DEVELOPMENT

Cutting out a pattern

Protein degradation is a key regulator of the cell cycle, inflammation and transcription — but little is known about its role in development. Zhu and Kirschner now report in *Developmental Cell* the identification of *Xom* — a developmental gene that is regulated by proteolysis.

In a screen to identify gene products that are differentially degraded before and after the onset of midblastula transition, the authors found that two proteins matched these criteria: an unknown protein and Xom, a homeobox transcription factor, which was stable during early gastrulation but subsequently degraded. Further experiments showed that Xom's degradation depended on the ubiquitin–proteasome pathway.

It turned out that one of two PEST domains (proline-, glutamic acid- and aspartic acid-, serine- or threonine-rich regions) present in Xom was required for Xom degradation. Interestingly, this so-called 'Xom destruction motif' (XDM) resembles the glycogen synthase kinase 3 (GSK3) consensus phosphorylation site, which is conserved in the known substrates for GSK3-dependent proteolysis such as β-catenin.

Within the XDM, the authors found two phosphorylation sites — Ser140 and Ser144. A peptide of the phosphorylated XDM blocked Xom degradation. Paradoxically so did the unphosphorylated peptide, implying that the embryonic extract used in these assays contained a kinase activity, although this turned out not to be GSK3. By contrast, the same peptide in which Ser140 and Ser144 were mutated to Alanine residues was unable to block Xom degradation.

Using *in vitro* binding assays, Zhu and Kirschner found that the E3 ubiquitin ligase Skp1–Cullin–F-box complex (SCF) containing the F-box protein βTRCP is responsible for Xom degradation. And they were able to show that a dominant-negative βTRCP mutation blocked Xom degradation *in vivo*. Intriguingly, this E3 ubiquitin ligase complex also mediates the phosphorylation-dependent degradation of β-catenin.

So, what is the role of Xom degradation in early *Xenopus* development? Bone-morphogenetic protein 4 (BMP4) is a ventral morphogen that acts together with Xom in an auto-activating feedback loop — Xom is activated by BMP and vice versa. In addition, Xom inhibits the transcriptional activity of dorsal-specific genes that, in turn, inhibit BMP activity. Zhu and Kirschner hypothesized that proteolysis of Xom might be needed to cease Xom-mediated repression of dorsal-specific genes such as *goosecoid* during early gastrulation. If true, the auto-activating circuit would be eliminated, leading to dorso–ventral asymmetry in the mesoderm, and loss of BMP expression on the dorsal side and high expression on the ventral side.

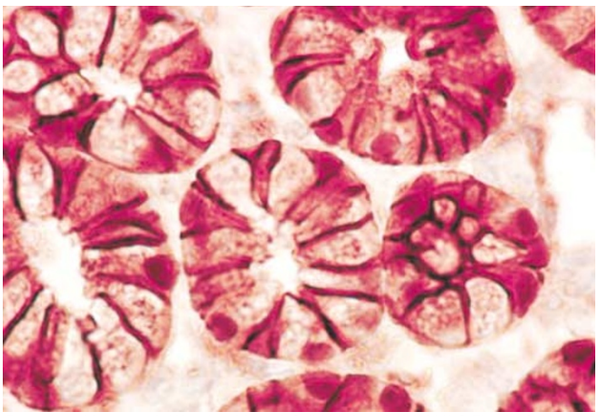
Indeed, luciferase reporter assays showed that non-degradable Xom is about 20 times better at inhibiting transcriptional activation of *goosecoid* than wild-type Xom. Consistent with this, embryos with non-degradable Xom have head truncation — typical of an enhanced ventralized phenotype — and this effect is restricted to the dorsal site of the embryos.

On the basis of these findings, Zhu and Kirschner propose that correct dorso–ventral BMP patterning in the mesoderm during gastrulation depends on specifically timed proteolysis of Xom. It is currently unclear how Xom is stabilized in the early gastrulation stage and what turns on Xom degradation later in gastrulation.

Arianne Heinrichs

References and links

ORIGINAL RESEARCH PAPER Zhu, Z. & Kirschner, M. Regulated proteolysis of Xom mediates dorsoventral pattern formation during early *Xenopus* development. *Dev. Cell* **3**, 557–568 (2002)



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CANCER

Know your place

Activating mutations in the Wnt pathway are the only known genetic alterations that cause intestinal epithelial cells to develop premalignant lesions (polyps). Back-to-back reports in *Cell* by Clevers and colleagues now provide insight into how dysregulation of the Wnt pathway might cause colorectal cancer (CRC).

Activation of the Wnt pathway induces the translocation of β-catenin to the nucleus to interact with TCF transcription factors. To understand the role of β-catenin–TCF complexes in CRC, the authors used dominant-negative TCF-4 (dnTCF-4) to inhibit TCF transactivation in CRC cell lines; this caused cell-cycle arrest in G1. Analysis of complementary DNA from the dnTCF-4-expressing CRC cells showed small subsets of genes that were up- and downregulated. Those that were downregulated were normally expressed in the proliferative compartment of colon crypts, whereas genes that were markedly upregulated localized to the top of the crypts (where differentiated cells are usually found), or were absent when polyps arose.

Of the genes upregulated by dnTCF-4, the cyclin-dependent kinase inhibitor $p21^{CIP1/WAF1}$ was the only cell-cycle regulator. When the authors induced $p21^{CIP1/WAF1}$ in CRC cells, G1 arrest and differentiation occurred. Conversely, *c-MYC* was the only dnTCF-4-downregulated gene that overrode the growth arrest induced by dnTCF-4 or $p21^{CIP1/WAF1}$, by binding to and repressing the promoter of $p21^{CIP1/WAF1}$. So levels of $p21^{CIP1/WAF1}$ are key in regulating differentiation or proliferation.

In the second paper, the role of Eph–ephrin signalling in mediating cell positioning in the small intestine was studied. EphB2 and EphB3 were downregulated in response to TCF-4 inhibition. In wild-type embryos, both genes are expressed in the proliferative intervillus pockets, whereas the ephrin-B1 ligand is expressed on adjacent differentiated villus cells. The authors propose that the interaction between cells at the boundary of these two compartments restricts cell intermingling in newborns, consistent with established roles for Eph–ephrin signalling. In adults, the pattern of EphB–ephrin-B1 expression is more complex. And in polyps, high levels of EphB2 and EphB3, but not ephrin-B ligands, were expressed. Ephrin-B1-expressing normal cells surrounded, but didn't mix with, EphB-expressing polyps. So, an attractive model put forward by the authors is that "β-catenin–TCF signalling couples cell positioning with cell proliferation, cell-cycle arrest and differentiation."

Katrin Bussell

References and links

ORIGINAL RESEARCH PAPERS van de Wetering, M. *et al.* The β-catenin/TCF-4 complex imposes a crypt progenitor phenotype on colorectal cancer cells. *Cell* **111**, 241–250 (2002) | Batlle, E. *et al.* β-catenin and TCF mediate cell positioning in the intestinal epithelium by controlling the expression of EphB/ephrinB. *Cell* **111**, 251–263 (2002)