EMBRYONIC STEM CELLS: THE ROUTE TO EXPERIMENTAL MAMMALIAN GENETICS

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Embryonic stem cells, the totipotential cells from mouse embryos may be maintained in tissue culture and retain their potential colonise the mouse germ line. Embryonic Stem Cells provide a link between *in vitro* tissue-culture cellular manipulations and whole, normal embryonic development and thus a route to genetic manipulation of mammals.

They have therefore allowed a comprehensive series of methods to be developed for direct experimental genetics in mice which provide an experimental arm for mammalian genome projects both in elucidation of function and in gene discovery. The availability of these cells in large number in tissue culture allows genetic manipulation, screening and/or selection before reconstitution into the breeding animal. Homologous recombination may be used to target specific genes leading to their complete, or partial inactivation. Subtle mutations may also be introduced as may reporter genes at an endogenous chromosomal site of the target gene. ES cells also offer an advantageous route for random mutagenesis by insertional mutation so that the mutated site is molecularly tagged. Mutation events may be screened and/or selected either before or after introduction into the mouse (Evans, 1996). Experiments in gene targeting include investigation of genes of unknown function and the creation of animal models of human disease.

The proto-oncogene c-mos produces a cytoplasmic serine/threonine kinase which is implicated in meiotic maturation events in spermatogenesis and oogenesis. A knockout null mutation at this locus gave rise to apparently normally healthy adult mice. Males are fully fertile but female mice showed a reduced fertility which was associated with the development of ovarian cysts. These proved to be the result of parthenogenetic activation of unfertilised oocytes which fail to arrest after the second meiotic division (Colledge et al., 1994). In subsequent studies it was shown that MAP kinase is not activated in $mos^{-/-}$ oocytes and that they do indeed arrest but only transiently (Verlhac et al., 1996).

Purple acid phosphatase is a metalloenzyme specific to activated macrophages and osteoclasts (Hayman et al., 1996). Homozygous null mice are healthy but show a deficit in endochondrial ossification and a deficit in bone matrix resorption.

In addition to mere gene ablation it is useful to include a reporter of function of the endogenous locus. By the introduction of a lac-z construct into the endogenous Hox-11 transcription unit we were able to show, in contrast to earlier observations, that $Hox11^{-/-}$ mice did not fail in spleen genesis but in the maintenance of the spleen primordium. In the absence of Hox-11 the spleen cells undergo apoptosis (Dear et al., 1995).

We have used mice with mutations in the cftr gene locus as animal models of cystic fibrosis. Although the syndrome differs between mouse and man these animals have proved invaluable in a series of studies elucidating the physiological consequences of the lack of a functional cftr channel and also as models for testing gene therapeutic approaches to the disease (Hyde et al., 1993; Colledge et al., 1995).

Gene targeting is a relatively lengthy process and is limited to investigation of known cloned loci. ES cells may also be used as vectors for random insertional mutation. Gene trap vectors allow gene transcript associated events to be selected or screened in tissue culture as well as in the whole animal. The generation of indexed libraries of mutated ES cells will facilitate future experiments.

References

Evans, M.J. (1996). The power of embryonic stem cell transgenesis for experimental mammalian genetics. Endocrinology and Metabolism 3 (Suppl. A): 45-52.
Colledge et al. (1994). Disruption of *c-mos* causes parthenogenetic development of unfertilized mouse eggs. Nature 370: 65-68.
Verlhac et al. (1996). MOS is required for MAP kinase activation and is involved in microtubule organization during meiotic maturation in the mouse. Development 122: 815-822.
Hayman et al. (1996). Mice lacking tartrate-resistant acid phosphatase (Acp5) have disrupted endochondral ossification and mild osteopetrosis. Development 122: 3151-3162.
Dear et al. (1995). The *HOX11* gene is essential for cell survival during spleen development. Development 121: 2909-2915.
Hyde et al. (1993). Correction of the ion transport defect in cystic fibrosis transgenic mice by gene therapy. Nature 362: 250-255.
Colledge et al. (1995). Generation and characterization of a ΔF508 cystic fibrosis mouse model. Nature Genetics 10: 445-452.