

Creating a translocation

Engineering interchromosomal translocations in the mouse

The site-specific recombinases (SSRs), Cre and FLP, have added a variety of possibilities to the field of genome engineering. Two articles in this issue of *EMBO Reports* (Buchholz *et al.*, 2000; Collins *et al.*, 2000) now extend the repertoire of SSR-driven genomic alterations in the mouse by adding possibly the most difficult task ever asked of these enzymes, namely translocation between non-homologous chromosomes. Aiming at establishing better mouse models of human leukemias, the two groups introduced *loxP* sites into the mouse at the relevant positions on two chromosomes and show that, upon Cre expression, it is possible to detect the heterologous translocation at reasonable frequencies. Although FLP-mediated translocation between homologous chromosomes in *Drosophila* was one of the first applications of site-specific recombination in genome engineering (Golic, 1991), the findings reported in this issue were by no means an expected advance along a linear pathway. Scepticism had lingered over the possibility that an SSR-driven non-homologous translocation could be achieved in the mouse at a detectable frequency. It was clear from other ('easier') genome engineering exercises that efficiency dropped as a function of the distance between SSR target-sites (Ramirez-Solis *et al.*, 1995; Ringrose *et al.*, 1999; Zheng *et al.*, 2000). Additionally, evidence indicating that reasonable efficiencies of SSR translocations require the pairing of homologous chromosomes achieved during mitosis (Golic and Golic, 1996) or meiosis (Herault *et al.*, 1998) was obtained in both flies and mice. A further source of doubt arose with the emergence of the chromosomal territory model of nuclear organization (Figure 1) (Lamond and Earnshaw, 1998; Zink and Cremer, 1998). How would the two SSR target sites, buried in distinct and possibly distant chromosomal territories, ever manage to find each other for the crucial handshake?

Hints for optimism, but also pessimism, came from pioneering experiments with Cre-mediated translocations between non-homologous chromosomes in mouse embryonic stem cells (Smith *et al.*, 1995; Van Deursen *et al.*, 1995). In spite of the success at achieving translocations, the very low efficiencies observed and the fact that these experiments were limited to cells in culture gave rise to legitimate doubts as to whether the result could be recapitulated in a living mouse. The work of Collins *et al.* (2000) and Buchholz *et al.* (2000) now dismisses these doubts. At the same time, their accomplishments prompt questions regarding how the two SSR target sites come together

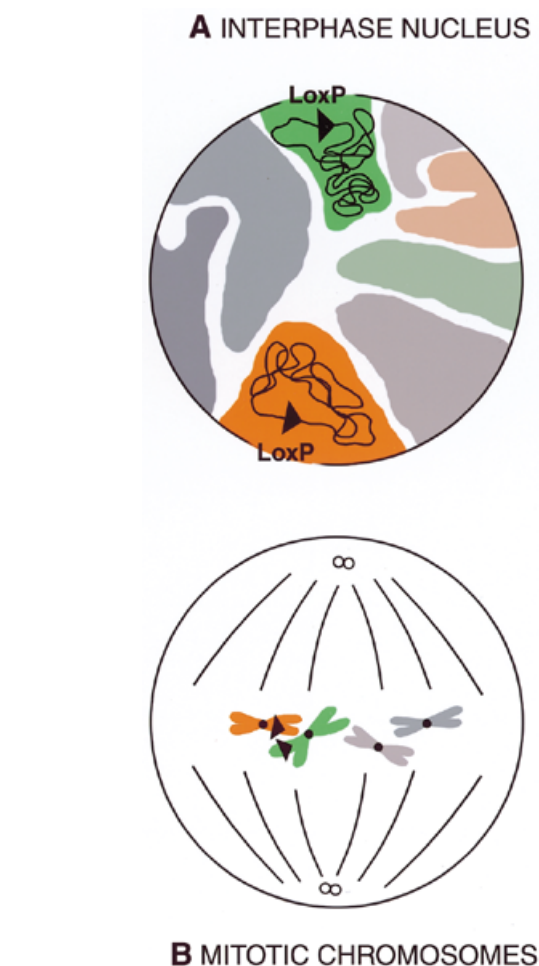


Fig. 1. (A) According to the chromosome territory model, during interphase each chromosome resides in a unique region within the nucleus, thus separating the two *LoxP* sites into different chromosomal territories (colored sectors). (B) During chromosomal resortment at mitotic metaphase, the two *LoxP* sites can stochastically come in close contact for Cre-mediated translocation to occur.

given the territorial organization of the nucleus. More work is needed to address this issue, but it is possible that global reshuffling of chromosomal positions may occur during mitosis, thus enabling pairing between two *loxP* sites. This scenario predicts that the rate of non-homologous SSR translocations will be higher among actively cycling cell populations. Alternatively, if recombination happens in interphase or resting cells, its frequency would be predicted to mirror the relative orientation of the two chromosomal territories in a given cell population.

Beyond the substantial technical achievement and inherent interest, what motivated these brave but risky enterprises considering that technically easier ways to make mouse models are

available? As is well illustrated by the path taken by Rabbitts and co-workers, the goal is to create the best possible mouse model. Previously this group had created a mouse model of the same leukemia by a knock-in strategy to the *Mll* (mixed-lineage leukemia) locus (Corral *et al.*, 1996; Dobson *et al.*, 1999). Although a good model was obtained, a knock-in strategy does not include the balanced translocation and does not imitate the cell-type-specific, somatic aspects of a tumorigenic translocation. The possibility that both expression products of a translocation can alter the disease phenotype is well illustrated by work on mouse models of the PML-RAR α (promyelocytic leukemia-retinoic acid receptor α) leukemogenic translocation. Using conventional transgenesis, it was shown that expression of the non-leukemogenic RAR α -PML translocation product affects the phenotype of PML-RAR α leukemias (Pollock *et al.*, 1999). This important observation emphasizes the need to create the most authentic models possible. Similarly, the drawbacks of creating a model where all cells carry the tumorigenic translocation product from the start of development are illustrated by work with the AML-ETO (acute myeloid leukemia-eighty-two-one) fusion product. When introduced as a fusion knock-in into the AML locus, the AML-ETO fusion protein is embryonically lethal (Yergeau *et al.*, 1997; Okuda *et al.*, 1998), thus precluding the creation of a simple model and underlying the *loxP* translocation efforts of Buchholz *et al.* (2000). Furthermore, *loxP* translocation strategies should permit cell-type-specific and temporal inductions of balanced translocations when combined with appropriate Cre-expressing mouse lines. This will provide an unprecedented opportunity to investigate leukemogenic fusion protein function at different stages throughout the development of the hematopoietic lineages.

The results of Collins *et al.* (2000) and Buchholz *et al.* (2000) represent another chapter in the remarkable story of SSRs in genome engineering. While we wait for the development of the leukemias as final proof of the applicability of this approach to disease modeling, other questions can be contemplated. Amongst these are how translocation frequencies relate to the levels of Cre expression, the relative positions of the targeted chromosomal territories and the rate of target cell proliferation.

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