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Transgenic Technologies Based on Transposons
Transgenní technologie založené na transpozonech

Bachelor´s thesis

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Prohlášení:

Prohlašuji, že jsem závěrečnou práci zpracovala samostatně a že jsem uvedla všechny použité informační zdroje a literaturu. Tato práce ani její podstatná část nebyla předložena k získání jiného nebo stejného akademického titulu.

V Praze, 16.5.2014

Ivana Dobiášovská

Abstract

Genenetic engineering is one of the leading technologies in biological research. Transgenesis, one of the most important genetic engineering technologies, enables to study genetic aspects of organismal systems and thus helps us to better understand to the functional characteristics of genomes. Transposons are naturally occuring mobile genetic elements, which can be used to artificially integrate transgenes into host cell genomes. Catalysis of this essential step during transgenesis makes from transposons an useful genetic tool.The aim of this work is to present eukaryotic DNA transposons that transpose in a cut-and-paste-fashion, together with particular mechanisms affecting their function, that can be used as gene delivery system.

Key words:
DNA transposons, transgenesis, Sleeping Beauty, PiggyBac, Tol2

Abstrakt

Genové inženýrství je jednou z vedoucích technologií biologického výzkumu. Transgeneze, jedna z nejvýznamnější technik genového inženýrství, umožňuje studium genetických aspektů organismálních systémů a napomáhá tak pochopení funkce a struktury genomů. Transposony jsou přirozeně se vyskytující mobilní genetické elementy, které mohou být uměle využity k začlenění transgenu do genomu hostitelských buněk. Katalýza tohoto zásadního kroku v průběhu transgeneze činí transposony užitečným genetickým nástrojem. Práce usiluje o prezentaci eukaryotických DNA transposonů využívajích cut-and-paste mechanismus integrace, společně s významnými mechanismy ovlivňujícími jejich funkci jako možnosti vhodných systémů pro genový přenos.

Klíčová slova:
DNA transposony, transgeneze, Sleeping Beauty, PiggyBac, Tol2

Glossary

BAC	bacterial artificial chromosome
DBD	DNA binding domain
DR	direct repeat
ESC	embryonic stem cell
IR	inverted repeat
OPI	overproduction inhibition
PB	PiggyBac
SB	Sleeping Beauty
TIR	terminal inverted repeat
TALE	transcription activator-like effector
ZNF	zinc finger

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1. Introduction

Genetic engineering is one of the leading technologies in biological research. Beginning with the first alteration of a bacterial plasmid, which was newly created from two different individual plasmids by *in vitro* joining of restriction endonuclease-generated fragments (Cohen et al. 1973), this technology developed rapidly over the last decades.

Transgenesis as one of the genetic engineering technologies enables the introduction of stable inherited DNA into the organism (Gordon and Ruddle 1981), became a frequently used and widespread research tool. The ability to integrate a specific DNA sequence into the genome of a living system is fundamental to investigate funcional characterisation of genomes.

To provide stable genomic insertion of a transgene into the genome, DNA transposons are employed as effective and practical systems. Several transposon systems have been successfully used in plants, invertebrates and also in vertebrate animal models, for various purposes - such as a vector or an insertional mutagen.

2. Transposons as a natural component of genomes

Transposons are moveable fragments of DNA with the ability to change position and spread within the host genome without the requirement for sequence homology between the element and target site, firstly reported and described by Barbara McClintock (McClintock 1948). They can actively transpose or remain inactive, due to mutations or epigenetic silencing by the host. These entities, capable of efficient genomic insertion as natural DNA transfer vehicles, are widely present as a variably large fraction in nearly all prokaryotic and eukaryotic genomes together with other transposable elements (Figure 1). They constitute at least 45 % of the human genome (Lander et al. 2001) and 37.5 % of the mouse genome (Waterston et al. 2002) which is in contrast to absence of any known transposable element in the genome of *Plasmodium falciparum* (Gardner et al. 2002).

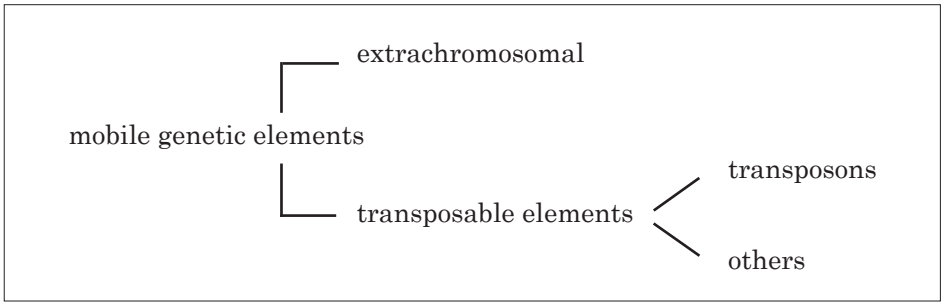

Figure 1: Mobile genetic elements
Trsnsposable elements are DNA sequences, capable of movement within a genome from one location to another. Transposons contitute a group of transposable elements.

Transposons can be viewed as parasitic sequences, adapted to the host expression machinery with the aim to spread within genomes. The mechanism of transposition, the process of transposon excision followed by integration, is facilitated by the transposase enzyme (reviewed in Skipper et al. 2013). Natural structure of a transposon as single unit consists of a transposase gene, flanked by terminal inverted repeats (TIRs) that carry transposase binding sites (Figure 2). However, under laboratory conditions it is possible to employ transposons as bi-component systems. DNA sequence of interest is placed between the transposon TIRs and mobilisation is facilitated by transposase supplemented in *trans* (Figure 3), often in the form of an expression plasmid or mRNA synthetized *in vitro* (reviewed in Ivics et al. 2010).

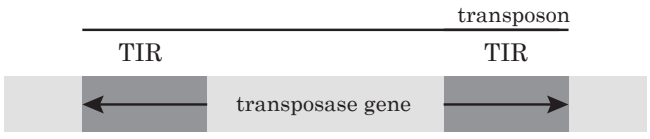

Figure 2: Natural transposon structure, based on Ivics et al. 2010

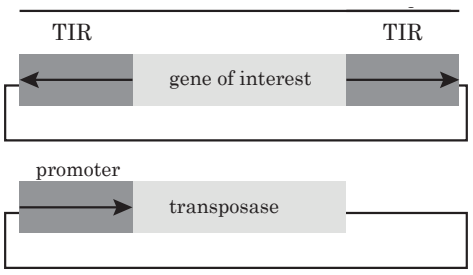

Figure 3: Transposons as bi-component systems, based on Ivics et al. 2010

2.1. Types of transposons

Due to increasing knowledge of genome constitution, number and diversity of known transposons and their genetic organisation is quickly increasing and it is still not easy to define objective criteria required to describe transposon classification clearly, particularly on lower levels. Nevertheless, transposons utilized in transgenic technologies share the same features and all of the transposons discussed further belong to the group of eukaryotic DNA transposons class I, which includes those that transpose by cut-and-paste mechanism via a double strand DNA intermediate (Wicker et al. 2007) shown in context of other eukaryotic transposons in figure 4.

