Genetica (2010) 138:473–484
DOI 10.1007/s10709-009-9391-x

Gene therapy vectors: the prospects and potentials of the cut-and-paste transposons

Corentin Claeys Bouuaert · Ronald M. Chalmers

Received: 20 March 2009 / Accepted: 14 July 2009 / Published online: 2 August 2009

Abstract Gene therapy applications require efficient tools for the stable delivery of genetic information into eukaryotic genomes. Most current gene delivery strategies are based on viral vectors. However, a number of drawbacks, such as the limited cargo capacity, host immune response and mutational risks, highlight the need for alternative gene delivery tools. A comprehensive gene therapy tool kit should contain a range of vectors and techniques that can be adapted to different targets and purposes. Transposons provide a potentially powerful approach. However, transposons encompass a large number of different molecular mechanisms, some of which are better suited to gene delivery applications than others. Here, we consider the range and potentials of the various mechanisms, focusing on the cut-and-paste transposons as one of the more promising avenues towards gene therapy applications. Several cut-and-paste transposition systems are currently under development. We will first consider the mechanisms of *piggyBac* and the hAT family elements Tol*1* and Tol*2*, before focusing on the *mariner* family elements including Mos*1*, Himar*1* and Hsmar*1*.

Keywords Gene therapy · Transposon · Transposition · Genetic disease · DNA recombination · Transformation

Abbreviations

cDNA	Complementary DNA
EMSA	Electrophoretic mobility shift assay
HTH	Helix-turn-helix
IR	Inverted repeat
kb	Kilobase
LTR	Long-terminal-repeat
NLS	Nuclear localization signal
OPI	Over-production inhibition
PEC	Paired-end complex
Rep	Replication
RSS	Recombination signal sequences
RT	Reverse-transcription
SEC	Single-end complex
Tnp	Transposon
TP	Target-primed
Txn	Transcription

C. Claeys Bouuaert · R. M. Chalmers (✉)
School of Biomedical Sciences, University of Nottingham, Queen's Medical Centre, Nottingham NG7 2UH, UK
e-mail: chalmers@bioch.ox.ac.uk

Introduction

To date, most gene therapy trials have used viral vectors for permanent or transient transfer of nucleic acids (Edelstein et al. 2007; Atkinson and Chalmers 2009). Viruses are attractive tools because they are well adapted to deliver their genetic cargo with high efficiency. However, most viral vectors also present serious problems. For example, although retroviruses provide a stable and long term expression of transgenes by integrating into euchromatin, this attractive feature is directly linked to one of their disadvantage which is the oncogenic risk related to their insertion (Bushman 2007). Indeed, some retroviruses have a preference for integration sites near transcriptional start sites or within introns (Laufs et al. 2004). Clearly, these properties are less than desirable in gene therapy applications.

Adenoviruses have two main advantages. They can deliver DNA into dividing or quiescent cells, and they are

unlikely to cause oncogenic transformation since they do not integrate their DNA. However, they fail to provide stable long term expression of their cargo and they are liable to evoke a host immune response (Young et al. 2006; McCaffrey et al. 2008). The immune response is especially problematic due to the repeated doses required to maintain a long term therapeutic effect. A third type of viral vector, derived from adeno-associated viruses, provides a reduced immune response and stable transmission of the cargo due to site specific integration. However, they have a very limited cargo capacity and may cause chromosomal breaks (Buning et al. 2008; Schaffer et al. 2008; Atkinson and Chalmers 2009).

The cargo of viral vectors is limited by the amount of material that can be packaged into the viral particle. This is typically in the range of 8–10 kb and limits their ability to deliver genes with large native control regions and/or introns (Mhashilkar et al. 2001). This restriction has prompted a search for non-viral alternatives that will provide an additional set of tools for gene therapy applications. These include site specific recombination systems, phage integrases and transposons (Gorman and Bullock 2000; Groth and Calos 2004; Ivics and Izsvak 2006). Here we will review the range of molecular mechanisms adopted by the DNA transposons, and consider their merits and potentials in gene therapy applications. We will focus on the cut-and-paste transposons, particularly members of the *mariner* family which are among the most promising candidates. Sleeping Beauty, a distant member of the *mariner* family, has been extensively reviewed as a good gene delivery system (Fernando and Fletcher 2006; Ivics and Izsvak 2006).

Transposons: a menagerie of mechanisms

Two great classes of transposable elements can be distinguished according to whether or not they use an RNA intermediate (Fig. 1). Transposition of Class I elements, or retrotransposons, requires the reverse-transcription of an RNA intermediate by a transposon-encoded enzyme (Curcio and Derbyshire 2003). Class II elements, or DNA transposons, transpose directly without an RNA intermediate. However, the sharp distinction between the two classes of elements can become slightly blurred if the mechanism of the long-terminal-repeat (LTR) and the tyrosine (Y)-retrotransposons are considered in detail (Fig. 1a, b). Following reverse transcription of the RNA intermediate, the cDNA is integrated using mechanisms shared with the Class II DNA transposons. Non-LTR or target-primed (TP)-retrotransposons in contrast combine a reverse transcriptase and endonuclease activity to copy their RNA directly into the target (Fig. 1c). We will now focus on the class II mechanisms and their relative merits as gene delivery tools.

