We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists



186,000

200M



Our authors are among the

TOP 1% most cited scientists





WEB OF SCIENCE

Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us? Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected. For more information visit www.intechopen.com



DNA Elements Tetris: A Strategy for Gene Correction

Colette Bastie and Florence Rouleux-Bonnin

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/62382

Abstract

Transposable elements (TEs) are mobile genetic sequences that are able to move in the genome from one location to another. TEs were first regarded as junk or selfish DNA, as they comprise the largest molecular class within most metazoan genomes having no genomic function. It was necessary to wait until whole genome sequencing to provide new insights about the origin, diversity, and impact of TEs on the genome function. Thus, due to advances in molecular technology, TEs have been shown to create new regulatory sequence networks. Although nowadays most TEs present in the human genome are silenced, particularly DNA transposons, it does not mean that these sequences are dead. In this review, we detail how DNA transposons could be emphasized to create a new tool for gene correction. DNA-based transposon vectors are derived from three models: Sleeping Beauty, piggyBac, and Tol2, which all work via a "cut-and-paste" mechanism where transposase enzyme is alone able to catalyze the transposition process, which means integrating the genes of interest in chromosomal DNA. Limitations and improvements of the systems are discussed, particularly the latest way to target a specific integration site, showing that the DNA transposon-derived system and its engineering, are powerful tools for gene correction.

Keywords: transposon, piggyBac, Sleeping Beauty, gene transfer, Molecular engineering

1. Introduction

1.1. Transposable elements (TEs) in the genome: a brief history from their discovery to their biotechnological use in gene transfer

TEs, also described as "jumping genes," were first discovered in maize by Barbara McClintock in the 1940s. TEs are discrete pieces of DNA that are able to move from one site to another



© 2016 The Author(s). Licensee InTech. This chapter is distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. within one genome. This new concept, which suggested that the genome was not a final design but was rather able to evolve, to rearrange, was first met with criticism. However, a large body of evidence has accumulated over the last 60 years not only on the categorization and classification of TEs [1] but also on the understanding of their mechanisms. The ability to accurately identify and classify these sequences is critical to understand their impact on host genomes. Pioneers such as Finnegan [2] classified TEs into two classes based on their mechanism of transposition (**Figure 1**). Class I elements transpose by reverse transcription using an RNA intermediate: they are named retrotransposons. Three kinds of enzyme, RNA polymerase, reverse transcriptase, and integrase, are used for transposition. Class II elements directly transpose from DNA to DNA: they are named DNA transposons and just one enzyme, the transposase, is needed.



Figure 1. Classes I and II transposable elements (TEs, in green). Class I transposon or RNA transposon: three enzymes are necessary to transpose (1: RNA polymerase, 2: reverse transcriptase and 3: integrase). This mechanism is called "copy-and-paste" and gives rise to two identical copies ; one in the donor site and one in the target site. Class II transposon or DNA transposon: only one enzyme, the transposase, catalyzes the excision and the integration processes. The mechanism is named "cut-and-paste" and translocates the TE element in the target site leaving a free TE donor site. Inverted terminal repeats (ITRs) are drawned in red.

Piégu et al. [1] clearly detailed the necessity to update this classification. TEs are widely distributed in prokaryotic and eukaryotic genomes and represent a variable fraction accounting for 8% in chicken to 85% in maize. After an initial phase of sudden episodic bursts, the invasion step, TEs proliferate and accumulate mutations. Finally, transposition is tolerated by the genome at a reduced rate. Some TE insertions contribute with new genes, exons, or

regulator regions. This has been called the exaptation [3] and domestication [4] processes. However, for a significant amount of time, TEs were primarily considered as "junk or selfish DNA" that played no significant role in genome evolution [5]. The modern-day view of TEs is that they can generate genomic instability and reconfigure gene expression networks in both germline and somatic cells. This comprehensive view came with significant advances in sequencing technologies and the development of bioinformatics tools. One of the most unexpected insights is that almost half of our DNA is derived from TEs and 75% of our genome is transcribed (ENCODE project [6]). Therefore, as an integral part of the genome, the dynamic presence of TEs will be a major force to naturally reshape genomes. Several researchers have found examples of concordant timing between bursts of transposition or massive extinction and speciation events. For example, Lynch et al. [7] noticed how transposons transformed the uterine regulatory landscape during the evolution of mammalian pregnancy and Britten [8] reviewed the importance of Alu inserts on brain growth. Thus, TEs are "spam" coming from the dark ages and nowadays a small proportion of retroelements (<0.05%) remains able to transpose in humans [9]. However, no evidence of DNA transposon families was found active in the human genome during the later phase of the primate Radiation, 37 million years ago [10]. The last active DNA transposons were from the hAT superfamily, the Tc1/mariner, and the piggyBac families. This suggests that three sources of transposase were silenced at the same evolutionary period. As previously discussed, although transposons have been silenced, it does not mean that they are dead sequences for the genome and they constitute new regulatory networks.

Thus, DNA TEs present distinguishing features, making them attractive as gene transfer tools. Indeed, they are not infectious, as they are able to mobilize DNA in a single genome and are ubiquitous. From the natural architecture of DNA transposons, a secure and easy system has been designed (**Figure 2**).



Figure 2. From natural transposon to engineered pseudo-transposon. a) In the natural transposon, the transposase ORF (green rectangle) is delineated by the two ITRs (red arrows). b) In engineered pseudo-transposon, the transposase ORF is replaced by the cassette of the gene of interest. Transposase should therefore be delivered in parallel either in DNA, mRNA or protein form.

Briefly, the transposon is naturally delineated by two inverted terminal repeats (ITRs) framing the unique transposase open reading frame (ORF). The transposase recognizes the ITRs and catalyzes the excision and integration processes (**Figure 1**). After engineering, the transposase ORF is replaced by the gene of interest cassette and the enzyme is brought independently (**Figure 2**). The transposase is then able to integrate any gene of interest, without crossmobilization between transposon families, as the ITR sequences are highly specific for each transposase. From this global conception of the transposon tool, numerous technological aspects have been explored, finally resulting in an attractive gene integrative system to modify the human genome.

2. Transposon-based strategies

Various transposon-based strategies are available to obtain efficient transgene integration while maintaining safety and cell integrity. First, it depends on the transposase used to govern the efficacy of the integration process. Second, it depends on the way the transposase and the transgene would be delivered. Some use only one plasmid carrying the transposase expression cassette and the transgene construct. Other strategies rely on using one helper molecule carrying the transposase under gene, mRNA, or protein form and one donor plasmid that brings the gene of interest delineated by two ITRs.

2.1. Different types of transposase

For genome engineering, two strategies have been developed: find a transposase in any other species that works in humans or create a new one considering that nowadays no DNA transposons are found active in mammalian genomes. After the identification of efficient transposases for gene correction, their activities have been dissected and optimized.

2.1.1. The three musketeers

For decades, three main transposases have been developed with the aim of gene correction: Sleeping Beauty (SB), piggyBac (PB) and Tol2. In 1997, the SB transposase was artificially reconstructed from partial ancestral copies of a transposase gene identified in salmonid *Salmo* sp. [11]. The Tol2 and piggyBac transposases have been found to be active in their natural host. The piggyBac transposase was isolated from the cabbage looper moth *Trichoplusia ni*, and the developed tool is active in human and mice cells [12]. Tol2 was isolated from the Japanese medaka fish *Oryzias latipes* [13]. It is active in vertebrate cells including zebrafish, chicken, mouse and human.

Following their discovery, various optimizations were carried out to increase their transposition efficiency. The development of the SB100x transposase [14], characterized by a 100-fold greater efficacy than the natural SB, stands as an important step of transposase optimization. Comparatively, in 2011, a hyperactive piggyBac transposase was found with 17- and 9-fold increases in excision and integration, respectively [15], and a codon-optimized PB (mPB) was also developed [16]. Following this, the efficacy of this hyperactive PB (hyPb or 7PB) was compared to SB100x by luciferase *in vivo* expression. Mice injected with m7pB had 10 times

greater luciferase expression than those injected with SB100x [17]. Currently, no optimization studies have been carried out On the Tol2 enzyme since it is higly sensitive to molecular engineering [1].

2.1.2. Transposases confer specific properties to the system

Naturally, each transposase governs the integration of the pseudo-transposon using their own target site. The integration site for the SB transposon is TA, whereas it is TTAA for the PB transposon and 8-bp target duplication for the Tol2 transposon. After integration, these target sites are duplicates on either side of the newly integrated pseudo-transposon. Besides this specific transposition signature, the SB, PB, and Tol2 transposases confer specific properties to the system, such as cargo size capacity, overproduction inhibition (OPI), and reversibility with or without footprint.

2.1.2.1. Cargo size capacity

The distance between ITRs delineates the cassette transgene and defines the cargo size capacity. The more this distance is important, the less the transposase is efficient for excision and integration. However, the constant optimization of the enzymes improved considerably the efficacy of the system.

For now, the SB transposase initially allowed the transposition of only 10-kb transposon [18]. Beyond this size, the transposition rate is abolished. In 2014, Turchiano et al. [19] suggested to change its configuration, permitting the use of SB transposon until 18 kb but with a reduced efficiency. To date, the PB transposon offers the higher cargo size capacity with a natural high activity with 14.3-kb transgenes [12]. The hyPB transposase allows transposition of transgenes up to 100 kb in mouse ES cells [20]. In contrast, Tol2 does not show decrease of transposition efficacy until 10-kb transposon [21], and its activity has been proven until 66 kb [22]. However, few studies have directly compared the transposition efficacy of the transposases in an identical system [23].

Raising cargo size capacity opens new perspectives in gene correction. For example, in muscular dystrophy, disease is induced by the dystrophin mutation. Adding the full-length cDNA of the dystrophin, 11-kb length, has been proven complicated using viral gene transfer. Recently, the full-length dystrophin cDNA has been successfully integrated in mesangioblasts from a dystrophic dog model using the PB transposon tool [24].

2.1.2.2. Overproduction inhibition

As previously discussed, the transposase is brought independently to the pseudo-transposon, and the ratio between the enzyme and the pseudo-transposon turns out to be important to establish. On the one hand, transposases act by creating double-stranded breaks so the amount of transposase used must be the lowest possible to avoid genotoxicity. On the other hand, it is necessary to have enough transposase for having high transposition rate. Unexpectedly, increasing the amount of transposase does not result in more transposition activity. Indeed, even if at low level the transposition rate increases with the amount of transposase until a

maximum value, it is abolished above. This phenomenon is called OPI and depends on the studied model and the type of transposase [25]. In other cases, the transposition rate is saturated, without decrease, and a plateau is observed. The OPI has been well documented for a long time concerning the SB transposase [26]. However, concerning the PB and Tol2 transposases, the OPI is not as clear. For example, the PB transposase showed an OPI phenomenon in HeLa cells [16], but a stabilization of the activity was demonstrated in HEK293 [27] or mouse ES cells [28]. Similarly, for its Tol2 transposase, OPI or stabilization has been observed [16,21]. The molecular mechanism of this phenomenon is not still clearly established. Numerous hypotheses have been subjected and reviewed in Ref. [25].

