# Systems for the Analysis of Developmental Control Genes of the Mouse

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by

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## Summary

The in vitro culture of pluripotential embryonic stem (ES) cells provides a system for the identification and analysis of genes which regulate stem cell self-renewal and differentiation. Since ES cells also provide a vehicle for alteration of the mouse germline, developmental control genes can further be analysed in vivo, in the context of the mouse. The overall aim of work reported in this thesis was to develop systems suitable for use in ES cells, both in vitro and in vivo, for the analysis of genes which regulate embryonic developmental decisions.

The first aim of work presented in this thesis was to characterise further an inducible expression system designed for use in ES cells in vitro. Expression vectors (Whyatt, 1991), based on the interferon- (IFN) inducible promoter of the human 6-16 gene, were designed to permit the controlled expression of genes in ES cells, which regulate stem cell self-renewal, differentiation and death. In this project the efficacy of the vectors was assessed in stable ES cell lines. Treatment of ES cells with IFN did not compromise ES cell pluripotence or germline competence and stable ES cell lines were responsive to IFN treatment. The vectors were able to direct dose dependent, IFN-inducible, heterologous gene expression in the absence of detectable basal expression within the context of the mouse genome. The ability of the vectors to direct precisely controllable levels of transgene expression in the absence of basal expression, makes them ideal for the analysis of genes such as developmental control genes which are likely to be biologically active at very low thresholds and may exert alternate biological effects at different concentrations.

Predetermined genetic alterations can be introduced into the mouse genome and germline by the technique of gene targeting in ES cells (Thomas and Capecchi, 1987). The second aim of work presented in this thesis was to develop a gene targeting system for the efficient, repeated introduction of precise, subtle alterations into the genome of ES cells for the analysis of developmental control genes in vivo. The stable tag-exchange gene targeting approach, developed in this work, is based on the two step targeting approach, known as the tag-and-exchange approach, first described by Reid and co-workers (1990). In the tag-and-exchange

approach the locus to be altered is tagged in an initial round of gene targeting (tag step gene targeting) with a positive/negative selection cassette. Replacement of the tag cassette with the alteration of interest in a subsequent round of gene targeting (exchange step gene targeting) provides direct selection for homologous recombination of the exchange step vector. This theoretically improves the efficiency of exchange step gene targeting by the elimination of non-homologous recombinants and permits the introduction of subtle alterations into the tagged site. However, the efficiency of the tag-and-exchange approach is compromised by a high background of lines which survive exchange step gene targeting due to physical loss of the tag gene by means other than homologous recombination (Askew *et al.*, 1993; Stacey *et al.*, 1994; Wu, 1994). The stable tag-exchange approach was designed to overcome this background by the inclusion of an additional selection cassette on the tag step vector. This positive selection cassette, termed the stability cassette, remains on the tagged allele during exchange step gene targeting, providing selection for the presence of the tagged allele.

The stable tag-exchange system was analysed at the Leukaemia Inhibitory Factor (LIF) locus in E14TG2a ($HPRT^-$) ES cells. The LIF gene product has been implicated in diverse developmental processes including haematopoiesis, osteogenesis, tumorigenesis, wound healing and embryonic development. The replacement vector used in tag step gene targeting carried the HPRT and *neo* genes as the tag and stability cassettes respectively, separated by almost five kilobases of homologous DNA. The tag step vector was introduced into the LIF locus with an efficiency of almost seventeen percent. All homologous recombinants contained both the HPRT and *neo* genes correctly integrated at the LIF locus, regardless of whether selection for HPRT or HPRT and *neo* was applied. This finding implies that multiple, separate regions of non-homology, for which selection can not be applied, may be introduced efficiently into the mouse genome on a single replacement vector.

Direct comparison of the unmodified tag-and-exchange approach with that of the stable tag-exchange approach was carried out at the LIF locus. The efficiency of the exchange step of the stable tag-exchange approach was almost ninety percent at the LIF locus compared to only twenty one percent when the tag-and-exchange approach was used. The improved efficiency of the stable tag-exchange approach makes it suitable for the repeated introduction of alterations into the mouse genome. Selection for the stability cassette during exchange step gene targeting improved the gene targeting efficiency through elimination of lines which had undergone physical loss of the tag gene by means other than homologous recombination. The mechanism responsible for this loss was gene conversion, in which both the tag and stability cassettes were accurately removed from the tagged LIF locus.

Three different isoforms of the LIF gene are known to exist (Rathjen *et al.*, 1990a; B. Haines, P. Rathjen personal communication) which are generated from transcripts carrying alternate first exons. Evidence suggests that the isoforms of LIF may play biologically distinct roles. In the exchange step of the stable tag-exchange approach, a β-galactosidase reporter gene was introduced into the unique exons of LIF or a common exon of LIF, to allow visualisation of the cellular sites of expression of each and all LIF isoforms in mice generated from these cells. Reporter gene expression in the exchange step targeted ES cell lines reflected the known in vitro pattern of LIF expression. In addition, these targeted lines retained germline competence. Identification of the cellular sites of expression of each LIF isoform in the mouse, should lead to a detailed understanding of the normal roles of each of these proteins during developmental processes.

The systems presented in this thesis provide valuable tools with which to exploit the ES cell system for the analysis of genes which regulate developmental decisions both in early and later embryogenesis.