The replicative DNA transposons

The DNA transposons can be further divided according to whether the mechanisms involve replication of the element. The most thoroughly characterized replicative transposon is bacteriophage Mu (Mizuuchi 1992; Baker 1993; Lavoie and Chaconas 1996; Gueguen et al. 2005). In the first step of the reaction the transposon end is nicked to expose the 3′-hydroxyl which is transferred to the target site (Fig. 1d). The second strand of the transposon is uncleaved and therefore remains attached to the donor site. This so-called ‘Shapiro intermediate’ resembles a double replication fork onto which the transposases recruit the host replication machinery (Shapiro 1979). Passage of the replication forks through the transposon generates a cointegrate product in which the donor backbone and the target are linked by two copies of the transposon. Cointegrates are further processed by element-encoded recombination machinery to generate the final products.

IS911 uses a different replicative mechanism (Fig. 1e). The first chemical step of the reaction is the same, exposing the 3′-end of the transposon. However, instead of integrating at a target site, the 3′-OH attacks the opposite end of the transposon, producing a figure-of-eight structure that upon replication yields a circular transposon intermediate (Duval-Valentin et al. 2004).

The mechanism of IS608 transposition does not directly involve replication. Yet it is intimately and inextricably associated with the replication machinery (Ronning et al. 2005; Barabas et al. 2008; Guynet et al. 2008). Unusually, the transposase does not recognize the double stranded from of the transposon end. Instead, it interacts with the single stranded DNA exposed on the lagging strand by passage of a replication fork (Fig. 1f). In higher eukaryotes, the helitrons probably use rolling-circle replication to generate the recombination intermediate, but this has not yet been reconstituted in vitro (Kapitonov and Jurka 2001).

Even though many of the replicative transposons are highly efficient, they depend on too many different components, often host specific, that limit their usefulness in gene delivery applications. In contrast, the non-replicative transposons are generally less complicated and many require only a single transposase protein encoded by the element itself.

The non-replicative tyrosine- and serine-transposases

Some non-replicative transposases are members of the tyrosine and serine recombinase families (Fig. 1g). These enzymes use covalent phospho-tyrosine and phospho-serine

