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# Vectors for gene therapy: A place for DNA transposon

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## ABSTRACT

**Gene therapy offers important perspectives in current and future medicine but suffers from imperfect vectors for the delivery of the therapeutic gene. Most preclinical and clinical trials have been based on the use of viral vectors, which have evident advantages but also some serious disadvantages. In the past decade the use of DNA transposon-based systems for gene delivery has emerged as a non-viral alternative. DNA transposon vector engineering remains largely in a preclinical phase but some interesting results have been obtained. This mini-review aims to provide the current state of the art on DNA transposon vectors used in a gene therapy perspective.**

**Keywords:** Transposable Element; Gene Delivery; Non-Viral Vectors; Piggybac; Sleeping Beauty

## 1. INTRODUCTION

Gene therapy has emerged as a promising method to cure genetic diseases or at least improve the status of patients. If the idea of replacing an "abnormal" dysfunctional gene by its "normal" functional counterpart may look simple, technical difficulties are often encountered such as the stable and targeted insertion of the therapeutic gene into the host genome. Several gene therapy strategies can be distinguished that address different kinds of pathologies. Recessive monogenic hereditary diseases, such as cystic fibrosis and Duchenne's muscular dystrophy, could be treated by the addition of the "normal" gene counterpart that will produce a sufficient amount of functional protein to reverse the disease. However, some other monogenic defects will require the replacement of the "abnormal" gene by its "normal" allele and not solely its addition. In some cases of acquired and/or polygenic diseases such as diabetes, the replacement of one of the deleterious alleles or the addition of one "normal" gene may be sufficient to fight the disease. Moreover, in complex pathologies that mix genetic predisposition with environmental factors (cancer diseases for example), different gene therapy strategies may be useful, such as the extinction of a targeted gene expression or the introduction of a suicide gene. The common challenge is to introduce the new DNA durably into the genome of pathologic cells without disturbing the regulation of "normal" gene expression. This is the main objective of vector development. Over 1340 human gene therapy clinical trials were completed in 28 countries between 1989 and 2007 [1]. However, very few phase-II and phase-III clinical trials have been conducted that mainly use viruses as vectors [2]. Following a brief description of virus-based vectors, the advantages offered by DNA transposons for gene therapy will be presented with some examples of successful applications.

## 2. VIRUS-BASED VECTORS: PROMISE AND DISAPPOINTMENT

Virus-based vectors are the most commonly used gene delivery system because of their ability to integrate the therapeutic gene into the host genome and ensure stable and long term gene expression. The second advantage of virus-based vectors is the combination of the DNA vector with a highly efficient DNA delivery system in the cell, *i.e.* the natural infection process. The main virus-based vectors used are derived from retroviruses (especially lentiviruses), adenoviruses and adeno-associated viruses [2,3]. For example, 68% of the gene therapy clinical trials completed in to date used virus-based vectors [1,2]. Unfortunately some fatal adverse events have clouded the sky. In 1999, a patient treated for ornithine transcarbamylase deficiency with an adenoviral gene transfer developed a fatal systemic inflammatory response syndrome [4]. In 2000, a French clinical trial using a retrovirus-based gene therapy on children suffering from X-linked severe combined immunodeficiency syndrome (X-SCID) seemed more promising, and 10 patients appeared successfully cured [5]. However, two years later two of the ten children treated developed T-cell lymphoma and one died [6]. This major adverse event was

subsequently explained by the insertion of the retroviral vector in the vicinity of the LMO-2 gene that encodes a crucial regulator of haematopoeisis [7]. This insertional mutagenesis event resulted in the dysregulation of LMO-2 gene expression that induced T-cell proliferation and then lymphoma [7,8]. So, virus-based vectors may have genotoxic effects, caused by deregulation of gene expression at the transcriptional or posttranscriptional level. In particular, integration of retroviral (lentiviral or gamma-retroviral) vectors within transcribed genes has a significant potential to affect their expression by interfering with splicing and polyadenylation of primary transcripts [9].

