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 (Keq = 3 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. JCB

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

Acyl cycles move Ras

Acyl cycles move Ras 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. JCB

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 translocation 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.” JCB

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