HIGHLIGHTS

TECHNOLOGY

Kitting out the rat

A model organism that lacks reliable genetic modification techniques is rather like a tool box without a spanner — somewhat lacking in a key piece of kit. Although the first transgenic rat was reported 12 years ago, genetically modifying the rat genome is still far from routine, predominantly because rat embryonic stem cells have not yet been successfully derived or cultured. Now, Kent Hamra and colleagues have sidestepped this problem by generating transgenic rats from male germline stem cells. They show that, when transplanted into sterile rats, these cells can form functional spermatozoa. Moreover, they can also be easily transformed with a lentiviral vector and subsequently used to produce transgenic offspring.

The authors obtained spermatogonial stem cells (SSCs) from primary cultures of rat spermatogenic cells — taken from *lacZ*-expressing male rats — using a two-step procedure. In step one, cells cultured for 2.5 days were plated onto a collagen matrix for 4 hours. Germ cells and somatic cells were then differentiated from each other by assaying for the expression of DAZL, a germ-cell-specific marker, and vimentin, a somatic cell marker. After harvesting DAZL+ cells — which remained unattached to the collagen matrix — Hamra *et al.* transplanted them into the testes of sterile male rats. Around 50% of the offspring of these rats carried the *lacZ* transgene, which they transmitted to the following generation at Mendelian ratios. Step two involved another enrichment step on a laminin matrix, which provided the authors with a population of cells that were ~97% DAZL+ and could colonize rat testes with greater efficiency.

These laminin-enriched cells were next transduced in culture with a lentiviral vector that carried an *EGFP* reporter gene. The transformed cells were transplanted into three male rats, where they colonized the seminiferous tubules, as shown by the expression of *EGFP* ~200 days after transplantation. Although only one of these males proved to be fertile, he sired 44 pups, five of which received two copies of the transgene, eight of which received one. The site of transgene integration was unique in each line, and seven lines genotyped so far have transmitted the transgene to ~50% of their progeny. Transgenic animals were also generated from SSCs cultured for four days, and SSCs cultured for seven days retained stem-cell function, possibly providing a window of time for the selection of drug-resistant targeted cells.

Much like a good tool, to be really useful, genetic modification techniques need to be simple, efficient and reliable. This approach appears to meet these requirements — but we'll know for sure once it is in general use.

Jane Alfred

References and links

ORIGINAL RESEARCH PAPER Hamra, F. K. *et al.* Production of transgenic rats by lentiviral transduction of male germ-line stem cells. *Proc. Natl Acad. Sci. USA* 21 October 2002 (10.1073/pnas222561399)

WEB SITE
David Garbers' lab:
http://www.swmed.edu/home_pages/pharmacology/greencenter/garberslab.html