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Non-viral therapeutic cell engineering with the *Sleeping Beauty* transposon system

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Widespread treatment of human diseases with gene therapies necessitates the development of gene transfer vectors that integrate genetic information effectively, safely and economically. Indeed, significant efforts have been devoted to engineer novel tools that (i) achieve high-level stable gene transfer at low toxicity to the host cell; (ii) induce low levels of genotoxicity and possess a 'safe' integration profile with a high proportion of integrations into safe genomic locations; and (iii) are associated with acceptable cost per treatment, and scalable/exportable vector production to serve large numbers of patients. Two decades after the discovery of the *Sleeping Beauty* (SB) transposon, it has been transformed into a vector system that is fulfilling these requirements. Here we review recent developments in vectorization of SB as a tool for gene therapy, and highlight clinical development of the SB system towards hematopoietic stem cell gene therapy and cancer immunotherapy.

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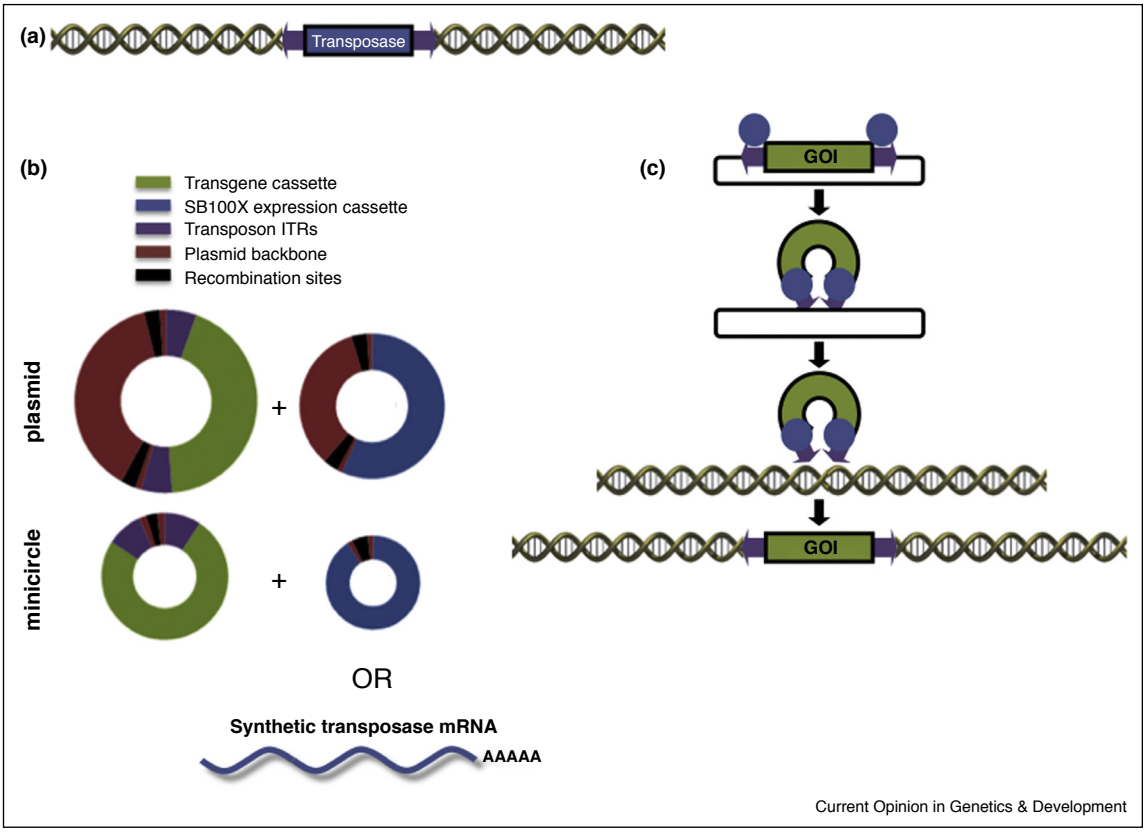
Non-viral gene transfer using the *Sleeping Beauty* transposon and recent developments towards clinical applications

Transposable elements (DNA transposons and retrotransposons) are genetic elements with the ability to change their positions within the genome. For simplicity, we will be referring to DNA transposons as 'transposons' throughout this article. Transposons are mobile ('jumping') units of DNA encoding a gene for a transposase enzyme flanked by inverted terminal repeats (ITRs) that contain the sites where the transposase binds (Figure 1a). A crucial point in turning transposons into genetic vectors is the possibility of separating these two functions (the ITRs and the transposase) to establish a two-component system: one supplying the transposase and the other carrying a DNA sequence of interest between the ITRs (Figure 1b). Transposition is achieved through a cut-and-paste mechanism, during which the transposase enzyme mediates the excision of the element from its donor plasmid, and then reintegrates the transposon construct into a chromosomal locus (Figure 1c). Such a system represents an easily controllable DNA delivery vehicle that has a vast potential for diverse applications in genetic engineering, including gene therapies.