Despite their recognized disadvantages, adenoviruses and retroviruses remained the two most widely-used vectors for gene therapy to date [1,2]. The adeno-associated virus (AAV) displays particularities making it a safer virus-vector [3]. AAV is a parvovirus, the proliferation of which depends on a helper virus, usually an adenovirus or a herpes virus [10]. Wild-type AAV is nonpathogenic with low immunogenicity and integrates into a specific point of the genome (19q 13qter) at high frequency and without genotoxicity to the host [11-14]. However this integration specificity is lost with engineered AAV, especially when increased cargo capacity is tried. Several AAV-based gene therapy trials have been launched for cancers, Parkinson's disease, Alzheimer's disease, and genetic diseases such as Leber's congenital amaurosis (LCA). LCA is a common cause of blindness in infants and children and two AAV-based gene therapy trails reported improved vision without serious adverse effects [15,16]. Nevertheless the main drawback of AAV is its limited cargo capacity. Furthermore both AAV and adenoviruses remain largely episomal, requiring repeated delivery that may compromise efficacy [17] and might induce a severe immune response [18]. Furthermore the presence of AAV-neutralizing antibodies prevalent in humans can hamper the clinical success of AAV-based gene therapy. For a more comprehensive review of pros and cons of viral vectors the reader may refer to Patel and Misra, 2011 [19].

The fatal adverse effects associated with adenoviruses and retroviruses and the limited cargo capacity of AAV prompted the investigation of non-viral vectors, especially those based on DNA transposons.

## 3. DNA TRANSPOSON-BASED VECTORS

DNA transposons are a group of transposable elements present in most (if not all) organisms from bacteria to mammals [20], but poorly represented in the human genome [21]. The main families of DNA transposons (*Tc*1/*mariner*, *hAT* and *PiggyBac*) have a simple structure with a unique gene flanked by inverted terminal repeats (**Figure 1(a)**). Their mobility is supported by the transposon-

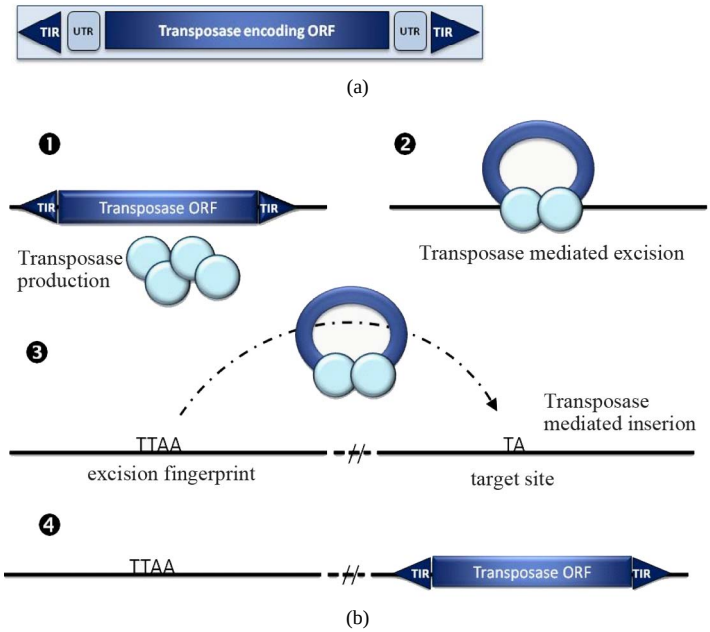


**Figure 1.** (a) Structure and (b) simplified "cut and paste" transposition mechanism of a DNA transposon. ORF, open reading frame; TIR, terminal inverted repeats; UTR, untranslated region. The light blue circles in B represent the transposase proteins encoded by the transposon ORF (b1). Transposase dimer (or more) binds to ITR (b2) and excises transposon DNA, then the transpososome is displaced (b3) to be integrated in another genomic site (b4).

