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Introduction

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Conditional gene targeting in mice ("Knockout: round 2") was first reported 8 yr ago on DNA polymerase β gene *(1)*. A literature search of PubMed reveals that studies using Cre/loxP system have been sporadic from 1981 to 1994 with only one to five reports annually. This number increases steadily from 16 in 1995 to 138 in 2002 (so far). Owing to the feasibility of homologous recombination, the relative simplicity of the Cre/loxP model, and, most important, the need to develop conditional gene targeting to bypass prenatal lethality and other intrinsic defects with conventional gene knockout strategy, results using this technique in recent years have been very encouraging. Tens if not hundreds of target genes have been flanked by loxP (floxed), even greater numbers of Cre transgenic mice have been reported. (Readers are referred to a useful web-based resource provided by Dr. A. Nagy, Mount Sinai Hospital, Toronto, at www.mshri.on.ca/nagy/default.htm.) We feel it's time to highlight some early success stories in order to aid future applications of the technique. Thus, six groups of investigators have been invited to review four different aspects of the system: general technical perspectives in articles 1 and 2; an example of tissue-specific knockout of a hormone gene in articles 3 and 4; a tissue-specific knockout of a hormone receptor gene in article 5; and applications of Cre/loxP system in studying ontogeny of the endocrine pancreas, in article 6.

Dr. B. Sauer was a pioneer in the initial development of the Cre/loxP tools and their fine tuning in application to mouse genetics *(2–5)*. In fact, he has already reviewed several aspects of the model in recent years *(6–9)*. His new contribution outlines historical background of the Cre/loxP system and explained targeted integration (genomic knock-ins) of exogenous DNA into mammalian genome. Drs. Misra and Duncan have discussed targeted integration of transgenes into mouse genome via homologous recombination in embryonic stem (ES) cells, a departure from traditional random genetic insertion in fertilized eggs following microinjection of the DNA. Although it is technically cumbersome due to the use of ES cells and the targeting vectors, they discuss ways of introducing transgenes into specific genomic loci and how these approaches can be coupled with tetraploid complementation to rapidly generate clonal transgenic mouse embryos. A major advantage is that we can control the site of transgene integration and copy numbers and have guaranteed expression of a foreign gene.

Of course, the Cre/loxP model is far from perfect. A relative low rate of success in achieving target gene recombination with high efficiency and tissue specificity is a major obstacle, although hardly reported. Some side effects caused by the model itself have been reported. For instance, naturally occurring, pseudo-loxP sites are present in mammalian genome that can serve as substrates for Cre recombinase *(10)*. It is also reported that Cre expression in developing spermatids can lead to chromosomal rearrangements and male sterility in mice *(11)*. Furthermore, Cre expression in cultured cells caused markedly reduced proliferation due to chromosome aberrations *(12)*. This toxicity effect is proportional to the level of Cre activity. Prolonged, low levels of Cre activity permit recombination without concomitant toxicity *(12)*. It urges for careful titration of Cre activity and critical analysis of the animal phenotype.

Two parallel articles evaluate the role of liver-derived insulin-like growth factor I (IGF-I) in animal growth, development, and normal metabolism. One was achieved using albumin promoter to drive hepatocyte-specific expression of the Cre transgene and therefore IGF-I gene inactivation *(13,14)*. Another has used tetracycline-inducible Cre expression and achieved liver- as well as spleen-specific IGF-I gene knockout *(15)*. Because lack of splenic IGF-I gene expression causes virtually no effect on overall body supply of IGF-I, the second model has created almost identical results. Both have achieved greater than 95% of liver gene inactivation and 75% reduction in circulating IGF-I level. Both studies demonstrate that liver-derived IGF-I is not essential to overall postnatal growth, although most recently its effects on normal bone growth and metabolism have been more carefully analyzed *(16,17)*. Finally, but not the least, both models point out a more prominent role of locally produced IGF-I, acting via paracrine/autocrine, in promoting postnatal growth. How growth hormone achieves its control on local IGF-I production remains to be further explored using new models that are yet to be developed.

Insulin receptor gene has become the best target of the Cre/loxP system in the hands of Dr. CR Kahn and his associates. In last 5 yr or so, they have systemically inactivated the gene in all major insulin targets including muscle, liver,

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adipose, brain, and pancreatic islet β-cells *(18–22)*. Not only are they successful technically, each of these studies has advanced our understanding of such important issues as insulin resistance, insulin secretion, food intake, obesity, and type 2 diabetes. The review by Drs. Kulkarni and Okada, through examples of two nonclassical insulin target tissues, gives us a flavor of what they have accomplished.

Dr. P. L. Herrera has studied the ontogeny of the endocrine pancreas using targeted expression of toxigenes, thus ablation of specific islet cell types *(23)*. When Cre/loxP tools became available, he was successful in labeling endocrine progenitor cells through expression of Cre recombinase driven by specific promoters *(24)*. This approach allowed him to demonstrate for the first time that pancreatic islet α- and β-cells are independently derived from progenitor populations, in contrast to the notion that β-cells were derived from glucagon-expressing progenitors. The Cre-mediated genetic cell labeling has great implications not only on pancreatic islet study but also in analyzing ontogeny of other heterogeneous cell populations such as many endocrine organs.

I believe that these choices are excellent in reflecting the cutting edge research, although we were unable to include many other important examples and some unique approaches such as the dominant negative gene targeting *(25)*. Every contributor has suffered personal sacrifices to make this issue a success. It's our sincere hope that the field of conditional gene targeting will proceed with even higher speed, and with caution in interpretation, and will flourish further.

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