DEVELOPMENTAL BIOLOGY

Cracking conditional transgenesis

Knocking out genes is sometimes like using a sledge hammer to crack open a nut — a lethal knockout phenotype that prevents a mutant animal from developing ends up destroying the very thing you are after. Conditional transgenesis provides developmental biologists with a more delicate tool for dissecting gene function. But, as shown by two groups studying *Fgf8* in the limb, interpreting the results of conditional gene inactivation requires a sound knowledge of where, and to what extent, a gene has been inactivated.

Fgf8 and other fibroblast growth factor genes (such as *Fgf4*) are expressed in the apical ectodermal ridge (AER), which covers the distal tip of the developing limb bud. *Fgf8* is the only *Fgf* to be expressed throughout the AER (see left image), and it can induce limb development when expressed ectopically. It might also regulate sonic hedgehog (*Shh*) expression in the developing limb bud (the right image shows the posterior domain of *Shh* expression (arrow) together with AER *Fgf8* expression (arrowhead)). But what happens to limb development when you knock out *Fgf8*? This has been hard to answer because *Fgf8* is essential for early mouse development. So, to bypass the knockout's lethal phenotype, two groups inactivated *Fgf8* in the developing mouse limb bud using Cre-recombinase technology.

Moon and Capecchi opted for a transgene that expressed *Cre* under the control of the retinoic acid receptor-β promoter (*RARCre*). This promoter drives *Cre* expression in the early mouse forelimb before the onset of *Fgf8* expression, and so completely abolishes forelimb *Fgf8* expression. However, this transgene is expressed very weakly in the hindlimb so *Fgf8* remains expressed there. Conversely, Lewandoski *et al.* expressed *Cre* under the control of the *Msx2* promoter, which drives expression in the hindlimb AER before the onset of *Fgf8* expression. In forelimb buds, however, this promoter only produces widespread *Cre* expression once *Fgf8* expression has been turned on, allowing *Fgf8* to be transiently expressed in developing forelimbs.

The conditional mutant mice produced by both groups developed limb abnormalities but were otherwise normal. Because the forelimbs of *RARCre–Fgf8* mice and the hindlimbs of *Msx2Cre–Fgf8* mice never express *Fgf8*, one might expect them to have equivalent skeletal defects, but this is not the case. Conditional *Fgf8* inactivation affects forelimbs more severely than it affects hindlimbs. This might be due to differences in the timing of *Fgf8* and *Fgf4* expression during limb development — the interval between the onset of the expression of *Fgf8* and then *Fgf4* is almost twice as long in the forelimb as it is in the hindlimb bud, perhaps making the forelimb more dependent on *Fgf8* expression than the hindlimb.

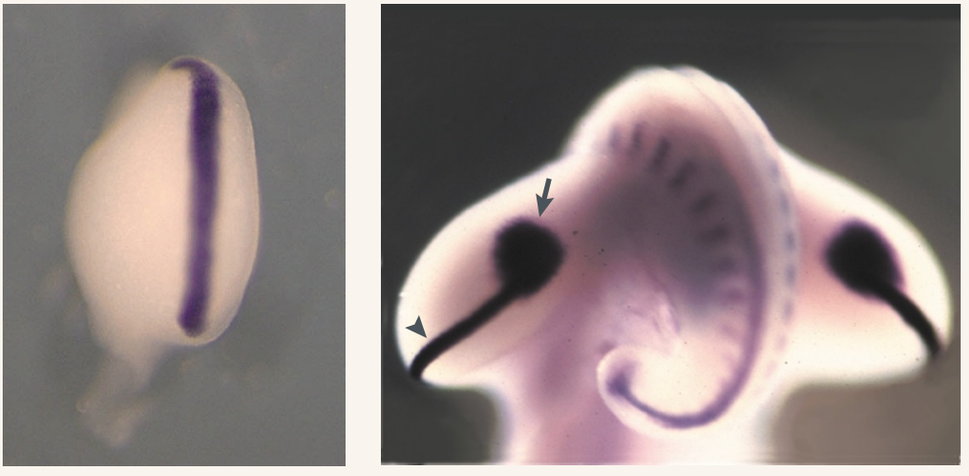
Using conditional transgenesis, these papers are the first to show that an AER-expressed *Fgf*, *Fgf8*, is necessary for the development of all the limb's segments. This fast-developing technique is likely to further the analysis of many essential genes, and may soon consign the sledge-hammer method for cracking their function to history.

Jane Alfred

References and Links

ORIGINAL RESEARCH PAPERS Moon, A. M. & Capecchi, M. R. Fgf8 is required for outgrowth and patterning of the limbs. *Nature Genet.* **26**, 455–459 (2000) | Lewandoski, M. *et al.* Fgf8 signalling from the AER is essential for normal limb development. *Nature Genet.* **26**, 460–463 (2000)

WEB SITE Hox net — limb development



Courtesy of Mark Lewandoski (left) and Phil Crossley (right), University of California, San Francisco, USA.

WEB WATCH

The rat pack

For decades, the rat has been used as a model system for studying human physiology and disease. In particular, there are excellent rat models for multifactorial diseases, the genetic components of which are being hunted down with increasing vigour. Consequently, there is strong motivation to develop genetic and genomic resources for the rat, so that the wealth of phenotypic and physiological data can be exploited in genetic analyses.

The data and resources are accumulating rapidly — the rat genome is scheduled to be sequenced to at least fourfold coverage by the end of 2002 — and to provide centralized access to this information, the Rat Genome Database (RGD) was launched in June, 2000. RGD is the result of an international collaboration of rat researchers and is hosted at the Medical College of Wisconsin.

The information available at RGD includes maps (genetic and physical), genes, ESTs, simple-sequence length polymorphisms and phenotypic data for 48 important inbred rat strains. Rat genes are linked to human and mouse homologues and to related information, such as NCBI's LocusLink, the Ratmap database and the Rat Gene Index at The Institute for Genomic Research.

RGD also provides tools for data analysis, such as Metagene. Users can submit genome sequence to Metagene and the sequence is analysed by seven popular gene-prediction algorithms. Results are aligned for all packages, allowing the user to compare the output and to assess the statistical significance of the predicted coding regions. And if you're stuck for a rat person to talk to, the Rat Community Forum, also hosted by the Medical College of Wisconsin, is a good first port of call.

Mark Patterson