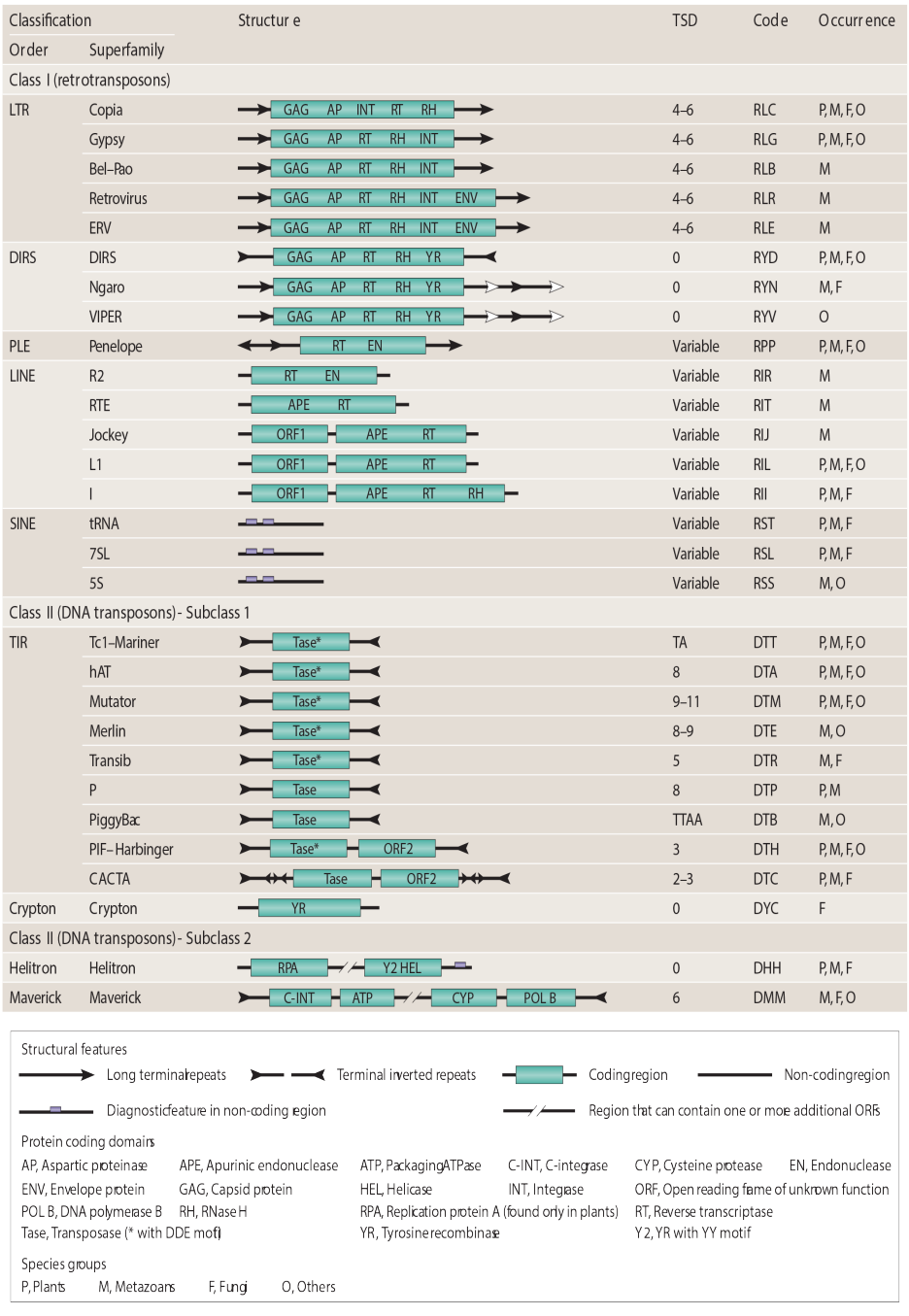

Figure 4: Classification of eukaryotic transposons, adopted from Wicker et al. 2007

2.2. Transposition mechanism

The transposition of DNA transposons is facilitated by a DNA recombination reaction achieved via a DNA intermediate. The mechanism of transpositon is facilitated in a cut-and paste fashion, the transposon is cut out of its location and is inserted into a new location.

To the contrary, in the case of the copy-and-paste mechanism, the original transposon remains at the original site, while its copy is inserted elsewhere. The integration of copy-and-paste transposon displays a similar mechanism that includes DNA breaks and ligation as for cut-and-paste mechanism, such as was compared by Craig (Craig 1995). However, copy-and-paste transposons are not widely used for delivery of exogenous genes. Attempts were made with retrotransposons on the model organism *Saccharomyces cerevisiae* (Xu and Boeke 1990), in gene therapy (Chakraborty et al. 1993) or as gene delivery vectors in plants (Hou et al. 2010), but they have never become widely used (Palazzoli et al. 2010).

Transposition process of DNA transposons is catalyzed by a highly diverse group of recombinases called transposases, DNA binding enzymes from the superfamily of polynucleotidyl transferases. The catalytic domain of all eukarotic cut-and-paste transposase superfamilies contains the acidic amino acid triad DDE consisting of two aspartic acid residues and a glutamic acid residue or DDD consisting of three aspartic acid residues (Yuan and Wessler 2011). Generally, the transposase recognizes the terminal inverted repeats flanking the DNA transposon. These repeats contain binding sites for the transposase, which binds and excises the element out of the donor position forming the synaptic complex. The tranposon bound by the transposase is excised and inserted elsewhere into a new acceptor site, shown in figure 5 (Skipper et al. 2013).

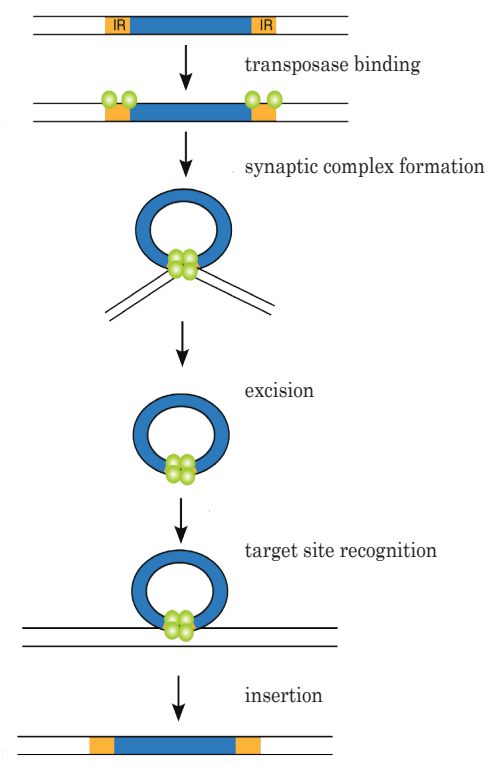

Figure 5: Cut-and-paste transposition, adopted from Skipper et al. 2013

Genes encoding transposases are the most prevalent genes identified in sequenced genomes, indicating the success of transposons in their ability to spread and persist throughout genomes in the course of evolution (Aziz et al. 2010). Catalyzed insertion of foreign DNA into host chromosomes together with the stable integration, render the cut-and-paste DNA transposons a suitable tool in gene transfer applications.

3. Transposons as a genetic tool

3.1. Main transposon systems used in transgenic technologies

Transposons are utilized as bi-component systems, consisting of delivered transgene flanked by transposon inverted repeats and transposase supplemented from a different molecule - in *trans*. There are many transposons systems which differ in their biological properties suitable for utilisation. Some of the features specific for other transposon systems, such as for Mos1, Hsmar1, P-elements or Ac/Ds elements are mentioned further in the following chapter. However Sleeping Beauty, PiggyBac and Tol2 currently represent the most promising transposon systems for transgenesis.

3.1.1. Sleeping Beauty

The Sleeping Beauty (SB) is a synthetic transposon system, that was constructed based on data of Tc1/mariner transposon superfamily found in salmonid fish (Radice et al. 1994). The Tc1-like element was chosen as substrate transposon from the *Tanichthys albonubes* which differs only in 3.8 % from the salmodid fish consensus. The transposase gene was constructed based on molecular phylogenetic data. Elimination of inactive mutations from consensus sequence found in salmonid fish led to reconstitution of transposase ability to provide a cut-and-paste transposition mechanism. Functional domains were identified as the conserved motifs with lower percentage of mutations compared to the regions in between, so this system could be the same or probably equal to an ancient Tc1/mariner system. SB was a first system shown to be functional in mammalian cells (Ivics et al. 1997).

The transposon is flanked by two inverted repeats (IR) and at its termini with two direct repeats (DR) per each IR (Figure 6), referred as left and right inner and outer DR, both required for transposition (Ivics et al. 1997). However, repeats are not equal and excision can be provided only at the outer DRs, started by a double strand break (Cui et al. 2002). Critical for excision are the first and the third nucleotides at the DRs termini (Liu et al. 2004).

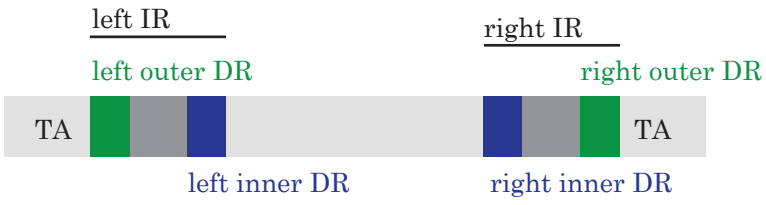

Figure 6: Sleeping Beauty structure, based on Ivics et al. 1997

Target site selection of the SB transposon is non-random, at the primary DNA sequence level SB always integrates into the TA dinucleotides, with preference for the palindromic AT-repeats and for insertion into the central TA (Vigdal et al. 2002). This integration leads to the creation of flanking TA-dinucleotides on the transposon ends and although the excision could occur when the TA on only one side of the transposon is present, activity of the process is extremely diminished. Thus, TA dinucleotides flanking the transposon could be viewed as a necessary requirement for the transposition process (Liu et al. 2004).

On the chromosomal level, SB does not exhibit any preference in the target choice, it exhibits a fairly random integration profile, however with affinity to bendable DNA structures (Vigdal et al. 2002; Huang et al. 2010). Excision of the element begins with a single strand nick that generates a free 3´OH reactive group, the cleavage of second strand is staggered, resulting in 3´overhangs at the transposon ends. These exposed 3´OH ends then facilitate integration into target site by nucleophilic attack, leading to an inserted transposon flanked by single stranded gaps. Repair of these gaps leads to the TA target site duplication and repair of the broken donor site by ends re-joining creates a footprint - a characteristic mark of transposon presence (Figure 7). Footprints generated by the SB at its excision sites are different depending on cell type, as a consequence of different types of host DNA repair machineries (Liu et al. 2004).