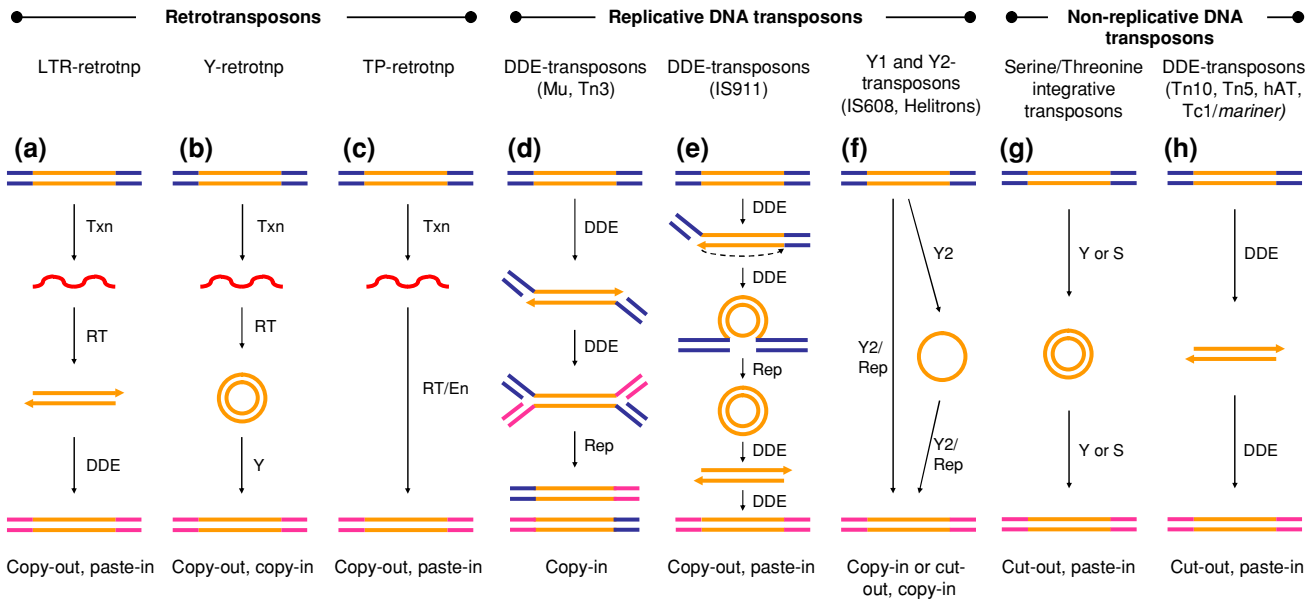

Fig. 1 The molecular mechanisms of transposition. DNA is represented by *straight lines*. RNA transcripts are *wavy lines*. The DNA flanking the transposon insertion is dark (*top*) and the target DNA is light (*bottom*). Reactive 3′-OH groups at the transposon ends are represented as *arrowheads*. Retrotransposons reverse-transcribe their RNA generated by transcription (Txn). **a** The linear cDNA intermediate of long-terminal repeat (LTR)-retrotransposons and retroviruses is integrated into the target using a DDE transposase. **b** Tyrosine (Y)-retrotransposons integrate the circular cDNA using a Y-transposase. **c** Target-primed (TP)-retrotransposons, or non-LTR-retrotransposons, use a reverse-transcriptase (RT) to copy the RNA directly into a target that was nicked by a transposon-encoded endonuclease (En). **d** Bacteriophage Mu transposes *via* a replicative mechanism. Mu transposase nicks the 3′-ends of the element and joins them to the target generating a Shapiro intermediate. The element is then duplicated using the 3′-OH of the target to prime DNA-replication (Rep) resulting in a product called a cointegrate. **e** IS*911*, an IS*3*-like element, initiates catalysis by liberating the 3-OH at the transposon end which subsequently attacks the 5′-phosphate of the same DNA strand. Passage of the replication machinery generates a circular double stranded DNA intermediate. The 3′-OH liberated by double strand cleavage are used as nucleophiles in the subsequent integration step. **f** Y1 and Y2-transposons use tyrosine residues to catalyse transposition of a single DNA strand by a mechanism probably related to rolling-circle replication. Two models have been proposed to describe the mechanism of transposition of these elements, according to whether donor and target cleavages are sequential (cut-out; copy-in) or concerted (copy-in). **g** Cut-and-paste transposition of Y- and S-integrative elements involves covalent phosphotyrosine or phospho-serine intermediates, respectively. These elements, related to either site-specific tyrosine or serine recombinases, use different mechanisms but generate similar DNA products and intermediates. Excision restores the original empty site and generates a circular DNA intermediate which is inserted into the target by a reversal of the excision step. **h** DDE cut-and-paste transposases generate double-strand breaks at both transposon ends. The 3′-ends of the excised element are used as nucleophiles to attack phosphodiester bonds at the target site. Concerted nucleophilic attack of both transposon ends result in transposon integration. This figure was inspired by (Curcio and Derbyshire 2003)

intermediates to break and rejoin DNA strands (Grindley et al. 2006). Transposon excision produces a circular DNA intermediate. Following target capture the enzyme catalyses a second reaction leading to the transposon insertion.

None of the serine or tyrosine transposase have been adapted for gene delivery applications in eukaryotes. However, some related integrase and invertase systems have been more widely used (Atkinson and Chalmers 2009). For example, the *lox*-Cre system, derived from bacteriophage P1, is a site-specific tyrosine-recombinase that functions well in higher eukaryotic cells. It is very efficient, but lacks directionality and therefore catalyzes integration and excision. In contrast, bacteriophage C31 integrase, a site-specific serine-recombinase, is highly directional, favoring integration over excision. Although these are both efficient systems, they are hampered by the absence of the respective target sites in the human genome, and by recombination at pseudo-sites that presumably promote illegitimate recombination events with the vector and between different pseudo-sites.

The non-replicative DNA transposons

The cut-and-paste DNA transposons have a conceptually simple mechanism (Fig. 2). They are usually flanked by short inverted repeats (IRs) that are the recognition sites for a single transposase protein, encoded by the element itself. The element is excised by double strand cleavage at both transposon ends, followed by integration at a target site which in most cases is selected essentially at random. Insertion at staggered position into the target site generates 5′ gaps that, after repair by the host machinery, result in short direct repeats flanking the transposon ends (Haren et al. 1999). Well known examples of cut-and-paste transposons

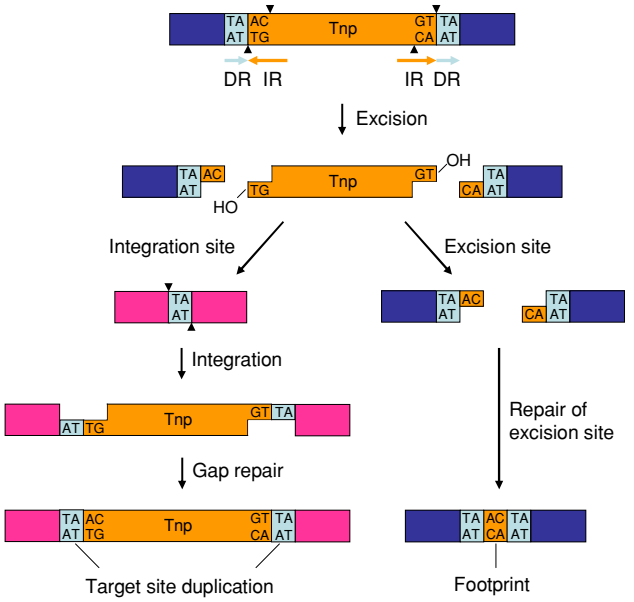

Fig. 2 The *Tc1-mariner* cut-and-paste transposition reaction. A *mariner* transposon (Tpn) is flanked by short terminal inverted repeats (IR). The direct repeat (DR) created by duplication of the TA dinucleotide target site is shown. The *mariner* elements are excised by transposase-mediated double-strand breaks at the ends of the IRs. DNA cleavage liberates the 3′-OH at the transposon end while the 5′-end is recessed two or three bases within the element. The free 3′-OH at both transposon ends subsequently attack the phosphodiester bond 5′ of a TA dinucleotide. The single strand gaps resulting from transposon integration are repaired by host-encoded enzymes and lead to the duplication of the target site. The excision site may also be repaired by host machinery and can in some cases include the terminal nucleotides of the transposon inverted repeats, generating transposon-specific footprints

