Sticking to PIP₂

The PIP₂ phospholipid is required for calcium-dependent exocytosis in at least some secretory cells, but its exact function has remained obscure. Now, Jihong Bai, Ward Tucker, and Edwin Chapman (University of Wisconsin, Madison, WI) find that PIP₂ is a plasma membrane dock for synaptotagmin-1 (syt), a transmembrane protein localized in secretory vesicle membranes, when calcium is absent. This dock may ensure speedy and directed fusion in response to calcium influx.

The syt dock has two calcium-binding domains in its cytoplasmic region, called C₂A and C₂B. “What we discovered is that there are two modes of binding to PIP₂ mediated by the C₂B domain of syt,” says Chapman. In the absence of calcium, syt binds PIP₂ weakly, lying on its side so that C₂B contacts the PIP₂ head group. Once the C₂A and C₂B domains bind to calcium, however, the protein flips over to allow the opposite face of C₂B to bind to PIP₂.

In this conformation, syt inserts membrane penetration prongs into the plasma membrane, potentially facilitating fusion with the secretory vesicle membrane and accelerating exocytosis. Thus, when calcium enters the cell, syt is already poised so that the first membrane C₂B encounters is the plasma membrane. In other words, the syt-PIP₂ interaction essentially steers the synaptic vesicle to the plasma membrane in preparation for exocytosis. ■

Reference: Bai, J., et al. 2004. Nat. Struct. Mol. Biol. 10.1038/nsmb709.

Parsing p53 activity

Vertebrate p53 has many talents, including the ability to induce apoptosis and cell cycle arrest in response to DNA damage. At least one of these talents seems to be crucial for suppressing tumor formation, as mice that lack p53 develop early onset T-cell lymphomas and usually die young. New results from Geng Liu, Guillermina Lozano, and colleagues (M.D. Anderson Cancer Center, Houston, Texas) suggest that, for tumor initiation, delay trumps death.

The group made mice with a p53 point mutation that leaves the protein with the ability to delay cell cycle but not induce apoptosis. This mutation causes a much less severe phenotype than p53 deletions, with no delay in tumor onset and only rare cases of lymphoma. They then showed that the decreased tumorigenesis is a result of the p53 cell cycle activity’s ability to maintain genome stability. Most of the tumor cells from p53 null mice were aneuploid, but those from mice with the point mutation remained diploid. The point mutation cells also had two centrosomes during division, whereas the null cells had multiple centrosomes that led to large chromosomal breaks and missegregations.

“If you look at the literature, it is almost dogma that the apoptosis function of p53 is the crucial activity for preventing tumorigenesis,” says Lozano. “Our data says that the cell cycle arrest function of p53 is as pivotal.” The tumors that do develop, however, tend to be aggressive, which supports the idea that apoptosis is critical for controlling tumor progression. ■

Reference: Liu, G., et al. 2004. Nat. Genet. 36:63–68.

RNAi-mediated DNA silencing

After embracing RNAi’s ability to silence and degrade transcripts, biologists have eyed reports that RNAi is required for heterochromatin assembly with skepticism. With new results showing that siRNAs are essential for targeting a heterochromatin-associated protein complex to DNA, André Verdel, Danesh Moazed (Harvard Medical School, Boston, Massachusetts), Shiv Grewal (National Cancer Institute, Bethesda, Maryland), and colleagues think that skepticism is now unfounded. “It leaves no doubt that RNAi is very directly involved in heterochromatin formation,” says Moazed.

Previous work showed that deletion of factors required for RNAi disrupted heterochromatin formation in yeast, implying that RNAi was involved in transcriptional gene silencing. Now, the authors have purified an RNAi effector complex (RITS) that contains Ago1 (the homologue of the siRNA-associated protein Argonaute), a heterochromatin associated protein Chp1, a novel protein Tas3, and small siRNAs complementary to centromeric DNA repeats.

Mutations of Tas3 disrupt heterochromatin formation and block association of methylated histone-3 with DNA, a phenotype that resembles the previously described mutants of Ago1 and Chp1. Targeting the RITS complex to DNA requires siRNAs—in yeast strains lacking Dicer, the enzyme that produces siRNAs, the proteins associate but fail to find chromatin. RITS may recruit histone modifying enzymes to chromatin and initiate heterochromatin assembly. Although Verdel et al. expect these associations to be transient, they are working to detect them directly. ■

Reference: Verdel, A., et al. 2004. Science. 10.1126/science.1093686.