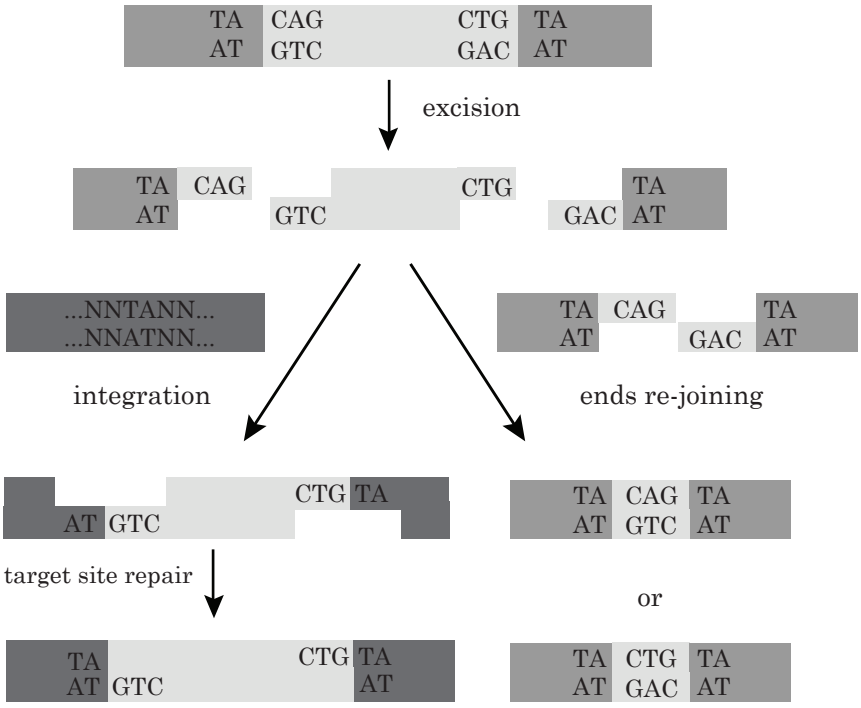

Figure 7: Sleeping Beauty tranposition mechanism, based on Liu et al. 2004

The SB has so far only been used in vertebrates with exception of the chordate *Ciona intestinalis* (Hozumi et al. 2013). Cargo-capacity is relatively proportional to transposon length, since transposition efficiency is decreasing with increasing transposon size (Izsvák et al. 2000). Nevertheless, it is usually possible to reach satisfying levels of the transposition with usage of codon-optimized, hyperactive forms of transposases, such as SB100X which has 100-fold enhanced transposition efficiency compared to originally reported SB transposase (Mátés et al. 2009).

Due to the absence of any related sequence to the SB in mammalian genomes, potential cross-mobilisation between endogenous and exogenously introduced transposons is excluded. This enables utilisation of the SB in precise tasks of mammalian genetics, such as in gene therapy. Without the difficult to detect off-target DNA cleavages and toxicity resulting from the emergency response of the host cell to the introduced double strand breaks, transposon mediated technologies can be employed in the challenging field of treatment of genetically caused human diseases. The SB transposon system rapidly progressed from its development to the application in humans in clinical trials. First reported successful preclinical gene therapy using non-viral vector system was in mouse model of haemophilia B (Yant et al. 2000) and up today, there are three first-in-human clinical trials using SB transposons in treatment of CD19+ B-cell malignancies (Maiti et al. 2013)

3.1.2 PiggyBac

PiggyBac (PB), as a founder member of the transposon piggyBac superfamily, was originally isolated from the cabbage looper moth *Trichoplusia ni* (Lepidoptera) as a naturally active cut-and-paste DNA transposon in this insect species (Fraser and Clszczon 1996). It exhibits innate specificity for the TTAA target sites and excision occurs precisely, without leaving a footprint in the donor sequence and thus allowing for the donor site to be fully restored to its pre-transposon state (Fraser et al. 1995).

The restoration of the donor site is made possible to the specific cleavage leading to complementary TTAA overhangs on the 5´donor DNA ends, which are ligated together by the cell repair mechanisms after the transposon excision. Excision of the transposon is initialized by single strand nicks on the 3´ ends of the transposon, leaving 3´ transposon ends flanked with the reactive OH groups. This 3´ OH then attacks the 5´ complementary ends of TTAA sequence flanking the donor DNA providing the formation of hairpin intermediate structures on the transposon ends. Despite being 4 bp away, the attacked phosphodiester bond on the complementary 5´ strand is relatively near the reactive 3´ OH group because of a twist of the DNA helix. Hairpins are then resolved by the transposase, generating TTAA overhangs on the 5´ transposon ends and leaving a reactive OH groups on 3´ transposon ends. This promotes the covalent joining

of 3´ OH transposon end to the target TTAA/AATT sequence. Incurred single strand gaps in the site of insertion are consequently repaired, leading to the duplication of the target site (Figure 8). The TTAA sequence is an essential requirement for the transposon excision and integration, respectively, as the sequence that is recognized by the transposase enzyme (Mitra et al. 2008).

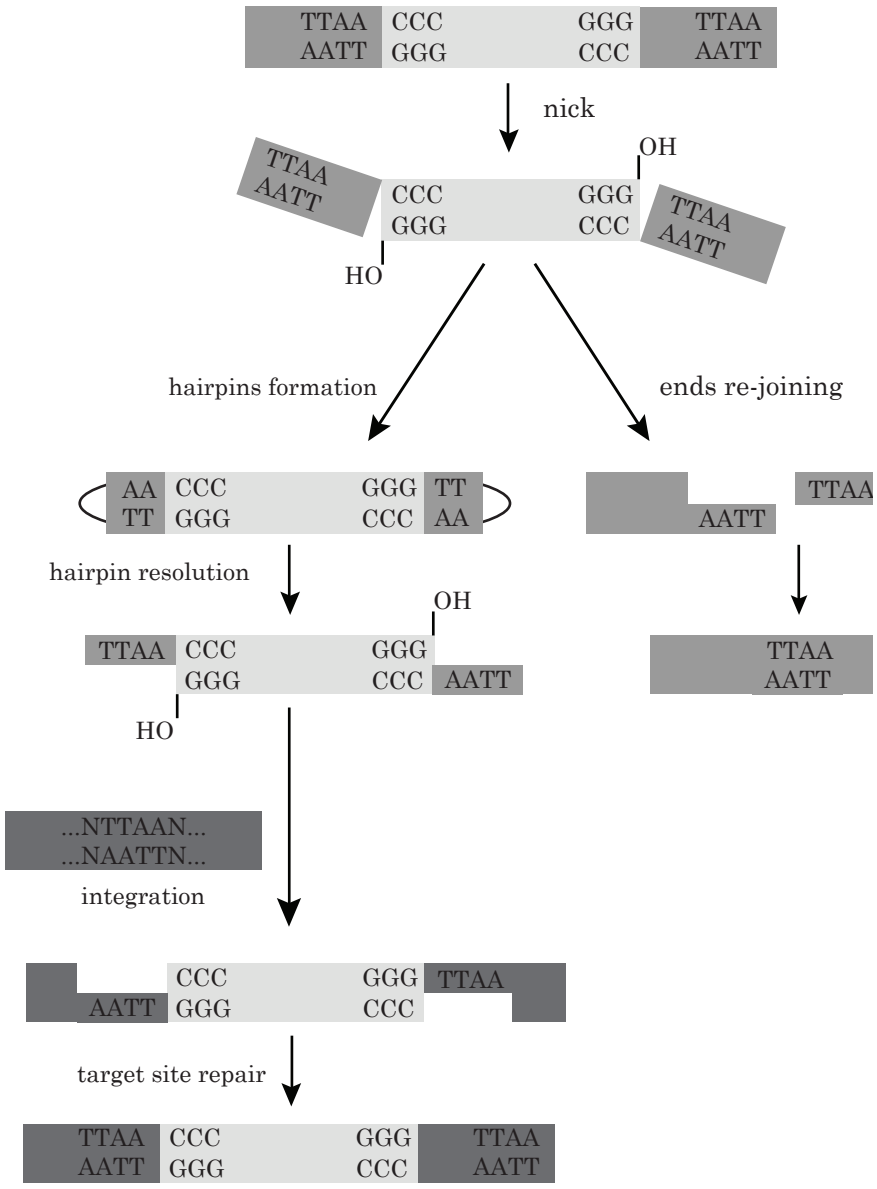

Figure 8: PiggyBac integration mechanism, adapted from Mitra et al. 2008

At the genomic level, PB exhibit a non-random integration profile, with preferred insertions to the actively transcribed genes and towards the transcriptional start sites. However targeting of the intragenic regions and the preference range is variable depending on the cell type (Galvan et al. 2009; Wilson et al. 2007).

From its initial utilisation in insects due to its origin, application of PB quickly spread to a wide range of species and acquired many modifications.

From the *Drosophila* (Hacker et al. 2003), through the challenging *Plasmodium falciparum* (Balu et al. 2005) or vertebrates like mice (Ding et al. 2005) up to the TALE-directed transposition in the human cells (Owens et al. 2013). This wide utilization of PB system in many cell types could indicate lower degree of requirement for host-encoded factors to enable efficient transposition, but this field is relatively unexplored.

The cargo sequence could be quite large, such as even 100 kb, as was tested in mouse embryonic stem cells (ESCs) (Li et al. 2011) or 150 kb as was tested in transgenesis mediated by bacterial artificial chromosomes in human ESCs (Rostovskaya et al. 2012). Nevertheless, transposon size (distance between TIRs) can negatively affect the ratio of successful transposition (Wang et al. 2014). To increase the gene transfer efficiency are utilized codon-optimized hyperactive transposases, such as the HyPBase (Yusa et al. 2011).

3.1.3 Tol2

The cut-and-paste DNA transposon Tol2 from the hAT superfamily was firstly identified as an active element in the medaka fish *Oryzias latipes* (Koga et al. 1996). Subsequently, following the identification of functional transposase, it was developed into a useful transposon-based method (Kawakami et al. 2000).

The transposon excision is initiated by a 5´ donor strand nick, one nucleotide beside the transposon end, leaving this nucleotide connected to the 5´ end of the transposon. This nick generates an exposed 3´ OH on the top donor strand, which joins to the bottom strand of the donor DNA on the 3´ end of the transposon and makes the hairpin formation on the ends of the donor site. This leads to another exposed reactive OH, on the 3´ end of the transposon, which is consequently covalently joined by transposase to the target DNA (Figure 9). Insertion is accompanied by the 8 bp target site duplication, which is a Tol2 characteristic footprint (Zhou et al. 2004).