include Tn*5* and Tn*10* in prokaryotes, and the Tc*1*/*mariner* and hAT families in higher eukaryotes.

The mariner *elements*

The Tc*1*/*mariner* superfamily is a particularly successful group of cut-and-paste transposons, first discovered in *Caenorhabditis elegans* and *Drosophila mauritiana*, respectively (Emmons et al. 1983; Jacobson et al. 1986). Members of this family were subsequently found in what was, at the dawn of the genomic era, an astonishingly large range of eukaryotic species, including plants, nematodes, fungi and animals (Robertson 1993; Plasterk et al. 1999). The most surprising aspect of these discoveries was that closely related *mariner* elements were found in more distantly related species, and vice versa. This showed that horizontal transfer of *mariner* elements is common on evolutionary time scales. Indeed, the *mariner* elements require frequent horizontal transfer as they persist only for a short time after entering a genome. Active elements tend to be swamped by more active deletion-derivatives that arise during amplification e.g., (Buisine et al. 2002; Liu et al. 2007). To date only three naturally active *mariner* elements have been discovered (Munoz-Lopez et al. 2008).

Horizontal transfer of transposons is easily understood in single celled organisms where conjugation and natural transformation are frequent. However, the barriers to horizontal transfer must be considerable in multicellular organisms where the element must succeed in reaching the germ line. Encouragingly, the high frequency of horizontal transfer also suggests that *mariner* transposons are probably independent of host-specific accessory factors, a very desirable property for gene delivery applications.

The chemistry of cut-and-paste transposition

The transposases belong to a large group of proteins, including the retroviral integrases and the Holiday junction resolvase RuvC, that contain an RNase H-like core structure (Nowotny et al. 2005). Members of this superfamily share a similar chemistry, using two metal ions in the active site to catalyze phosphoryl transfer reactions (Yang et al. 2006). The metal ions are coordinated by a conserved triad of acidic amino acid residues. In the transposases and retroviral integrases these residues are present as a DDE motif, a feature that gives its name to this group of proteins. However, the *mariner* elements are unique amongst the cut-and-paste transposons in having a DDD catalytic triad. This chemically conservative glutamate to aspartate substitution was probably an ancient event since the *mariner* elements can no longer tolerate reversion to DDE (Lohe et al. 1997).

In most members of the RNase H superfamily the activity is confined to a single strand of their respective nucleic acid substrates. Thus, RNase H binds a double stranded substrate and nicks one of the strands. This is also true for some of the transposases. For example, the replicative transposon phage Mu nicks one strand, leaving the other completely intact (Fig. 1d). Even some of the cut-and-paste transposases uphold this rule and cut only one strand at the transposon end. Tn7 transposase, for example, nicks the first strand, but the other is cut using an entirely different protein related to the type II restriction enzymes (Peters and Craig 2001). Other cut-and-paste transposases, however, are unusual and cleave both strands of DNA at the transposon end. This is achieved using an elegant DNA hairpin intermediate.

The forward hairpin reaction

Cleavage of the bacterial transposons Tn*10* and Tn*5* is initiated by a nick that exposes the 3′-OH at the end of the transposon (Sakai et al. 1995; Chalmers and Kleckner

1996; Fig. 3a). This group is then used as a nucleophile to the attack the opposite strand. This transesterification reaction generates a hairpin structure at the transposon end and a double strand break on the flanking DNA that liberates the transposon from the donor site (Kennedy et al. 1998; Bhasin et al. 1999; Crellin et al. 2004; Bischerour and Chalmers 2007, 2009). The hairpin is resolved by a second hydrolysis reaction, yielding the 3′-OH and 5′-P groups on the transposon end. The regenerated 3′-OH is finally joined to the target site by a second transesterification reaction. All four phosphoryl transfer reactions are carried out by a single active site (Bolland and Kleckner 1996). This alternation of hydrolysis and transesterification reactions requires a degree of flexibility in the active site. However, the movement of the components is probably kept to a minimum because the product of one reaction is always the substrate of the next. For example, the 3′-OH produced by the first hydrolysis is used to attack the phosphodiester bond on the opposite strand in the first transesterification. We will refer to this polarity as the forward hairpin reaction because the first nick generates the 3′-OH group that is eventually transferred to the target site. Although this forward hairpin mechanism is best characterized in the prokaryotic elements, it has recently been found in the eukaryotic *piggyBac* transposon (Mitra et al. 2008).

