MOUSE GENETICS

What a screen!

Most yeast, fly and worm geneticists know how useful modifier genetic screens can be; screening for mutants that enhance or suppress a certain phenotype can be used to build up the signalling cascades that underlie that trait. Marina Carpinelli, Doug Hilton and colleagues now show that suppressor screens are equally applicable to vertebrate models in general and to mice in particular.

The authors focussed on a mouse model of thrombocytopenia — a disease that is caused by a lack of blood platelets and that is seen in $Mpl^{-/-}$ animals. They treated 300 male, *Mpl* homozygous knockout mice with the mutagen *N*-ethyl-*N*-nitrosourea (ENU). These mutagenized mice were then crossed with isogenic knockout females. A total of 5 of the 1,575 F_1 offspring from these crosses suppressed the mutant phenotype: their platelet counts far exceeded those of their thrombocytopenic parents. Subsequent crosses with untreated $Mpl^{-/-}$ mice showed that two of the suppressed mice (*Plt3* and *Plt4*) harboured dominant ENU-induced mutations.

So, without too much trouble, Carpinelli *et al.* were able to use a large-scale suppressor screen to isolate new mutant mice that are relevant to the study of the platelet-production pathway. However, it is their subsequent work that shows just how useful mutants isolated from suppressor screens can be. First, they intercrossed mice that were heterozygous for the new mutations to obtain homozygotes in an $Mpl^{-/-}$ background. These mice had platelet levels that were above normal, in contrast to the below wild-type levels in the heterozygotes, which clearly indicated that *Plt3* and *Plt4* are semi-dominant mutations.

Further crosses of *Plt3*/*Plt4* compound heterozygotes to $Mpl^{-/-}$ mice showed that the two mutations are tightly linked and possibly allelic. Using standard segregation analysis of 148 microsatellite markers, the authors localized the mutations to a region on chromosome 10 that contains *cMyb*, a gene that was previously linked to elevated platelet levels. They then identified candidate mutations in *cMyb*, in both *Plt3* and *Plt4* mice, that caused single amino-acid substitutions in functionally significant regions of the cMyb protein. Follow-up protein transactivation assays confirmed that these mutations reduce the activity of cMyb. Coupled with the finding that *cMyb* heterozygous knockout mice have higher platelet levels than wild-type homozygotes, these studies provide compelling evidence that the *cMyb* mutations underlie the *Plt3* and *Plt4* gain-of-function phenotypes.

Detailed haematological analyses of heterozygous and homozygous mutants showed that *Plt3* and *Plt4* increase the production of megakaryocytes and their progenitors, from which platelets are derived. So, we now have a more complete picture of the role of *cMyb* in haematopoiesis, but the real beauty of mutants that are identified from a supressor screen is that their epistatic interactions with the original mutant can be genetically analysed. In this case, Carpinelli *et al.* clearly showed that the effect of mutations in *cMyb* on platelet and megakaryocyte levels was independent of the *Mpl* genotype. So, the semi-dominance of mutations in *cMyb*, regardless of the genetic background, indicates that downregulation of *cMyb* is probably an important step in platelet production and that the gene is in the same signalling pathway as *Mpl*.

It seems to have taken vertebrate geneticists some time to catch on to the allure of suppressor screens. However, if the success of this initial application of the approach is anything to go on, it seems likely that we will soon see many more examples.

Nick Campbell

References and links

ORIGINAL RESEARCH PAPER Carpinelli, M. R. & Hilton, D. J. *et al.* Suppressor screen in $Mpl^{-/-}$ mice: *c-Myb* mutation causes supraphysiological production of platelets in the absence of thrombopoietin signaling. *Proc. Natl Acad. Sci. USA* 7 Apr 2004 (doi:10.1073/pnas.0401496)

WEB SITE

The art and design of genetic screens: http://www.nature.com/nrg/focus/screens

IN THE NEWS

Life without men

Dandelions do it all the time, aphids do it occasionally, and now mice can do it too, but only in the laboratory. Parthenogenesis — the reproduction of a gamete without fertilization — has been achieved in mice by Tomohiro Kono and colleagues, who announced the birth of live mice that were derived from two mothers but no father.

"This achievement, published in *Nature*, might be seen as being of comparable significance to the birth in 1996 of Dolly the sheep. And it may prove almost as controversial" (*Financial Times*).

The researchers knocked out a gene in an immature mouse egg to give it a 'male-like' imprinted character, and then combined the cell's genetic material with that of a normal mature egg. "From around 600 eggs, only two live mice were born" (*Financial Times*). "One mouse, named Kaguya, after a Japanese folk tale in which a princess is born from a bamboo stump, grew to adulthood and has become a mother herself — though by conventional means" (*Washington Post*).

"The practical implications are obscure, since the method is even more complex, inefficient and unsafe than cloning", said Ian Wilmut (*Daily Telegraph*). But could such a 'virgin birth' occur in humans too? "Researchers were quick to head off suggestions that the technique could be used to treat infertility" (*BBC News Online)* or that it "makes men obsolete" (*Daily Telegraph*). However, the use of the technique might "circumvent the political and ethical obstacles to using stem cells" (*BBC News Online)*.

Whatever its application, this work is "sure to stimulate conversation about the intrinsic importance of male–female pairing, at both the biological and the social level" (*Washington Post*).

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