8 bp target site can vary in nucleotide composition, however preferred are AT-rich regions (Huang et al. 2010). On the genomic level Tol2 has a tendency to integrate into the transcription units (Kondrychyn et al. 2009) and near the transcriptional start sites (Huang et al. 2010).

Although less efficient than SB or PB, this transposon system was active in all vertebrate cells tested so far. However, in the focus of the Tol2 transposon are fish models mainly, especially the zebrafish. Tol2 is capable to mediate integration of even quite large transgenes. Facilitating the insertion of 66 kb sequences into zebrafish and mice, Tol2 was the first transposon system demonstrated to mediate BAC transgenesis (Suster et al. 2009). Stable integration of BAC transgenes into the zebrafish genome was observed for 230 kb long inserts as well (Suster et al. 2011).

Overproduction inhibition was not proved for Tol2, in contrast to the SB or the PB. Another indisputable advantage is that the Tol2, as one of the few transposon systems, belongs to the hAT superfamily. This means it can be used with other transposons from different families, providing the same task, more variants of the transposon systems could be delivered at the same or different time to the same cell without a risk of a cross-mobilisation (Balciunas et al. 2006).

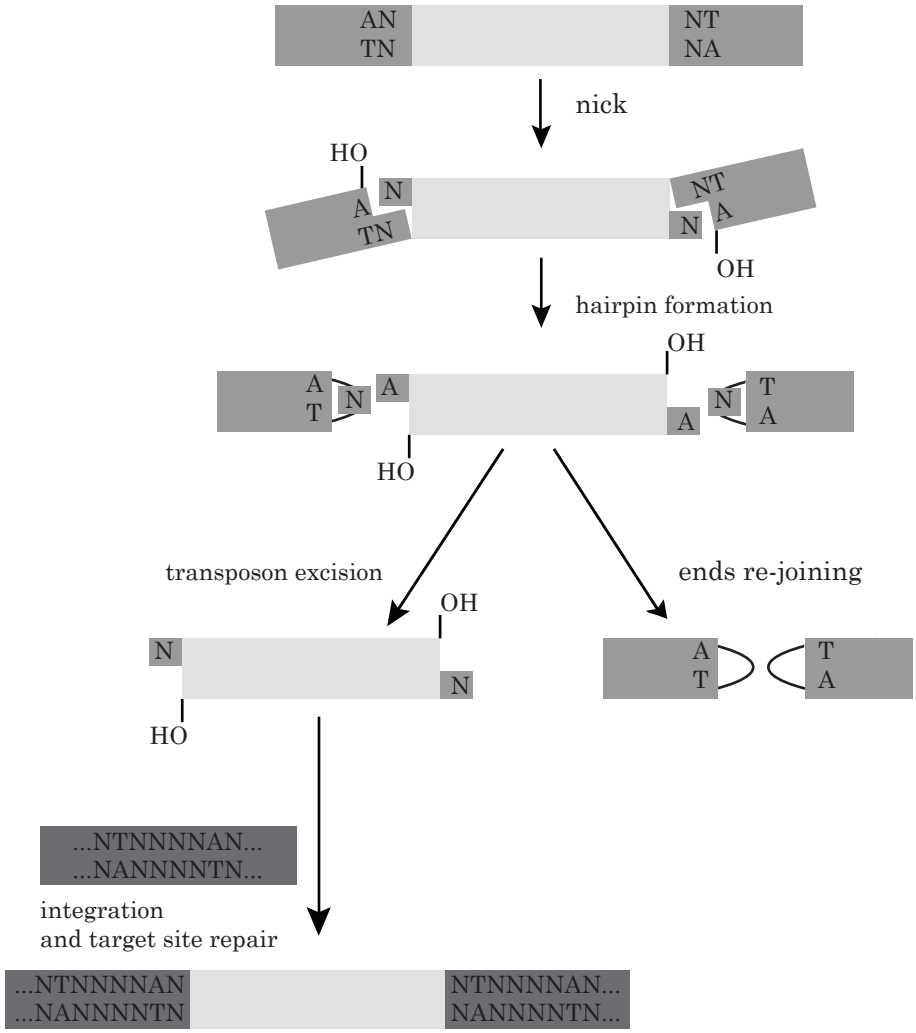

Figure 9: Tol2 integration mechanism, based on Zhou et al. 2004

3.2. Examples of application

Since the whole bi-componet transposon-transposase system can be delivered separately at different times, temporal control of the transposase expression facilitates control over the transposon integration, remobilisation and re-excision.

Transposon-mediated reprogramming of somatic cells to induced pluripotent stem cells (iPSC) encourages the progress of cell-based regenerative medicine. Transgene

expression of transctription factors *Oct4*, *Sox2*, *Klf4* and *Myc* delivered by the PB transposon mediated reprogramming of mouse and human fibroblasts to iPSC with the capability of building functional adult tissues. After the complete reprogramming, integrated transgenes were permanently removed by re-expressing transposase. Since the PB excision does not leave a footprint, iPSCs remain any genetic alteration and can be used in therapeutic applications (Yusa et al. 2009; Woltjen et al. 2009).

Transposons have a stable postion as a genetic tool within several species of model organism, for example the zebrafish *Danio rerio*. Tol2 mediated transposition in zebrafish has been employed to various purposes, for example for insertional mutagenesis by the transposon-mediated enhancer trap (Nagayoshi et al. 2008), phenotypic rescue experiments (Takeuchi et al. 2010), Gal4-UAS gene and enhancer trapping (Asakawa et al. 2009), protein trap mutagenesis (Clark et al. 2011), tissue-specific cell labelling and ablation (Davison et al. 2007) or expresssion of specific peptides (Peng et al. 2010).

Even Tol2 mediated trasgenesis of bacterial artificial chromosomes (BACs) were performed. BACs are DNA constructs, based on a functional fertility plasmids used for transforming and cloning usually in *Escherichia coli,* capable to carry large cargo (O'Connor et al. 1989). BACs performed generation of stable transgenic zebrafish lines to recapitulate endogenous expression patterns are considered as a reliable alternative to in-situ hybridisation in tissues difficult to access for probes and antibiodies (Bussmann et al. 2011).

Utilisation of BACs enables usage of large fragments, such as large promoters and complete genes with all the necessary cis-regulatory elements to study gene regulation and function. Tol2 mediated transgenesis of BACs facilitated generation of zebrafish stable transgenic lines to recapitulate endogenous expression patterns. This could be viewed as a reliable alternative to in-situ hybridisation in even tissues difficult to access for probes and antibodies (Bussmann et al. 2011; Suster et al. 2011). BAC transgenesis with usage of Tol2 transposon results in clean integrations into zebrafish genome and transmission of the transgene to the germline at a rate about 15 %. With this simplifing of BAC transgenesis, transgenic fish were obtained within 3-4 month and labor involved in BAC transgenesis was extremely reduced (Suster et al. 2011)

Potential in therapeutic approaches arised also with modification of human ESCs by engineered variant of PB, ePB. This system capable of delivery of insert up to 18 kb transgenes showed transgene expression in almost 90 % of transfected cells. PB directed human ESC differentiated into neuronal precursors and neurons, followed by transgenes removal without leaving any footprint owe to PB utilisation, confirms the ability of transposons to create fully reversible transgenesis (Lacoste et al. 2009).

3.3. Specific features affecting transposon behaviour

Utilizing different features and specific properties of different transposons helps to overcome possible obstacles during transgenesis. Careful choice of tranposon components has significant influence to the rate of successful transposition.

3.3.1. Aberrant transposition

3.3.1.1. Autointegration

Process of the autointegration occurs when the excised transposon targets itself and integrates into its own sequence leading to intramolecular transposition and self-disruption (Benjamin and Kleckner 1989). Thus this suicidal process could seriously affect the ratio of the delivered transgene transposition (Figure 10).

Under the standard conditions in vitro, the autointegration frequency is estimated to be higher than 90 % in the mariner transposition (Claeys Bouuaert and Chalmers 2010), however different transposons may vary according to the presence of cis transposon target sites suitable for traposition, or up to the transposon length. The frequency of transposition is affected by the transposon length itself already before the excision step, nevertheless autointegration can contribute to the unsuccessful tranposition (Wang et al. 2014). Regulation of the autointegration was well characterized as an interaction between the transposon and host factors in some of bacterial transposons, such as a Tn10 (Wardle et al. 2005), but detailed information about it is still lacking in case of eukaryotic transposons, with only exception for the supposed analogies.

Autointegration was observed for the Mos1 transposon (Sinzelle et al. 2008), for the Hsmar1 transposons (Claeys Bouuaert and Chalmers 2010) or with comparable frequences and higher efficiency for longer tranposons in the SB and PB systems. In case of the SB, autointegration proceed even in the zebrafish, despite fish is the SB natural cellular environment (Wang et al. 2014).

Successful transposition rates in the natural hosts could be attributed to possible recruitment of host-encoded factors, which may protect transposon against self-disruption through association with the pre-integration complex. As a plausible host-encoded factor influencing fate of SB and PB in tested heterologous environments was identified barrier-to-autointegration factor BANF1. This factor probably could be recruited into a higher order protein complexes involved in conserved cellular mechanisms, such as was proved for the HMGXB4 transcription factor, which interacts with the given SB tranposase in mammalian cells (Wang et al. 2014).