2.1.2.3. Integration is reversible

In some conditions, the desired integration needs to be reversed. The transposase could then been readded with the aim of excising the pseudo-transposon from its chromosomal location. The excision of SB pseudo-transposons drives a footprint signature creating a 5-bp insertion [29]. Tol2 transposase excisions have been less investigated, but they could leave a short insertion or deletion [30]. In contrast, PB transposases have the particularity to carry out this excision without leaving a footprint in the genomic sequence. This property has been extensively exploited in induced pluripotent stem cells (iPSC) generation [31–33]. For more security, it is possible to use an engineered PB transposase in which the integration efficacy is abolished while conserving its excision property [34].

2.2. Design of the coupled pseudo-transposon/transposase architecture

Besides the intrinsic particularities of the transposases, the cellular delivery system is crucial. In a first system, called "cis" configuration, only one plasmid carries both the transposase and the gene of interest. The second way, termed "trans" configuration, is based on the principle of separately bringing the gene of interest on one plasmid, "donor" plasmid, and the transposase under a "helper" plasmid or mRNA or protein form.

2.2.1. "Cis" versus "trans" configurations

In the cis configuration, only one plasmid needs to be prepared. This confers easier manipulation and high efficacy, but three drawbacks need to be overcome. First, the pseudo-transposon/transposase ratio is fixed, conferring less flexibility to the system. Second, the plasmid backbone could be integrated as well as, third, the transposase gene. Even if the pseudotransposon/transposase ratio is fixed, working on promoters has brought flexibility. Indeed, Mikkelsen et al. [35] compared the efficiency of their helper-independent SB vector depending on 11 different promoters used for driving the transposase gene and they observed the OPI phenomenon with the strongest promoter.

In the "trans" configuration, two molecules are used, one carrying the gene of interest and one bringing the transposase either in DNA, RNA, or protein forms. The trans configuration offers naturally more flexibility than the cis one. On the one hand, this approach gives the advantage to modulate the molecular ratio between the transposase and the pseudo-transposon. On the

other hand, this approach gives the possibility to introduce several independent pseudotransposons [36] in their inducible systems. Only one constraint has been detailed: transposases are able to catalyze integration more efficiently with a circular donor plasmid than with a linear one [37].

Type of system	System architecture	Risk of transposase integration	Reference
Cis delivery: one plasmid containing the transposase and the transposon	Conventional cis architecture prom Tnpase p(A) ITR 5' prom GOI p(A) ITR 3'	High	[47,48]
	Promoter of the transposase is between the ITRs p(A) Tnpase ITR 3' prom prom GOI p(A) ITR 5'	Low and inefficient transposase	[46]
	Transposase and pseudo-transposon share the same polyadenylation signal prom Tnpase ITR 5' prom GOI p(A) ITR 3'	Low and inefficient transposase	[44,49]
Trans delivery: two separate molecules	Conventional trans architecture: two separate plasmids ITR 5' prom GOI p(A) ITR 3' + prom Tnpase p(A)	Low	[48,50]
	Pseudo-transposon + transposase as mRNA	Not	[51–54]
	Pseudo-transposon + transposase as protein	Not	[55–57]

Table 1. Different configurations to deliver transposase and pseudo-transposon and their consequences. Transposase molecules are in green whatever is the molecule type. Pseudo-transposon molecule is drawned in blue. GOI, gene of interest; p(A), polyadenylation signal; Prom, promoter; Tnpase, transposase; ITR, Inverted terminal repeats.

2.2.2. Risks and solutions associated to each strategy

2.2.2.1. Risk of linearized backbone integration

After excision of the gene of interest, the backbone thereby linearized is more prone to be integrated by a nontransposition process [38], whatever the cis or trans configuration used. This undesired integration exposes the problem of the presence of bacterial sequence such as resistance gene or bacterial replication origin. This has been correlated with the amount of transfected transposase [38] and with the size of the transgene [39]. To avoid this, Wilson's team suggested to use a suicide gene in the plasmid backbone, [40] or to select cells expressing green fluorescent protein (GFP) present in the backbone donor plasmid [38]. Other authors

suggested using DNA minicircles [41]. Interestingly, they also observed an increased efficacy with DNA minicircles compared to standard plasmid for the same transgene size in several cell lines. However, keeping only the pseudo-transposon as linearized donor plasmid showed no efficacy with SB transposase [42] and a low one with the PB transposase [37].

2.2.2.2. Risk of transposase gene integration

The presence of the transposase gene within the plasmid generates risk of its own integration and *per se* a risk of sustained transposase expression. The consequence could be saltatory remobilization of the integrated transgene [43]. To limit the effect of sustained transposase expression, a self-inactivated transposase gene has been obtained by including either the promoter [44,45] or the polyadenylation signal [46] between the ITRs (**Table 1**). Indeed, in primary human T cells, authors identified an active SB transposase ORF only in one clone out of 94, but a bulk analysis showed up to 0.047 transposase copy integrated per cell [50]. This still has not been evaluated for the PB and Tol2 transposases. Nevertheless, it is possible to completely abolish its integration by introducing transposase under mRNA or protein form (**Table 1**) [51]. mRNA or protein forms allow a one-shot transposition process, thanks to the time-restricted transposase expression.

For example, mRNA transposase expression peaked at 18 h after transfection [58]. Galla et al. [52] demonstrated less cell mortality with the mRNA transposase than an integrative form. Bire et al. [51] showed that the mRNA transposase gave less double-stranded break formation and less copy transgene integration. Moreover, no integrations of the transposase mRNA have been highlighted [51]. These considerations have been confirmed *in vivo* [53], as detailed in the end of this chapter.

Using the protein transposase offers also a short window of expression. Cai et al. [55] recently used the transposase protein associated with viral polyprotein. They observed a high number of transgene expressing cells, with a few number of integrated transgene copies per genome. Aiming to limit viral particle uses, recombinant transposase protein was fused with the cell penetrating peptide (CPP) [56] or transposase was delivered with a free CPP [57]. For now, no *in vivo* evaluations have been found in the bibliographic database.

3. Editing the genome: the final step after a long journey through the cell

Genome editing includes all methods aimed to modify the genome by introducing new DNA sequences or by correcting existing genomic sequences. The journey begins with the ability to enter into the cell, evade the immune response, and, after crossing the nuclear barrier, integrate the gene of interest into the DNA genome.

3.1. Cross the cellular membrane and escape immune response

As free DNA delivery did not show efficient results, both transposase and pseudo-transposon need to be driven into the cell using different gene delivery strategies, either using a carrier

(viral particles or chemical agent) or using a physical method. According to the method selected, it is important to consider all parameters of cellular defense against the entry of the foreign DNA.

3.1.1. Viral hybrid systems

The viral-transposon hybrid systems take advantage of the natural properties of viral proteins to enter into the cell. For example, as early as 2006, a hybrid HSV amplicon-SB transposase vector was used in a central nervous system development study [59]. Since that time, several studies have been developed on hybrid transposase systems (reviewed in Refs. [60,61]) that use adenovirus [62–64], adeno-associated virus [65], baculovirus [66], or nonintegrative lentivirus [67,68] particles.

3.1.2. Chemical agents

Chemical agents have been developed with the aim of condensating DNA and thereby avoiding any viral derived systems. However, it turns out to be more controversial than expected with respect to the immune escape [69]. Indeed, these nanovehicles enter into the cell essentially *via* the endosomal pathway [70,71] and therefore expose foreign DNA to the endosomal Toll-like receptors. Among all available chemical carriers, the polyethylenimine (PEI) polymers appear to be the most used in transposon systems. Indeed, the PEI improve endosomal escape through the "proton sponge" mechanism. For example, in 2009, Kang et al. [72] used the PB transposase-based system with the PEI as a transfection reagent for ovarian cancer treatment in a mouse model. Further examples have been realized both *in vitro* [73] and *in vivo* [36,74].

3.1.3. Physical gene transfer

Finally, plasmid DNA could be driven by physical methods. In this case, the plasmid traffic does not go through the endosome and thereby escapes Toll receptors. One such method, electroporation, turned out to be highly efficient to transfect otherwise hard to transfect cells such as dendritic cells and human hematopoietic or embryonic cells [75–77]. Depending on the cell type used, the results may be controversial. Ley et al. [73] compared transposition efficiency in PEI-transfected versus electroporated mesoangioblasts and were not able to obtain efficient long-term expression in muscle after *in vivo* electroporation.

Other physicals methods have therefore been developed. For example, ultrasound targeted microbubble destruction (UTMD) results in pore formation on the cell membrane after ultrasonic waves application. Recently, two *in vivo* studies have been carried out with clinical perspectives [78,79]. In parallel to UTMD, the hydrodynamic (HD) injection has been applied to transfer the clotting factor VIII [80]. However, they are proinflammatory consequences inducing a lack of transgene expression. To circumvent this drawback, Doherty et al. [81] suggested to induce transient transgene repression, thereby preventing the priming of transgene-specific T cells.

3.2. Cross the nuclear barrier and transgene integration

3.2.1. The transposase is driven to the nucleus

For an efficient transposition, the transposase needs to be localized into the nucleus at the same time as the pseudo-transposon DNA.

The transposases contain a nuclear localization signal, driving them to the nucleus [82]. An engineered PB transposase have been developed for increasing its localization within the nucleoli by adding a nucleolus-predominant (NP) signal peptide from HIV-1 TAT protein [83]. With this NP-mPB, a three- to fourfold increase in PB transposition rate, in both murine and human cells, was observed.

From the pseudo-transposon point of view, its nuclear targeting is also essential. Thus, DNA nuclear targeting sequences (DTS) might be added to the plasmid backbone. These DTS consist, for example, to a 72-bp sequence from the SV40 enhancer and act as a sequence driver [84].

3.2.2. Integration profile of the gene of interest

All transposon systems have less integration bias than viruses, as previously described [85–88]. However, it is important to note that there are some differences within transposon systems [89]. The SB transposase is known to allow the more random integration [90], with approximately 35% into RefSeq sequences. It has been notified that the SB transposition has an affinity for the heterochromatin topology [91]. In contrast, the Tol2 and PB transposases are not considered to allow random integration. Indeed, the PB transposase shows a bias towards integration of the transgene into CpG islands and transcriptional start site, with approximately 49% into RefSeq sequences [16,27], and the Tol2 transposase presents a strong bias for the intergenic regions [92].

