GENE REGULATION

Wake-up call for *Sleeping Beauty*

By tinkering with the transposon *Sleeping Beauty*, researchers have developed new, highly effective strategies for performing mammalian mutagenesis screens.

When it comes to mutational analysis, transposons have long been the sweetheart of the fly-and-worm genetics set, but for various technical reasons, took some time to make a splash in vertebrate research. A dramatic jump forward came in 1997, when University of Minnesota researcher Perry Hackett identified numerous copies of a salmon transposase gene that had been rendered inactive through frameshifts, deletions and so forth. Hackett and his colleagues aligned these mutated transposases and used the resulting consensus as a template for a functional transposon (Ivics *et al.*, 1997). "They took one copy and fixed all the mutations that had accumulated during the last—what they estimate is 14 million years," explains David Largaespada, a current colleague of Hackett's. "And that's why they called the enzyme *Sleeping Beauty*, because they resurrected it from millions of years of evolutionary sleep."

This revived transposon can undergo cut-and-paste chromosomal transposition in many vertebrate species, although low efficiency has limited the range of practical applications. Nevertheless, Largaespada recognized its potential, and in a recent article in *Nature* (Collier *et al.*, 2005) describes the successful application of *Sleeping Beauty* (SB) as a tool for solid tumor gene discovery in mice. His group engineered an SB variant, T2/Onc, capable of generating loss of function or gain of function mutations after insertion into a gene (**Fig. 1**). Mice with chromosomal insertions containing multiple concatemerized copies of T2/Onc were bred against transgenic mice ubiquitously expressing an SB-derived transposase, SB10. Transposition events were readily detected, but no increase in tumor formation was observed until the same cross was performed against an *Arf*$^{-/-}$ background, a cancer-predisposing genotype.

The resulting mice had a wide range of different tumor types. Largaespada's team sequenced the transposon insertion sites for many samples, and identified 54 sites for which SB integration seemed statistically likely to be relevant to the cancer phenotype. The majority represented new loci, according to Largaespada: "It looks like SB mutagenesis is yielding genes that have not been seen in retroviral mutagenesis screens, even for the same tumor type,… which means that everything that's been done with [virus], we might consider doing with SB, and we'll get an overlapping but distinct set of genes."

This was only the beginning, however; after this work, coauthor Adam Dupuy moved to the National Cancer Institute to do his postdoc with Largaespada's former mentors, Nancy Jenkins and Neal Copeland, who also recognized the potential of SB for the study of solid tumors. "Retroviruses primarily only induce hematopoietic cancer or mammary cancer, and so they're really powerful tools for identifying cancer genes in those two tumor types," says Copeland, "[but] solid cancers are the cancers that most people die from." They went another step forward, working with SB11, a highly optimized transposase developed in the Hackett lab, and created knock-in mice that expressed it from the *Rosa26* locus, ensuring consistent and ubiquitous expression. They also worked with an enhanced T2/Onc variant, using founder lines with far more transposon copies—as many as 350. As described in a second *Nature* article (Dupuy *et al.*, 2005), these modifications permitted even more widespread transposition throughout the genome, revealing several putative oncogenes and even tumors with multiple gene insertions that seem to partially delineate cancer-related pathways.

Jenkins and Copeland reaped considerable benefits from their modified system, but some issues still clearly need to be addressed. "We expressed the transposase from a locus where it's expressed all over the place, and in that case we mainly got hematopoietic tumors again," says Copeland. "That seems to be the default." Ubiquitous transposase activity also leads to a large number of developmentally fatal mutations, limiting the quality and quantity of data. Both research groups believe that restricting the expression of transposase in a tissue-specific—or even temporal—manner with the Cre recombinase system will make this assay more efficient, broadening the possibilities for identifying key genes involved in cancer initiation and progression in a wide range of tumor types. "Not only that," says Copeland, "you [can] get cooperating genes because you get more than one hit per tumor, and you get networks." Jenkins adds, "It's the first step… but I do think it offers real potential for the next generation of identification of pathways."

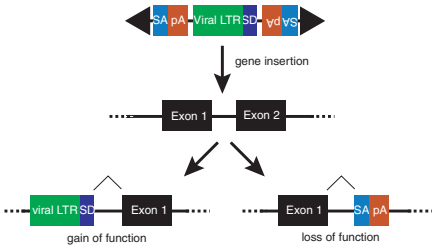

Figure 1 | The T2/Onc transposon. Between the essential flanking transposition repeats, this *Sleeping Beauty* variant contains a viral promoter (LTR) with a splice donor (SD) to induce gain-of-function phenotypes upon integration upstream of a gene, and polyadenylation sequences (pA) linked with splice acceptors (SA) to induce loss-of-function phenotypes when integrated into the middle of a gene.

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RESEARCH PAPERS

Collier, L.S. *et al.* Cancer gene discovery in solid tumors using transposon-based somatic mutagenesis in the mouse. *Nature* **436**, 272–276 (2005).

Dupuy, A.J. *et al.* Mammalian mutagenesis using a highly mobile somatic *Sleeping Beauty* transposon system. *Nature* **436**, 221–226 (2005).

Ivics, Z. *et al.* Molecular reconstruction of *Sleeping Beauty*, a *Tc1*-like transposon from fish, and its transposition in human cells. *Cell* **91**, 501–510 (1997).