DNA Elements Tetris: A Strategy for Gene Correction

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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 the 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...
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 drawn 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](http://dx.doi.org/10.5772/62382)

**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 cross-mobilization 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 highly 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 pseudo-transposon/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 pseudo-transposons [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 | High | [47,48] |
| | Promoter of the transposase is between the ITRs | Low and inefficient transposase | [46] |
| | Transposase and pseudo-transposon share the same polyadenylation signal | Low and inefficient transposase | [44,49] |
| Trans delivery: two separate molecules | Conventional trans architecture: two separate plasmids | 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 physical 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. Conversely, the transgene copy might also influence surrounding sequences according to the integration site. To counter 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 boundary 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, predeter‐
mend," 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, tran‐
scription 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. 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 β4 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 cell type                     | Reference                          |
|----------------------------------|-------------|--------|----------------------------------------------|-----------------------------------|
| 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  | PB     | 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     | hIDUA or hGUSB | SB | MPS I NOD/SCID mice                          | [136,154,155]                     |
| (MPS I)                          |             |        |                                              |                                   |
| 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 rats                | [158]                             |
| Junctional epidermolysis bullosa (JEB) | LAMB3     | SB     | Epidermal holoclones from JEB patients       | [159]                             |
| Vaccination                      |             |        |                                              |                                   |
| Immunization against non-self protein | eGFP    | PB     | Mice                                         | [160]                             |
| Regenerative medicine            |             |        |                                              |                                   |
| iPSC generation                  | SOX2, OCT4, KLF4, c-MYC | PB | Fibroblasts, melanoma cells, HDDPC          | [32,143,161–164]                  |
| Diabetes                         | Nks2.2 or insulin | PB/SB | STZ-rat pancreas                             | [78,165]                          |
| Retinal degeneration             | PEDF        | SB     | IPE and RPE cells                            | [166]                             |
| Acute myocardial infarction      | TB4 or GLP1 | PB     | Rat heart                                    | [79,140]                          |
| Cancerology                      |             |        |                                              |                                   |
### Table 2. Application fields of transposon-based gene correction.

| Disease                        | Transgene            | Tnase | Animal model or cell type                     | Reference            |
|-------------------------------|----------------------|-------|-----------------------------------------------|----------------------|
| Angiogenesis-dependent tumors | sFlt-1 or statin-AE  | SB    | Tumor engrafts in mice                        | [66,137,167]         |
| Cervical cancer               | HSV-tk               | PB    | Cervical cancer xenografts                    | [72]                 |
| Ovarian adenocarcinoma        | HSV-tk               | PB    | Cell line                                     | [168]                |
| Melanoma                      | TRAIL and IFNγ       | PB    | 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 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]                |

Tnase, 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 α1-antitrypsin; HDDPC, primary human deciduous tooth dental pulp cells; HER2, human epidermal growth factor receptor 2; hGSTB, β-glucuronidase; 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; IFNγ, interferon γ; IGF-1R, insulin-like growth factor-1 receptor; HIK, 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 epithelium-derived factor; PEP, 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 β4; Tnpase, transposase; TRAIL, TNF-related apoptosis-inducing ligand.

### 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.

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