Review

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piggyBac-ing models and new therapeutic strategies

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DNA transposons offer an efficient nonviral method of permanently modifying the genomes of mammalian cells. The *piggyBac* transposon system has proven effective in genomic engineering of mammalian cells for preclinical applications, including gene discovery, simultaneous multiplexed genome modification, animal transgenesis, gene transfer *in vivo* achieving long-term gene expression in animals, and the genetic modification of clinically relevant cell types, such as induced pluripotent stem cells and human T lymphocytes. *piggyBac* has many desirable features, including seamless excision of transposons from the genomic DNA and the potential to target integration events to desired DNA sequences. In this review, we explore these recent applications and also highlight the unique advantages of using *piggyBac* for developing new molecular therapeutic strategies.

piggyBac: a versatile tool for biotechnology

DNA-based transposon systems first emerged as efficient tools for genome engineering of mammalian cells after the *Sleeping Beauty* transposon system was resurrected from the genome of the medaka fish [1]. Transposon DNA vectors can be engineered for a variety of purposes, including transgenesis, gene therapy, gene trapping, or insertion of other DNA elements into the genomes of cells (see Glossary). The *piggyBac* transposon system is naturally active and was first discovered in insect cells while propagating Baculovirus in the TN-386 cell line, from the cabbage looper moth *Trichoplusia ni* of the order Lepidoptera [2,3]. The cause of unexpected viral plaques was the insertion of novel class II mobile DNA elements in the Baculovirus. The inserted mobile DNA was being carried 'piggyback' by the virus, so it was named *piggyBac*; the capitalized and shortened '*Bac*' part of the name signifies its Baculovirus-related discovery. Following its discovery in 1983, *piggyBac* (formerly named IFP2) was studied mainly in insects. However, it was first shown to be efficient in gene transfer in mice in 2005 [4]. Since then, *piggyBac* has been used for genomic modification of human cells [5] and for a variety of applications. These include mammalian transgenesis, mutagenesis, *ex vivo* modification of clinically relevant cell types, and gene transfer in mammals *in vivo*. Such applications have opened new areas of research that will hopefully lead to new therapeutic strategies for human disease.

piggyBac-based gene transfer or mobilization is carried out through a 'cut and paste' mechanism (Box 1). For most applications, the *piggyBac* transposase and *piggyBac* transposon are carried on two separate plasmids (*trans*). It is also possible to deliver the transposase and transposon on the same plasmid (*cis)* with the transposase gene encoded outside of the transposon inverted terminal repeat elements (IRs). When the *piggyBac* transposase protein is expressed in mammalian cells, it binds to the inverted repeats of the transposon, nicking the DNA and freeing a 3′ hydroxyl group at both ends of the transposon. This results in hydrophilic attack of the flanking TTAA sequence and hairpin formation (Box 1), freeing the transposon from its plasmid backbone [6]. The plasmid backbone is then repaired by host cell factors by ligation of the complementary TTAA overhangs. *piggyBac* transposase locates TTAA sequences in the genomic DNA of the mammalian cells. Through hairpin resolution of the transposon and hydrophilic attack of the genomic DNA by 3′ hydroxyl groups on the transposon, a staggered four base-pair (bp) cut in the genomic DNA is produced, creating a transient double-strand (ds) break with TTAA overhangs on both sides of the break. The transposon is then inserted into the genomic DNA at the TTAA site, resulting in a duplication of this

Glossary

Seamless excision: after *piggyBac* excises the transposon from DNA, it seamlessly generates the original *piggyBac* target site >95% of the time. This characteristic of *piggyBac* has enabled it to be used to integrate DNA sequences (such as those harboring antibiotic resistance or transcription factors), which can then be removed by re-expression of the transposase.
Targeted integration: efforts are underway to target *piggyBac* integration into user-defined chromosomal loci. Native *piggyBac* integration is not targeted or site specific. Targeting *piggyBac* integration would improve its safety and efficiency in gene transfer applications.
Transgene: the transposon can be engineered to carry one or more gene(s) of interest, which can be inserted into the genomic DNA. The transgene typically carries its own promoter. Alternatively, the transgene can be replaced with a gene-trapping cassette for gene discovery. A variety of DNA sequences can be inserted into the transposon sequence for multiple different applications.
Transposon: self-mobilizing pieces of nucleic acids. Here, we refer to a specific subclass of transposons, the DNA transposons that use a 'cut-and-paste' nonreplicating mechanism of transposition. When the transposase is expressed, the transposon is excised from its current location (plasmid, virus, or genomic DNA harboring the transposon) and moves (or transposes) to a new location. Therefore, transposons can be used to transfer genes into a cell or tissue (by using plasmid or viral DNA to carry the transposon into the cell) or to move transposons throughout the genome (such as in gene discovery applications).

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Box 1. The mechanism of *piggyBac* transposition

For illustration purposes (Figure I), transposition is demonstrated from plasmid DNA into the genome, although it could also be from one genomic locus to another. Once transposase is expressed (Figure I, red circles), it binds to the *piggyBac* IRs and induces nicking and 3′ hydrophilic attack of the TTAA ends. Hairpin formation occurs with transposon excision. The transposon is then integrated and joined into genomic DNA at a TTAA nucleotide sequence, resulting in TTAA target site duplication at the genomic locus. If precise excision is desired, transposase can be re-expressed and the transposon can be excised, thereby recreating the original TTAA target site at the original locus.