The reverse hairpin reaction

The hAT family of transposons is named after three founding members; *hobo*, *Activator* and Tam*3*. The family includes *Tol2* which is widely used in gene delivery applications, and *hermes* where the molecular mechanism has been determined in vitro (Zhou et al. 2004). In these transposons the hairpin mechanism has a reversed polarity (Fig. 3b). The reverse hairpin mechanism was originally deduced from the structure of *Tam3* excision footprints (Coen et al. 1989). However, it was first directly demonstrated in the immune system V(D)J recombination where it is catalyzed by RAG1, a protein distantly related to the *Transib* transposase (Jones and Gellert 2004; Kapitonov and Jurka 2005).

The reverse hairpin is initiated by nicking the 5′-end of the element (Fig. 3b). The second step is similar to the bacterial elements in that the 3′-OH attacks the opposite strand. However, since the 3′-OH is located in the flanking DNA, the hairpin is likewise on the flanking DNA. This reaction liberates the 3′-OH on the transposon end which is now available for integration at the target site. Indeed, integration of the 3′ transposon end at the target site is the one mechanistic feature that unites all of the DDE cut-and-paste transposons.

The reversed polarity of the hairpin reaction allows eukaryotic elements to carry out transposition with one less phosphoryl transfer reaction than the bacterial elements which are obliged to resolve the hairpin. However, this is probably achieved at a cost. The active site can no longer alternate between hydrolysis and transesterification reactions. Instead, the transesterification that creates the hairpin is followed immediately by a second transesterification of the 3′-OH to the target site. Compared to the more elegant forward hairpin reaction, this probably requires more extensive conformational changes in the active site as the flanking 3′-OH is exchanged for the 3′-OH on the transposon end.

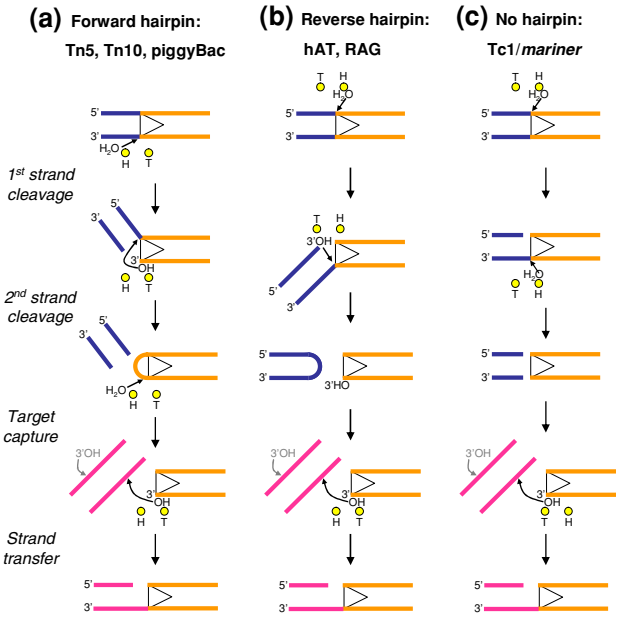

Fig. 3 Three mechanisms for DNA cleavage in the cut-and-paste transposons. Cut-and-paste transposons use different mechanisms to generate double strand breaks at their ends. Here, single transposon ends are represented as double stranded DNA. Catalysis involves two Mg^{2+} ions represented as *spheres* labeled H and T indicating whether the ion is involved in hydrolysis or transesterification steps, respectively. **a** Tn*5* and Tn*10* in bacteria and *piggyBac* in eukaryotes initiate catalysis by hydrolyzing the terminal phosphodiester bond of the transposon transferred strand. The resulting 3′-OH is used as a nucleophile to attack the opposite DNA strand in a transesterification reaction that generates a double strand break on the flanking DNA and a hairpin structure at the transposon end. The hairpin is then resolved by a second hydrolysis reaction, regenerating the terminal 3′-OH which then attacks a phosphodiester bond on the target site. **b** Excision mediated by eukaryotic hAT elements and RAG proteins use a reverse hairpin intermediate. The non-transferred strand is cleaved first by a transposase-mediated hydrolysis reaction. The resulting 3′-OH flanking the transposon end is then used as a nucleophile in the attack of the opposite strand giving a hairpin structure on the flanking DNA. This transesterification reaction liberates the 3′-OH on the transposon end which is used for the integration step. **c** Members of the Tc*1*/*mariner* superfamily appear to perform excision without using a hairpin intermediate. As for hAT elements and V(D)J recombination the first nick is on the non-transferred strand, but the second strand break likely occurs by a second hydrolysis reaction. This figure was inspired by (Yang et al. 2006)

A third way: mariner cleavage without a hairpin

The first step of *mariner* cleavage is similar to the majority of other eukaryotic transposons that use the reverse hairpin mechanism (Fig. 3c). The first nick exposes the 5′-P, which in this case is usually two or three bases within the element. This is followed by a second nick that generates the 3′-OH at the transposon end (Lampe et al. 1996; Dawson and Finnegan 2003; Lipkow et al. 2004b; Liu et al. 2007). Surprisingly, this second strand cleavage reaction does not appear to involve a hairpin intermediate. Hairpins could not be detected in reactions reconstituted in vitro (Dawson and Finnegan 2003). More convincingly, accurate second strand cleavage was detected in substrates lacking the flanking 3′-OH which provides the nucleophile for the hairpin reaction (Richardson et al. 2006).

