Transgenics at breaking-point

In this issue of *Cancer Cell*, Forster et al. (2003) have generated mice that recapitulate both the mechanism (sporadic somatic translocation) and the consequences (expression of two translocation fusion genes) leading to an accurate leukemia model.

Translocations are one of the characteristic genetic changes found in virtually all hematopoietic and other malignancies. Cloning of recurrent translocation breakpoints and the identification of the native genes involved has provided important insights into the role of the fusion genes in the process of malignant transformation. The genes participating in these events are involved in the regulation of differentiation, development, cell cycle, and cell death. The fusion genes generated by translocations are selected in tumors because their products have acquired enhanced, unregulated, or novel properties.

Translocations at 11q23 involve the 5′ end of the *MLL* gene which is fused to one of a multitude of potential partners, the identity of which determines the etiology of the subsequent leukemia in humans. While most of the *MLL* fusion genes give rise to either a myeloid or a mixed lineage leukemia, the translocation t(9:11) between the *MLL* and *ENL* genes is unusual in promoting both myeloid leukemia and a mixed myeloid/lymphoid leukemia (Ayton and Cleary, 2001). The mouse model developed for the *MLL-ENL* translocation described by Forster et al. (2003) is striking in its genetic elegance and its ability to model many key aspects of the human disease.

The properties of leukemia-associated fusion genes have been examined in a variety of ways in mice. The earliest approaches used transgenic expression of cDNA versions of these fusion genes (Bernardi et al., 2002; Figure 1A). Although the oncogenic properties of some fusions can be demonstrated in this way, the results are variable. The fusion is often not expressed at the correct level or in the correct tissue compartment for disease development. Overexpression of the fusion transgene during development often results in nonauthentic or lethal phenotypes that invalidate or complicate the use of the mouse line as a model for the intended disease.

The use of the P1 phage derived recombinase, Cre, for genetic manipulation of mice has provided a means to refine these crude transgenic models (Kuhn and Torres, 2002). By expressing Cre as a transgene, spatial and temporal restrictions can be imposed so that the expression of the fusion gene is limited to compartments where both transgenes are/were expressed. For example

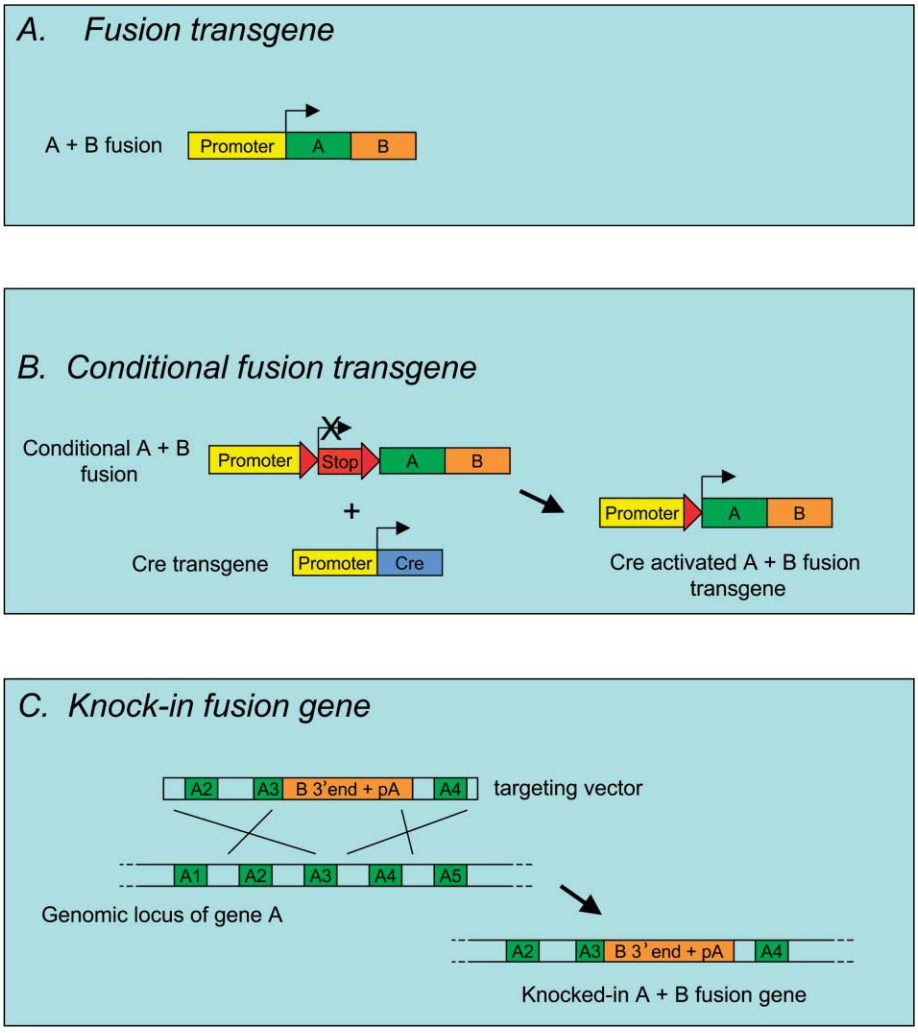

Figure 1. Expression of fusion genes using fusion transgene, conditional fusion transgene, and knockin fusion transgene

A: Fusion transgene. A fusion gene between partners A and B can be used as a transgene in mice. The spatial distribution, temporal activation, and level of expression of germline copies of the transgene are determined by the heterologous promoter/enhancer and the integration site(s) of the transgene.