Interestingly, this global integration profile could be affected by various parameters, such as the transposase variant [93] or the cell type [94].

In addition, it is important to note that, for now, studies have been essentially established in *in vitro* models and no predictions could be drawn regarding the *in vivo* integration profile. Indeed, after *in vivo* UTMD transfection, the pseudo-transposon showed a significant bias of transgene integration into chromosome 14 [49], but no bias was observed in their *in vitro* control.

4. Side effect of the transgene integration system

The newly integrated foreign DNA is considered as an invader by the cell. This leads to postintegrative transgene silencing. Conversly, the transgene copy might also influence surrounding sequences according to the integration site. To conter these mutual side-effects numerous strategies have been developed.

4.1. Communication mechanisms between the transgene and the genome

During their evolution, transposons have been made extinct by at least chromatin condensation and by RNA interference (RNAi) induction.

The transcriptional regulation includes DNA CpG methylation and histone modifications. It has been confirmed that the transgene expression could be restored by a demethylating agent such as 5-aza-2'-deoxycytidine or by a histone deacetylase inhibitor such as trichostatin A [95]. However, it is easier to avoid the induction of upstream gene silencing. To this end, working with a methylated pseudo-transposon plasmid unlike an unmethylated one showed more transposition rate with the SB transposase [96]. Curiously, when the SB, PB, and Tol2 transposase systems are directly compared, the integrated transgene is less silenced if integrated by the PB transposase [97].

The role of RNAi in posttranscriptional silencing of exogenous DNA transposons remains unclear. One study demonstrated that, in the absence of an efficient cellular RNAi system, by establishing p19 protein knockdown cells, the number of colonies is increased [98]. Nonetheless, the mechanism is still not elucidated.

Besides the host-to-transgene effect, a transgene-to-host effect, driving perturbations in sequences surrounding the transgene by DNA methylation modulation, has been highlighted [99]. A further study investigated the expression levels of host genes neighboring the SB transposon and underlined variations depending on the chromosomal location of the transgene [100]. Therefore, solutions allowing a complete isolation of the transgene should be developed.

4.2. Overcoming the host regulation for a sustained expression

In gene correction, maintaining the expression level of the transgene and limiting host genome perturbations are crucial for having an efficient therapeutic effect.

4.2.1. Matrix attachment region (MAR)

The human MAR elements are natural elements of the eukaryotic genome, which mediate the structural organization of the chromatin domains. When included in a transposon plasmid, they do not affect the number of transposed transgene copies but rather increase the transgene expression per integrated copy [101]. Moreover, when the MAR element is included in the transposase vector, an increased transposition efficacy has been observed [102].

4.2.2. Insulators

Insulators are short DNA sequences naturally present in the genome and act as genetic boundary elements. In a recent study, four different insulators (cHS4, D4Z4, CTCF, and CTF/ NF1) were compared and showed that D4Z4 and CTF/NF1 had insulator functions when combined with transposition [51]. The protective effect of the cHS4 insulator has been demonstrated by a strong diminution of the activation of a nearby promoter [103] and by a prolonged fluorescent marker expression [104,105]. Some equivalent studies corroborated this

role in clinically relevant cells as well as primary hematopoietic CD34⁺ cells [106]. Moreover, cHS4 insulators abolished the RNAi pathway effects regulating transposon-derived transgene expression by epigenetic silencing [98]. Nevertheless, for an optimal boundarie effect of insulators, it is necessary to consider the model used. Indeed, the size of the pseudo-transposon increased by the insulator or steric hindrance of transposase action [103] could also influence the transgene expression.

5. Going further

For many years, researchers have provided elements for a better understanding of their mechanism and have given solutions for the optimal use of these systems. Here, we recall promising leads for further work in this area: targeting a specific site within the genome and targeting a specific tissue at the body scale.

5.1. Targeting a specific site within the genome

Replacing a defective gene or introducing a gene of interest into a completely safe, predetermined, specific genomic site is the ideal approach for gene correction. This potential locus could be defined by numerous criteria determined by its position from gene, miRNA, transcription unit, or ultraconserved region. All of these aspects have been recently reviewed [107].

5.1.1. Transposon targeting strategies

The SB, PB, and Tol2 transposases have short integration target sites: TA, TTAA, and 8-bp sequences, respectively. Thus, transposon-derived systems should be optimized by combining the transposase to a system able to target a specific DNA sequence, such as a DNA-binding domain (DBD). The first strategy uses a fusion protein containing both the transposase and a DBD. In the second method, a fusion protein is constructed between a DBD and a protein, which is able to specifically recruit the transposase. To date, only one protein is known to be able to interact with the SB transposase, which is named N-57 [108]. Finally, another solution is based on a fusion protein between two DBD, one recognizing a genomic sequence and one specific to a sequence inserted within the pseudo-transposon plasmid. Few parameters of this third approach have been explored in a mammalian model [108]. Considerations of these three strategies have been recently reviewed [109], and we herein detail only chimeric transposases.

The proof-of-concept has been demonstrated by studying intraplasmic integration using the PB transposase fused to the Gal4 domain [110]. However, the system revealed to be more restrictive than expected both in the conservation of the transposition activity and the ability to restrict integration in the targeted locus. Therefore, the transposition activity might be affected by the DBD fusion. Indeed, the DBD Gal4 (a zinc finger domain, ZF) has been tested in fusion to the Tol2, SB11, and PB transposases. The number of chromosomal integrations of the transposon is abolished with Gal4-Tol2 and Gal4-SB11, but no loss of efficiency was observed for the Gal4-PB transposase [111]. Some studies have been carry out to analyze the parameters of this loss of activity, such as the sequence surrounding the targeted site [108], the

orientation of the fusion [112], or the choice of the linker [113]. The DBD type has also been evaluated in their ability to avoid off-target integration. With the Gal4-PB transposase, transposition occurred at 23% within 0.8 kb of Gal4 site compared to 5% for the native transposase [114]. However, for improvement of the targeting, artificial ZFs have been created by assembling six ZF domains to create a polydactyl protein capable of targeting a unique sequence of 18 bp [115]. For example, the sequence targeting with these artificial ZF allowed 44.3% of integration events near the CHK2-ZF site [116]. Comparatively, when the Sp1 ZF is fused with the PB transposase, which preferentially binds the CG-rich motif, the integration increased near the CpG islands (25.7% versus 10.5% with the native PB transposase) but without modification regarding the integration into the RefSeq genes [117].

5.1.2. Other systems allowing a targeting integration

In 2011, the discovery of the CRISPR/Cas9 system revolutionized the gene transfer because of its ability to drive the transgene in its physiological site, but no studies directly compared the efficiency of both transposon and CRISPR/Cas9-based systems. It has been supposed that this system arises from casposon in the evolutionary tree. Casposons are mobile cryptic sequences present in Achaea and bacteria, and two independent studies described this superfamily of mobile elements by linking transposon and CRISPR/Cas systems [118,119].

Recently, a combinatory approach was developed, in which the correction is realized gene by gene (CRISPR/Cas9 role) and temporarily needed sequences are removed from the genome (transposase role). This method has been applied for gene correction of β -thalassemia [120] and to create iPSC with deletion into the CCR5 gene [121].

5.2. Targeting a specific tissue at the organism scale

For *in vivo* application of gene correction, it is important to express the transgene of interest only in the organ, tissue, or cell types in which the transgene expression is required. The design of the transgene vector is essential and might contain specific elements such as tissue-specific promoter or regulatory sequences. The second option is to deliver the system only in the specific cells.

5.2.1. Design of the transgene vector for in vivo applications

In the ideal gene transfer, the transgene is expressed in the same conditions, as it is in physiological conditions. Indeed, overexpression of the transgene or expression in a nontarget cell could improve cytotoxicity, induce its clearance by the immune system, and increase its gene silencing (reviewed in Ref. [122]). With this aim, vectors have been designed in such a way as promoters or regulatory sequences are chosen for restricting the expression of the gene of interest only in the cells of interest. Tissue-specific promoters control gene expression in a tissue-dependent manner or according to the development stage of the cells. In plasmid design, several approaches are available such as using a promoter regulating an endogenous gene expressed in one type of cell (minimal promoter) or combining numerous enhancers to a minimal promoter. In the first case, the transposon is under a native promoter. For example, endothelin-1 [123] allows a decreased GFP expression in a nonendothelial cell line while maintaining the expression level in endothelial cell lines. When the targeted cell type is the final point of a differentiation lineage, it seems essential to have the expression of the therapeutic protein only in the differentiated state, such as promoters capable of restricting β -globin expression in differentiated erythroid cells from transfected proerythroid cells [124]. In cancer therapy, a study based on the SB transposition showed that the HSV-TK transgene driven by a telomerase reverse transcriptase promoter increased death rate in cancer cell lines compared to fibroblast cell lines [125].

The second approach is based on constructions containing a minimal promoter with specific enhancers. For example, the SB transposon system has been used for the introduction of the telomerase gene driven by a combination of the transthyretin (TTR) gene promoter/enhancer, the human alcohol dehydrogenase gene promoter, and the SV40 enhancer [126]. The authors observed an induced transcriptional activity only in hepatocytes. In an *in vivo* study, the authors developed a TTR minimal promoter coupled to a hepatocyte-specific cis-regulatory module, driving the clotting factor IX for correction of hemophilia B [127]. This promoter has also been combined with a PB transposon-mediated gene transfer and confirmed the high efficiency of the transgene construct [128].

5.2.2. Limiting the ectopic integrations by tissue targeting

For improvement of tissue targeting, two major routes have been developed, either administration of *ex vivo* premodified cells of interest or direct delivery of the integrative system, containing the transgene, to the whole organism.

5.2.2.1. Administration route for ex vivo modified cells

The delivery of premodified cells to a patient was extensively carried out in adoptive cell transfer of immune cells expressing an artificial T-cell receptor (TCR) designed to target an antigen. Briefly, T cells are removed from a patient and transformed to express the artificial TCR (also named chimeric antigen receptor or CAR). After amplification, modified T cells are intravenously readministrated to the organism. In the field of transposon technology, this approach has been used in several applications. For example, a human epidermal growth factor receptor 2-specific CAR was introduced into cytotoxic T cells, thanks to the PB transposase [129]. More recently, T lymphocytes were modified to express the CD19-CAR transgene, and after 7 days of coculture, CAR T cells eradicated all CD19⁺ tumor cells *in vitro* [130]. In lower proportions, the Tol2 transposase has also been used for the integration of a CD19-CAR into T cells [131]. However, production of CD19-CAR T cells usually uses SB transposase and clinical trials are currently under investigation [132]. The authors detailed their protocol for manufacturing clinical-grade CD19-specific T cells [76].