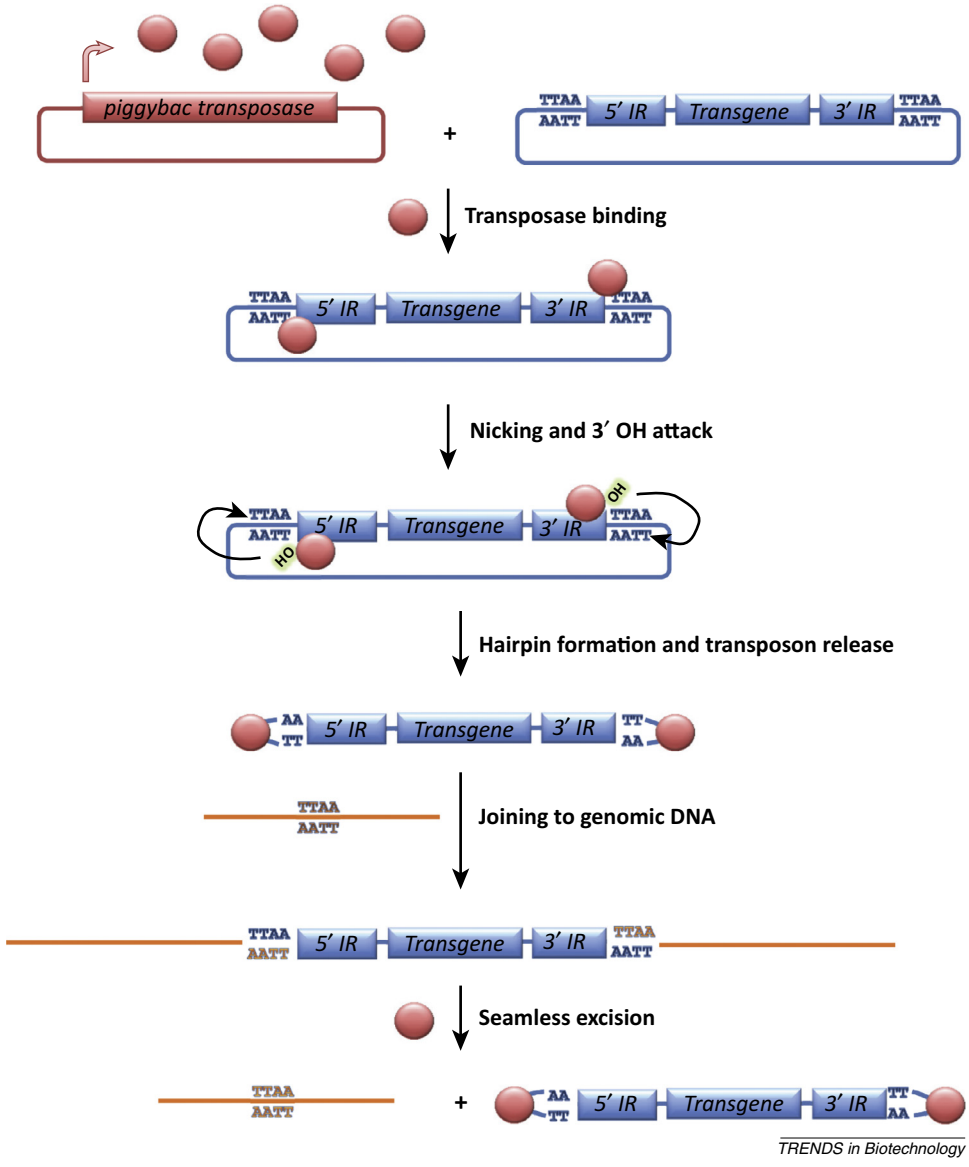

Figure I. Steps of piggyBac transposition. Abbreviation: IR, inverted repeats.

TTAA, such that a TTAA is found on both sides of the transposon. Upon excision of the transposon by *piggyBac* transposase, which can be induced and selected for to rid the cells of the transgene, the single-stranded TTAAs are religated to reform a single TTAA. Thus, the unique mechanism of *piggyBac* transposition results in a unique advantage: seamless excision of the transposon sequence (Box 2). This phenomenon has already been put to great advantage to generate transgene-free iPSC [7,8]. The *piggyBac* transposon system has proven important for several versatile biotechnology applications (Figure 1). *piggyBac* enables greater cargo capacity compared with commonly used viral vectors for gene transfer and, compared with *Sleeping Beauty*, it has a different genomic target sequence, a higher likelihood of hitting genes, and does not suffer from overproduction inhibition (Table 1).

Gene discovery via insertional mutagenesis

piggyBac has been used for insertional mutagenesis to evaluate various pathways both in tissue culture *ex vivo* and in live animals *in vivo*. Transposon integrations can be recovered and mapped to identify genes that have been misregulated (either activated or inactivated), resulting in the phenotype of the mutant cell or organism [9]. Given