The mechanism of second strand cleavage in *mariner* elements remains unknown. One possibility is that two monomers of transposase are required to cleave the two strands of DNA at each transposon end. The stoichiometry of the active complexes have not been unequivocally demonstrated, however, there is some evidence for a dimer of transposase at each transposon end (Lipkow et al. 2004b; Auge-Gouillou et al. 2005a).

Subunit architecture and mechanistic constraints

The transposase subunit architecture can place important constraints on the products of transposition reactions. For example, cleavage and/or integration events involving only one end of the transposon are highly undesirable as they are likely to be detrimental to the survival of both the transposon and the host cell. Transposons exert control over single ended events by performing the reaction within a paired ends complex (PEC), or transpososome, in which the transposon ends are held together in a synapsis by the transposase. In all transposons that have been investigated, double strand cleavage is dependent on the formation of this complex.

The synaptic complex of the prokaryotic transposons has a '*trans* architecture' in which the transposon end is cleaved by the transposase monomer bound to the opposite end (Aldaz et al. 1996; Savilahti and Mizuuchi 1996; Davies et al. 2000). Since one monomer of transposase binds to each transposon end independently, single ended events are precluded. Although the *trans* architecture provides an absolute mechanistic bar against cleavage at an unsynapsed transposon end, single end events can still occur within a synaptic complex for stochastic reasons. However, such aberrant events probably occur at a low level and most synaptic complexes once assembled complete the entire transposition reaction e.g., (Chalmers and Kleckner 1996).

In contrast to the prokaryotic elements described above, some of the eukaryotic transposases, including RAG, hAT and *mariner*, appear to exist in solution as multimers prior to binding the transposon ends (Rodgers et al. 1996; Hickman et al. 2000; CCB and RC, unpublished). Very little is known about transpososome assembly in these eukaryotic elements, but binding of a transposase multimer to a single transposon end provides the possibility of single end events. Single end events have been confirmed for RAG recombination. Even though the RSS are cleaved in *trans*, binding of the recombinases as a dimer from solution allows cleavage to start before synapsis. However, only the first nick is completed as the hairpin step is coupled to synapsis by conformational changes in the complex (West and Lieber 1998; Kim et al. 1999; Yu and Lieber 2000).

Several lines of evidence suggest that first strand cleavage in *mariner* could also be independent of synapsis. Hartl and colleagues, working in vivo, recovered mutations in the Mos*1* IRs at an unexpectedly high frequency and suggested that these 'self-inflicted wounds' were the result of transposase acting on a single transposon end (Lohe et al. 2000). This hypothesis was further supported by the first strand nicking detected in gel-purified single end complexes of the related Himar*1* transposase (Lipkow et al. 2004b).

Analysis of *mariner* transposition reactions reconstituted on short linear fragments of DNA revealed a variety of single-end complexes (SECs) and PECs (Lipkow et al. 2004b; Auge-Gouillou et al. 2005b). Although the precise composition of these complexes remains uncertain, they show that PECs and single unsynapsed transposon ends can bind different numbers of transposase monomers.

Multimerization of the transposase has been proposed to provide a mechanism to down-regulate Mos*1* transposition in vivo (Lohe and Hartl 1996). The mechanism is called over-production inhibition (OPI), and is based on the observation that the rate of transposition in *Drosophila* decreases when the number of copies of the element increases beyond a certain point. A negative feedback mechanism was proposed in which the transposase produced by an ever-increasing number of elements culminates in a concentration-dependent aggregation of the transposase and the assembly of non-productive complexes.

In vitro titrations of Himar*1* and Hsmar*1* transposase concentrations were consistent with OPI. Transposition activity reaches a plateau and then declines with increasing transposase concentration (Lampe et al. 1998; Tosi and Beverley 2000; Lipkow et al. 2004a; CCB and RC, manuscript in preparation). Analysis of the complexes revealed a progressive increase in the multimeric state of the protein, culminating in 'super-shifted' complexes and aggregates. The architecture and stoichiometry of the complexes

remains uncertain. Also uncertain is the relationship between the observed complexes and the OPI phenomenon. Nevertheless, these observations suggest that high transposition efficiency will not be achieved simply by producing the highest level of transposase. Gene therapy applications will instead require fine tuning of the balance between the transposase concentration and the substrate.

Transposons as gene delivery vehicles

Transposons have been one of the most useful tools in bacterial genetics. Soon after their discovery they were used to generate insertion mutation libraries, but the number of applications has since expanded. In the post-genomic era transposons have been used in DNA sequencing applications, as polymorphic markers for genomic display and for delivering sequence bar codes. More recently transposons have been developed as gene delivery vehicles and offer the prospect to repair mutations in gene therapy applications. How well suited are they to these goals, and what are the future limits of their potential?