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encoded transposase enzyme in a "cut and paste" mechanism (**Figure 1(b)**). This simple genome integration mechanism makes DNA transposons very attractive as gene delivery tools. To achieve this goal a two-plasmid system should be developed consisting of a helper plasmid expressing the transposase and a donor plasmid with the terminal repeat sequence flanking the gene of interest (**Figure 2**) [22-24]. Using this system, several DNA transposons, such as *P* element, *PiggyBac*, *Tol*2, *Hsmar*1 and *Sleeping Beauty*, have been utilized for gene delivery and mutagenesis in invertebrate and vertebrate cells [24-33]. Among the DNA transposons, *PiggyBac*, *Tol*2 and *Sleeping Beauty* have been evaluated for gene therapy in animal experiments, primary cell gene delivery and a few pre-clinical trials [34-37]. The advantages and disadvantages of DNA transposon-based gene delivery will be discussed below with respect to cargo capacity, efficiency, gene silencing and targeted insertion with special emphasis on *PiggyBac*, *Tol*2 and *Sleeping Beauty*.

### 3.1. Cargo Capacity

A large cargo capacity is desired for therapeutic application and DNA transposon-based vectors appear as good candidates with respect to this criterion. Indeed *Tol*2 and *PiggyBac* vectors are able to integrate up to 11 and 9.1 kb of foreign DNA respectively [28,38]. Furthermore the large cargo capacity of *Tol*2 was further evidenced with a ~70 kb BAC transfer in mouse and zebrafish genomes [39]. By contrast the transposition activity of wild type *Sleeping Beauty* is significantly decreased when foreign DNA exceeds the initial 1.7 kb size [40].

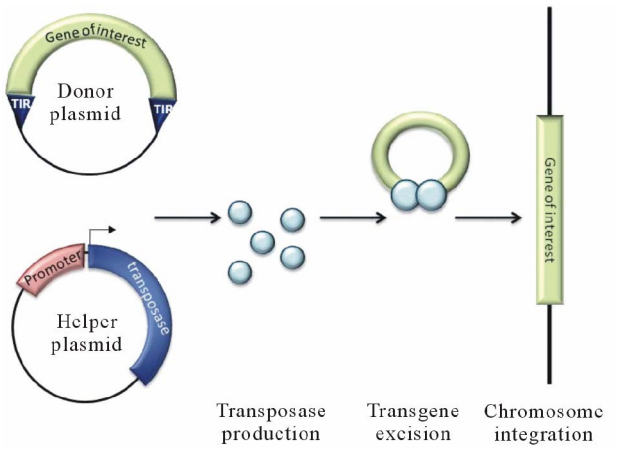


**Figure 2.** Schematic view of a DNA-transposon system for gene delivery. Both donor and helper plasmids are transfected into the host cells. The helper plasmid is an expression vector that allows transposase production and the donor plasmid bears the gene of interest that will be integrated into the host genome by the transposase. The light blue circles represent the transposase proteins produced from helper plasmid.

### 3.2. Transposition Efficiency

The transposition efficiency of *PiggyBac*, *Tol*2 and a mutant *Sleeping Beauty* transposase (SB11) was compared in several human cell lines. *Tol*2 and *PiggyBac* were highly active in all cell lines tested while SB11 was obviously less efficient and marginally or totally inactive in two cell lines, *i.e.* HeLa and H1299, respectively [30]. However, a new hyperactive *Sleeping Beauty* transposase (SB100X) displays transposition efficiency comparable to *PiggyBac* under non-restrictive conditions [41]. So, transposase mutagenesis may be an interesting way to increase DNA transposon-based vector efficiency as shown for *Sleeping Beauty* hyperactive mutants [41,42] and hyperactive or mouse-codon optimized *PiggyBac* [43,44].

