IN THE NEWS

Unifying to study division

European researchers from 11 institutes have joined forces, funded by an €8.5 million grant from the European Union, to get to grips with one of the most fundamental scientific processes — how cells divide.

In the words of Dr Jan-Michael Peters, from the Research Institute of Molecular Pathology in Vienna, "We have set very ambitious goals, which no single research partner could have tackled alone. By bringing together a group of excellent European scientists who contribute expertise in rather diverse areas, we can hope to solve a complex biological puzzle" (*CORDIS News*, 16 July 2004).

The integrative 'MitoCheck' consortium aims to carry out an audit of all the genes that are required during cell division to understand better this precisely choreographed process. Using RNA interference, ~20,000 genes will be individually silenced in cells, and the resulting effects on mitosis will be analysed by filming the cells. Scientists already know that kinases are important in regulating mitosis, so they hope to examine how these enzymes influence the products of all the genes tested.

MitoCheck will also study the subcellular localization of these gene products and the involvement of a subset of them in protein complexes. As cell division must be so tightly regulated — with obvious consequences for cancer and fertility should this fail — the information could be used for diagnostic or therapeutic purposes. And, "Because the concept behind this project could be applied to other areas, it will have an impact on European cell biology far beyond the cell cycle community." (http://www.mitocheck.org).

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DEVELOPMENTAL BIOLOGY

Putting up barriers

If it wasn't for the generation of an in-built barrier, the phrase 'spiralling out of control' could easily be applied to the positive-feedback loop that drives limb outgrowth in vertebrates. Such a barrier has now been discovered by Scherz *et al.* and their findings are reported in *Science*.

Usually, Sonic hedgehog (Shh) in the zone of polarizing activity (ZPA) of the posterior mesenchyme in the limb bud maintains the expression of several fibroblast growth factors (Fgfs), including *Fgf4*, in the apical ectodermal ridge (AER) by upregulating *Gremlin* in the adjoining mesenchyme. Gremlin antagonizes bone morphogenetic proteins (Bmps), which normally downregulate *Fgf4* — so *Fgf4* expression is maintained. Fgfs, in turn, maintain *Shh* expression. That is, until embryonic day 6 (E6), when *Shh* is downregulated and *Fgf4* and *Gremlin* are no longer expressed. At this point, cells proliferate more slowly in the limb — so this breakdown of the Shh–Fgf loop is essential for regulating limb size.

To figure out how the breakdown occurs, the authors ectopically expressed each of the individual components of the loop in chick embryos just before E6 to see if they could maintain expression of the other genes that would normally be downregulated. Overexpressing either *Fgf4* or *Gremlin* could maintain expression of the other genes, which indicated that cells had not lost responsiveness to either of these signals. However, when a bead soaked in Shh was implanted in the posterior limb, neither *Gremlin* nor *Fgf4* expression was maintained. This ties in with the known occurrence of a zone of exclusion of *Gremlin* expression in the posterior limb — the cells in this zone cannot express *Gremlin* in response to Shh.

When the authors marked the descendants of cells that expressed *Shh*, they found them to expand anteriorly into a domain that resembled the *Gremlin*-free domain. Indeed, the cells that formerly expressed *Shh* did not express *Gremlin*. So Scherz *et al.* proposed that, as limb outgrowth progresses, the proliferating *Shh* descendants form a barrier between the Shh source and the cells that respond to Shh by expressing *Gremlin*. Initially, Shh can diffuse across a small barrier, but as this barrier enlarges and is filled with *Gremlin*-negative *Shh* descendants, such diffusion is no longer possible. Failure to express *Gremlin* results in the failure to antagonize Bmps, and so *Fgf4* is downregulated.

To test their hypothesis, the authors physically removed the 'barrier' cells and rejoined the remaining posterior cells to the remainder of the anterior limb at E5. The feedback loop then operated for longer than in wild-type organisms and the limbs grew until they resembled those of the wild type in both structure and size. The proposed explanation is simple: removal of the barrier removes the obstruction to *Gremlin* induction and the feedback loop is restored — until the barrier forms again. One question to ask is why the *Shh* descendants cannot induce *Gremlin*. Another is whether the downregulation of other *Fgf* genes, such as *Fgf9* and *Fgf17*, occurs through the same mechanism, although the chances are that it does.

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References and links

ORIGINAL RESEARCH PAPER Scherz, P. J. *et al.* The limb bud Shh–Fgf feedback loop is terminated by expansion of former ZPA cells. *Science* **305**, 396–399 (2004)