TARGETED TRANSGENESIS AND THE 186 SITE-SPECIFIC RECOMBINATION SYSTEM

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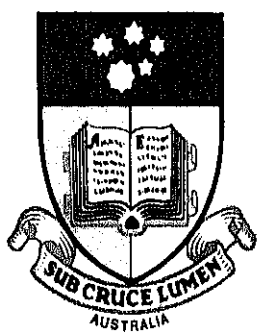

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Summary

Efforts are being made to find alternative and more efficient means of generating transgenic livestock, to perform precise genetic modifications in mammals, other than the mouse, such as a gene "knock-out", and to insert a new gene or replace a defective gene, as required for successful gene therapy in humans.

Current methods for producing transgenic farm animals rely on the use of microinjection into fertilised eggs which has several inherent disadvantages as the site of insertion is random, the copy number uncontrollable and gene expression is subject to position effects from the surrounding DNA context. In contrast, homologous recombination to generate transgenic mice has been very successful in contributing to our understanding of basic biological phenomena such as mammalian physiology and development, and for producing animal models of human disease. Currently this technology is dependent on the availability of embryonic stem cells which do not yet exist for any livestock species. Although homologous recombination has been tried in fertilised mouse eggs the success rate is too low for consideration as a routine method. The Cre/*lox* and FLP/FRT recombinase systems have been shown to operate in eukaryotic systems via microinjection into fertilised mouse eggs (excision), but the drawback with these systems is their non-directionality, one protein directs both excision and integration.

Nuclear transfer has been used to produce cloned sheep (Dolly), cows and mice from adult cells. Combining the techniques of homologous recombination in a foetal primary cell line, and nuclear transfer, Schnieke's team (1997) successfully produced transgenic sheep. At present the technology is inefficient. Furthermore, successful homologous recombination demands construction of a targeting vector containing vast regions of isogenic homology (species specific), a time consuming and onerous task.

The temperate coliphages like λ, P2 and 186 can enter either a lytic or lysogenic lifecycle upon infection of their *Escherichia coli* host. As different combinations of proteins mediate the excisive and integrative pathways these systems have the directionality lacking in the one protein systems like Cre and FLP. However, none have been shown to function in a eukaryotic environment. The recombinase systems may offer an alternative route to homologous recombination for the initial introduction of the target site, and that is by using closely matching, naturally occurring target sites in combination with an altered integrase, one could bypass the homologous recombination step altogether

The aim of the work described in this thesis was to characterise the 186 integration reaction and, to conduct preliminary investigations into the possible use of such a system for targeted transgenesis in livestock species. In Chapter 2 the *in vitro* requirements for 186 integrative site-specific recombination were investigated. Chapter 3 details characterisation of the 186-*att*B site in which I located its precise position, sites of strand cross-over, order of strand exchange and conducted preliminary mutational analysis to identify bases important for recombination. Chapter 4 describes *in vitro* mouse genomic DNA recombination experiments using naked DNA and chromatin containing *att*B sites. Lastly, *in vivo* investigations were conducted whereby active 186-intasomes were microinjected into fertilised mouse eggs containing genomic copies of 186-*att*B. The resultant progeny were then analysed for 186-mediated integration events.

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