It is also possible to reimplant modified cells *in situ* after their encapsulation. In this aim, Fjord-Larsen et al. [133] developed a model in which a new clinical-grade cell line expresses a high level of neural growth factor after striatum implantation.

The administration of already modified cells increases the security of the transfer system. However, applications are, for now, restricted to cells easy to collect and reimplant to a patient. For less accessible tissue or organs, targeting methods are more often driven by a direct administration of the transgene.

5.2.2.2. Administration route for transposon DNA system

The administration of the therapeutic gene, associated with the transposase, needs a delivery method able to drive them into the organ or tissue of interest. To this end, two strategies have been developed. The first one takes advantages of specific administration route properties, whereas the second one uses vehicles expressing receptors capable of specific recognition of the targeting tissue.

It has been demonstrated that all gene delivery methods do not present an equal distribution in the different organs. For example, the HD injection is known to target the liver at 95%, as detailed by Bell et al. [134]. In agreement, Herweijer and Wolff [135] showed that transgene expression was also found in others organs such as the heart, spleen, and kidneys at levels approximately 100-fold lower than in the liver. This liver targeting way has been applied in gene correction, and in 2007, Aronovich et al. showed a model of correction of mucopolysaccharidosis mice by SB-mediated transgene α -L-iduridase (IDUA) transposition [136]. They mentioned a persistent expression of IDUA in plasma for almost 10 weeks after injection. In cancer therapy, liver metastasis of colorectal cancer was reduced after antiangiogenic genes were integrated by the SB transposase [137].

As a complement, the DNA transposon could also been administrated after complexation to a targeting vehicle. After an intravenous administration, Kren et al. [47] highlighted a hepatocyte-specific integration of the transgene when condensated with coated nanocapsules. Comparatively, the transgene complexed to the PEI showed an expression in the lung, not observed after HD injection [138]. More specifically, within the lung, the polyplexes are addressed into pneumocytes and no transgene expression was detected within the conducting airways [139].

Coupling specific administration route and nanocapsules is the future way. In this aim, the UTMD gene delivery method allows mediating the site-specific delivery of transposons. Briefly, the transgene is intravenously injected and cell penetration occurs at the targeted organ by acoustic cavitation [49]. This approach has been used for the transposition of the Nkx2.2 transcriptional factor to the pancreas by the PB system [78] or for the transposition of the thymosine β 4 gene, or the glucagon-like peptide-1 one, to the heart [79,140].

In gene correction, targeting the tissue of interest is essential for reflecting physiological conditions. Compared to viral transduction, the transposon systems are more customizable and numerous possibilities are available for users. Depending on the tissue to target, it is possible to play at the same time on the promoter, the administration route, and the presence of targeting molecules.

6. Therapy applications of transposase tools

Some technological aspects previously discussed offer a suitable transposon toolbox to gene correction. Transposon-based systems allow first the transgene integration in a large range of clinically relevant target cells, including hematopoietic stem cells [141], mesenchymal stromal cells [142], iPSC [143], and lymphoid T cells [131]. Transposon-mediated correction could therefore be used in a large-scale application, such as treatment of inherited disorders, cancer, and tissue degeneration (**Table 2**).

Disease	Transgene	Tnpase	Animal model or	Reference
			cell type	
Inherited disorders				
Hemophilia A	FVIII	SB/PB	Hemophilic A mice	[47,80,144–146]
Hemophilia B	FIX	SB/PB	Hemophilic B mice or dogs	[42,62,128,147]
Huntington's disease	siRNA-htt	SB	Human cell lines	[148]
Duchene muscular dystrophy	Dystrophin	РВ	Dog dystrophic mesoangioblast	[24]
Tyrosinemia type I	Fah	SB	FAH-deficient mice	[149–151]
Sickle cell disease	HO-1 or IHK	SB	Mice	[152,153]
Mucopolysaccharidosis type I (MPS I)	hIDUA or hGUSB	SB	MPS I NOD/SCID mice	[136,154,155]
α -Antitrypsin deficiency	hAAT	PB	Mice and iPSC	[93,156]
Fanconi anemia type C	FA-C	SB	Human lymphoblastoid cells	[157]
Crigler-Najjar syndrome type 1	hUGT1A1	SB	Hepa1 cell line and gunn rate	5 [158]
Junctional epidermolysis bullosa (JEB)	LAMB3	SB	Epidermal holoclones from JEB patients	[159]
Vaccination				
Immunization against non-self protein	eGFP	РВ	Mice	[160]
Regenerative medicine				
iPSC generation	SOX2, OCT4, KLF4, and <i>c-</i> MYC	РВ	Fibroblasts, melanoma cells, HDDPC	[32,143,161–164]
Diabetes	Nkx2.2 or insulin	PB/SB	STZ-rat pancreas	[78,165]
Retinal degeneration	PEDF	SB	IPE and RPE cells	[166]
Acute myocardial infraction	TB4 or GLP1	PB	Rat heart	[79,140]
Cancerology				

Disease	Transgene	Tnpase	Animal model or	Reference
			cell type	
Angiogenesis-dependent tumors	sFlt-1 or statin-AE	SB	Tumor engrafts in mice	[66,137,167]
Cervical cancer	HSV-tk	РВ	Cervical cancer xenografts	[72]
Ovarian adenocarcinoma	HSV-tk	РВ	Cell line	[168]
Melanoma	TRAIL and IFN γ	РВ	ADSC	[169]
Adoptive T-cell therapy	CD19-CAR, HER2-CAR, or IL-11-CAR	SB, PB or Tol2	Human T cells and clinical trials	[40,77,129,130,132, 170–179]
Pulmonary and other diseases				
Pulmonary fibrosis	miR-29 or hIDO	SB	Bleomycin-induced pulmonary fibrosis mice or rats	[180,181]
Pulmonary hypertension	eNOS	SB	Monocrotaline-induced pulmonary hypertension rats	[182]
Acute cellular injury	hTERT	SB	Primary hepatocytes	[126]
Unilateral ureteral obstruction	IGF-1R	PB	Mice	[183]

Tnpase, transposase ; ADSC, adipose-derived mesenchymal stem cells; eGFP, enhanced GFP; eNOS, endothelial nitric oxide synthase; FA-C, Fanconi anemia complementation group C; Fah, fumaryl-acetoacetate hydrolase; FVIII, clotting factor, factor VIII; hAAT, human α 1-antitrypsin; HDDPC, primary human deciduous tooth dental pulp cells; HER2, human epidermal growth factor receptor 2; hGUSB, β -glucoronidase; hIDO, human indoleamine-2,3-dioxygenase; hIDUA, human α -L-iduronidase; HO-1, heme oxygenase-1; HSV-tk, herpes simplex virus thymidine kinase; hTERT, human telomerase reverse transcriptase; htt, huntingtin; hUGT1A1, human uridine diphosphoglucuronate glucuronosyltransferase 1A1; IFN γ , interferon γ ; IGF-1R, insulin-like growth factor-1 receptor; IHK, antisickling globin; IL-11, interleukin-11; IPE, iris epithelial cells; KLF4, Krüppel-like factor 4; LAMB3, laminin B3 subunit of laminin 5; Nkx2.2, NK-type homeodomain transcription factor; OCT4, octamer-binding transcription factor 4; PEDF, pigment epithelial cells; sFlt-1, soluble fms-like tyrosine kinase-1; SOX2, SRY (sex-determining region Y) box 2; statin-AE, angiostatin-endostatin fusion gene; STZ, streptozotocin; TB4, thymosin β 4; Tnpase, transposase; TRAIL, TNF-related apoptosis-inducing ligand.

Table 2. Application fields of transposon-based gene correction.

7. Conclusion

Transposons have naturally drawn genomes since the first forms of life. Scientists have taken advantage of their properties with the aim of constantly updating the safety of this nonviral tool for gene transfer. With the other integrative systems derived from casposons, such as CRISPR/Cas9, we dispose of complementary tools for reshaping the genome. Latest discoveries have open new horizons, but a long road is still ahead.

Acknowledgements

This work has been supported by La Ligue Contre le Cancer.

Author details

Colette Bastie and Florence Rouleux-Bonnin*

*Address all correspondence to: florence.bonnin@univ-tours.fr

LNOX GICC UMR CNRS 7292, Department of Medicine, Bâtiment Dutrochet, 10 boulevard Tonnellé, 37032 TOURS, France

References

- Piégu B, Bire S, Arensburger P, Bigot Y. A survey of transposable element classification systems—A call for a fundamental update to meet the challenge of their diversity and complexity. Mol Phylogenet Evol. 2015;86:90–109.
- [2] Finnegan DJ. Eukaryotic transposable elements and genome evolution. Trends Genet. 1989;5:103–7.
- [3] de Souza FSJ, Franchini LF, Rubinstein M. Exaptation of transposable elements into novel cis-regulatory elements: is the evidence always strong? Mol Biol Evol. 2013;30:1239–51.
- [4] Alzohairy AM, Gyulai G, Jansen RK, Bahieldin A. Transposable elements domesticated and neofunctionalized by eukaryotic genomes. Plasmid. 2013;69:1–15.
- [5] Doolittle WF, Sapienza C. Selfish genes, the phenotype paradigm and genome evolution. Nature. 1980;284:601–3.
- [6] Birney E, Stamatoyannopoulos JA, Dutta A, GuigóRR, Gingeras TR, Margulies EH, et al. Identification and analysis of functional elements in 1% of the human genome by the ENCODE pilot project. Nature. 2007;447:799–816.
- [7] Lynch VJ, Leclerc RD, May G, Wagner GP. Transposon-mediated rewiring of gene regulatory networks contributed to the evolution of pregnancy in mammals. Nat Genet. 2011;43:1154–9.