Although most transposons became dormant over the course of evolution in vertebrates, it was possible to reconstruct an active sequence from ancient inactive transposon sequences isolated from fish genomes. This transposon was named *Sleeping Beauty* (SB) after the Grimm brothers' famous fairy tale [1]. SB was the first transposon ever shown to be capable of efficient transposition in vertebrate cells, and it opened entirely new avenues for genetic engineering, including gene therapies (recently reviewed in [2–7]). Indeed, the SB transposon system has been successfully adapted to render sustained expression of therapeutic transgenes for treatment of a variety of animal disease models following both *ex vivo* and *in vivo* gene delivery (recently reviewed in [4–6]). A transposon-based gene delivery system has the advantage of combining the favorable features of viral vectors with those of naked DNA molecules, namely (i) permanent insertion of transgene constructs into the genome by the transposition mechanism leads to sustained (potentially life-long) and efficient transgene expression in preclinical animal models [8]; (ii) transposon vectors can be maintained and propagated as plasmid DNA, which makes vector manufacture simple, scalable and cost-effective; (iii) transposons can deliver larger genetic cargoes than viruses [9]; and (iv) transposon-based vectors have an attractive safety profile [10–14].

Implementation of the SB transposon system for gene therapy has been, however, hampered by a low efficiency of plasmid DNA delivery into primary human cells in general. Nucleofection — an advanced technique of electroporation [15,16] — can significantly facilitate the delivery of transposon-based vectors; this has been achieved in $CD34^+$ hematopoietic stem and progenitor cells (HSPCs) [17–20] and primary T cells [21–23,24•].

Figure 1



(a) The *Sleeping Beauty* transposon system. **(a)** Autonomous transposable elements consist of inverted terminal repeats (ITRs, black arrows) that flank the transposase gene (blue). **(b)** Bi-component, *trans*-arrangement transposon vector system for delivering transgenes that are typically maintained in plasmids. MC vectors encoding the transposon or the transposase component of the SB system are derived from parental plasmids by intramolecular recombination. The parental transposon plasmid carries a transgene cassette (green) flanked by the ITRs of SB, plasmid backbone sequences (red) and recombination sites (black). The parental transposase plasmid carries a CMV-SB100X expression cassette (blue), plasmid backbone sequences (red) and recombination sites (black). MCs have a markedly reduced size and lack most of the plasmid backbone sequences. Transposon cassettes can be mobilized by transposase supplied as *in vitro*-transcribed mRNA. **(c)** Cut-and-paste DNA transposition. The transposition process is depicted by using a standard plasmid as an example. The transposon carrying a gene-of-interest (GOI) is excised from the donor plasmid and is integrated in the genome by the transposase.

However, nucleofection of naked plasmid DNA compromises cell viability [25], at least partly because unmethylated CpG dinucleotide motifs present in the bacterial backbone of conventional plasmid vectors can trigger immunogenic responses against foreign DNA [26–28]. In addition, the presence of an antibiotic resistance gene typically present in plasmid vectors raises safety concerns in the context of gene therapy.

Two recent developments that address both the efficiency and safety of SB gene delivery are the use of mRNA-encoded SB transposase and minicircle (MC) vectors to encode transposase/transposon (Figure 1b). Both are key milestones towards the clinical use of SB transposition. MCs are supercoiled minimal expression cassettes that are derived from parental plasmids via an intramolecular recombination process, during which the majority of bacterial backbone sequences are depleted from the vector [29–31]. The MC vectors are therefore significantly reduced in size and, as a consequence, have been shown to enhance gene delivery into a variety of cell lines *in vitro* [29,32,33], and into the liver [34,35], lungs [36], muscle [32,37], and tumors [33,37] *in vivo*. The improved potential of MC DNA-based delivery was also evidenced in preclinical gene therapy of mucopolysaccharidosis type I [35] and familial hypercholesterolemia [38].

By applying mRNA for intracellular delivery in therapeutically relevant cells *ex vivo*, some hurdles of gene transfer typical for DNA-based vectors can be avoided. For example, nucleofection of primary human cells, including HSPCs and T cells, with mRNA was shown to cause significantly reduced cellular toxicity as compared to nucleofection with plasmid DNA [39]. Second, upon