SUMOYLATION

On the move

In the social amoeba *Dictyostelium discoideum*, cyclic AMP activates a mitogen-activated protein kinase (MAPK) cascade, which operates through MAPK and extracellular signal-regulated kinase (ERK) kinase — MEK — to induce chemotaxis and cell aggregation. Here, Firtel's group shows that MEK SUMOylation and ubiquitylation are necessary for regulating chemotaxis.

Using *mek1*-null cells, the authors showed that a tagged version of MEK1 complemented the null phenotype (defective chemotaxis and small-aggregate formation), but that cAMP also shifted the electrophoretic mobility of MEK1 — with the maximal change occurring 5–15 seconds after stimulation. This result, plus the presence of two putative SUMOylation sites, led the authors to ask if MEK1 was SUMOylated. Biochemical studies showed that it was, but only on one of the sites — lysine (K) 105. A K105R (arginine) mutation ($MEK1^{K105R}$) couldn't complement the chemotaxis defect of *mek1*-null cells, which implied that SUMOylation was needed for MEK to function properly.

So what is SUMOylation doing? One function, it seems, is to translocate MEK to the cell cortex in response to cAMP. Normally, a proportion of MEK moves to the cell cortex — again, maximally after 5–15 seconds — concomitant with the ability to detect SUMO immunoreactivity here and with a decrease in the amount of MEK in the nucleus. But this didn't occur in $MEK1^{K105R}$ cells.

The authors then investigated whether there is a relationship between MEK activation and SUMOylation — that is, would SUMOylation translocate non-activatable MEK? The answer was no; non-activatable MEK stayed in the nucleus and was not SUMOylated. Conversely, constitutively activated MEK was found mainly in the cytosol and was constitutively SUMOylated.

In searching for MEK1-interacting proteins, Firtel's group identified MEK1-interacting protein (MIP1), which contains a RING finger that is common in SUMO ligases (enzymes that add SUMO to their target proteins). But whereas MIP1 did not mediate MEK SUMOylation (MEK1 was still strongly SUMOylated in *mip1*-null cells), MIP1 turned out to be an E3 ubiquitin ligase. Indeed, a higher molecular weight MEK 'smear' that was seen after prolonged cAMP stimulation (3–30 minutes) was ubiquitin immunoreactive.

Further investigation showed that SUMOylation is not required for MEK1 ubiquitylation and that, in contrast to SUMOylation, ubiquitylation does not require MEK1 activation. So the authors propose a situation in which, in response to cAMP, MEK1 is activated, SUMOylated and transported to the cell cortex. MEK1 inactivation — by an unknown process — precedes deSUMOylation and re-localization to the nucleus, where it is ubiquitylated, and thereby terminates the signal.

Katrin Bussell

References and links

ORIGINAL RESEARCH PAPER Sobko, A., Ma, H. & Firtel, R. A. Regulated SUMOylation and ubiquitination of DdMEK1 is required for proper chemotaxis. *Dev. Cell* **2**, 745–756 (2002)
FURTHER READING Müller, S. *et al.* SUMO, ubiquitin's mysterious cousin. *Nature Rev. Mol. Cell Biol.* **2**, 202–210 (2001)
WEB SITES
Rick Firtel's laboratory:
http://www-biology.ucsd.edu/faculty/firtel.html
Encyclopedia of Life Sciences: http://www.els.net
Slime moulds

CYTOSKELETON

Formin' filaments

When it comes to actin-filament assembly, the Arp2/3 complex seems to have received all the attention recently. In budding yeast, though, the formins Bni1 and Bnr1 promote the assembly of actin cables (bundles of actin filaments) when Arp2/3 is absent, and in *Science Express*, Pruyne and colleagues report that formins might directly nucleate unbranched actin filaments.

Bni1 is made up of a Rho-binding domain, a formin homology-1 (FH1) domain, an FH2 domain and a carboxy-terminal extension. Expression of Bni1 that lacks the first domain (Bni1FH1FH2COOH) can induce filament assembly *in vivo*, so the authors attempted to reconstitute this *in vitro*. They succeeded — Bni1FH1FH2COOH nucleated actin filaments in actin-polymerization assays. Nucleating activity was dose-dependent on Bni1FH1FH2 (deletion of the carboxy-terminal extension had no effect) but, in contrast to Arp2/3-mediated nucleation, was not enhanced by pre-formed actin filaments. Further analysis showed that FH2 alone could nucleate actin, but that FH1, which binds profilin (a nucleation enhancer) *in vivo*, is probably important too.

Actin filaments have their own polarity in the form of opposing barbed and pointed ends, and Pruyne and colleagues wanted to know at which end Bni1FH1FH2 was mediating filament growth. Bni1FH1FH2-stimulated filament growth was sensitive to cytochalasin B — an inhibitor of barbed-end filament growth — which indicates that Bni1FH1FH2-nucleated filaments grow from the barbed end. Furthermore, the authors found that Bni1FH1FH2 localized with assembling filaments at the barbed end. Notably, electron-microscopy examination showed all the filaments to be long and unbranched.

As Bni1FH1FH2 nucleates unbranched actin filaments and remains associated with the growing end of barbed filaments, the authors propose that these unique properties help Bni1 to establish the polarity of growing actin filaments, a feature that could apply to actin dynamics in other eukaryotes.

Katrin Bussell

References and links

ORIGINAL RESEARCH PAPER Pruyne, D. *et al.* Role of formins in actin assembly: nucleation and barbed end association. *Science Express* 2002 June 6 (DOI 10.1126/science.1072309)
WEB SITES
Anthony Bretscher's laboratory:
http://www.mbg.cornell.edu/bretscher/bretscher.html
Encyclopedia of Life Sciences:
http://www.els.net
Actin and Actin filaments