- [8] Britten RJ. Transposable element insertions have strongly affected human evolution. Proc Natl Acad Sci. 2010;107:19945–8.
- [9] Muotri AR, Marchetto MCN, Coufal NG, Gage FH. The necessary junk: new functions for transposable elements. Hum Mol Genet. 2007;16:R159–67.
- [10] Pace JKI, Feschotte C. The evolutionary history of human DNA transposons: evidence for intense activity in the primate lineage. Genome Res. 2007;17:422–32.
- [11] Ivics Z, Hackett PB, Plasterk RH, Izsvák Z. Molecular reconstruction of Sleeping Beauty, a Tc1-like transposon from fish, and its transposition in human cells. Cell. 1997;91:501– 10.
- [12] Ding S, Wu X, Li G, Han M, Zhuang Y, Xu T. Efficient transposition of the piggyBac (PB) transposon in mammalian cells and mice. Cell. 2005;122:473–83.
- [13] Kawakami K, Noda T. Transposition of the Tol2 element, an Ac-like element from the Japanese medaka fish *Oryzias latipes*, in mouse embryonic stem cells. Genetics. 2004;166:895–9.
- [14] Mátés L, Chuah MKL, Belay E, Jerchow B, Manoj N, Acosta-Sanchez A, et al. Molecular evolution of a novel hyperactive Sleeping Beauty transposase enables robust stable gene transfer in vertebrates. Nat Genet. 2009;41:753–61.
- [15] Yusa K, Zhou L, Li MA, Bradley A, Craig NL. A hyperactive piggyBac transposase for mammalian applications. Proc Natl Acad Sci. 2011;108:1531–6.
- [16] Grabundzija I, Irgang M, Mátés L, Belay E, Matrai J, Gogol-Döring A, et al. Comparative analysis of transposable element vector systems in human cells. Mol Ther. 2010;18:1200–9.
- [17] Doherty JE, Huye LE, Yusa K, Zhou L, Craig NL, Wilson MH. Hyperactive piggyBac gene transfer in human cells and *in vivo*. Hum Gene Ther. 2012;23:311–20.
- [18] Zayed H, Izsvák Z, Walisko O, Ivics Z. Development of hyperactive Sleeping Beauty transposon vectors by mutational analysis. Mol Ther. 2004;9:292–304.
- [19] Turchiano G, Latella MC, Gogol-Döring A, Cattoglio C, Mavilio F, Izsvák Z, et al. Genomic analysis of Sleeping Beauty transposon integration in human somatic cells. PLoS One. 2014;9:e112712.
- [20] Li MA, Turner DJ, Ning Z, Yusa K, Liang Q, Eckert S, et al. Mobilization of giant piggyBac transposons in the mouse genome. Nucleic Acids Res. 2011;39:e148.
- [21] Balciunas D, Wangensteen KJ, Wilber A, Bell J, Geurts A, Sivasubbu S, et al. Harnessing a high cargo-capacity transposon for genetic applications in vertebrates. PLoS Genet. 2006;2:e169.
- [22] Suster ML, Sumiyama K, Kawakami K. Transposon-mediated BAC transgenesis in zebrafish and mice. BMC Genomics. 2009;10:477.

- [23] Rostovskaya M, Fu J, Obst M, Baer I, Weidlich S, Wang H, et al. Transposon-mediated BAC transgenesis in human ES cells. Nucleic Acids Res. 2012;40:e150.
- [24] Loperfido M, Jarmin S, Dastidar S, Di Matteo M, Perini I, Moore M, et al. piggyBac transposons expressing full-length human dystrophin enable genetic correction of dystrophic mesoangioblasts. Nucleic Acids Res. 2015 Dec 17;44(2):744–60.
- [25] Bire S, Casteret S, Arnaoty A, Piégu B, Lecomte T, Bigot Y. Transposase concentration controls transposition activity: myth or reality? Gene. 2013;530:165–71.
- [26] Lohe AR, Hart DL. Autoregulation of mariner transposase activity by overproduction and dominant-negative complementation. Mol Biol Evol. 1996;13:549–55.
- [27] Wilson MH, Coates CJ, George AL. PiggyBac transposon-mediated gene transfer in human cells. Mol Ther. 2007;15:139–45.
- [28] Cadiñanos J, Bradley A. Generation of an inducible and optimized piggyBac transposon system. Nucleic Acids Res. 2007;35:e87.
- [29] Liu G, Aronovich EL, Cui Z, Whitley CB, Hackett PB. Excision of Sleeping Beauty transposons: parameters and applications to gene therapy. J Gene Med. 2004;6:574–83.
- [30] Huang P, Xu L, Liang W, Tam CI, Zhang Y, Qi F, et al. Genomic deletion induced by Tol2 transposon excision in zebrafish. Nucleic Acids Res. 2013;41:e36.
- [31] Igawa K, Kokubu C, Yusa K, Horie K, Yoshimura Y, Yamauchi K, et al. Removal of reprogramming transgenes improves the tissue reconstitution potential of keratinocytes generated from human induced pluripotent stem cells. Stem Cells Transl Med. 2014;3:992–1001.
- [32] Talluri TR, Kumar D, Glage S, Garrels W, Ivics Z, Debowski K, et al. Non-viral reprogramming of fibroblasts into induced pluripotent stem cells by Sleeping Beauty and piggyBac transposons. Biochem Biophys Res Commun. 2014;450:581–7.
- [33] Hu K. All roads lead to induced pluripotent stem cells: the technologies of iPSC generation. Stem Cells Dev. 2014;23:1285–300.
- [34] Li X, Burnight ER, Cooney AL, Malani N, Brady T, Sander JD, et al. piggyBac transposase tools for genome engineering. Proc Natl Acad Sci. 2013;110:E2279–87.
- [35] Mikkelsen JG, Yant SR, Meuse L, Huang Z, Xu H, Kay MA, et al. Helper-independent Sleeping Beauty transposon-transposase vectors for efficient nonviral gene delivery and persistent gene expression *in vivo*. Mol Ther. 2003;8:654–65.
- [36] Saridey SK, Liu L, Doherty JE, Kaja A, Galvan DL, Fletcher BS, et al. PiggyBac transposon-based inducible gene expression *in vivo* after somatic cell gene transfer. Mol Ther. 2009;17:2115–20.

- [37] Nakanishi H, Higuchi Y, Kawakami S, Yamashita F, Hashida M. Comparison of piggyBac transposition efficiency between linear and circular donor vectors in mammalian cells. J Biotechnol. 2011;154:205–8.
- [38] Saha S, Woodard LE, Charron EM, Welch RC, Rooney CM, Wilson MH. Evaluating the potential for undesired genomic effects of the piggyBac transposon system in human cells. Nucleic Acids Res. 2015;43:1770–82.
- [39] Nakazawa Y, Saha S, Galvan DL, Huye L, Rollins L, Rooney CM, et al. Evaluation of long-term transgene expression in piggyBac-modified human T lymphocytes. J Immunother. 2013;36:3–10.
- [40] Nakazawa Y, Huye LE, Dotti G, Foster AE, Vera JF, Manuri PR, et al. Optimization of the PiggyBac transposon system for the sustained genetic modification of human T lymphocytes. J Immunother. 2009;32:826–36.
- [41] Sharma N, Cai Y, Bak RO, Jakobsen MR, Schrøder LD, Mikkelsen JG. Efficient Sleeping Beauty DNA transposition from DNA minicircles. Mol Ther Nucleic Acids. 2013;2:e74.
- [42] Yant SR, Ehrhardt A, Mikkelsen JG, Meuse L, Pham T, Kay MA. Transposition from a gutless adeno-transposon vector stabilizes transgene expression *in vivo*. Nat Biotechnol. 2002;20:999–1005.
- [43] Li MA, Pettitt SJ, Eckert S, Ning Z, Rice S, Cadinanos J, et al. The piggyBac transposon displays local and distant reintegration preferences and can cause mutations at noncanonical integration sites. Mol Cell Biol. 2013;33:1317–30.
- [44] Urschitz J, Kawasumi M, Owens J, Morozumi K, Yamashiro H, Stoytchev I, et al. Helper-independent piggyBac plasmids for gene delivery approaches: strategies for avoiding potential genotoxic effects. Proc Natl Acad Sci. 2010;107:8117–22.
- [45] Urschitz J, Moisyadi S. Transpositional transgenesis with piggyBac. Mob Genet Elements. 2013;3:e25167.
- [46] Chakraborty S, Ji H, Chen J, Gersbach CA, Leong KW. Vector modifications to eliminate transposase expression following piggyBac-mediated transgenesis. Sci Rep. 2014;4:7403.
- [47] Kren BT, Unger GM, Sjeklocha L, Trossen AA, Korman V, Diethelm-Okita BM, et al. Nanocapsule-delivered Sleeping Beauty mediates therapeutic Factor VIII expression in liver sinusoidal endothelial cells of hemophilia A mice. J Clin Invest. 2009;119:2086– 99.
- [48] Huang X, Wilber AC, Bao L, Tuong D, Tolar J, Orchard PJ, et al. Stable gene transfer and expression in human primary T cells by the Sleeping Beauty transposon system. Blood. 2006;107:483–91.

- [49] Anderson CD, Urschitz J, Khemmani M, Owens JB, Moisyadi S, Shohet RV, et al. Ultrasound directs a transposase system for durable hepatic gene delivery in mice. Ultrasound Med Biol. 2013;39:2351–61.
- [50] Huang X, Haley K, Wong M, Guo H, Lu C, Wilber A, et al. Unexpectedly high copy number of random integration but low frequency of persistent expression of the Sleeping Beauty transposase following trans delivery in primary human T cells. Hum Gene Ther. 2010;14:1–14.
- [51] Bire S, Ley D, Casteret S, Mermod N, Bigot Y, Rouleux-Bonnin F. Optimization of the piggyBac transposon using mRNA and insulators: toward a more reliable gene delivery system. PLoS One. 2013;8:e82559.
- [52] Galla M, Schambach A, Falk CS, Maetzig T, Kuehle J, Lange K, et al. Avoiding cytotoxicity of transposases by dose-controlled mRNA delivery. Nucleic Acids Res. 2011;39:7147–60.
- [53] Bell JB, Aronovich EL, Schreifels JM, Beadnell TC, Hackett PB. Duration of expression and activity of Sleeping Beauty transposase in mouse liver following hydrodynamic DNA delivery. Mol Ther. 2010;18:1796–802.
- [54] Wilber A, Frandsen JL, Geurts JL, Largaespada DA, Hackett PB, McIvor RS. RNA as a source of transposase for Sleeping Beauty-mediated gene insertion and expression in somatic cells and tissues. Mol Ther. 2006;13:625–30.
- [55] Cai Y, Bak RO, Krogh LB, Staunstrup NH, Moldt B, Corydon TJ, et al. DNA transposition by protein transduction of the piggyBac transposase from lentiviral Gag precursors. Nucleic Acids Res. 2014;42:e28.
- [56] Lee C-Y, Li J-F, Liou J-S, Charng Y-C, Huang Y-W, Lee H-J. A gene delivery system for human cells mediated by both a cell-penetrating peptide and a piggyBac transposase. Biomaterials. 2011;32:6264–76.
- [57] Järver P, Fernaeus S, EL-Andaloussi S, Tjörnhammar M-L, Langel Ü. Co-transduction of Sleeping Beauty transposase and donor plasmid via a cell-penetrating peptide: a simple one step method. Int J Pept Res Ther. 2007;14:58–63.
- [58] Bire S, Gosset D, Jégot G, Midoux P, Pichon C, Rouleux-Bonnin F. Exogenous mRNA delivery and bioavailability in gene transfer mediated by piggyBac transposition. BMC Biotechnol. 2013;13:75.
- [59] Bowers WJ, Mastrangelo MA, Howard DF, Southerland HA, Maguire-Zeiss KA, Federoff HJ. Neuronal precursor-restricted transduction via in utero CNS gene delivery of a novel bipartite HSV amplicon/transposase hybrid vector. Mol Ther. 2006;13:580– 8.
- [60] Müther N, Noske N, Ehrhardt A. Viral hybrid vectors for somatic integration—are they the better solution? Viruses. 2009;1:1295–324.