B: Conditional fusion transgene. The activity of the fusion transgene can be refined by making it dependent on the expression of Cre. The fusion transgene is not expressed until the transcriptional stop cassette between the promoter and fusion gene is deleted by Cre, determined by its heterologous promoter. loxP sites are shown as red triangles.

C: Knockin fusion gene. A fusion gene can be generated in the appropriate chromosomal context of one partner (gene A) by "knocking in" the appropriate part of the fusion partner (gene B) into the genomic locus of gene A. The fusion gene is present in the germline, and its expression during development can lead to developmental abnormalities.

(Figure 1B), expression of Cre leads to deletion of a transcriptional stop sequence between a fusion transgene and its promoter. This enables mice to proceed through embryogenesis with certain transgenes that cause developmental problems. However, in the tissues in which Cre is expressed, the transgene may still be expressed in an unregulated way.

The deficiencies of randomly integrated transgenic constructs have been largely overcome with the development of embryonic stem cell technology. A fusion gene(s) can be constructed in an appropriate chromosomal context (Figure 1C). This is most readily achieved by targeting (knocking in) the 3′ part of the fusion gene into the genomic locus of the other partner. Although such an allele is likely to be expressed at the appropriate level, it can be difficult to transmit this type of allele into the mouse germline, because of the developmental problems caused by activity of the fusion gene in an entire tissue compartment (Corral et al., 1996). This can be overcome if the fusion gene can be conditionally activated with Cre (Figure 2A).

All of these transgenic and targeting approaches suffer from one major limitation; namely, they produce a field of cells expressing the fusion gene within a specific tissue compartment. However, cancer develops in an entirely different way: a single cell which undergoes a translocation will clonally expand in a microenvironment which is initially composed of normal cells.

A more normal "physiological" cellular context can be achieved by transducing bone marrow progenitor cells with the relevant transgene and transferring them back into the mouse to develop in vivo (Figure 2B). This approach has been successfully employed to model MLL-associated translocations. Pertinent to the current study, this approach has recently been applied to the *Mll-Enl* fusion gene (Zeisig et al., 2003). Syngeneic mice receiving transduced bone marrow cells developed an aggressive disease. This technique has the added advantage that the mixed lymphoid/myeloid component of the human leukemia can be artificially modeled by inducing the lymphoid differentiation pathway by treating the transduced cells with flt3 ligand.

Forster et al. (2003) have solved this problem in a different and altogether more elegant way, and in doing so have taken cancer modeling to a new mechanistic level (Figure 2C). In their experiments, *loxP* sites were targeted to positions in the mouse corresponding to the breakpoint observed in MLL and ENL

