DEVELOPMENT

All shapes and sizes

The small GTPase Rho is best known for its influence on cell shape, but a report in *Developmental Cell* by Settleman's group now shows that it might control cell size too.

In mammalian cells, a class of GTPase activating proteins — GAPs — are the main negative regulators of Rho, and p190-B RhoGAP seems to be among the most potent of these *in vivo*. Settleman's group disrupted the p190-B RhoGAP gene in mice, which resulted in a range of defects and death immediately after birth. What was striking, though, was a 30% reduction in mouse size and an intriguing phenotype similarity to that seen in mice that lack the transcription factor cyclic AMP responsive element binding protein (Creb). Further analysis showed that levels of phosphorylated Creb were reduced in the p190-B RhoGAP mutants, indicating a possible connection between these proteins.

Mutant embryo cells were found to be 30% smaller than wild-type cells, which probably accounts for the decreased tissue size. As p190-B RhoGAP$^{-/-}$ cells were smaller than the same cells expressing constitutively active Creb, Creb probably functions downstream of p190-B RhoGAP. Furthermore, transfection of wild-type cells with constitutively active Creb produced abnormally large cells, whereas a dominant-negative Creb construct, or a constitutively active form of Rho (RhoV14), which mimics the levels of active Rho that are seen in p190-B RhoGAP$^{-/-}$ cells, resulted in smaller cells.

Signalling by the insulin growth factor (IGF) pathway regulates the size of cells, so the group studied the effect of IGF on Creb phosphorylation in p190-B RhoGAP$^{-/-}$ cells and found it was reduced compared to wild-type cells. The RhoV14 construct also had the same effect. One Rho effector — Rho-kinase (ROK) — antagonizes insulin signalling by associating with the insulin receptor substrate, so could this account for the decrease in cell size? Adding a ROK inhibitor restored Creb phosphorylation and cell size in p190-B RhoGAP$^{-/-}$ cells, so ROK is important in regulating cell size. Further analysis showed that phosphorylation of c-Jun-amino-terminal kinase and p38 — two kinases that are downstream of IGF signalling — is reduced in p190-B RhoGAP$^{-/-}$ cells. As both of these kinases activate Creb, this might explain how Rho signalling affects cell size as well as shape.

Katrin Bussell

References and links

ORIGINAL RESEARCH PAPER Sordella, R. *et al.* Modulation of CREB activity by the Rho GTPase regulates cell and organism size during mouse embryonic development. *Dev. Cell* **2**, 553–565 (2002)

CELL CYCLE

Under arrest!

During the development of a vertebrate oocyte, there's a pause in metaphase of meiosis II to prevent parthenogenetic activation — only when the egg has been fertilized can development resume. This arrest is maintained by the so-called 'cytostatic factor' (CSF) and, reporting in *Nature*, Julie Reimann and Peter Jackson now describe the long-sought mediator of CSF activity.

The existence of the CSF was proposed by Yoshio Masui and Clement Markert in 1971. Then, in 1989, George Vande Woude and colleagues showed that the Mos signalling pathway is a critical component of CSF activity. Subsequent studies found that Mos is indeed needed to establish CSF arrest, but that it is not required to maintain it.

Enter Emi1. Last year, Jackson's group showed that this protein inhibits the anaphase-promoting complex (APC)/Cdc20 in *Xenopus laevis*. This is significant because the APC-mediated degradation of cyclin B/Cdc2 is the trigger that releases eggs from metaphase arrest. Cdc20 fits into this picture because it is required for the activation of APC after fertilization (see diagram).

Fertilization leads to an increase in the levels of cytoplasmic Ca^{2+} and, ultimately, release from CSF-mediated arrest. So, to test whether Emi1 is indeed a component of CSF, the authors checked whether its overexpression is enough to prevent release from metaphase arrest in the presence of Ca^{2+}. Sure enough, the addition of purified Emi1 to arrested eggs ('CSF extracts') prevented the Ca^{2+}-induced destruction of cyclin B. The authors then showed that this effect did not require the Mos pathway.

Reimann and Jackson next asked whether Emi1 is needed to maintain the metaphase arrest. They depleted Emi1 from CSF extracts using magnetic beads coupled to anti-Emi1 antibodies, and observed the destruction of cyclin B and release from arrest in these extracts. When purified Emi1 was added back to these extracts, the remaining cyclin B was stabilized and metaphase arrest resumed. Moreover, pre-incubation with a carboxy-terminal fragment of Emi1, which can inhibit the activation of APC, rescued cyclin B stability and metaphase arrest, indicating that these effects indeed occur through APC.

If Emi1 inhibits the Cdc20-mediated activation of APC, then addition of excess Cdc20 might be expected to override the inhibitor effect of Emi1. And this is what the authors saw — Cdc20 induced the spontaneous degradation of cyclin B in a dose-dependent fashion. But what acts upstream of Cdc20? And how is the Emi1-dependent inhibition removed after fertilization?

On fertilization, the Ca^{2+} signal is transduced by calmodulin-dependent protein kinase II (CaMKII). So the authors wondered whether the activation of CaMKII might lead to a change in the interaction between Emi1 and Cdc20. They found that, just 2.5 minutes after the addition of Ca^{2+}, the binding of endogenous Emi1 and Cdc20 to one another was blocked. This was accompanied by a rapid increase in the electrophoretic mobility of Cdc20, which is consistent with dephosphorylation.

Is the mystery finally solved then? Not quite. We still do not know how Emi1 is activated during oocyte maturation, or whether it is involved at an earlier stage too (possibly under the control of the Mos pathway). But this study takes us a big step closer to understanding this very elusive factor.

Alison Mitchell

References and links

ORIGINAL RESEARCH PAPER Reimann, J. D. R. & Jackson, P. K. Emi1 is required for cytostatic factor arrest in vertebrate cells. *Nature* **416**, 850–854 (2002)

FURTHER READING Masui, Y. The elusive cytostatic factor in the animal egg. *Nature Rev. Mol. Cell Biol.* **1**, 228–232 (2000)