Conceivable utilisation of host-encoded factors to provide the precise transposition can negatively affect the accuracy of the transposition in user-defined host, which could be too evolutionary distant to the transposon natural environment and thus lack factors that can be recruited. In this manner, experimental conditions can bias transposition quality or even applicability of a given transposon (Wang et al. 2014).

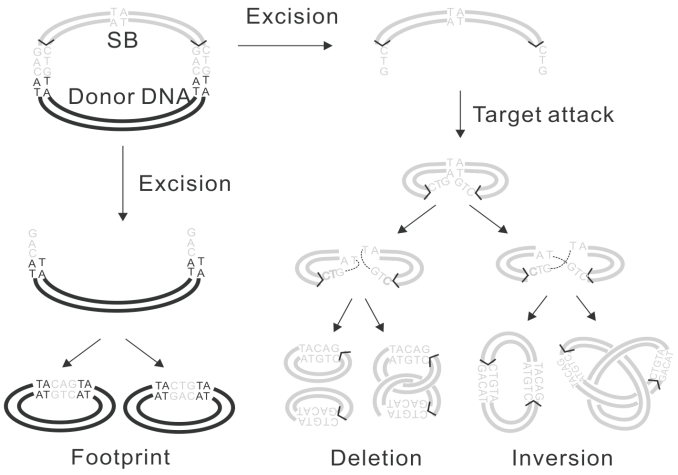

Figure 10: Autointegration. Autointegrative transposition events can be rescued in the form of either two deletion circles or a single inversion circle, depending on the topology of the strand attack. Adopted from Wang et al. 2014.

3.3.1.2. Incorrect recognition of the transposon ends

Misunderstanding of the transposase in the TIRs recognition may leads to the pseudo-transposition results, such as a single-ended transposition. This aberrant event involves a transposase interacting with a single transposon end, which is transposed either inside (autointegration) or outside the original transposon with the second end remained in the donor locus - does not participating in transposition process.

The SB and the PB were noticed to be capable of excision of the whole transposon and further transposition with one of the IRs missing in low rates (Izsvák et al. 2002; Wang et al. 2014). However, single-ended transposition with one of the TIRs remained in the donor site was observed only for the PB system so far (Wang et al. 2014).

Transposase misunderstanding in the TIRs recognition can even involve utilization of two ends from two separate transposons, which could be integrated into the same target site (Wang et al. 2014), but sufficiency of experimental data to the broad understanding are still lacking. Although these rare excision mistakes should not affect proper delivery of the transgene in general, they can be genotoxic during the transposon remobilisation or re-excision in case of the intended transient presence of the transgene.

3.3.2. Overproduction inhibition

Phenomenon of the overproduction inhibition (OPI) refers to the natural autoregulation mechanism, when excessive levels of the transposase production leads to reduced levels of the overall excision activity, detected firstly for the wild-type transposases *in vivo* in transgenic *Drosophila* (Lohe and Hartl 1996) and *in vitro* even before (Chalmers and Kleckner 1994).

Emloyment of the transposon systems, often with the transposase driven by strong promoters or utilisation of genetically engineered transposases to obtain high levels of the transition activity, over-production inhibition could affect the success of the transgene integration. Nevertheless, this self-regulatory mechanism, showing difference among different transposon families, is not yet fully understood and experimental results are inconsistent. Observed were three cases of the transposition behaviour according to the increasing concentration rate of the transposase to the transposon. Constant increasing of the transposition activity with no evidence of OPI (Jaillet et al. 2012), increasing transposition up to the maximum value of the transposase concentration and consequent OPI detected above this threshold (Lohe and Hartl 1996; Staunstrup et al. 2011) or increasing transposition up to the maximum value and then stable transposition keeping on this maximum (Wilson et al. 2007).

Identified results differ also for the usage of the same transposon system, such as for the PB. OPI was not observed during the usage of the PB system in mouse embryonic stem cells, despite the used transposase was codon-optimized to provide greater transposition levels than the original transposase (Cadiñanos and Bradley 2007). Nevertheless, OPI was observed for the case of PB system in vivo in mouse (Nakanishi et al. 2010).

These discrepancies possibly could be explained by cellular factors interacting with the transposase, which could be absent in mouse ES cells contrary to their presence in mouse *in vivo*, such as is deliberated by Bire et al. With presence of host-encoded co-factors, the transposase could be inhibited after it crosses the threshold for the transposition optimum value as expected defence, since the overblown transposition can make genotoxic effects to the host (Bire, Casteret, et al. 2013).

OPI also might be influenced by the transfection efficiency, such as might be in the PB transfection of HEK239 cells by Wu et al. - with maximal number of colonies about 4 000 and detected OPI (Wu et al. 2006) contrary to Wilson et al. - with maximal number of colonies about 200 and with no OPI present (Wilson et al. 2007). In both cases were used equivalent amounts of the transposases and colonies were counted after 14 days. However, transposon systems have been independently tested under possibly different additional conditions in different laboratories, so for more conclusions further investigation is needed.

Recently, computer predicted, assembly-site-occlusion model predominantly based on the Hsmar1, but with similar behaviour in testing of the SB and the PB, might suggest OPI control mechanism. Point of view is assuming the transposition amount controlled by ratio of the transposase to transposon ends instead of control by concentration of the transposase. With the rising concentration of the transposase bounding to transposon ends, number of unbound ends decrease. Thus the chance to find naked end needed to the synaptic complex formation is decreasing, which also leading to the down-regulated level of the transposition (Claeys Bouuaert et al. 2013). However, if behaviour of other transposons report to this system is question of further research.

3.3.3. Local hopping

Transposon movement is affected by the additional genomic features, including the distance from the transposon donor site. This trend is called a local hopping and reflects a tendency to choose a target site physically close to the transposon donor site, however significantly influenced by the physical accessibility of chromatin. Physically easy accessible sites in euchromatin are integration hot spots and are consequently independent of the donor locus, thus not affected by the local hopping (Li et al. 2013).

Local hopping seems to be a general property of transposon systems, but with variable length of local transposition intervals and variable frequency according to the used transposase, organism or even different genomic loci using the same transposon system, all the more so with different system respectively.

For Tol2 transposon, 4 Mbp range of local hopping within the same chromosome with 85% frequency was detected in mouse, using the codon-optimized iTol2 transposase for multicopy donor (Keng et al. 2009). On the other hand, 300 kbp range with the 9% local hopping frequency were observed in zebrafish (Urasaki et al. 2008) or up to 5 Mbp range with 60% frequency in *Xenopus tropicalis* (Lane et al. 2013), using a singlecopy donor and codon nonoptimized transposase.

However, different hopping patterns within various condition or hosts are not fully deciphered, which could be attendant on less comprehension of host-encoded factors that influence the transposition. Another instances of observed local hopping could be P elements (Tower et al. 1993) or Ac/Ds elements (Bancroft and Dean 1993). Inclination to choosing physically close donor sites to integration exhibit SB (Keng et al. 2005) and also PB (Li et al. 2013), despite previous investigations that PB is not touched by this phenomenon (Hacker et al. 2003; Ding et al. 2005). However the inconsistency of the PB tendency to local hopping could be assigned to the transiently expressed transposase instead of previous constitutive expression, with which effect of local hopping was not observed.

Local hopping is a useful feature during the investigation of genome regulatory architecture. Random inserted tranposons containing a reporter gene are forming overlapping blocks, allowing observation of long-range regulatory activities along chromosomes. Thanks to local hopping, it is possible to provide systematic analysis of regions of interest by reusing insertions obtained previously a thus focuse the view to the specific area (Ruf et al. 2011).

3.3.4. Transgene silencing

Transposons present endogenously have to face host defence mechanisms naturally trying to make them silenced to prevent any potential dangerous effects caused by the transposon spreading in the genome. One of these mechanisms is a heterochromatin formation by the dense CpG sites methylation, which is affecting most of the transposons present in eukaryotic genomes (Zemach et al. 2010).

Thus CpG methylation could also affect the transposition or sufficient expression of the integrated transgene. In mouse embryonic stem cells, the CpG methylated PB transposon decreased in its transposition efficiency 12-fold (Wang et al. 2008), further the SB was proved to be affected by the post-integrative transgene silencing in HeLa cells and its true integrative potential estimated to be 25 times higher than the previously reported because of not detected integrations due to the silencing.

Designation of the artificial sequence as a target for silencing could be a sequence rich in CpG dinucleotides, since also careful choice of transposon components, such as a promoter or cargo, maybe determinants of persistence of the transgene expression (Garrison et al. 2007). For example some eGFP variants appears to be sensitive to the CpG methylation due to its high level of CpG dinucleotides (Dalle et al. 2005).

To overcome this silencing effect without the transposon targeting to the continuously expressed site, it is possible to use genetic insulators. Employing of the insulator sequences flanking the transgene can protect transcribed regions against the spread of heterochromatin and protect their expression (Dalsgaard et al. 2009). As an example of utilized protection system can be mentioned 5´-HS4 chicken ß-globin, that is a sequence used as a genetic insulator (Bire, Ley, et al. 2013)

Despite of the silencing mechanism, transposition of the SB and other transposons sharing the same inverted repeat/direct repeat (IR/DR) structure of terminal repeats is unexpectedly enhanced by the CpG methylation, even more than 10-fold (Jursch et al. 2013). These terminal repeats are consisting of two binding sites for the transposase in the same orientation referred as direct repeats (DRs) on both sides of transposon (Izsvák et al. 1995), nevertheless methylation of the IR/DR contained CpG sites does not directly influence the transposition and even enhanced affinity of the transposase for the CpG-methylated

DNA was not proven. Since the CpG methylation is not required for the transposition, offered mechanism supporting the transposition enhancement is DNA condensation associated with the CpG methylation and subsequent heterochromatin formation. Proposed mechanism is possible aid of the synaptic complex formation by closer physical distance of transposase binding sites localized on TIRs, due to the heterochromatin arrangement (Jursch et al. 2013). Affinity of the SB transposase for the heterochromatin regions was already proved (Ikeda et al. 2007).

