DEVELOPMENTAL BIOLOGY

Becoming competent

The key functions of the pancreas are to regulate digestion and sugar metabolism. Our desire to understand the development of this organ is driven not only by the need to understand organ formation, but also by the hope that we can recapitulate it *in vitro*, thus creating a supply of insulin-producing cells for diabetes therapy. An important step in this direction is now provided by Kawaguchi and colleagues, who report that the pancreatic transcription factor Ptf1a is required in mice to commit cells to the pancreatic fate and for their subsequent proliferation and differentiation.

Ptf1a was previously implicated in the development of the exocrine pancreas — the portion that is responsible for secreting digestive enzymes. Little was known about *Ptf1a*-expressing cells during early pancreatic development, so the authors did careful recombination-based lineage tracing in mice in which the expression of a *lacZ* reporter was activated by the endogenous *Ptf1a* promoter in normal and in *Ptf1a*-deficient mice.

Because the activated *lacZ* allele is expressed independently of cell fate, the progeny of cells in which the *Ptf1a* promoter had been activated could be definitively identified. In the normal pancreas, Kawaguchi *et al.* found that cells from both the exocrine and the endocrine — hormone-producing — pancreas express *Ptf1a* early in their lineage history. Most importantly, they saw that *Ptf1a* deficiency causes large numbers of *lacZ*-expressing cells to appear in the intestinal epithelium. So, the presence or absence of *Ptf1a* seems to be crucial in determining how endodermal progenitors choose between organ fates.

As another test of whether *Ptf1a* is expressed in all pancreatic precursors, the authors used its promoter to drive the expression of *Pdx1* — a gene that is essential for pancreas formation. It turned out that *Ptf1a*-driven expression of *Pdx1* was sufficient to restore the formation of all pancreatic

EVO–DEVO

Jaws — the making of

They might not beat insects in number of species but, compared with all other animals that roam the Earth, vertebrates have certainly hit a winning formula. More sophisticated brains had some say in this, although a lesser known morphological development — the invention of upper and lower jaws — probably contributed to the success of this phylum. It might be some time before we're privy to the genetic basis of brain complexity; however, one paper now provides a developmental genetic model for the evolution of jaws. The explanation — as gleaned from knockout mice — lies in the peculiar expression pattern of a family of Distal-less homeobox (Dlx) factors along the proximal–distal axis of jaw precursor tissues.

Mice, like all mammals, have six Dlx genes (1–3 and 5–7), which are tandemly arranged in the genome. The genes are expressed in several head regions, but of importance to jaw development is their expression in the branchial arches (BAs) — segmentally repeated structures with proximal–distal polarity. In the first arch, the more proximal (maxillary) portion develops into the upper jaw and the more distal (mandibular) portion into the lower jaw. The Dlx genes are expressed in nested pairs along this axis — *Dlx1/2* are present along most of the axis, with *Dlx5/6* and *Dlx3/7* showing progressively more restricted distal expression. In previous work, mice in which either *Dlx1* or *Dlx2*, or both, were knocked out had only proximal jaw defects, which was attributed to the rescue of their distal functions by other genes in the family, notably *Dlx5*. The authors therefore asked what would happen if both *Dlx5* and *Dlx6* were knocked out — the prediction being that distal BAs would acquire proximal properties. Although *Dlx5/6*$^{-/-}$ mice die at birth, their embryonic phenotype was even more striking than expected. Analysis of morphology and gene expression shows that the loss of these two genes leads to the homeotic, mirror image transformation of the lower jaw into the upper jaw.

Although vertebrates have had jaws for over 400 million years, jawless vertebrates, such as lampreys, are still around. Curiously, Dlx genes are expressed in the lamprey BAs, but the pattern is not nested. This gives weight to the theory that the transition from jawless to jawed vertebrates depends on the particular Dlx distribution pattern. As the authors point out, the cell fates imparted by Dlx genes probably depend on interpreting the one or more positional signals that act upstream of them. Candidates for these molecules exist (fibroblast growth factor 8, for example), but

lineages in *Pdx1*$^{-/-}$ mice, indicating that *Ptf1a* might be expressed early in all pancreatic precursors. Immunohistochemical time-course studies confirmed that this expression is subsequently turned off in endocrine cells.