- [61] Skipper KA, Andersen PR, Sharma N, Mikkelsen JG. DNA transposon-based gene vehicles—scenes from an evolutionary drive. J Biomed Sci. 2013;20:92.
- [62] Hausl MA, Zhang W, Müther N, Rauschhuber C, Franck HG, Merricks EP, et al. Hyperactive Sleeping Beauty transposase enables persistent phenotypic correction in mice and a canine model for hemophilia B. Mol Ther. 2010;18:1896–906.
- [63] Zhang W, Muck-Hausl M, Wang J, Sun C, Gebbing M, Miskey C, et al. Integration profile and safety of an adenovirus hybrid-vector utilizing hyperactive Sleeping Beauty transposase for somatic integration. PLoS One. 2013;8:e75344.
- [64] Smith RP, Riordan JD, Feddersen CR, Dupuy AJ. A hybrid adenoviral vector system achieves efficient long-term gene expression in the liver via piggyBac transposition. Hum Gene Ther. 2015;26:377–85.
- [65] Zhang W, Solanki M, Müther N, Ebel M, Wang J, Sun C, et al. Hybrid adeno-associated viral vectors utilizing transposase-mediated somatic integration for stable transgene expression in human cells. PLoS One. 2013;8:e76771.
- [66] Luo W-Y, Shih Y-S, Hung C-L, Lo K-W, Chiang C-S, Lo W-H, et al. Development of the hybrid Sleeping Beauty: baculovirus vector for sustained gene expression and cancer therapy. Gene Ther. 2012;19:844–51.
- [67] Staunstrup NH, Moldt B, Mátés L, Villesen P, Jakobsen M, Ivics Z, et al. Hybrid lentivirus-transposon vectors with a random integration profile in human cells. Mol Ther. 2009;17:1205–14.
- [68] Vink CA, Gaspar HB, Gabriel R, Schmidt M, McIvor RS, Thrasher AJ, et al. Sleeping beauty transposition from nonintegrating lentivirus. Mol Ther. 2009;17:1197–204.
- [69] Sakurai H, Kawabata K, Sakurai F, Nakagawa S, Mizuguchi H. Innate immune response induced by gene delivery vectors. Int J Pharm. 2008;354:9–15.
- [70] Bieber T, Meissner W, Kostin S, Niemann A, Elsasser H-P. Intracellular route and transcriptional competence of polyethylenimine-DNA complexes. J Control Release. 2002;82:441–54.
- [71] Rejman J, Bragonzi A, Conese M. Role of clathrin- and caveolae-mediated endocytosis in gene transfer mediated by lipo- and polyplexes. Mol Ther. 2005;12:468–74.
- [72] Kang Y, Yu W, Sun Q, Zhang X, Jiang W, Wu C, et al. High-level transgene expression mediated by the piggyBac transposon enhances transgenic therapeutic effects in cervical cancer xenografts. Oncol Rep. 2010;24:897–907.
- [73] Ley D, Van Zwieten R, Puttini S, Iyer P, Cochard A, Mermod N. A PiggyBac-mediated approach for muscle gene transfer or cell therapy. Stem Cell Res. 2014;13:390–403.
- [74] Lin E-H, Keramidas M, Rome C, Chiu W-T, Wu C-W, Coll J-L, et al. Lifelong reporter gene imaging in the lungs of mice following polyethyleneimine-mediated Sleeping-Beauty transposon delivery. Biomaterials. 2011;32:1978–85.

- [75] Chicaybam L, Sodre AL, Curzio BA, Bonamino MH. An efficient low cost method for gene transfer to T lymphocytes. PLoS One. 2013;8:e60298.
- [76] Singh H, Figliola MJ, Dawson MJ, Olivares S, Zhang L, Yang G, et al. Manufacture of clinical-grade CD19-specific T cells stably expressing chimeric antigen receptor using Sleeping Beauty system and artificial antigen presenting cells. PLoS One. 2013;8:e64138.
- [77] Singh H, Huls H, Kebriaei P, Cooper LJN. A new approach to gene therapy using Sleeping Beauty to genetically modify clinical-grade T cells to target CD19. Immunol Rev. 2014;257:181–90.
- [78] Chen S, Shimoda M, Chen J, Matsumoto S, Grayburn PA, Paul A. Ectopic transgenic expression of NKX2.2 induces differentiation of adult pancreatic progenitors and mediates islet regeneration. Cell Cycle. 2012;11:1544–53.
- [79] Chen S, Shimoda M, Chen J, Grayburn PA. Stimulation of adult resident cardiac progenitor cells by durable myocardial expression of thymosin beta 4 with ultrasoundtargeted microbubble delivery. Gene Ther. 2013;20:225–33.
- [80] Staber JM, Pollpeter MJ, Arensdorf A, Sinn PL, Rutkowski DT, McCray PB. piggyBacmediated phenotypic correction of factor VIII deficiency. Mol Ther Methods Clin Dev. 2014;1:14042.
- [81] Doherty JE, Woodard LE, Bear AS, Foster AE, Wilson MH. An adaptable system for improving transposon-based gene expression *in vivo* via transient transgene repression. FASEB J. 2013;27:3753–62.
- [82] Keith JH, Fraser TS, Fraser MJ. Analysis of the piggyBac transposase reveals a functional nuclear targeting signal in the 94 c-terminal residues. BMC Mol Biol. 2008;9:72.
- [83] Hong J-B, Chou F-J, Ku AT, Fan H-H, Lee T-L, Huang Y-H, et al. A nucleolus-predominant piggyBac transposase, NP-mPB, mediates elevated transposition efficiency in mammalian cells. PLoS One. 2014;9:e89396.
- [84] Miller AM, Dean DA. Tissue-specific and transcription factor-mediated nuclear entry of DNA. Adv Drug Deliv Rev. 2009;61:603–13.
- [85] de Jong J, Akhtar W, Badhai J, Rust AG, Rad R, Hilkens J, et al. Chromatin landscapes of retroviral and transposon integration profiles. PLoS Genet. 2014;10:e1004250.
- [86] Li X, Ewis H, Hice RH, Malani N, Parker N, Zhou L, et al. A resurrected mammalian hAT transposable element and a closely related insect element are highly active in human cell culture. Proc Natl Acad Sci. 2013;110:E478–87.
- [87] Moldt B, Miskey C, Staunstrup NH, Gogol-Döring A, Bak RO, Sharma N, et al. Comparative genomic integration profiling of Sleeping Beauty transposons mobilized with high efficacy from integrase-defective lentiviral vectors in primary human cells. Mol Ther. 2011;19:1499–510.

- [88] Field A-C, Vink C, Gabriel R, Al-Subki R, Schmidt M, Goulden N, et al. Comparison of lentiviral and Sleeping Beauty mediated αβ T cell receptor gene transfer. PLoS One. 2013;8:e68201.
- [89] Huang X, Guo H, Tammana S, Jung Y-C, Mellgren E, Bassi P, et al. Gene transfer efficiency and genome-wide integration profiling of Sleeping Beauty, Tol2, and piggyBac transposons in human primary T cells. Mol Ther. 2010;18:1803–13.
- [90] Yant SR, Wu X, Huang Y, Garrison B, Burgess SM, Kay MA. High-resolution genomewide mapping of transposon integration in mammals. Mol Cell Biol. 2005;25:2085–94.
- [91] Ikeda R, Kokubu C, Yusa K, Keng VW, Horie K, Takeda J. Sleeping beauty transposase has an affinity for heterochromatin conformation. Mol Cell Biol. 2007;27:1665–76.
- [92] Meir Y-JJ, Weirauch MT, Yang H-S, Chung P-C, Yu RK, Wu SC-Y. Genome-wide target profiling of piggyBac and Tol2 in HEK 293: pros and cons for gene discovery and gene therapy. BMC Biotechnol. BioMed Central Ltd; 2011 Jan;11:28.
- [93] Burnight ER, Staber JM, Korsakov P, Li X, Brett BT, Scheetz TE, et al. A hyperactive transposase promotes persistent gene transfer of a piggyBac DNA transposon. Mol Ther Nucleic Acids. 2012;1:e50.
- [94] Galvan DL, Nakazawa Y, Kaja A, Kettlun C, Laurence JN, Rooney CM, et al. Genomewide mapping of PiggyBac transposon integrations in primary human T cells. J Immunother. 2009;32:837–44.
- [95] Garrison BS, Yant SR, Mikkelsen JG, Kay MA. Postintegrative gene silencing within the Sleeping Beauty transposition system. Mol Cell Biol. 2007;27:8824–33.
- [96] Yusa K, Takeda J, Horie K. Enhancement of Sleeping Beauty transposition by CpG methylation: possible role of heterochromatin formation. Mol Cell Biol. 2004;24:4004– 18.
- [97] Sharma N, Hollensen AK, Bak RO, Staunstrup NH, Schrøder LD, Mikkelsen JG. The impact of cHS4 insulators on DNA transposon vector mobilization and silencing in retinal pigment epithelium cells. PLoS One. 2012;7:e48421.
- [98] Rauschhuber C, Ehrhardt A. RNA interference is responsible for reduction of transgene expression after Sleeping Beauty transposase mediated somatic integration. PLoS One. 2012;7:e35389.
- [99] Park CW, Park J, Kren BT, Steer CJ. Sleeping Beauty transposition in the mouse genome is associated with changes in DNA methylation at the site of insertion. Genomics. 2006;88:204–13.
- [100] Zhu J, Park CW, Sjeklocha L, Kren BT, Steer CJ, Park CW, et al. High-level genomic integration, epigenetic changes, and expression of Sleeping Beauty transgene. Biochemistry. 2010;49:1507–21.