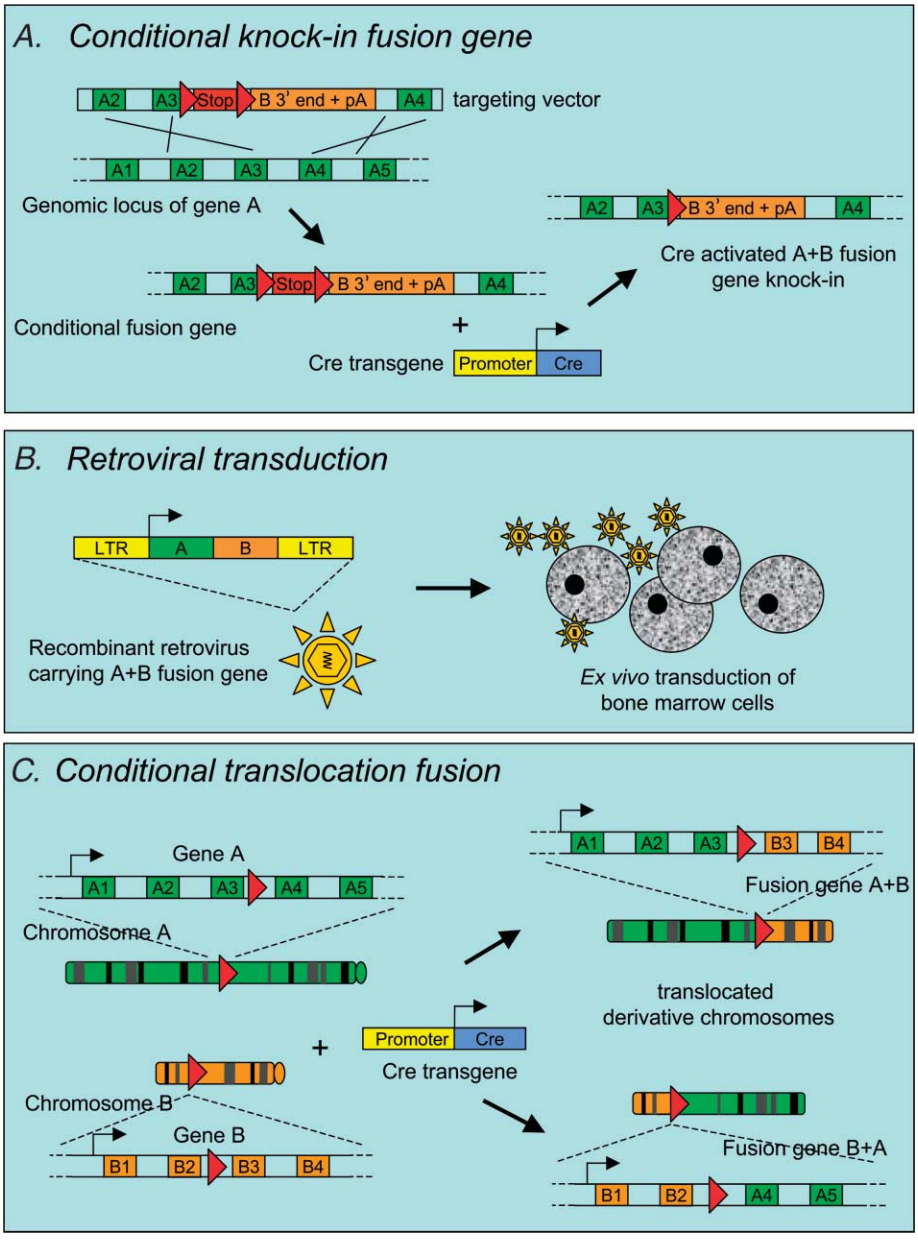

Figure 2. Expression of fusion genes using conditional knockin fusion gene, retroviral transduction of haematopoetic progenitor cells, and de novo reciprocal chromosomal translocation

A: Conditional knockin fusion gene. An additional layer of temporal and spatial control of the activity of a knockin fusion can be provided by a Cre transgene. The knockin fusion is not expressed until the "stop" cassette is removed by the activity of Cre, determined by a heterologous promoter. loxP sites are shown as red triangles.

B: Transduction of haematopoetic progenitor cells with retrovirus particles containing the fusion gene expression cassette in vitro. The transduced cells can be returned to adult mice in appropriate numbers and at a relevant time, avoiding many of the issues of inappropriate temporal expression.

C: Cre mediated interchromosomal translocation between *loxP* sites (red triangles) targeted to the desired breakpoint point positions. Both fusion gene partners are induced in authentic chromosomal locations. An appropriately expressed Cre transgene limits the translocation to an appropriate cellular compartment and the events are relatively rare, recapitulating the sporadic nature of translocation events in human cancer.

in human leukemia. They have utilized Cre to recombine *loxP* sites placed on different chromosomes (Van Deursen et al., 1995; Smith et al., 1995). This juxtaposition of genetic elements creates reciprocal fusion genes at the breakpoints in the context of their natural loci and under transcriptional control of their authentic promoters.

Although previous studies had indicated the ability to effect such de novo translocations within mice, disease phenotypes were not observed, ascribed by the authors to inappropriate levels and distribution of Cre transgene expression (Bucholz et al., 2000; Collins et al., 2000). The hematopoietic lineage expression of the *lmo-2 Cre* knockin mouse has the appropriate expression characteristics for use with the *Mll-Enl* translocation induction within the myeloid lineage. Expression of Cre leads to rapid onset, highly penetrant leukemogenesis, which is always associated with the induced translocation event. However, the mouse model only developed the myeloid component of the disease, with no evidence of lymphoid lineage markers being detected in leukemic cells. Whether the lack of mixed lymphoid/myeloid leukemia in these mice represents a technical inadequacy in the system or a basic difference in biology between mouse and man remains to be seen.

Although this study is of undoubted significance to understanding the development and potential treatment of this particular form of leukemia, the real highlight is its technical elegance and the likely applicability to the whole range of leukemias and sarcomas that develop as a consequence of chromosomal translocations (Bernardi et al., 2002). This is because it accurately replicates all of the functional events that lead to the development of the analogous human tumor: (1) the translocation occurs somatically, rather than being present throughout development; (2) the translocation occurs exclusively within a defined tissue compartment where the tumor develops in human; and (3) the translocation produces fusion genes at the two chromosomal breakpoints that accurately replicate those in the human cancer, as it is possible that either one, or indeed both, of the fusion genes might contribute to various aspects of disease development (Rego and Pandolfi, 2002). The judicious choice and empirical development and testing of Cre-expressing mouse lines may allow similar development of de novo translocation models for a whole range of different endpoints with specific target disease tissues.

There is one real limitation to this technique for modeling human translocations. Regions in the mouse genome which correspond to translocation endpoints in humans are not always in the same orientation with respect to the centromere. To avoid the creation of aberrant dicentric chromosomes, comparison of human and mouse genome sequences is essential to assess applicability of the technique to individual cases. In cases where the genes are not appropriately aligned, it is possible to invert one or other chromosome using chromosome engineering technology (Zheng et al., 1999), thus recreating the human chromosomal context.

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