3.4 Transposon targeting

Almost random DNA insertion into the recipient genome is great tool for transposon-mediated insertional mutagenesis in genetic screening. Cancer gene discovery in mice (Rad et al. 2010) or analysis of regulatory activities of mouse genome (Ruf et al. 2011) can be mentioned as excellent examples. However, random insertion can be associated with position effects and undesired insertional mutagenesis during the precise tasks, such as in gene therapy.

The position effect can generate substantial variation of the transgene expression among lines, associated with silencing or enhancing of the transgene owe to the local chromatin environment in the insertion place, such as was observed in telomeric regions (Gao et al. 2007; Pedram et al. 2006).

Undesired random integration is currently conquered via several approaches of the site-directed DNA integration using the fusion protein constructions or utilisation of the local hopping, which results into the integration near to the donor site (Keng et al. 2005). Targeting, defined in the transposon-based technologies as a biased insertion in the meaning of enrichment transpositions adjacent to the DNA binding domain (DBD) target sequence, is highly tested on members of the Tc1/mariner transposon family, mostly on the SB system, owe its well characterization.

To estimate the transposition directivity is used an inter-plasmid assay, constituted from the donor plasmid containing the transposon sequence and antibiotic resistance, the acceptor plasmid containing the DBD recognition site plus another antibiotic resistance and the helper plasmid coding the transposase or fusion proteins. Plasmids are simultaneously inserted to the cell and selection only on complete transfer into the acceptor plasmid is provided (Ivics et al. 1997).

Modification could be done to the transposase, by the adding a new DBD, alternative is indirect targeting either the transposon or the transposase by DBD fused to the transposon/transposase-binding protein. Another possibility is fusion of two DBDs when one DBD binds to the targeted integration site and the second DBD binds a sequence inserted into the transposon (Ivics et al. 2007).

However, DBD joined to the transposase can reduce the transposase catalytic activity up to the complete loss and inability of the transposition reaction. Inhibition degree is quite various, from the complete deprivation up to the nearly no detectable inhibition.

For example, complete loss of the transposase function was observed in case of addition of the Sp1 human zinc finger DBD to the C-terminus of the hyperactive SB12 transposase, which is responsible for the DNA cleavage and strand transfer. On the other hand, when the the same DBD was added to the N-terminus, observed transposition activity was similar to the native, non-hyperactive SB transposase. Moreover addition of this DBD to the SB transposase N-terminus reduced the occurrence of the over-production inhibition phenomenon as an added value. Observed reduced catalytic activity after the binding of DBD to the transposase C-terminus did not change even with the addition of another nuclear localization signal (Wilson et al. 2005) or the change of the peptide linker between DBD and transposase. So, extremely diminished transposition activity after the fusion of DBD to the transposase C-terminus seems to be a general trend, which could be connected with the catalytic activity of the C-terminal domain (Yant et al. 2007).

As DNA binding proteins with the desired flexibility and specificity, in the sense that they can be designed to target any sequence, has been used user-defined synthetic zinc finger proteins (Wilson et al. 2005) and transcription activator-like effectors (Owens et al. 2013a). Utilization of zinc finger proteins (ZNF) and transcription activator-like-effectors proteins (TALE) as a programmable sequence specific DNA-binding modules already proved its potential in the connection with nucleases-based technologies including zinc-finger nucleases (Kim et al. 1996) and transcription activator-like effector nucleases - TALENs (Boch et al. 2009). Targeting of the genomic site by the ZNF is limited by the sequence requirements needed, however ZNFs were proved to make an inserting bias to the targeting into the desired sequence. This was possible in the plasmid and also in the cell context due to the improved transpositional activity by the using of hyperactive transposases to overcome normally diminished transposition efficiency caused by the ZNF fusion.

In some cases, better results in transposition efficiency were obtained by the fusion including a protein linker between the transposase and the ZNF domain instead of the direct fusion. For example for the hyperactive transposase SB100X fused by the protein linker to the ZNF domain engineered to target human L1 element, the transposase shows about nearly 40 % of transpositional activity compared to the unfused control SB100X (Voigt et al. 2012). Nevertheless, data indications of the linker impact to the transposition efficiency are inconsistent. Contrary to the mentioned profitable influence of the linker length with SB100X, no influence of the linker length was observed with the SB12 transposase fused to the ZNF202 (Wilson et al. 2005).

According to the proposed flexing mode, physical constraints can restrain targeted

transposition. That means the case, when the transposase is too tightly bounded to the target DNA, which makes the catalysis of the transposition reaction impossible (Yant et al. 2007).

However different transposases react to the protein fusions differently and variations appear also determined by the origin of cells. Contrary to the weak targeting efficiency in mammalian cells, the Gal4 domain specifically recognizing the UAS sequence was succesfully used in the site-directed transposition in insect models. The Gal4-Mos1 construct extend 96 % of insertions to the TA dinucleotide 954 bp away from the UAS target sequence and Gal4-piggyBac construct gave 67 % of insertions to the target 912 bp from the UAS, both in interplasmid system tranfected to Aedes aegypti embryos (Maragathavally et al. 2006).

Influence to the targeting has also user-defined target site, which needs to be accessible for the transpositional complex and in the balance with the transposase preferences for insertions. This is ideally flanked by favoured sequences (Voigt et al. 2012), such as TA-dinucleotides in case of SB (Liu et al. 2005), in all used aproaches.

Usage of the TALE domain as DBD is a relatively recent investigation and is affected by the low efficiency of integration to regions adjacent to the DBD recognition site. In investigated TALE-directed piggyBac transposition was observed approximately 0.014 % cells received targeted insertions from the set of stably transfected cells during usage of the fusion of a TALE DBD through protein linker to the hyperactive PB transposase. Utilisation of the tethering of the TALE to the delivery plasmid backbone using the Gal4/UAS system leads to the 0.010 % of cells with targeted insertion. Despite tethering of the whole system via the TALE DBD, targeting to the desired sequence, linked to the Gal4, which bounded to UAS sites incorporated in the plasmid backbone (Figure 11), transposition rate was not improved.

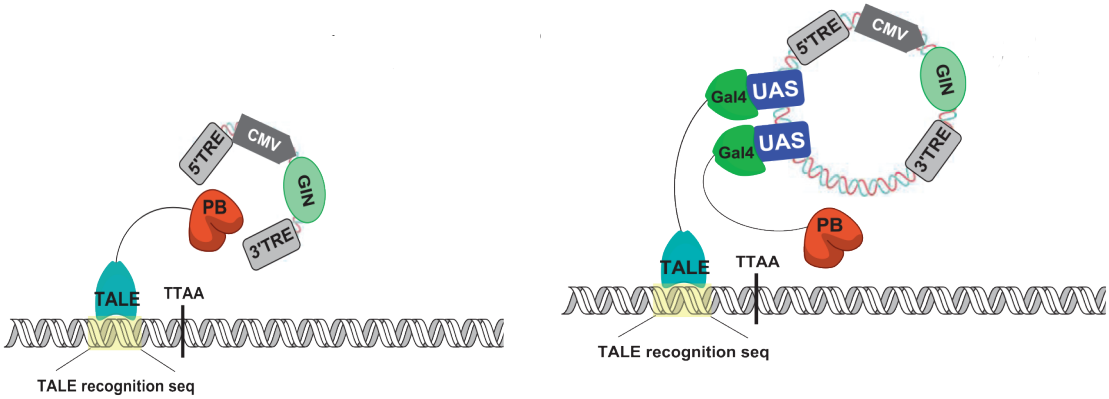

Figure 8: TALE-constructs for transpon targeting, adapted from Owens et al. 2013b left - a hyperactive PB transposase covalently linked to a TALE, designed to bind a specific sequence right - a double-DBD protein including TALE linked to Gal4, tethering of the plasmid to the target is mediated by Gal4 binding to UAS sites found on the plasmid backbone and TALE binding to the genomic recognition sequence

The functional targeted transposition was also done by the combination of both approaches in the single transfection and the closest insertion was only 24 bp distant from the TALE-DBD, encouraging further investigation of the system. Because transposases in fusion proteins still retain their natural ability to bind the target DNA independent from the fused DBD, authors also anticipate suggestions to improvements. For example making the localization/binding of used transposon system to the target sequence a necessary event for the transposition, ideally together with the inhibition by binding of off-target sequences, can significantly improve whole system (Owens et al. 2013).

4. Conclusion

Transgenic technologies based on transposon are potent approaches that contribute to advances in molecular genetics and consequently help to understand functional characterisation of genomes. Transposons can be suitable used as gene trapping vectors, that can express a reporter molecule in a context dependent manner and thus provide insight functional genomics through gene discovery, studying of gene expression and gene regulation, for instance via gene or promoter trapping. Ongoing attempts with transposons in reverse genetics, manipulating known genes and connecting the certain DNA sequence with specific effects on the phenotype, open a wide range of applications in targeted genome engineering, including human therapy.

Careful choice of the tranposon system according to its specific features, such as is overproduction inhibion, local hopping or sensitivity to autointegration and transgene silencing, has significant influence to the rate of successful transposition.