### 3.3. Gene Silencing and Stability of Gene Insertion

Any transgene introduced into the host genome is a potential target for the position effect that causes transgene silencing. This phenomenon is observed particularly in viral vectors but also appears in plasmid and DNA-transposon vectors. However, in the study by Grabundzija *et al.* transgene silencing observed following DNA-based gene delivery using *Tol*2, *PiggyBac* or SB100X, was found to be very low (3.8% vs 26.5% in control) [45]. This result has to be moderated since earlier results reported progressive post-integrative gene silencing after *Sleeping Beauty* transposition [46]. Furthermore post-integration transgene silencing may be higher than previously observed as suggested by Meir and Wu [47] indicating 63% for *Sleeping Beauty*, 46% for *Tol*2 and 20% for *PiggyBac*. A comparative study between viral vectors and non viral vectors used under the same conditions may be of interest to decide whether DNA transposon-based vectors have advantages or not with respect to the gene silencing effect [47]. Interestingly, reducing the terminal repeats of *PiggyBac* to 40 bp and 67 bp from 245 bp and 311 bp of 3' and 5' terminal repeats respectively in the commonly used *PiggyBac*, enhances *Piggy Bac*'s transposition activity [48]. This suggests a possible interaction between epigenetic silencing factors and sequences present in the common *PiggyBac* but not in its shorter form. Furthermore, this shorter *PiggyBac*, called micro-*PiggyBac*, lacks many activator sequences initially present in the 3' *PiggyBac* terminal repeats that influence neighboring gene expression [49].

In addition, transgene delivery without drug selection is advantageous for therapeutic purposes but may interfere with stable insertion. A recent study compared the stable insertion ability of the *Tol*2, *PiggyBac* and *Sleeping Beauty* DNA-transposon gene delivery systems in human peripheral blood and umbilical cord blood-derived

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T-cells [50]. This study showed that *PiggyBac* is the most efficient of the three DNA transposon systems in mediating stable gene insertion.

### 3.4. Insertion Site Targeting

Site targeting is a crucial point in gene transfer and especially in the human gene therapy perspective. Most of the integrating viral vectors have integration site preferences leading to an integration site bias that may explain some adverse effects such as gene silencing or activation of cancer-related genes [51-54]. By contrast, genome-wide targeting profiles of *PiggyBac*, *Sleeping Beauty* and *Tol*2 in various human cell lines and human primary cells clearly demonstrate that the three transposon systems display a lower frequency in targeting near cancer-related genes and less bias in targeting active genes compared to viral-based vectors [29,45,48,50,55, 56]. A detailed analysis of this aspect is available in Meir and Wu (2011) [47]. The ability of *PiggyBac* transposase to be molecularly engineered without a loss of activity allows the construction of *PiggyBac* transposase chimeras with a DNA binding domain, such as the zinc finger DNA binding domain that would recognize specific and safe integration sites.

## 4. PHENOTYPIC CORRECTION IN DISEASE MODELS AND PRECLINICAL TRIALS

Stable chromosomal insertion can be achieved from a transposon-based delivery system which results in robust, long-term expression of a therapeutic transgene in certain primary stem cells as well as *in vivo* (**Table 1**). This includes extended expression of human clotting factor IX in factor IX-deficient mice [57], in vivo correction of murine tyrosinemia type I [58], sustainable correction of junctional epidermolysis bullosa [59], long term expression of factor VIII and phenotypic correction in hemophilic mice [60-62], correction of lung allograft fibrosis

**Table 1.** Examples of successful DNA transposon-based gene delivery in disease models.

| Disease | Delivered gene | Model organism or cell type | DNA-transposon used | References |
|---|---|---|---|---|
| Hemophilia A | Human clotting factor VIII | Mouse | *Sleeping Beauty* | [60-62] |
| Hemophilia B | Human clotting factor IX | Mouse | *Sleeping Beauty* | [57] |
| Inherited type I tyrosinemia | hFAH[a] | Mouse | *Sleeping Beauty* | [58] |
| Glioblastoma | sEGF-R[b] and AE-FP[c] | Mouse | *Sleeping Beauty* | [69] |
| Junctional epidermolysis bullosa | hLAMB-3[d] | Human patient derived cells | *Sleeping Beauty* | [59] |
| Pulmonary hypertension | eNOS[e] | rat | *Sleeping Beauty* | [97] |
| Lung allograft fibrosis | hIDO[f] | rat | *Sleeping Beauty* | [63] |
| Mucopolysaccharidosis, type I and VII | hIDUA[g] and/or hGUSB[h] | Mouse | *Sleeping Beauty* | [64,65] |
| Crigler-Najjar syndrome, type I | hUGT1A[i] | rat | *Sleeping Beauty* | [66] |
| Ovarian cancer | suicide gene[j] | Mouse ovarian tumor | *PiggyBac* | [70] |
| Ovarian cancer | suicide gene[j] | Human ovarian adenocarcinoma cell line | *PiggyBac* | [72] |
| B-lymphoid malignancies | CAR[k] | Mouse primary T cells | *Sleeping Beauty* | [77] |
| B-lymphoid malignancies | CAR[k] | Primary human T cells/mouse tumor | *Sleeping Beauty* | [98] |
| Solid tumor (murine xenograft of) | HER2[l]-CAR[k] | Human peripheral blood mononuclear cells | *PiggyBac* | [71] |
| Fanconi anemia, type C | FANC-C[m] | Human lymphoblastoid cells | *Sleeping Beauty* | [67] |
| Skin inflammation | Human $\beta$1 and $\alpha$2 integrin | Pig | *Sleeping Beauty* | [99] |
| Osteosarcoma lung metastase | CAR[k] and IL-11R$\alpha$[n] | T cells/mouse tissue | *Sleeping Beauty* | [100] |
| Pulmonary fibrosis | miR-29[o] | Mouse lung tissue | *Sleeping Beauty* | [101] |

