membrane (PM), as shown by Oliver Rocks, Alfred Wittinghofer, Philippe Bastiaens (EMBL, Heidelberg, Germany), and colleagues.

These locales are the homes of two Ras isoforms, Nras and Hras, both of which are modified with the acyl group palmitate. Now it is shown that transport to the PM is not a one-way trip for Ras, which cycles back to the Golgi.

De- and repalmitoylation drive this cycling. Ras that could not be palmitoylated was found on all membranes. Less stable palmitates, which were more rapidly removed, favored Golgi localization. Other palmitoylated peptides cycled similarly.

The results suggest that palmitoylation occurs at the Golgi and temporarily anchors it there. Some of this pool is sent via the exocytic pathway to the PM, where the palmitate group is eventually removed. The low affinity of this de-palmitoylated Ras for membranes ensures that it does not accumulate on non-Golgi membranes.

“[Ras] is just in sampling mode,” says Bastiaens, “until it encounters the palmitoylation activity.”

Activity did not affect Ras cycling, but the authors found that growth factors first activated Ras at the PM. The Golgi pool was activated with kinetics that reflect the speed of Ras retrograde transport. Nras (with its one palmitate) thus beat Hras (which has two) to the Golgi, giving the isoforms distinct signaling capabilities.

Reference: Rocks, O., et al. 2005. Science. doi: 10.1126/science.1105654.

Two vesicle pools for neurons

Yildirim Sara, Ege Kavalali, and colleagues (University of Texas Southwestern Medical Center, Dallas, TX) show that nerve terminals possess two independent vesicle populations: one for activity-dependent neurotransmitter release, and one for spontaneous release.

Activity-dependent release is the typical action potential–generating mechanism. But occasionally a vesicle leaks its contents without provocation. Most scientists figured these events—which affect synaptic development and inhibit transition in dendrites—reflect the occasional escape of a vesicle primed for activity-dependent release. But the new results reveal that spontaneously released vesicles comprise a pool of their own.

The two pools were distinguished by their filling mechanism: vesicles loaded with dyes by spontaneous endocytosis were then unloaded more rapidly by spontaneous release than by stimulated release. Activity-dependent endocytosis filled vesicles that were more rapidly unloaded by stimulated release. Blocking neurotransmitter refilling into vesicles at rest only affected spontaneous release, suggesting that the pools do not intermix.

Spontaneous vesicles may be defective in fusion yet occasionally fuse where and when they should not. The two pools looked the same by EM and were similarly localized, so the differences probably lie in lipid or protein content. How the differences originate is unclear. “There could be two recycling pathways” such as local and endosomal routes, says Kavalali.

“Or [the spontaneous pool] might just be the use-dependent accumulation of defective vesicles over time.”

Reference: Sara, Y., et al. 2005. Neuron. 45:563–573.
The learning matrix

The old dog now has an excuse for failing to learn new tricks. Research from Karen Christopherson, Erik Ullian, Ben Barres (Stanford University, Stanford, CA), and colleagues may help to explain why the young make better learners. The work identifies a youth-related factor that is needed for synapse formation.

Learning depends in part on new synapse formation. But neurons make few synapses in the absence of astrocytes, which seem to secrete a synaptogenic factor. Christopherson et al. now show that this synaptogenic activity is thrombospondin (TSP)-1 and –2.

To find the synaptic helpers, the authors fractionated astrocyte-conditioned medium. From this soup, TSPs were both necessary and sufficient for neurons to form synapses in vitro. TSPs are extracellular matrix proteins that alter cell adhesions by binding to other matrix proteins or to membrane receptors. It is not clear how TSPs build synapses, but they boost synaptic protein localization. TSPs may activate signaling pathways via receptors on the neuronal cell body, or they may act more locally to reorganize synaptic proteins. There are many known TSP receptors; identification of the relevant ones should help to resolve this question.

The TSP-induced synapses looked normal, but they lacked functional AMPA receptors on the postsynaptic side. As functional synapses are made in the presence of live astrocytes, the findings suggest that the missing effect is due to a second, unidentified, astrocyte-derived factor.

TSP is around at the right time and place to regulate synaptogenesis in the developing brain. The authors found that TSP expression was strong in the postnatal mouse brain but was turned off in adults. Mutant mice lacking TSP-1 and -2 were missing 40% of the synaptic connections of their wild-type counterparts. Humans express much more TSP than do other primates. Perhaps this difference is one reason why Earth is not the planet of the apes. JCB

Reference: Christopherson, K.S., et al. 2005. Cell. 120:421–433.

Cells do not relive youth

A few cells in the imaginal disc of the fly larva have the exceptional ability to transdeterminate (TD)—that is, to change fate—upon injury and regeneration. For example, injured leg precursors may form a wing instead. TD cells have many stem cell–like qualities. As stem cell multipotency is thought to be characteristic of "young" cells, it has been suggested that TD is preceded by cellular rejuvenation. Now, research from Anne Sustar and Gerold Schubiger (University of Washington, Seattle, WA) shows that no such fountain of youth is necessary. Instead, cells first adopt an unusual cell cycle profile before TD.

Using injuries or ectopic expression of Wg (a Wnt mitogen that induces TD) and a wing-specific reporter, the authors isolated transdetermining cells from imaginal discs. Young cells normally cycle more rapidly than do older cells, but TD cells kept the same relatively slow doubling time that they had before the injury or Wnt induction. In this sense, at least, they were not rejuvenated.

Though division timing was unaffected, TD cells did transiently alter their cell cycle phasing; they passed quickly through G1 and lagged in S phase. This phase change preceded TD. A longer S phase might allow time for chromatin changes that are needed for the different cell fate, and the authors propose that Wg induces chromatin remodeling proteins such as Polycomb.

Cells with the unusually long S phase were also larger than non-TD cells. Bursts of biosynthetic activity may be the driving force for the phase changes and subsequent developmental plasticity, but this remains to be shown. Forcing growth and division by overexpressing insulin pathways induced some fate changes but did not induce TD to wing. Wg may also have morphogenic properties. "We can speculate," says Schubiger, "that Wg targets TD cells and acts as a mitogen. But we believe that only sustained Wg signaling causes a change [in cell fate]."

Reference: Sustar, A., and G. Schubiger. 2005. Cell. 120:383–393.