SIGNALLING

PIKE fishing

Phospholipase C-γ (PLC-γ) is an important mediator of the mitogenic actions of several growth factor receptors; overexpression of the PLC-γ1 isoform induces DNA synthesis and tumorigenicity. Its phospholipase activity, though, isn't required for this — it is the Src-homology-3 (SH3) domain that is responsible, but the mechanism underlying this action has so far remained unsolved. Now, Snyder and his colleagues report that PLC-γ1 — through its SH3 domain — has physiological guanine nucleotide exchange factor (GEF) activity towards a nuclear GTPase that activates nuclear phosphatidyl inositol 3′-kinase (PI3K) activity.

The nuclear GTPase in question is PI3K enhancer (PIKE), identified two years ago. PIKE has proline-rich domains, so pull-down assays were used to 'fish' for potential binding partners — proteins that contain SH3 domains. PLC-γ was found to interact strongly with the third proline-rich domain of PIKE, so the authors then tested whether PLC-γ affected PIKE's GEF activity. They showed that the SH3 domain of PLC-γ (residues 790–850) stimulated GTP loading and GDP dissociation, whereas a proline to leucine mutation at 842 abolished this. If PLC-γ does indeed have GEF activity then, like other GEFs, it should bind strongly and then dissociate from its target GTPase — in this case, PIKE — which indeed it did.

PIKE is a nuclear protein, so can PLC-γ be found in the nucleus, too? Cell fractionation studies and immunofluorescent staining confirmed that stimulation with nerve growth factor (NGF) increased the level of PLC-γ in the nucleus. So how does stimulation with NGF and subsequent nuclear translocation of PLC-γ regulate the activities of PIKE and PI3K? In cells transfected with wild-type PLC-γ and treated with NGF, PIKE activity and nuclear PI3K activity were markedly enhanced. Deletion of the SH2 or catalytic domains of PLC-γ had no effect on this, but deleting or mutating the SH3 domain blocked the activation of both proteins.

Next, the authors showed that the SH3 domain of PLC-γ is necessary for mitogenesis in response to growth factors. This is consistent with previous findings in which cells containing SH3-domain-deletion constructs failed to grow in the absence of serum. Together, these results provide a mechanism by which PLC-γ — through PIKE — can mediate mitogenic actions in the absence of its lipase activity, and further contribute to the possibility that the SH3 domain is not just a binding motif, but in fact has some enzymatic activity.

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References and links

ORIGINAL RESEARCH PAPER Ye, K. *et al.* Phospholipase C-γ1 is a physiological guanine nucleotide exchange factor for the nuclear GTPase PIKE. *Nature* **415**, 541–544 (2002)

FURTHER READING Ye, K. *et al.* PIKE: A nuclear GTPase that enhances PI3Kinase activity and is regulated by protein 4.1N. *Cell* **103**, 919–930 (2000)

STRUCTURE WATCH

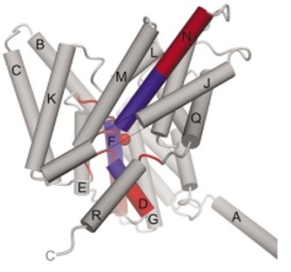
A common theme

SNAREs — an evolutionarily conserved superfamily of membrane-associated proteins — are essential for intracellular membrane fusion in all eukaryotes. They assemble into tight complexes that connect membranes and might induce fusion. Much of our understanding of these proteins is based on the crystal structure of the neuronal SNARE core complex, which is an unusually long bundle of four α-helices (provided by three different SNAREs) connected by 16 layers of mainly hydrophobic amino acids. But are these structural features common to all SNAREs?

Schneider and colleagues have now solved the 1.9 Å-resolution crystal structure of an endosomal SNARE core complex, in which four different SNAREs each contribute a single helix to make a four-helix bundle. In terms of overall structure and interactions, this bundle is surprisingly similar to the neuronal core complex, despite limited sequence homology. Although there are subtle structural variations that characterize SNARE subfamilies, the authors propose that the core complex structure is conserved to a greater extent than would be expected on the basis of sequence similarity, and that the structure is closely linked to the general role of SNAREs in membrane fusion. They suggest that evidence supporting other structural arrangements of the core complex should be re-examined.

REFERENCE Antonin, W. *et al.* Crystal structure of the endosomal SNARE complex reveals common structural principles of all SNAREs. *Nature Struct. Biol.* **9**, 107–111 (2002)

How to be selective



ClC chloride channels — found in prokaryotes and eukaryotes — catalyse the flow of Cl^- across cell membranes. It has been difficult to understand many of the properties of these channels — for example, their selectivity — in the absence of a high-resolution structure. However, MacKinnon and co-workers have now determined the crystal structures of two prokaryotic ClC Cl^- channels from *Salmonella typhimurium* and *Escherichia coli* to 3.0 and 3.5 Å resolution, respectively.

Both structures reveal a homodimeric membrane protein containing two identical, independent pores, one in each subunit. The arrangement of α-helices within each subunit forms a selectivity filter, in which a single Cl^- ion is stabilized by electrostatic interactions with helix dipoles and by contacts with main-chain nitrogen atoms and side-chain hydroxyl groups. Cl^- does not directly contact a full positive charge from a lysine or arginine, which, the authors suggest, would cause it to bind too tightly. They propose that the use of partial charges to stabilize Cl^- permits rapid conduction rates.

The authors also identified a glutamate side chain that projects into the selectivity filter. They propose that the glutamate might 'block' the ion pathway until Cl^- enters the pore and induces a conformational change that displaces this residue.

REFERENCE Dutzler, R. *et al.* X-ray structure of a ClC chloride channel at 3.0 Å reveals the molecular basis of anion selectivity. *Nature* **415**, 287–294 (2002)