SIGNALLING

Making waves

The small GTPase Rab5 has well-established roles in membrane trafficking — for example, in receptor endocytosis. However, as Di Fiore and colleagues now show in *Nature*, it seems that Rab5 is also a signalling GTPase. They show that Rab5 is essential for a form of receptor tyrosine kinase (RTK)-induced actin remodelling that produces waves/circular ruffles.

The signalling events that lead to circular ruffling have remained unclear, despite the fact that the formation of another type of ruffle — cell-edge ruffles — is known to involve an RTK–Ras–Rac linear cascade. However, from previous work, it was believed that Ras/Rac activation together with further RTK-triggered pathways might be required to induce circular ruffling.

Di Fiore and co-workers began by showing that a dominant-negative Rab5 mutant inhibited the RTK-dependent induction of circular ruffling: these effects were not the result of inhibiting endocytosis. They further dissected the signalling pathway that is involved in circular ruffling using various mutant proteins, and found that three independent signals — from Rab5, the Ras–phosphatidylinositol-3-kinase pathway and Rac — are simultaneously required to induce this ruffling.

To further understand the signals that connect Rab5 to circular ruffling, the authors carried out a structure–function analysis of the Rab5-specific GTPase-activating-protein (GAP) RN-tre. They showed that two independent functions of RN-tre — the GAP function and a function that resides in the carboxyl terminus — are linked to the formation of circular ruffles. So, what is the function of the latter region?

The authors used mass spectrometry to identify proteins that interact with the carboxyl terminus of RN-tre, and they found actinin-4 — an actin-binding protein that crosslinks filamentous (F)-actin. They showed that RNA interference against RN-tre or actinin-4 markedly impaired the circular ruffling that is induced by RTK activation. In addition, overexpressing actinin-4 could rescue the inhibitory effect of an RN-tre mutant on RTK-induced circular ruffling. It therefore seems that actinin-4 functions downstream of Rab5 and RN-tre in the RTK-dependent induction of circular ruffling.

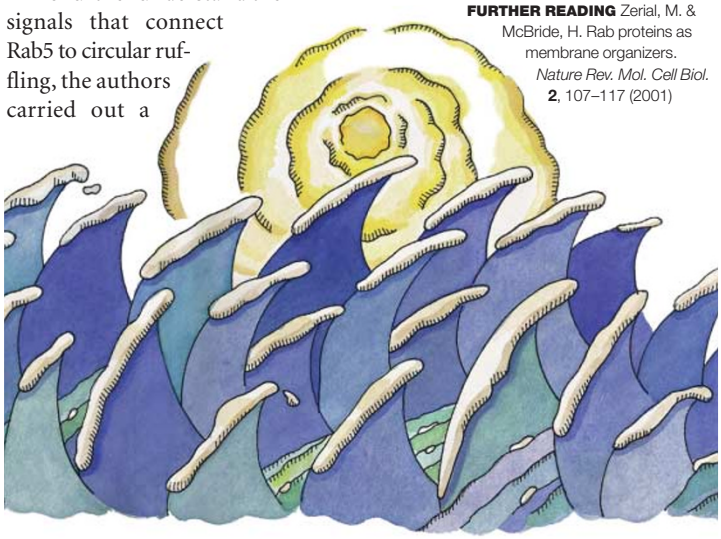
In the final part of their study, Di Fiore and colleagues showed that RN-tre can bind to F-actin and actinin-4 simultaneously. So, this work has established Rab5 as a signalling GTPase and the authors propose "…that RN-tre establishes a three-pronged connection with Rab5, F-actin and actinin-4", which might "…aid crosslinking of actin fibres into actin networks at the plasma membrane".

Rachel Smallridge

References and links

ORIGINAL RESEARCH PAPER Lanzetti, L. *et al.* Rab5 is a signalling GTPase involved in actin remodelling by receptor tyrosine kinases. *Nature* **429**, 309–314 (2004)

FURTHER READING Zerial, M. & McBride, H. Rab proteins as membrane organizers. *Nature Rev. Mol. Cell Biol.* **2**, 107–117 (2001)



STRUCTURE WATCH

Compacted by condensin'

Aspects of chromosome compaction have been well characterized, yet little is known about the higher orders of DNA compaction. However, two papers — from the Hirano group in *Current Biology* and from Bustamante and colleagues in *Science* — now describe the compaction of DNA by the *Xenopus laevis* condensin-I complex and by the *Escherichia coli* condensin MukBEF, respectively.

Using single-molecule manipulation techniques, the Hirano group showed that DNA is reversibly compacted against a weak stretching force in the presence of condensin I and hydrolysable ATP. Increasing the stretching force resulted in large, discrete increases in the DNA end-to-end extension, and the size of the increases led this group to propose that condensin I compacts DNA by introducing loops along the DNA. Also using single-molecule techniques, Bustamante and co-workers showed that MukBEF cooperatively compacts DNA into a repetitive, stable structure in an ATP-binding-dependent manner. Extending the DNA caused it to expand in a series of repetitive steps, which, surprisingly, were identical in every subsequent experiment. In addition, DNA compaction after this extension occurred in the absence of ATP and free MukBEF. Consequently, these authors propose that MukBEF requires ATP binding to form force-insensitive, intermolecular contacts and to polymerize along DNA. In addition, they propose that force-sensitive, intramolecular contacts between the two heads of each MukBEF molecule loop the DNA and induce compaction.

REFERENCES Strick, T. R. *et al.* Real-time detection of single-molecule DNA compaction by condensin I. *Curr. Biol.* **14**, 874–880 (2004) | Case, R. B. *et al.* The bacterial condensin MukBEF compacts DNA into a repetitive, stable structure. *Science* 3 June 2004 (doi:10.1126/science.1098225)

Get into shape

The coordinated action of serine/threonine kinases and phosphatases is important for the control of many biological processes, but the human genome encodes far more serine/threonine kinases than phosphatases. To overcome this problem, substrate specificity can be conferred on phosphatases through their interactions with regulatory proteins. For example, protein phosphatase-1 (PP1) has many cellular functions — its substrate specificity is regulated by proteins that are generally unrelated, but that usually bind to PP1 through a canonical PP1-binding motif (RVxF). In myosin phosphatase, PP1 interacts with MYPT1, which regulates the function of PP1 in smooth muscle relaxation. And, in *Nature*, Dominguez and colleagues now show how PP1–MYPT1 interactions control PP1 activity.

The authors describe the 2.7-Å resolution crystal structure of PP1 bound to the amino-terminal domain of MYPT1. They found that, overall, the structure of PP1 in this complex is similar to that seen in other PP1-containing complexes. However, they noted that PP1 interactions with structural elements of MYPT1 that are amino- and carboxy-terminal of its RVxF motif lead to a marked reshaping of the PP1 catalytic cleft. This reshaping contributes to the increased myosin specificity of the PP1–MYPT1 complex, which means that this work "…has general implications for the control of PP1 activity by other regulatory subunits".

REFERENCE Terrak, M. *et al.* Structural basis of protein phosphatase 1 regulation. *Nature* 26 May 2004 (doi:10.1038/nature02582)