EXOCYTOSIS

Rapid-response unit

In an emergency situation, a speedy response is essential — whether it's at the level of an ambulance responding to an emergency phone call or a reflex response of the nervous system to a painful stimulus. Nerves communicate with one another by the Ca^{2+}-induced exocytosis of synaptic vesicles and, in *Nature Structural & Molecular Biology*, Chapman and colleagues now provide insights into how this event can occur so rapidly.

Synaptotagmin I is anchored to synaptic-vesicle membranes by its transmembrane domain, and it can sense Ca^{2+} levels through its two cytoplasmic C2 domains (C2A and C2B). This Ca^{2+} sensing is thought to be important for the Ca^{2+}-induced fusion of docked vesicles. A putative effector of synaptotagmin-I function is phosphatidylinositol-4,5-bisphosphate ($PtdIns(4,5)P_2$), which has recently been shown to be selectively localized to the plasma membrane. Chapman and co-workers therefore studied the interaction of synaptotagmin I with $PtdIns(4,5)P_2$-containing membranes.

The authors first assessed the ability of the fragments C2A, C2B and C2A–C2B to interact with liposomes containing varying amounts of $PtdIns(4,5)P_2$. In the presence of Ca^{2+}, C2A only interacted with liposomes containing high levels of $PtdIns(4,5)P_2$, which might not be physiologically relevant, whereas C2A–C2B and C2B interacted with liposomes containing low levels of $PtdIns(4,5)P_2$. Notably, the latter two fragments also interacted with these liposomes in the absence of Ca^{2+}, which indicates that C2B might bind to $PtdIns(4,5)P_2$ before the Ca^{2+} signal *in vivo*.

In response to Ca^{2+}, the Ca^{2+}-binding loops of the C2A and C2B domains have been shown to penetrate membrane bilayers, and Chapman and colleagues inserted fluorescent probes into the four loops of C2A–C2B to see if this is the case for $PtdIns(4,5)P_2$-containing membranes. They used liposomes containing $PtdIns(4,5)P_2$ and membrane-embedded, fluorescence-quenching labels to show that, in response to Ca^{2+}, three of the four loops of C2A–C2B penetrate the bilayer. By contrast, in the absence of Ca^{2+}, C2A–C2B binding occurs in the absence of quenching, which indicates that Ca^{2+}-independent binding does not require membrane penetration. In fact, the authors showed that this binding occurs through a polybasic region on the side of C2B.

So, does this 'pre-binding' increase the speed of the Ca^{2+}-induced response of synaptotagmin I? When the authors premixed C2A–C2B with $PtdIns(4,5)P_2$-containing liposomes in the absence of Ca^{2+}, the response of C2A–C2B to Ca^{2+} occurred with submillisecond kinetics and was more rapid than when the premixing step was omitted. Furthermore, they showed that $PtdIns(4,5)P_2$ steers synaptotagmin I towards $PtdIns(4,5)P_2$-containing membranes. Synaptotagmin I reconstituted into proteoliposomes interacts preferentially with $PtdIns(4,5)P_2$-containing membranes in *trans*; this interaction occurs in the absence of Ca^{2+} and is enhanced by Ca^{2+}.

Chapman and co-workers therefore propose a model in which the side of the C2B domain first steers synaptotagmin I to $PtdIns(4,5)P_2$-containing membranes by interacting with $PtdIns(4,5)P_2$ in a Ca^{2+}-independent manner. As a result of this pre-binding, in response to Ca^{2+}, the C2 domains only need to be reorientated, so Ca^{2+}-binding loops can penetrate the bilayer rapidly. Synaptotagmin-I–$PtdIns(4,5)P_2$ interactions are therefore required to steer the membrane-penetration activity of synaptotagmin I towards the plasma membrane, as well as to increase the speed of the Ca^{2+}-induced response of synaptotagmin I.

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References and links

ORIGINAL RESEARCH PAPER Bai, J. *et al.* PIP_2 increases the speed of response of synaptotagmin and steers its membrane-penetration activity toward the plasma membrane. *Nature Struct. Mol. Biol.* 29 Dec 2003 (doi:10.1038/nsmb709)

IN BRIEF

NUCLEAR EXPORT

Nuclear export of microRNA precursors.

Lund, E. *et al. Science* 20 November 2003 (doi:10.1126/science.1090599)

Nuclear and cytoplasmic RNase-III-like endonucleases are involved in the biogenesis of microRNAs (miRNAs) in mammalian cells. Pre-miRNAs are generated in the nucleus by Drosha, but need to be processed further in the cytoplasm by Dicer. In this report, Kund *et al.* show that exportin-5 (Exp5), which binds minihelix-containing RNAs, stimulates pre-miRNA export in a RanGTP-dependent way. Efficient nuclear export requires pre-miRNAs to be correctly processed, and the binding is direct, as adaptor proteins did not enhance the Exp5–pre-miRNA association.

PHAGOCYTOSIS

Phosphatidylserine receptor is required for clearance of apoptotic cells.

Li, M. O. *et al. Science* **302**, 1560–1563 (2003)

Cell corpse engulfment mediated by *C. elegans* phosphatidylserine receptor through CED-5 and CED-12.

Wang, X. *et al. Science* **302**, 1563–1566 (2003)

Exposure of the normally inner-leaflet-localized phospholipid phosphatidylserine on the surface of dying cells usually signals phagocytosis, but exactly how was unclear until now. Wang *et al.* have characterized a *C. elegans* phosphatidylserine receptor (PSR)-1. Mutating *psr-1* compromised the engulfment of cell corpses, whereas re-expressing *psr-1* restored corpse engulfment. The authors found that PSR-1 probably functions upstream of a ternary complex (CED-2, CED-5 and CED-12) that regulates the small GTPase CED-10 to control cell-corpse engulfment. Li *et al.* studied the effects of deleting the PSR in mice. PSR-deficient mice died shortly after birth from impaired respiration. A certain amount of apoptosis is involved in lung morphogenesis, and the authors found that phagocytosis of apoptotic cells occurred in wild-type, but not PSR-deficient, lungs. In some of the PSR-deficient mice, brain cells also overproliferated, and there were increased numbers of apoptotic cells and macrophages. So, dying cells might normally bind to macrophages to induce/enhance apoptosis.

CYTOSKELETON

Regulation of cell polarity and protrusion formation by targeting RhoA for degradation.

Wang, H.-R. *et al. Science* **302**, 1775–1779 (2003)

Until now, ubiquitin ligases have not been reported to regulate cell shape, motility or polarity. But the authors of this paper show that Smurf1, a HECT-domain E3 ubiquitin ligase, regulates protrusive activity and polarity in fibroblasts. Small interfering RNA against Smurf1 decreases cell motility and restores a non-transformed morphology to tumour cells. Protein kinase Cγ, a component of the PAR6–PKCγ complex, recruits Smurf1 to filopodia and lamellipodia, where it locally mediates Rho degradation by ubiquitylation. This probably inhibits inappropriate stress-fibre formation during remodelling of protrusions.