Utilizing modified and optimized transposons from different superfamilies and their specific properties gives a broad range to overcome possible obstacles in the given host. The risk of potential cross-mobilisation of endogenous transposons is excluded, because lack of sufficient level of sequence similarity, with preserved option of transgene inducibility and reversibility by controlled delivery of transposase. Their unique attribute of active integration mechanism, catalysis of the most crucial step in transgene delivery is making from transposon technologies potent tool of genetic engineering even in host with the low level of homologous recombination.

5. References

Asakawa, K. & Kawakami, K., 2009. The Tol2-mediated Gal4-UAS method for gene and enhancer trapping in zebrafish. Methods (San Diego, Calif.), 49(3), pp.275–81.

Aziz, R.K., Breitbart, M. & Edwards, R. a, 2010. Transposases are the most abundant, most ubiquitous genes in nature. Nucleic acids research, 38(13), pp.4207–17.

Balciunas, D. et al., 2006. Harnessing a high cargo-capacity transposon for genetic applications in vertebrates. PLoS genetics, 2(11), p.e169.

Balu, B. et al., 2005. High-efficiency transformation of Plasmodium falciparum by the lepidopteran transposable element piggyBac. Proceedings of the National Academy of Sciences of the United States of America, 102(45), pp.16391–6.

Bancroft, I. & Dean, C., 1993. Transposition pattern of the maize element Ds in Arabidopsis thaliana. Genetics, 134, pp.1221–1229Benjamin, H. & Kleckner, N., 1989. Intramolecular transposition by Tn10. Cell, 59, pp. 373-383.

Bire, S., Ley, D., et al., 2013. Optimization of the piggyBac transposon using mRNA and insulators: toward a more reliable gene delivery system. PloS one, 8(12), p.e82559.

Bire, S., Casteret, S., et al., 2013. Transposase concentration controls transposition activity: myth or reality? Gene, 530(2), pp.165–71.

Boch, J. et al., 2009. Breaking the code of DNA binding specificity of TAL-type III effectors. Science (New York, N.Y.), 326(5959), pp.1509–12.

Bussmann, J. & Schulte-Merker, S., 2011. Rapid BAC selection for tol2-mediated transgenesis in zebrafish. Development (Cambridge, England), 138(19), pp.4327–32.

Cadiñanos, J. & Bradley, A., 2007. Generation of an inducible and optimized piggyBac transposon system. Nucleic acids research, 35(12), p.e87.

Claeys Bouuaert, C. et al., 2013. The autoregulation of a eukaryotic DNA transposon. eLife, 2, p.e00668.

Claeys Bouuaert, C. & Chalmers, R., 2010. Transposition of the human Hsmar1 transposon: rate-limiting steps and the importance of the flanking TA dinucleotide in second strand cleavage. Nucleic acids research, 38(1), pp.190–202.

Clark, K.J. et al., 2011. In vivo protein trapping produces a functional expression codex of the vertebrate proteome. Nature methods, 8(6), pp.506–15.

Cohen, S.N. et al., 1973. Construction of biologically functional bacterial plasmids in vitro. Proceedings of the National Academy of Sciences of the United States of America, 70(11), pp.3240–4.

Craig, N., 1995. Unity in transposition reactions. Science, 2(October), pp.2–3.

Cui, Z. et al., 2002. Structure–Function Analysis of the Inverted Terminal Repeats of the Sleeping Beauty Transposon. Journal of Molecular Biology, 318(5), pp.1221–1235.

Dalle, B. et al., 2005. eGFP reporter genes silence LCRbeta-globin transgene expression via CpG dinucleotides. Molecular therapy : the journal of the American Society of Gene Therapy, 11(4), pp.591–9.

Dalsgaard, T. et al., 2009. Shielding of sleeping beauty DNA transposon-delivered transgene cassettes by heterologous insulators in early embryonal cells. Molecular therapy : the journal of the American Society of Gene Therapy, 17(1), pp.121–30.

Davison, J., Akitake, C. & Goll, M., 2007. Transactivation from Gal4-VP16 transgenic insertions for tissue-specific cell labeling and ablation in zebrafish. Developmental Biology, 304(2), pp.811–824.

Ding, S. et al., 2005. Efficient transposition of the piggyBac (PB) transposon in mammalian cells and mice. Cell, 122(3), pp.473–83.

Fraser, M. & Clszczon, T., 1996. Precise excision of TTAA-specific lepidopteran transposons piggyBac (IFP2) and tagalong (TFP3) from the baculovirus genome in cell lines from two species of Lepidoptera. Insect Molecular Biology, 5(2), pp.141–151.

Fraser, M. & Clszczon, T., 1996. Precise excision of TTAA-specific lepidopteran transposons piggyBac (IFP2) and tagalong (TFP3) from the baculovirus genome in cell lines from two species of Lepidoptera. Insect Molecular Biology 5, pp.141–151.

Galvan, D., Nakazawa, Y. & Kaja, A., 2009. Genome-wide mapping of PiggyBac transposon integrations in primary human T cells. Journal of immunotherapy, 32(8), pp 837-844.

Gao, Q. et al., 2007. Telomeric transgenes are silenced in adult mouse tissues and embryo fibroblasts but are expressed in embryonic stem cells. Stem cells (Dayton, Ohio), 25(12), pp.3085–92.

Gardner, M.J. et al., 2002. Genome sequence of the human malaria parasite Plasmodium falciparum. Nature, 419(6906), pp.498–511.

Garrison, B.S. et al., 2007. Postintegrative gene silencing within the Sleeping Beauty transposition system. Molecular and cellular biology, 27(24), pp.8824–33.

Gordon, J.W. & Ruddle, F.H., 1981. Integration and Stable Germ Line Transmission of Genes Injected into Mouse Pronuclei. Science (New York, N.Y.), 214, pp.1244–1246.

Hacker, U. et al., 2003. piggyBac-based insertional mutagenesis in the presence of stably integrated P elements in Drosophila. Proceedings of the National Academy of Sciences of the United States of America, 100(13), pp.7720–5.

Hou, Y. et al., 2010. Retrotransposon vectors for gene delivery in plants. Mobile DNA, 1(1), p.19.

Hozumi, A. et al., 2013. Germline transgenesis of the chordate Ciona intestinalis with hyperactive variants of sleeping beauty transposable element. Developmental dynamics : an official publication of the American Association of Anatomists, 242(1).

Huang, X. et al., 2010. Gene transfer efficiency and genome-wide integration profiling of Sleeping Beauty, Tol2, and piggyBac transposons in human primary T cells. Molecular therapy : the journal of the American Society of Gene Therapy, 18(10), pp.1803–13.

Chakraborty, A. et al., 1993. Synthetic retrotransposon vectors for gene therapy. The FASEB journal, 58, pp.8500–8502.

Chalmers, R.M. & Kleckner, N., 1994. Tn10/IS10 transposase purification, activation, and in vitro reaction. The Journal of biological chemistry, 269(11), pp.8029–35.

Ikeda, R. et al., 2007. Sleeping beauty transposase has an affinity for heterochromatin conformation. Molecular and cellular biology, 27(5), pp.1665–76.

Ivics, Z. et al., 1997. Molecular reconstruction of Sleeping Beauty, a Tc1-like transposon from fish, and its transposition in human cells. Cell, 91(4), pp.501–10.

Ivics, Z. et al., 2007. Targeted Sleeping Beauty transposition in human cells. Molecular therapy : the journal of the American Society of Gene Therapy, 15(6), pp.1137–44.

Ivics, Z. & Izsvák, Z., 2010. The expanding universe of transposon technologies for gene and cell engineering. Mobile DNA, 1(1), p.25.

Izsvák, Z. et al., 2002. Involvement of a bifunctional, paired-like DNA-binding domain and a transpositional enhancer in Sleeping Beauty transposition. The Journal of biological chemistry, 277(37), pp.34581–8.

Izsvák, Z., Ivics, Z. & Hackett, P.B., 1995. Characterization of a Tc1-like transposable element in zebrafish (Danio rerio). Molecular & general genetics : MGG, 247(3), pp.312–22.

Izsvák, Z., Ivics, Z. & Plasterk, R.H., 2000. Sleeping Beauty, a wide host-range transposon vector for genetic transformation in vertebrates. Journal of molecular biology, 302(1), pp.93–102.

Jaillet, J. et al., 2012. Regulation of mariner transposition: the peculiar case of Mos1. PloS one, 7(8), p.e43365.

.
Jursch, T. et al., 2013. Regulation of DNA transposition by CpG methylation and chromatin structure in human cells. Mobile DNA, 4(1), p.15.

Kawakami, K., Shima, a & Kawakami, N., 2000. Identification of a functional transposase of the Tol2 element, an Ac-like element from the Japanese medaka fish, and its transposition in the zebrafish germ lineage. Proceedings of the National Academy of Sciences of the United States of America, 97(21), pp.11403–8.

Keng, V. et al., 2005. Region-specific saturation germline mutagenesis in mice using the Sleeping Beauty transposon system. Nature methods, 2(10), pp.763–769.

Keng, V.W. et al., 2009. Efficient transposition of Tol2 in the mouse germline. Genetics, 183(4), pp.1565–73.

Kim, Y.G., Cha, J. & Chandrasegaran, S., 1996. Hybrid restriction enzymes: zinc finger fusions to Fok I cleavage domain. Proceedings of the National Academy of Sciences of the United States of America, 93(3), pp.1156–60.