a. hFAH, human fumaryl acetoacetate hydroxylase. b. sEGF-R, soluble endothelium growth factor receptor. c. AE-FP, angiotensin-endothelin fusion protein. d. hLAMB-3, human laminin-$\beta$3. e. eNOS, endothelial nitric oxide synthase. f. hIDO, human indoleamine-2-3-dioxygenase. g. hIDUA, human $\alpha$-L-iduronase. h. hGUSB, human $\beta$-glucuronidase. i. hUGT1A, uridinediphosphoglucuronate glucuronosyl transferase-1A1. j. Herpes simplex thymidine kinase. k. CAR, chimeric antigen receptor. l. HER2, human epidermal growth factor 2. m. FANC-C, Fanconi anemia gene C. n. IL-11R$\alpha$, interleukin-11 receptor $\alpha$; o. miR-29, microRNA-29.

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[63], prolonged expression of lysosomal enzymes in mouse liver and correction of mucopolysaccharidosis [64, 65]; long term reduction of jaundice in Gunn rats [66], correction of Fanconi anemia type C in human patient-derived cells [67], and stable integration and continuous expression of pigment epithelium-derived factor gene in retinal and iris pigment epithelial cells [68]. Another example of successful *Sleeping Beauty*-based gene delivery is given by the antiangiogenic gene therapy of mice bearing intracranial human glioblastoma, which causes tumor regression and improves animal survival [69]. The *PiggyBac* system was also used in cancer cell gene therapy [70,71]. For example, Kang *et al.* applied a gene-directed enzyme prodrug therapy using a *PiggyBac*-based vector to treat mouse ovarian cancer and human ovarian adenocarcinoma cells [70,72].

In the *ex vivo* gene delivery approach, the therapeutic gene construct is introduced into a selected cell population, removed from the patient and then the treated cells are infused back into the same patient. Efficient transfection and stable transgene expression using (*Sleeping Beauty* or *PiggyBac*) DNA-transposon based systems were achieved in several types of human stem cells, including cord blood-derived $CD34^+$ hematopoietic progenitors [41, 73-76], primary T cells [50,71,77-81], and human embryonic stem cells [82,83]. So, transposon-based gene delivery systems such as *Sleeping Beauty* [78,81,83] or *PiggyBac* [71,79,80] allow stable gene transfer into T cells and an efficient expression of the therapeutic transgene.

The first clinical application of the *Sleeping Beauty* system was tested using autologous T cells genetically modified to redirect specificity to B-lineage malignancies [84]. The first generation *Sleeping Beauty* vector phase-I clinical trials carried a chimeric antigen receptor (CAR) to render the T cells cytotoxic specifically towards $CD19^+$ B-lineages tumors (reviewed in [85]. In the next generation of clinical trials the chimeric antigen receptor carried by *Sleeping Beauty* vectors combined with a CD28-CD3-z fusion to provide T cells with an endodomain to achieve CD19-specific activation events of cytolysis, cytokine production, and proliferation [86]. In addition Torikai *et al.*, 2012 recently reported an important step towards eliminating the need to generate patient-specific T cells by using genetically-edited T cells that express CAR without the endogenous $\alpha\beta$ T cell receptor and are therefore suitable for administration in multiple recipients [87].

