The complex life of a GEF

Receptor tyrosine kinases (RTKs) that are bound to appropriate growth factors activate PI3K, which initiates Rac-induced cytoskeletal alterations. The signaling steps between PI3K and Rac are uncovered on page 17 in an article by Innocenti et al. that reveals the importance of complexes in activating guanine nucleotide exchange factors (GEFs).

Genetic evidence has supported the involvement of the GEF Sos-1 for stimulating Rac activity in response to growth factors. Sos-1 is found in complex with Abi1 and Eps8, and interference with any of these proteins is known to block actin remodeling in response to RTK activation. Innocenti et al. now show biochemically that Sos-1 is indeed the GEF that activates Rac in response to PI3K.

Full GEF activation was a stepwise process. The physical presence of PI3K itself stimulated low levels of the Sos-1 GEF activity, perhaps by inducing a conformational change in Sos-1. PI3K gets to the Sos-1 complex through Abi1. PI3K bound to Abi1 in vitro and colocalized with Abi1 and Eps8 in PDGF-induced membrane ruffles.

PIP3, the product of PI3K, further stimulated the basal Sos-1 GEF activity. Thus, strong GEF activity would be unleashed only by activation of PI3K by growth factor-stimulated RTKs. As expected, inhibition of Sos-1 GEF activity by interfering with the interaction of PI3K and Abi1 blocked PDGF-induced membrane ruffling. The assembly of the tetrameric complex therefore allows for a number of biological consequences important for growth factor responses, including two levels of Sos-1 activation and the recruitment of both PI3K and PIP3 to sites where they are most needed for actin reorganization.

FRET-ing over scaffolds

Opposites really do attract. Results on page 101 by Oliveria et al. show that, although they perform opposing functions, a kinase and a phosphatase linked to synaptic plasticity are close neighbors at the plasma membrane. The two are brought together by the scaffolding protein AKAP79 into a complex that may be important for efficient learning.

The close proximity of the three proteins is uncovered by a powerful microscopy technique, fluorescence resonance energy transfer (FRET). Using this technique in COS cells, the group shows that protein kinase A (PKA) and the phosphatase calcineurin (CaN) bind to sites on membrane-targeted AKAP79 that are spaced only nanometers apart. In neurons, AKAP79 is known to associate with SAP97, a scaffolding protein that links the signaling complex to glutamate receptors. Now, using standard immuno-fluorescence techniques, the authors show that expression of SAP97 in COS cells brings a complex of PKA-AKAP-CaN and SAP97 to the plasma membrane.

This complex may also be assembled at synapses in neurons, indicating that scaffolds coordinate colocalization of proteins that do not necessarily interact, but rather regulate common downstream targets—in this case, glutamate receptors. The proximity of the proteins—not necessarily to each other but to their common target—is expected to regulate signaling during long-term potentiation or depression by increasing both efficiency (i.e., cAMP or Ca2+ increases will more rapidly activate PKA or CaN, respectively) and specificity (e.g., only Ca2+ increases that occur near the receptors will activate calcineurin).

RNA travels with ZBP1

On page 77, Farina et al. get a handle on RNA localization machinery by proving that an RNA-binding protein is essential for both mRNA transport and cell motility.

The cell motility connection comes about because localization of the β-actin mRNA to the lamellae is required for cell polarity and motility in fibroblasts. The new results show that this localization depends on ZBP1, a protein associated with cytoplasmic granules that contain the actin mRNA. ZBP1 bound to the mRNA through two COOH-terminal KH domains that were required for granule formation and attachment to the actin cytoskeleton. NH2-terminal regions of ZBP1 were necessary for granule localization in the lamellae.

Dominant–negative ZBP1 constructs that mislocalized actin RNA inhibited fibroblast motility. Since mRNAs for some actin-associated proteins, such as ARP3, also contain ZBP1-binding sequences, the authors believe that ZBP1 may link several messages involved in motility to a transport complex. But ZBP1 may be more than just a scaffold: a ZBP1 homologue has been linked to translational repression of the insulin-related growth factor. Perhaps ZBP1 also ensures that the actin mRNA is not translated until it reaches its ultimate destination.

The group plans to purify ZBP1-associated proteins in the complex to identify the motor responsible for actin-based transport. ZBP1 is also known to be associated with microtubules in neurons, so it may connect to different motors depending on the cell type.