Making a knockout mouse: From stem cells to embryos

Janet Rossant

I began my research career in the UK as a graduate student in the mid 1970s with Richard Gardner, a pioneer in mammalian experimental embryology. It marked the beginning of my interest in the blastocyst as a model system to study early cell lineage development, a fascination that continues today as we uncover the signalling pathways and transcriptional networks that underlie lineage specification. Studying the mouse blastocyst has led me down many different winding paths over the years. None has been more exciting and career-changing than the period in the mid-to-late 1980s, when my lab (and many others) got caught up in the revolution in mammalian genetics brought about by the development of targeted mutagenesis in mouse embryonic stem cells (ESCs).

In 1985, I left a faculty position at Brock University, St Catharines, to join the newly formed Samuel Lunenfeld Research Institute at Mount Sinai Hospital in Toronto. Under the leadership of Lou Siminovitch and Alan Bernstein, the institute very rapidly gained a reputation for outstanding science and state-of-the-art approaches to studying molecular development and cancer. Soon after, Alex Joyner joined as a new faculty member, following a post-doctoral position with Gail Martin, where she had cloned the mouse engrailed genes. The discovery of vertebrate orthologues of fly developmental control genes, including the *Hox* genes and *engrailed,* changed mammalian developmental biology forever. I remember many earlier conferences where those of us studying the mouse would be relegated to the session after the banquet, as an esoteric subject with little insight to be gained from comparison with other species. Suddenly it became obvious that many of the underlying principles and genetic pathways of development were conserved.

I was very excited by the new research horizons opened by these developments. With Alex's arrival, our labs started working together on the next challenges — testing the function of these newly identified genes in mouse development and finding more ways to identify other key developmental genes. We knew the answer must lie with ESCs. In 1981, Martin Evans and Matt Kaufman in Cambridge and Gail Martin in San Francisco had separately isolated pluripotent stem cells directly from the mouse blastocyst. Allan Bradley and Liz Robertson, in Martin Evans's lab, and Achim Gossler in Rolf Kemler's lab then provided proof-of-principle studies demonstrating that any genetic alteration introduced into these cells in culture could be transmitted through the germ line in chimaeras. Alex introduced ESC concepts to Toronto from San Francisco. When Achim Gossler joined my lab as a post-doc and brought germline-competent ESCs, we had the starting material for a major assault on gene functional analysis in the mouse.

But how should we attack the problem? We knew about the promise of targeted mutagenesis by homologous recombination, developed in mammalian cells by Mario Capecchi and Oliver Smithies, but it had only been demonstrated to work in ESCs for genes such as *Hprt*, where one could select for mutation or correction of the gene. Would it be efficient enough to work for non-selectable genes such as engrailed? We pondered instead a random retroviral insertional approach, followed by a pooled polymerase chain reaction (PCR) assay to detect mutations in genes of interest. Achim and I also started to plan an enhancer trap approach, similarly to that developed in *Drosophila melanogaster*, to identify novel genes by expression of their associated enhancers in ESC chimaeras. However, when we heard from Oliver Smithies that he was developing a PCR-based approach to detect targeted mutations in non-selectable genes in ESCs, we abandoned the random insertional approach and put all our efforts into targeted mutagenesis with engrailed2 as the first target. We were fortunate to have Bill Skarnes join Alex's lab as a graduate student at that time. He developed the targeting vector for *En2*, which was at the same time developed into a gene trap reporter vector, using the *En2* splice acceptor to trap upstream exons in a *lacZ* fusion that would also disrupt the gene.

Alex and I started the targeting experiments, picking portions of hundreds of individual neomycin-resistant colonies and assaying for targeted integrations using PCR. We began without a PCR machine — moving tubes by hand from water-bath to water-bath. Hints of success spurred us to purchase the second PCR machine ever sold in Canada, and, as they say, the rest is history. We published one of the first reports of successful targeting of a non-selectable gene in ESCs in *Nature* in 1989, and reported approaches for enhancer and gene-trap screens in ESCs in *Science* in the same year.

The field of mammalian genetics was very rapidly transformed as more and more ways of manipulating the mouse germ line were developed by different groups. But it was a special time for me in Toronto, working in a great group of talented people, all with our own personalities and quirks, but all bringing our unique skills to make a successful team.

The efforts of the International Knockout Mouse Consortium have made knockout mouse ESCs available off the shelf for almost the entire mouse genome, relegating the generation of a targeted mutation to supplementary material in most papers. And it seems that classical targeted mutagenesis in ESCs may be rapidly superseded by exciting new genome editing tools. Mammalian genetics moves on and new horizons await.

COMPETING FINANCIAL INTERESTS
The author declares no competing financial interests.

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