- [101] Ley D, Harraghy N, Le Fourn V, Bire S, Girod P-A, Regamey A, et al. MAR elements and transposons for improved transgene integration and expression. PLoS One. 2013;8:e62784.
- [102] Sjeklocha L, Chen Y, Daly MC, Steer CJ, Kren BT. β-Globin matrix attachment region improves stable genomic expression of the Sleeping Beauty transposon. J Cell Biochem. 2011;112:2361–75.
- [103] Walisko O, Schorn A, Rolfs F, Devaraj A, Miskey C, Izsvák Z, et al. Transcriptional activities of the Sleeping Beauty transposon and shielding its genetic cargo with insulators. Mol Ther. 2008;16:359–69.
- [104] Dalsgaard T, Moldt B, Sharma N, Wolf G, Schmitz A, Pedersen FS, et al. Shielding of Sleeping Beauty DNA transposon-delivered transgene cassettes by heterologous insulators in early embryonal cells. Mol Ther. 2009;17:121–30.
- [105] Mossine VV, Waters JK, Hannink M, Mawhinney TP. piggyBac Transposon plus insulators overcome epigenetic silencing to provide for stable signaling pathway reporter cell lines. PLoS One. 2013;8:e85494.
- [106] Sjeklocha LM, Park C-W, Wong PY-P, Roney MJ, Belcher JD, Kaufman DS, et al. Erythroid-specific expression of β-globin from Sleeping Beauty-transduced human hematopoietic progenitor cells. PLoS One. 2011;6:e29110.
- [107] Bire S, Rouleux-bonnin F. Transgene Site-specific integration: problems and solutions. In: Renault S, Duchateau P, editors. Site-directed insertion of transgenes. Dordrecht, Netherlands: Springer; 2013:3–39.
- [108] Ivics Z, Katzer A, Stüwe EE, Fiedler D, Knespel S, Izsvák Z. Targeted Sleeping Beauty transposition in human cells. Mol Ther. 2007;15:1137–44.
- [109] Demattei M-V, Thomas X, Carnus E, Augé-Gouillou C, Renault S. Site-directed integration of transgenes: transposons revisited using DNA-binding-domain technol-ogies. Genetica. 2010;138:531–40.
- [110] Maragathavally KJ, Kaminski JM, Coates CJ. Chimeric Mos1 and piggyBac transposases result in site-directed integration. FASEB J. 2006;20:1880–2.
- [111] Wu SC-Y, Meir Y-JJ, Coates CJ, Handler AM, Pelczar P, Moisyadi S, et al. piggyBac is a flexible and highly active transposon as compared to Sleeping Beauty, Tol2, and Mos1 in mammalian cells. Proc Natl Acad Sci. 2006;103:15008–13.
- [112] Wilson MH, Kaminski JM, George AL. Functional zinc finger/Sleeping Beauty transposase chimeras exhibit attenuated overproduction inhibition. FEBS Lett. 2005;579:6205–9.
- [113] Yant SR, Huang Y, Akache B, Kay MA. Site-directed transposon integration in human cells. Nucleic Acids Res. 2007;35:e50.

- [114] Owens JB, Urschitz J, Stoytchev I, Dang NC, Stoytcheva Z, Belcaid M, et al. Chimeric piggyBac transposases for genomic targeting in human cells. Nucleic Acids Res. 2012;40:6978–91.
- [115] Mandell JG, Barbas CF. Zinc finger tools: custom DNA-binding domains for transcription factors and nucleases. Nucleic Acids Res. 2006;34:W516–23.
- [116] Kettlun C, Galvan DL, George AL, Kaja A, Wilson MH. Manipulating piggyBac transposon chromosomal integration site selection in human cells. Mol Ther. 2011;19:1636–44.
- [117] Wang H, Mayhew D, Chen X, Johnston M, Mitra RD. "Calling cards" for DNA-binding proteins in mammalian cells. Genetics. 2012;190:941–9.
- [118] Hickman AB, Dyda F. CRISPR-Cas immunity and mobile DNA: a new superfamily of DNA transposons encoding a Cas1 endonuclease. Mob DNA. 2014;5:23.
- [119] Krupovic M, Makarova KS, Forterre P, Prangishvili D, Koonin EV. Casposons: a new superfamily of self-synthesizing DNA transposons at the origin of prokaryotic CRISPR-Cas immunity. BMC Biol. 2014;12:36.
- [120] Xie F, Ye L, Chang JC, Beyer AI, Wang J, Muench MO, et al. Seamless gene correction of β-thalassemia mutations in patient-specific iPSCs using CRISPR/Cas9 and piggyBac. Genome Res. 2014;24:1526–33.
- [121] Ye L, Wang J, Beyer AI, Teque F, Cradick TJ, Qi Z, et al. Seamless modification of wildtype induced pluripotent stem cells to the natural CCR5∆32 mutation confers resistance to HIV infection. Proc Natl Acad Sci. 2014;111:9591–6.
- [122] Toscano MG, Romero Z, Muñoz P, Cobo M, Benabdellah K, Martin F. Physiological and tissue-specific vectors for treatment of inherited diseases. Gene Ther. 2011;18:117– 27.
- [123] Liu L, Sanz S, Heggestad AD, Antharam V, Notterpek L, Fletcher BS. Endothelial targeting of the Sleeping Beauty transposon within lung. Mol Ther. 2004;10:97–105.
- [124] Zhu J, Kren BT, Park CW, Bilgim R, Wong PY-P, Steer CJ. Erythroid-specific expression of beta-globin by the Sleeping Beauty transposon for Sickle cell disease. Biochemistry. 2007;46:6844–58.
- [125] Hong I-S, Lee H-Y, Kim H-P. Novel therapeutic approaches for various cancer types using a modified Sleeping Beauty-based gene delivery system. PLoS One. 2014;9:e86324.
- [126] Song JS, Kim HP, Rubin E. Development of a Sleeping Beauty-based telomerase gene delivery system for hepatocytes. Biosci Biotechnol Biochem. 2011;75:227–31.
- [127] Chuah MK, Petrus I, De Bleser P, Le Guiner C, Gernoux G, Adjali O, et al. Liver-specific transcriptional modules identified by genome-wide *in silico* analysis enable efficient gene therapy in mice and non-human primates. Mol Ther. 2014;22:1605–13.

- [128] Di Matteo M, Samara-Kuko E, Ward NJ, Waddington SN, Waddingon SN, McVey JH, et al. Hyperactive piggyBac transposons for sustained and robust liver-targeted gene therapy. Mol Ther. 2014;22:1614–24.
- [129] Nakazawa Y, Huye LE, Salsman VS, Leen AM, Ahmed N, Rollins L, et al. PiggyBacmediated cancer immunotherapy using EBV-specific cytotoxic T-cells expressing HER2-specific chimeric antigen receptor. Mol Ther. 2011;19:2133–43.
- [130] Saito S, Nakazawa Y, Sueki A, Matsuda K, Tanaka M, Yanagisawa R, et al. Antileukemic potency of piggyBac-mediated CD19-specific T cells against refractory Philadelphia chromosome-positive acute lymphoblastic leukemia. Cytotherapy. 2014;16:1257–69.
- [131] Tsukahara T, Iwase N, Kawakami K, Iwasaki M, Yamamoto C, Ohmine K, et al. The Tol2 transposon system mediates the genetic engineering of T-cells with CD19-specific chimeric antigen receptors for B-cell malignancies. Gene Ther. 2015;22:209–15.
- [132] Kebriaei P, Huls H, Jena B, Munsell M, Jackson R, Lee DA, et al. Infusing CD19-directed T cells to augment disease control in patients undergoing autologous hematopoietic stem-cell transplantation for advanced B-lymphoid malignancies. Hum Gene Ther. 2012;23:444–50.
- [133] Fjord-Larsen L, Kusk P, Emerich DF, Thanos C, Torp M, Bintz B, et al. Increased encapsulated cell biodelivery of nerve growth factor in the brain by transposon-mediated gene transfer. Gene Ther. 2012;19:1010–7.
- [134] Bell JB, Podetz-Pedersen KM, Aronovich EL, Belur LR, McIvor RS, Hackett PB. Preferential delivery of the Sleeping Beauty transposon system to livers of mice by hydrodynamic injection. Nat Protoc. 2007;2:3153–65.
- [135] Herweijer H, Wolff JA. Progress and prospects: naked DNA gene transfer and therapy. Gene Ther. 2003;10:453–8.
- [136] Aronovich EL, Bell JB, Belur LR, Gunther R, Koniar B, Erickson DCC, et al. Prolonged expression of a lysosomal enzyme in mouse liver after Sleeping Beauty transposonmediated gene delivery: implications for non-viral gene therapy of mucopolysaccharidoses. J Gene Med. 2007;9:403–15.
- [137] Belur LR, Podetz-Pedersen KM, Sorenson BS, Hsu AH, Parker JB, Carlson CS, et al. Inhibition of angiogenesis and suppression of colorectal cancer metastatic to the liver using the Sleeping Beauty transposon system. Mol Cancer. 2011;10:14.
- [138] Podetz-Pedersen KM, Bell JB, Steele TWJ, Wilber A, Shier WT, Belur LR, et al. Gene expression in lung and liver after intravenous infusion of polyethylenimine complexes of Sleeping Beauty transposons. Hum Gene Ther. 2010;21:210–20.
- [139] Belur L, Frandsen JL, Dupuy AJ, Ingbar DH, Largaespada DA, Hackett PB, et al. Gene insertion and long-term expression in lung mediated by the Sleeping Beauty transposon system. Mol Ther. 2003;8:501–7.