Koga, A. et al., 1996. Transposable element in fish. Nature.
Kondrychyn, I. et al., 2009. Genome-wide analysis of Tol2 transposon reintegration in zebrafish. BMC genomics, 10, p.418.

Lacoste, A., Berenshteyn, F. & Brivanlou, A.H., 2009. An efficient and reversible transposable system for gene delivery and lineage-specific differentiation in human embryonic stem cells. Cell stem cell, 5(3), pp.332–42.

Lander, E.S. et al., 2001. Initial sequencing and analysis of the human genome. Nature, 409(6822), pp.860–921.

Lane, M. a, Kimber, M. & Khokha, M.K., 2013. Breeding based remobilization of Tol2 transposon in Xenopus tropicalis. PloS one, 8(10), p.e76807.

Li, M.A. et al., 2011. Mobilization of giant piggyBac transposons in the mouse genome. Nucleic acids research, 39(22), p.e148.

Li, M.A. et al., 2013. The piggyBac transposon displays local and distant reintegration preferences and can cause mutations at noncanonical integration sites. Molecular and cellular biology, 33(7), pp.1317–30.

Liu, G. et al., 2005. Target-site preferences of Sleeping Beauty transposons. Journal of molecular biology, 346(1), pp.161–73.

Liu, G., Aronovich, E. & Cui, Z., 2004. Excision of Sleeping Beauty transposons: parameters and applications to gene therapy. The journal of gene medicine, 6(5), pp.574–583.

Lohe, A. & Hartl, D., 1996. Autoregulation of mariner transposase activity by overproduction and dominant-negative complementation. Molecular biology and evolution, pp.549–555.

Maiti, S., Huls, H. & Singh, H., 2013. Sleeping beauty system to redirect T-cell specificity for human applications. Journal of immunotherapy 36(2), pp.112–123.

Maragathavally, K.J., Kaminski, J.M. & Coates, C.J., 2006. Chimeric Mos1 and piggyBac transposases result in site-directed integration. FASEB journal : official publication of the Federation of American Societies for Experimental Biology, 20(11), pp.1880–2.

Mátés, L. et al., 2009. Molecular evolution of a novel hyperactive Sleeping Beauty transposase enables robust stable gene transfer in vertebrates. Nature genetics, 41(6), pp.753–61.

McClintock, B., 1948. Mutable loci in maize. Carnegie Institute Washington Year Book, 47.

Mitra, R., Fain-Thornton, J. & Craig, N.L., 2008. piggyBac can bypass DNA synthesis during cut and paste transposition. The EMBO journal, 27(7), pp.1097–109.

Nagayoshi, S. et al., 2008. Insertional mutagenesis by the Tol2 transposon-mediated enhancer trap approach generated mutations in two developmental genes: tcf7 and synembryn-like. Development (Cambridge, England), 135(1), pp.159–69.

Nakanishi, H. et al., 2010. piggyBac transposon-mediated long-term gene expression in mice. Molecular therapy : the journal of the American Society of Gene Therapy, 18(4), pp.707–14.

O'Connor, M., Peifer, M. & Bender, W., 1989. Construction of large DNA segments in Escherichia coli. Science, 244, pp.1307–1312

Owens, J.B. et al., 2013. Transcription activator like effector (TALE)-directed piggyBac transposition in human cells. Nucleic acids research, 41(19), pp.9197–207.

Palazzoli, F. et al., 2010. Transposon tools: worldwide landscape of intellectual property and technological developments. Genetica, 138(3), pp.285–99.

Pedram, M., Sprung, C. & Gao, Q., 2006. Telomere position effect and silencing of transgenes near telomeres in the mouse. Molecular and cellular biology, 26(5).

Peng, K.-C. et al., 2010. Using an improved Tol2 transposon system to produce transgenic zebrafish with epinecidin-1 which enhanced resistance to bacterial infection. Fish & shellfish immunology, 28(5-6), pp.905–17.

Rad, R. et al., 2010. PiggyBac transposon mutagenesis: a tool for cancer gene discovery in mice. Science (New York, N.Y.), 330(6007), pp.1104–7.

Radice, A. et al., 1994. Widespread occurrence of the Tc1 transposon family: Tc1-like transposons from teleost fish. MGG Molecular & General Genetics, 244(6).

Rostovskaya, M. et al., 2012. Transposon-mediated BAC transgenesis in human ES cells. Nucleic acids research, 40(19), p.e150.

Ruf, S. et al., 2011. Large-scale analysis of the regulatory architecture of the mouse genome with a transposon-associated sensor. Nature genetics, 43(4), pp.379–86.

Sinzelle, L. et al., 2008. Factors acting on Mos1 transposition efficiency. BMC molecular biology, 9, p.106.

Skipper, K.A. et al., 2013. DNA transposon-based gene vehicles - scenes from an evolutionary drive. Journal of biomedical science, 20, p.92.

Staunstrup, N.H. et al., 2011. A Sleeping Beauty DNA transposon-based genetic sensor for functional screening of vitamin D3 analogues. BMC biotechnology, 11(1), p.33.

Suster, M.L. et al., 2011. Transposon-mediated BAC transgenesis in zebrafish. Nature protocols, 6(12), pp.1998–2021.

Suster, M.L., Sumiyama, K. & Kawakami, K., 2009. Transposon-mediated BAC transgenesis in zebrafish and mice. BMC genomics, 10, p.477.

Takeuchi, M. et al., 2010. Efficient transient rescue of hematopoietic mutant phenotypes in zebrafish using Tol -mediated transgenesis. Development, Growth & Differentiation, pp.245–250.

Tower, J. et al., 1993. Preferential transposition of Drosophila P elements to nearby chromosomal sites. Genetics, 359, pp.347–359.

Urasaki, A., Asakawa, K. & Kawakami, K., 2008. Efficient transposition of the Tol2 transposable element from a single-copy donor in zebrafish. Proceedings of the National Academy of Sciences of the United States of America, 105(50), pp.19827–32.

Vigdal, T.J. et al., 2002. Common Physical Properties of DNA Affecting Target Site Selection of Sleeping Beauty and other Tc1/mariner Transposable Elements. Journal of Molecular Biology, 323(3), pp.441–452.

Voigt, K. et al., 2012. Retargeting sleeping beauty transposon insertions by engineered zinc finger DNA-binding domains. Molecular therapy : the journal of the American Society of Gene Therapy, 20(10), pp.1852–62.

Wang, W. et al., 2008. Chromosomal transposition of PiggyBac in mouse embryonic stem cells. Proceedings of the National Academy of Sciences of the United States of America, 105(27), pp.9290–5.

Wang, Y. et al., 2014. Suicidal Autointegration of Sleeping Beauty and piggyBac Transposons in Eukaryotic Cells. C. Feschotte, ed. PLoS genetics, 10(3), p.e1004103.

Wardle, S.J. et al., 2005. The global regulator H-NS acts directly on the transpososome to promote Tn10 transposition. , pp.2224–2235.

Waterston, R.H. et al., 2002. Initial sequencing and comparative analysis of the mouse genome. Nature, 420(6915), pp.520–62.

Wicker, T. et al., 2007. A unified classification system for eukaryotic transposable elements. Nature reviews. Genetics, 8(12), pp.973–82.

Wilson, M.H., Coates, C.J. & George, A.L., 2007. PiggyBac transposon-mediated gene transfer in human cells. Molecular therapy : the journal of the American Society of Gene Therapy, 15(1), pp.139–45.

Wilson, M.H., Kaminski, J.M. & George, A.L., 2005. Functional zinc finger/sleeping beauty transposase chimeras exhibit attenuated overproduction inhibition. FEBS letters, 27 (579), pp. 6205-9.

Woltjen, K. et al., 2009. piggyBac transposition reprograms fibroblasts to induced pluripotent stem cells. Nature, 458(7239), pp.766–70.

Wu, S.C.-Y. et al., 2006. piggyBac is a flexible and highly active transposon as compared to sleeping beauty, Tol2, and Mos1 in mammalian cells. Proceedings of the National Academy of Sciences of the United States of America, 103(41), pp.15008–13.

Xu, H. & Boeke, J., 1990. Localization of sequences required in cis for yeast Ty1 element transposition near the long terminal repeats: analysis of mini-Ty1 elements. Molecular and cellular biology, 10 (6), pp. 2695-702

Yant, S.R. et al., 2007. Site-directed transposon integration in human cells. Nucleic acids research, 35(7), p.e50.

Yant, S.R. et al., 2000. Somatic integration and long-term transgene expression in normal and haemophilic mice using a DNA transposon system. Nature genetics, 25(1), pp.35–41.

Yuan, Y.-W. & Wessler, S.R., 2011. The catalytic domain of all eukaryotic cut-and-paste transposase superfamilies. Proceedings of the National Academy of Sciences of the United States of America, 108(19), pp.7884–9.

Yusa, K. et al., 2011. A hyperactive piggyBac transposase for mammalian applications. Proceedings of the National Academy of Sciences of the United States of America, 108(4), pp.1531–6.

Yusa, K. et al., 2009. Generation of transgene-free induced pluripotent mouse stem cells by the piggyBac transposon. Nature methods, 6(5), pp.363–369.

Zemach, A. et al., 2010. Genome-wide evolutionary analysis of eukaryotic DNA methylation. Science (New York, N.Y.), 328(5980), pp.916–9.

Zhou, L. et al., 2004. Transposition of hAT elements links transposable elements and V(D)J recombination. Nature, 432(7020), pp.995–1001.