DEVELOPMENTAL BIOLOGY

Separating head from tail

During early embryonic development in vertebrates, a round clump of cells reshapes into an elongated structure with the future head at the anterior (A) end and the rump or tail at the opposite, posterior (P) end. This process, which is known as convergent extension, involves the narrowing and lengthening of a group of cells, the chordamesoderm cells, parallel to the A–P axis. A report in *Nature* now sheds light on how the cell movements that underlie convergent extension are aligned with the A–P axis in *Xenopus laevis* embryos.

By dissociating chordamesoderm cells, mixing them up and letting them reaggregate, Ninomiya *et al.* found that the cells rearranged themselves according to their original A or P position. In support of this intrinsic chordamesodermal A–P pattern, the *X. laevis brachyury* (*Xbra*) and *chordin* (*chd*) genes show a typical reverse expression pattern, with low A and high P *Xbra* expression, and the opposite pattern for *chd*.

So, what's the relevance of chordamesodermal A–P patterning? To probe this problem, the authors dissociated chordamesoderm cells from the A and P regions, reaggregated them separately, and then combined these aggregates. Combinations of identical aggregates remained round, whereas combined A and P aggregates became elongated along the A–P axis. A–P polarity therefore seems to be required for convergent extension.

At certain concentrations, the signalling molecule activin induces *chd* expression and reduces that of *Xbra*. Treating explants *in vitro* with graded doses of activin caused them to elongate, whereas the uniform exposure to high or low doses of activin meant that the explants remained spherical. Expression of *chd* and *Xbra* was more evenly distributed in uniformly exposed explants when compared with the graded explants, which showed an *Xbra–chd* expression gradient. So, graded activin signalling can establish A–P polarity and trigger

STEM CELLS

Altered fates

Stem-cell research has had more than its share of highs and lows. But now, reporting in *Nature*, Fred Gage and co-workers show that stem cells that are derived from the nervous system can be converted into endothelium-like cells in the absence of cell fusion.

Studies indicating that tissue-specific somatic stem cells can differentiate into other lineages have caused great excitement among researchers, as they promise an attractive potential resource for future regenerative therapies. But, in many cases, these phenotypic changes reflected the donation of genetic material through the formation of cell hybrids.

In view of these results, Gage and his team re-evaluated the developmental potential of stem cells that were derived from adult mouse brain, taking care to exclude fusion events. The authors harvested neural stem cells (NSCs) from mice that constitutively expressed green fluorescent protein (GFP), isolated single cells, and expanded them in culture to derive purified clonal cell lines. As NSCs and blood-vessel endothelia are often anatomical 'neighbours', the authors co-cultured these clones with GFP-negative primary human endothelial cells to ascertain if the NSCs could be induced to develop into endothelial cells. After 2–5 days of co-culture, 6% of the NSCs had developed an endothelial phenotype, as shown by positive staining for both GFP and an endothelium-specific cell-surface antigen, CD146. A purified, expanded population of these GFP/CD146-positive cells was then subjected to PCR after reverse transcription of RNA (RT-PCR), and to immunofluorescence and functional assays (such as capillary-network formation and electron-microscopic identification of specialized secretory vesicles). The results of these studies confirmed the acquisition of endothelium-specific gene expression, protein markers and functional properties. Importantly, these changes were accompanied by the decreased expression of certain neural-lineage-specific genes.

To exclude the possibility that cell fusion had occurred, Gage and co-workers adopted various strategies. First, they prepared metaphase chromosome spreads from NSC-derived endothelial cells. If cell fusion had occurred, microscopic examination would easily distinguish human chromosomes — which are metacentric and acrocentric — from the characteristic telocentric murine chromosomes. In fact, no human chromosomes were present. Next, they excluded initial fusion events by co-culturing NSCs with paraformaldehyde-treated human endothelial cells, which are unable to undergo cell fusion, but can mediate surface-receptor contact. After 5 days, 2–4% of these co-cultured GFP-containing NSCs bound to lectin — a feature of mouse endothelium. Similar endothelium-like cells were found in sections of embryonic brain after *in utero* transplantation of one clonal stem cell line, although *in vivo* conversion rates were lower than those observed *in vitro*. The authors propose that these results could reflect a novel mechanism of angiogenesis, in which cells that line blood vessels are derived from adjacent nervous tissue.

By showing that ectodermally-derived NSCs can differentiate into endothelial cells (which originate from mesoderm), this study revives the concept that NSCs have a relatively broad developmental potential that enables these versatile cells to adopt alternate germ cell layer affiliations.

Shannon Amoils

References and links

ORIGINAL RESEARCH PAPER Wurmser, A. E. *et al.* Cell fusion-independent differentiation of neural stem cells to the endothelial lineage. *Nature* **430**, 350–356 (2004)

convergent extension. According to the authors, a different signalling molecule, Nodal, rather than activin, is likely to be responsible for controlling A–P patterning *in vivo*.

In a final set of experiments, the impairment of the Wnt/planar cell polarity (PCP) pathway prevented the elongation of explants, but did not disturb the graded gene-expression pattern. So, Wnt/PCP signalling seems to control convergent extension independently of A–P polarity, which indicates that the two pathways probably function in parallel.