The authors' demonstration that *Ptf1a* expression is essential for cells to adopt and maintain pancreatic fate has important therapeutic potential. So far, attempts to form pancreatic precursors *in vitro*, which could be used to treat diabetes using transplantation therapy, have failed. It might be that with their discovery of the early role of *Ptf1a*, Kawaguchi and colleagues have uncovered one of the missing key players.

Magdalena Skipper

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just how many convergent signals it takes to make a vertebrate jaw remains to be seen.

Tanita Casci

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WEB SITE

John Rubenstein's lab:
http://www.ucsf.edu/jlrrlab

CANCER GENETICS

Dual control

Driving-instructors' cars are equipped with dual controls, but this might not always be a sensible safety feature. Cell-cycle exit and apoptosis are two crucial processes by which cells limit proliferation, so genes that control both of these would be a prime target for mutation in tumorigenesis. Now, Iswar Hariharan and colleagues have identified a *Drosophila* gene, *salvador* (*sav*), that regulates both of these processes; the human orthologue is also mutated in cancer cell lines.

The development of the *Drosophila* eye is tightly regulated — cell proliferation occurs throughout the larval stage, differentiation occurs during the late larval and pupal stages, and excess cells are eliminated by apoptosis. These characteristics make it an ideal system to screen for mutations that alter cell proliferation or apoptosis. The authors have identified mutations in at least 23 loci that, when homozygous mutant, cause an over-representation of mutant cells compared with wild-type cells, making them good candidates for tumour-suppressor genes. One of these, *sav*, was characterized further.

An increase in cell number could be caused by an increase in proliferation or a decrease in apoptosis, so both of these processes were examined in turn. In *sav* mutants, ectopic BrdU incorporation was observed posterior to the morphogenetic furrow — which moves from the posterior to anterior of the eye, causing cells to arrest, after which they synchronously enter S phase. This indicates that these cells continue to proliferate after wild-type cells have arrested. Flow-cytometry analysis confirmed that *sav* mutants are delayed in exiting the cell cycle.

However, this delay in cell-cycle exit is not sufficient to account for the increase in cell number, so might apoptosis also be inhibited in *sav* mutant cells? TUNEL analysis revealed that cell death seemed to be mostly confined to the wild-type regions of the eye. Hid and Rpr, which target the caspase inhibitor DIAP1, were unable to induce apoptosis in *sav*-mutant cells. DIAP1 protein levels remained high and the effector caspase Drice was not cleaved to generate the active form.

The *sav* gene was sequenced and contained two putative WW domains, which are involved in protein–protein interactions. The *warts* (*wts*) gene was also identified in the mutant screen and was shown to have a similar phenotype to *sav* in regulating cell-cycle exit and apoptosis. It contains five PPXY motifs, to which WW domains bind, and a precipitation experiment with GST-tagged Sav confirmed that Sav and Wts interact.

So, two genes have been identified that, when mutated, confer a selective advantage to cells. Might they be mutated in cancer cells? The human orthologue of *sav*, *WW45*, was sequenced in 52 tumour-derived cell lines, and *WW45* was altered in three of these. Two renal-cancer cell lines — ACHN and 786-O — had deletions in *WW45* that would inactivate the protein.

The authors have therefore identified a new potential tumour suppressor, and have proved, yet again, that *Drosophila* can be a useful model organism for cancer research.

Emma Greenwood, Associate Editor,
Nature Reviews Cancer

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WEB SITE

Iswar Hariharan's lab:
http://www.mgh.harvard.edu/depts/CancerCenter/hariharan.html