Host range and tissue specificity

In gene delivery applications it is important that the vector of choice is not restricted by the properties of the host cell. The lack of restrictions will facilitate targeting of the widest possible range of tissues under different stages of development. The cut-and-paste transposons have minimal host factor requirements, and most achieve transposition using only the cognate transposase. One exception is the *P* element which works efficiently in Drosophila and related species were it is dependent on the Mus309 gene (Rio 1990). Most other elements, however, are not restricted in this way, or may be modulated by host factors that are sufficiently widespread and conserved that they do not present a barrier to transposition. For example, the bacterial transposon Tn*10* is modulated by IHF, a site specific DNA bending protein found in most, if not all, gram negative bacteria. IHF binds immediately adjacent to the transposase recognition site where it stimulates excision and influences the choice of target site (Chalmers et al. 1998). In contrast, the related element Tn*5* is independent of IHF binding. Both elements are, however, modulated by the H-NS global regulator (Singh et al. 2008; Whitfield et al. 2009).

In eukaryotes, the fish transposon Sleeping Beauty is stimulated by the mouse DNA bending protein HMGB1 (Zayed et al. 2003). Clearly, ubiquitous host factors such as HMGB1 do not represent a significant barrier to transposition in different hosts or tissues. The insect transposon Himar*1* appears to be completely independent of host factors and is even active in a range of bacteria, where it has been widely used for mutagenesis applications (Rubin et al. 1999). However, the bacterial transposons seem to be profoundly restricted, and as yet none have been reported to function in higher eukaryotes. This restriction is not imposed by the bacterial history of these elements per se; the bacteriophage P1 Cre recombinase and phage C31 integrase function well in eukaryotes. The restriction is more likely to be mechanistic, imposed by the coupled transcription and translation in prokaryotes and the *cis* action of the bacterial transposases (Jain and Kleckner 1993).

Flexibility and engineering of transposon vectors

The *mariner* elements have a simple structure comprising a single gene encoding a transposase of about 350 amino acids, flanked by a pair of simple inverted repeats of about 30 bp in length. In principle, any sequence placed between the inverted repeats can be mobilized if the transposase is provided in *trans*. Unlike the viral vectors where the cargo is packaged inside virus particles, transposons are not limited by the size of the transgene to be delivered. In bacteria, entire metabolic pathways have been delivered on fragments of DNA as large as 60 kb (Fu et al. 2008). However, in practice larger transgenes are likely to be problematic as transposition efficiency declines as the distance between the inverted repeats increases (Atkinson and Chalmers 2009). Overall, the simplicity of transposons compared to viral vectors is likely to facilitate engineering of desirable features.

Transpososome preassembly

A number of different strategies have been developed to mobilize transposons in higher eukaryotes. The most common is to co-transfect cells with one plasmid encoding the transposon and another plasmid encoding a transposase expression cassette. An alternative is to prepare transposase mRNA which can be co-transfected or microinjected along with the transposon plasmid. In either case, the transposase, once synthesized, must interact with the transposon and assemble the transpososome. All of the chemical steps leading to successful transposition take place within this complex. The principle drawback of these strategies is that the ratio of transposase to transposon, which is critical for successful transposition, is not well controlled.

Tn*10* and Tn*5* are the most widely used transposon tools in prokaryotes. Tn*5* is a particularly tractable system where the efficiency of the reaction in vitro approaches 100%. The high efficiency of transpososome assembly has facilitated an electroporation technique for generating libraries of transposon insertions (Reznikoff et al. 2004). The

transpososomes are assembled in vitro where the absence of the catalytic metal ion, Mg^{2+}, prevents transposition until after the complexes have been electroporated into the bacterial cells. The *mariner* elements are similar to Tn*5* in their simplicity and the availability of an in vitro reaction. The preassembly of transpososomes is therefore highly feasible and will help to control the potential genotoxic effects of excessive amounts of transposase or transposon.

One potential limitation of the preassembly strategy for *mariner* elements is the instability of the transpososomes. The Mos*1*, Himar*1* and Hsmar*1* transpososomes have proven difficult, though not impossible, to detect in EMSA assays and are probably less stable than the equivalent complexes in Tn*5* and Tn*10* (Dawson and Finnegan 2003; Lipkow et al. 2004b; Auge-Gouillou et al. 2005b; Liu et al. 2007). However, EMSA conditions are harsh and it remains to be seen whether the *mariner* transpososomes will survive transfection into eukaryotic cells. One approach to increase the stability of the *mariner* transpososomes is to use pre-nicked or pre-cleaved transposon ends. Transposition reactions are energetically down-hill and the stability of the complexes generally increases as the reaction progresses. This has been observed for Hsmar*1* where the transpososome formed with the pre-cleaved transposon ends is detected in greater quantity than those assembled with uncleaved ends (Liu et al. 2007).