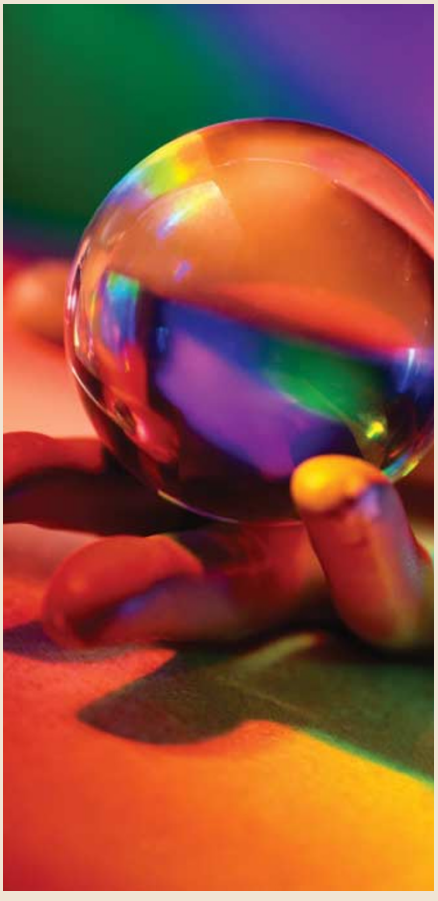
Together, these new findings pave the way for investigating the specific cellular properties that make polarized chordamesoderm cells intercalate, and converge and extend along the A–P axis, thereby separating head from tail.

Arianne Heinrichs

References and links

ORIGINAL RESEARCH PAPER Ninomiya, H. *et al.* Antero–posterior tissue polarity links mesoderm convergent extension to axial patterning. *Nature* **430**, 364–367 (2004)

FURTHER READING Keller, R. Heading away from the rump. *Nature* **430**, 305–306 (2004)



PROTEIN DEGRADATION

Be more choosy

The selection of substrates for degradation by the 26S proteasome is generally thought to lie at the level of ubiquitin-chain assembly. But, in *Cell*, Verma, Deshaies and colleagues now show that the proteins that link polyubiquitylated substrates to the proteasome can add a further layer of substrate selectivity.

The recruitment of polyubiquitylated substrates to the proteasome is essential for ubiquitin-selective degradation, but which protein recognizes the polyubiquitin chains? So far, three different proteins — Rpn10, Rad23 and Rpt5 — have been proposed to be involved in this recognition, and Verma, Deshaies and co-workers addressed the issue of substrate recruitment by studying the degradation of a polyubiquitylated yeast protein (UbSic1) *in vitro*.

They found that, whereas wild-type proteasomes degraded UbSic1 quickly, *rpn10*Δ or *rad23*Δ proteasomes were largely defective in this degradation. These defects could be rescued by the addition of an optimal amount of recombinant Rpn10 or Rad23 to either mutant proteasome, although the rescue of *rpn10*Δ proteasomes by recombinant Rad23 was weak. So, what does this mean?

Verma *et al.* found that the rescue of *rpn10*Δ proteasomes by recombinant Rad23 could be enhanced by the addition of the von Willebrand A (VWA) domain of Rpn10. This Rpn10 construct lacks its ubiquitin-interacting motif (UIM), which highlights two points.

First, the ubiquitin-binding domains of Rpn10 and Rad23 do not have to function sequentially — in fact, the authors found that Rad23 and the UIM of Rpn10 function redundantly, and in parallel, to recruit UbSic1 to proteasomes. Second, the VWA domain of Rpn10 seems to facilitate the Rad23-mediated degradation of UbSic1. Furthermore, as the VWA domain of Rpn10 was not required for the Rad23-dependent tethering of UbSic1 to the proteasome, the authors propose that it functions downstream of Rad23 to enable the productive engagement of proteasome-bound substrates by the degradation machinery.

Next, the authors confirmed their results *in vivo*. Consistent with their proposal that Sic1 can be targeted for degradation by Rad23 or the UIM of Rpn10, they showed that Sic1 was significantly stabilized in cells that were mutated for both Rad23 and the UIM of Rpn10 (Sic1 was degraded with normal kinetics in *rad23*Δ cells). Furthermore, consistent with the proposed facilitator function of Rpn10, Sic1 was more stable in *rpn10*Δ cells than in cells that were only mutated for the UIM of Rpn10 — that is, in the presence of Rad23, Sic1 is more stable in the absence of the VWA domain of Rpn10.

In the final part of this study, Verma, Deshaies and co-workers studied the degradation of other proteasome substrates and found that there was an unexpected degree of specificity in the requirement of substrates for different polyubiquitin-binding proteins. On the basis of their results, they propose that Rpn10, Rad23 and Dsk2 (a protein that is similar to Rad23), and possibly Rpt5 and Ufd1–Cdc48 (a complex that is involved in endoplasmic-reticulum-associated degradation) can function separately to recruit polyubiquitylated substrates to the proteasome. The challenges for the future are to elucidate how many recruitment pathways exist, to understand how substrates are targeted to the different pathways, and to determine whether the different pathways are differentially regulated.

Rachel Smallridge

References and links

ORIGINAL RESEARCH PAPER Verma, R. *et al.* Multiubiquitin chain receptors define a layer of substrate selectivity in the ubiquitin–proteasome system. *Cell* **118**, 99–110 (2004)

FURTHER READING Pickart, C. M. & Cohen, R. E. Proteasomes and their kin: proteases in the machine age. *Nature Rev. Mol. Cell. Biol.* **5**, 177–187 (2004)

WEB SITE

Ray Deshaies' laboratory: http://www.its.caltech.edu/~rjdlab/