## 5. DRAWBACKS AND FUTURE CHALLENGES

The DNA transposon-based gene delivery systems encounter some drawbacks which must be overcome, some being shared with other viral and non-viral vectors. The behavior of DNA transposon in different cell types remains largely unknown, especially regarding *PiggyBac* and *Tol*2. One might worry some cytotoxicity of transposase overproduction, leading to overproduction inhibition. Studies with *Sleeping Beauty* have been conducted in numerous cell types showing a cell-type dependent activity and the interaction with human factors such as HMGB1 and Miz-1 [24,36,45]. Unlike *Sleeping Beauty*, *PiggyBac* transposition seems to be cell type independent. However cytotoxic interaction of *PiggyBac* with cellular factors cannot be ruled out since a little overproduction inhibition could be observed [30,44,47]. The existence of *PiggyBac*-like elements in the human genome [21] raises the question of safety concerns. Indeed a trans-mobilization of endogenous elements by the engineered transposase might be mutagenic. This does not apply to Tc-1 like elements such as *Sleeping Beauty*, which have no close relatives in the human genome. This is also one interest to develop new vectors based on distant sequences (*e.g.* from crabs···) [22,88]. However the longer the foreign sequences introduced into the host genome, the greater the probability of evoking adverse consequences such as gene silencing and dysregulation of genes nearby. Therapeutic gene silencing and dysregulation of genes nearby are common drawback of DNA transposon-based vectors and viral vectors [3,47]. Epigenetic gene silencing was observed post-integration with *Sleeping Beauty* that was influenced by DNA methylation and histone deacetylation [46]. Epigenetic regulation of *PiggyBac* vector was also suggested by the results of Meir *et al.* [48].

Another concern shared by both non-viral and viral vectors, is the integration site specificity and its counterpart insertional mutagenesis. To overcome this point is the major challenge for gene therapy, and despite years of research, this has not been addressed to date. The addition of specific DNA binding domain, such as zinc-finger domain of known transcription factors, appears inefficient and reduces the transposition rate. Some outlook may come from artificial zinc-fingers which harbor modular structure and function making possible to recognize theoretically any sequence in the genome [37]. Finally, to my point of view, the major concern of DNA transposon-based delivery systems is to keep open the problem of DNA delivery into cells, with the drawbacks of all non-viral delivery systems (mainly toxicity and low efficiency). It is beyond the scope of this review to address the pros and cons of non-viral delivery systems (e.g. liposomes, polymers, electroporation···) but it is a great challenge to develop efficient and non-cytotoxic reagents for DNA delivery across the cell membrane. Maybe the future resides in the hybrid technology combining the natural ability of viruses to cross cell membranes with efficient genomic insertion of DNA-transposon. Several

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*Sleeping Beauty*-virus combinations have been tried with herpes virus [89] or lentivirus [90,91] in human cells and adenovirus in mouse liver [92,93].

## 6. CONCLUDING REMARKS

Because of their lower immunogenicity and the fact that they are less prone to gene silencing than viral vectors, DNA transposon-based vectors appear to be a more desirable therapeutic gene delivery system. *PiggyBac* has some advantages over *Sleeping Beauty* or *Tol*2 because *PiggyBac* transposase can be molecularly modified without substantially losing its activity [47]. By contrast, any modification done on *Tol*2 and *Sleeping Beauty* transposases drastically reduces or completely eliminates their enzymatic activity [94]. According to Meir and Wu, 2011, *PiggyBac* seems currently the most promising DNA transposon to obtain a highly efficient gene delivery system capable of targeting the therapeutic gene at a predefined safe location in the host genome where the transgene can be stably and faithfully expressed without disturbing global gene expression. However, this ambitious goal has not yet been achieved and much progress is still required. Finally, improvement of cargo capacity, delivery efficiency and expression stability/persistence may be achieved with chimeric vectors combining viral (*i.e.* baculovirus or adenovirus) and non viral (*i.e.* DNA transposon) elements such as those recently developed [95,96].

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