- [140] Chen S, Chen J, Huang P, Meng X-L, Clayton S, Shen J-S, et al. Myocardial regeneration in adriamycin cardiomyopathy by nuclear expression of GLP1 using ultrasound targeted microbubble destruction. Biochem Biophys Res Commun. 2015;458:823–9.
- [141] Xue X, Huang X, Nodland SE, Mátés L, Ma LL, Izsvák Z, et al. Stable gene transfer and expression in cord blood-derived CD34⁺ hematopoietic stem and progenitor cells by a hyperactive Sleeping Beauty transposon system. Gene. 2010;114:1319–30.
- [142] Ma K, Wang D-D, Lin Y, Wang J, Petrenko V, Mao C. Synergetic targeted delivery of Sleeping-Beauty transposon system to mesenchymal stem cells using LPD nanoparticles modified with a phage-displayed targeting peptide. Adv Funct Mater. 2013;23:1172–81.
- [143] Tsukiyama T, Asano R, Kawaguchi T, Kim N, Yamada M, Minami N, et al. Simple and efficient method for generation of induced pluripotent stem cells using piggyBac transposition of doxycycline-inducible factors and an EOS reporter system. Genes Cells. 2011;16:815–25.
- [144] Matsui H, Fujimoto N, Sasakawa N, Ohinata Y, Shima M, Yamanaka S, et al. Delivery of full-length factor VIII using a piggyBac transposon vector to correct a mouse model of hemophilia A. PLoS One. 2014;9:e104957.
- [145] Liu L, Mah C, Fletcher BS. Sustained FVIII expression and phenotypic correction of hemophilia A in neonatal mice using an endothelial-targeted Sleeping Beauty transposon. Mol Ther. 2006;13:1006–15.
- [146] Ohlfest JR, Frandsen JL, Fritz S, Lobitz PD, Perkinson SG, Clark KJ, et al. Phenotypic correction and long-term expression of factor VIII in hemophilic mice by immunotolerization and nonviral gene transfer using the Sleeping Beauty transposon system. Blood. 2005;105:2691–8.
- [147] Yant SR, Meuse L, Chiu W, Ivics Z, Izsvak Z, Kay MA. Somatic integration and long-term transgene expression in normal and haemophilic mice using a DNA transposon system. Nat Genet. 2000;25:35–41.
- [148] Chen ZJ, Kren BT, Wong PY-P, Low WC, Steer CJ. Sleeping Beauty-mediated downregulation of huntingtin expression by RNA interference. Biochem Biophys Res Commun. 2005;329:646–52.
- [149] Pan X-J, Ma Z-Z, Zhang Q-J, Fan L, Li Q-H. Sleeping Beauty transposon system is a reliable gene delivery tool for hereditary tyrosinaemia type 1 disease gene therapy: size of the foreign gene decides the timing of stable integration into the host chromosomes. J Int Med Res. 2012;40:1850–9.
- [150] Montini E, Held PK, Noll M, Morcinek N, Al-Dhalimy M, Finegold M, et al. *In vivo* correction of murine tyrosinemia type I by DNA-mediated transposition. Mol Ther. 2002;6:759–69.

- [151] Wilber A, Wangensteen KJ, Chen Y, Zhuo L, Frandsen JL, Bell JB, et al. Messenger RNA as a source of transposase for Sleeping Beauty transposon-mediated correction of hereditary tyrosinemia type I. Mol Ther. 2007;15:1280–7.
- [152] Belcher JD, Vineyard JV, Bruzzone CM, Chen C, Beckman JD, Nguyen J, et al. Heme oxygenase-1 gene delivery by Sleeping Beauty inhibits vascular stasis in a murine model of sickle cell disease. J Mol Med (Berl). 2010;88:665–75.
- [153] Sjeklocha LM, Wong PY-P, Belcher JD, Vercellotti GM, Steer CJ. β-Globin Sleeping Beauty transposon reduces red blood cell sickling in a patient-derived CD34(+)-based *in vitro* model. PLoS One. 2013;8:e80403.
- [154] Aronovich EL, Bell JB, Khan SA, Belur LR, Gunther R, Koniar B, et al. Systemic correction of storage disease in MPS I NOD/SCID mice using the Sleeping Beauty transposon system. Mol Ther. 2009;17:1136–44.
- [155] Aronovich EL, Hall BC, Bell JB, Mc Ivor RS, Hackett PB. Quantitative analysis of α-Liduronidase expression in immunocompetent mice treated with the Sleeping Beauty transposon system. PLoS One. 2013;8:e78161.
- [156] Yusa K, Rashid ST, Strick-Marchand H, Varela I, Liu P-Q, Paschon DE, et al. Targeted gene correction of *α*1-antitrypsin deficiency in induced pluripotent stem cells. Nature. 2011;478:391–4.
- [157] Hyland KA, Olson ER, Clark KJ, Aronovich EL, Hackett PB, Blazar BR, et al. Sleeping Beauty-mediated correction of Fanconi anemia type C. J Gene Med. 2011;13:462–9.
- [158] Wang X, Sarkar DP, Mani P, Steer CJ, Chen Y, Guha C, et al. Long-term reduction of jaundice in Gunn rats by nonviral liver-targeted delivery of Sleeping Beauty transposon. Hepatology. 2009;50:815–24.
- [159] Ortiz-Urda S, Lin Q, Yant SR, Keene D, Kay MA, Khavari PA. Sustainable correction of junctional epidermolysis bullosa via transposon-mediated nonviral gene transfer. Gene Ther. 2003;10:1099–104.
- [160] Bertino P, Urschitz J, Hoffmann FW, You BR, Rose AH, Park WH, et al. Vaccination with a piggyBac plasmid with transgene integration potential leads to sustained antigen expression and CD8(+) T cell responses. Vaccine. 2014;32:1670–7.
- [161] Martinez-Fernandez A, Nelson TJ, Reyes S, Alekseev AE, Secreto F, Perez-Terzic C, et al. iPS cell-derived cardiogenicity is hindered by sustained integration of reprogramming transgenes. Circ Cardiovasc Genet. 2014;7:667–76.
- [162] Tsukiyama T, Kato-Itoh M, Nakauchi H, Ohinata Y. A comprehensive system for generation and evaluation of induced pluripotent stem cells using piggyBac transposition. PLoS One. 2014;9:e92973.

- [163] Yin J, Fan Y, Qin D, Xiaocui Bian X, Bi X. Generation and characterization of virus-free reprogrammed melanoma cells by the piggyBac transposon. J Cancer Res Clin Oncol. 2013;139:1591–9.
- [164] Inada E, Saitoh I, Watanabe S, Aoki R, Miura H, Ohtsuka M, et al. PiggyBac transposonmediated gene delivery efficiently generates stable transfectants derived from cultured primary human deciduous tooth dental pulp cells (HDDPCs) and HDDPC-derived iPS cells. Int J Oral Sci. 2015;7:144–54.
- [165] He C-X, Shi D, Wu W-J, Ding Y-F, Feng D-M, Lu B, et al. Insulin expression in livers of diabetic mice mediated by hydrodynamics-based administration. World J Gastroenterol. 2004;10:567–72.
- [166] Johnen S, Izsvák Z, Stöcker M, Harmening N, Salz AK, Walter P, et al. Sleeping Beauty transposon-mediated transfection of retinal and iris pigment epithelial cells. Invest Ophthalmol Vis Sci. 2012;53:4787–96.
- [167] Ohlfest JR, Demorest ZL, Motooka Y, Vengco I, Oh S, Chen E, et al. Combinatorial antiangiogenic gene therapy by nonviral gene transfer using the Sleeping Beauty transposon causes tumor regression and improves survival in mice bearing intracranial human glioblastoma. Mol Ther. 2005;12:778–88.
- [168] Kang Y, Zhang X-Y, Jiang W, Wu C-Q, Chen C-M, Gu J-R, et al. The piggyBac transposon is an integrating non-viral gene transfer vector that enhances the efficiency of GDEPT. Cell Biol Int. 2009;33:509–15.
- [169] Bahrambeigi V, Ahmadi N, Moisyadi S, Urschitz J, Salehi R, Haghjooy Javanmard S. PhiC31/PiggyBac modified stromal stem cells: effect of interferon γ and/or tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) on murine melanoma. Mol Cancer. 2014;13:255.
- [170] Huang X, Guo H, Kang J, Choi S, Zhou TC, Tammana S, et al. Sleeping Beauty transposon-mediated engineering of human primary T cells for therapy of CD19⁺ lymphoid malignancies. Mol Ther. 2008;16:580–9.
- [171] Singh H, Manuri PR, Olivares S, Dara N, Dawson MJ, Huls H, et al. Redirecting specificity of T-cell populations for CD19 using the Sleeping Beauty system. Cancer Res. 2008;68:2961–71.
- [172] Maiti SN, Huls H, Singh H, Dawson M, Figliola M, Olivares S, et al. Sleeping beauty system to redirect T-cell specificity for human applications. J Immunother. 2013;36:112– 23.
- [173] Galvan DL, O'Neil RT, Foster AE, Huye L, Bear A, Rooney CM, et al. Anti-tumor effects after adoptive transfer of IL-12 transposon-modified murine splenocytes in the OT-Imelanoma mouse model. PLoS One. 2015;10:e0140744.
- [174] Ramanayake S, Bilmon I, Bishop D, Dubosq M-C, Blyth E, Clancy L, et al. Low-cost generation of Good Manufacturing Practice-grade CD19-specific chimeric antigen

receptor-expressing T cells using piggyBac gene transfer and patient-derived materials. Cytotherapy. 2015;17:1251–67.

- [175] Manuri PVR, Wilson MH, Maiti SN, Mi T, Singh H, Olivares S, et al. piggyBac transposon/transposase system to generate CD19-specific T cells for the treatment of Blineage malignancies. Hum Gene Ther. 2010;21:427–37.
- [176] Huye LE, Nakazawa Y, Patel MP, Yvon E, Sun J, Savoldo B, et al. Combining mTor inhibitors with rapamycin-resistant T cells: a two-pronged approach to tumor elimination. Mol Ther. 2011;19:2239–48.
- [177] Huang G, Yu L, Cooper LJN, Hollomon M, Huls H, Kleinerman ES. Genetically modified T cells targeting interleukin-11 receptor α-chain kill human osteosarcoma cells and induce the regression of established osteosarcoma lung metastases. Cancer Res. 2012;72:271–81.
- [178] Deniger DC, Yu J, Huls MH, Figliola MJ, Mi T, Maiti SN, et al. Sleeping Beauty transposition of chimeric antigen receptors targeting receptor tyrosine kinase-like orphan receptor-1 (ROR1) into diverse memory T-cell populations. PLoS One. 2015;10:e0128151.
- [179] Jin Z, Maiti S, Huls H, Singh H, Olivares S, Mátés L, et al. The hyperactive Sleeping Beauty transposase SB100X improves the genetic modification of T cells to express a chimeric antigen receptor. Gene Ther. 2011;18:849–56.
- [180] Xiao J, Meng X-M, Huang XR, Chung AC, Feng Y-L, Hui DS, et al. miR-29 inhibits bleomycin-induced pulmonary fibrosis in mice. Mol Ther. 2012;20:1251–60.
- [181] Liu H, Liu L, Fletcher BS, Visner GA. Sleeping Beauty-based gene therapy with indoleamine 2,3-dioxygenase inhibits lung allograft fibrosis. FASEB J. 2006;20:E1694– 703.
- [182] Liu L, Liu H, Visner G, Fletcher BS. Sleeping Beauty-mediated eNOS gene therapy attenuates monocrotaline-induced pulmonary hypertension in rats. FASEB J. 2006;20:2594–1596.
- [183] Liang M, Woodard LE, Liang A, Luo J, Wilson MH, Mitch WE, et al. Protective role of insulin-like growth factor-1 receptor in endothelial cells against unilateral ureteral obstruction-induced renal fibrosis. Am J Pathol. 2015;185:1234–50.