The risk of partial and aberrant transposition reactions

Undesirable genotoxic effects could arise from partial and aberrant transposition reactions. This issue has been investigated in Tn*10* and reaction products have been detected corresponding to transpososomes stalled at each stage of the reaction (Chalmers and Kleckner 1996). Products nicked or cleaved at a single transposon end are not necessarily problematic as they damage the donor DNA and not the target (Fig. 4a). Single end insertions, however, will yield a flap structure that must be repaired by the host and may promote illegitimate recombination (Fig. 4b). A more serious potential problem is bimolecular synapsis when the transpososome is assembled using a pair of transposon ends located on different molecules. Following cleavage, insertion of this structure will break the target chromosome with genotoxic consequences (Fig. 4c). Finally, insertion of the transposon into itself is a suicide event that would not lead to productive transfer of the transgene. If not controlled, these intramolecular insertions will reduce the efficiency of the gene delivery tool (Fig. 4d).

In Tn*10*, partial reactions constitute a relatively small proportion of the products (<1%). However, bimolecular synapsis is very efficient. Transpososome assembly is not subject to any kind of topological constraint and transposon ends in any relative orientation and on different donor molecules are synapsed with equal efficiency (Chalmers and Kleckner 1996). This issue has been addressed for the *mariner* transposon Hsmar*1* (CCB and RC, in preparation). Single end insertions were barely detectable and probably represent <0.1% of the reaction products. The level of bimolecular synapsis was also very low, and it appears that synapsis of transposon ends in Hsmar*1* is much more constrained than in Tn*10*. If other *mariner* elements such as Mos*1* are similar, which is likely, this is very promising for gene delivery applications.

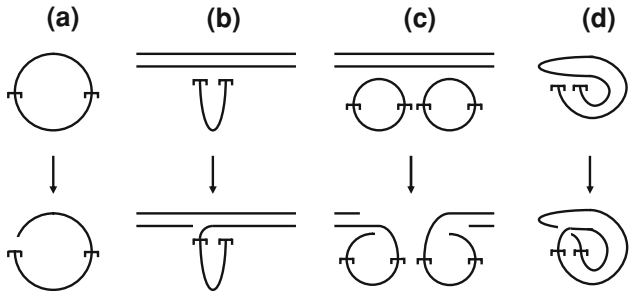

Fig. 4 Partial and aberrant reactions may affect the efficiency of transposon-based gene delivery tools. **a** Single-end cleavage of a circular transposon donor generates a linear DNA fragment. This would not present a danger in a gene therapy situation as this reaction only damages the donor DNA. It would, however, reduce the efficiency of gene delivery. **b** Single-end insertions damage the target DNA, creating a potentially genotoxic lesion. However, the host DNA repair machinery could probably deal with the product of this reaction. **c** Bimolecular synapsis of transposon ends on different donor molecules will break the target chromosome upon insertion. **d** Insertion of the excised transposon into itself would reduce the efficiency of the gene delivery process

Target site selection

Target site specificity varies greatly among different transposons. Retrotransposons, such as LINES and Alu, for example, tend to accumulate in heterochromatic regions either because they are targeted to these regions or because their presence induces heterochromatin formation. Nevertheless, they remain a significant cause of de novo mutations. Among the retroviruses, which use a transposition mechanism of integration, a number of preferred sites, or hot spots, have been documented. Insertions are over-represented near transcriptional start sites and in the first intron of genes (Laufs et al. 2004).

The minimal target site requirement for the *mariner* elements, including Sleeping Beauty, is the TA dinucleotide that is duplicated upon insertion. Local hotspots have been documented, presumably reflecting a more favorable DNA conformation, or a higher flexibility of the DNA favoring capture by the transpososome (Vigdal et al. 2002;

Geurts et al. 2006; Crenes et al. 2009). The target specificity on a wider, genomic scale, has not been thoroughly characterized. One source of target site bias sometimes observed in vivo is transposition into genetically linked loci. This may arise from diffusion barriers or if the transpososome can engage in productive target interactions before the cleavage step has been completed (Lipkow et al. 2004a). These issues are unlikely to affect transposon introduced from external sources.

A more significant bias may be a tendency to insert into transcriptionally active DNA. Mos*1*, like the transpositional retroviruses, may have an affinity for promoter regions as it was first detected as the *peach* insertion upstream of the *white* gene that controls eye color in the Drosophila host (Hartl 2001). In *Caenorhabditis elegans*, Mos*1* is also widely used for insertion mutagenesis, indicating its ability to insert into transcribed regions (Bessereau 2006). Of course, insertions that cause visible phenotypic changes, such as *peach*, or those promoting proliferation or engraftment, will tend to be documented most frequently. Silent insertions are not directly observable and they have never been systematically examined. Unbiased assessment of target specificity therefore requires a selectively neutral method for the recovery of insertions e.g., (Ivics et al. 2007).

Specific targeting of transposon insertions

The cut-and-paste transposon Tn*7* uses a specialized protein, TnsD, to target its insertions to a unique site in the bacterial chromosome (Peters and Craig 2001). This site, known as the attachment site, provides very high target specificity, comparable to that enjoyed by the Cre recombinase and phage C31 integrase. There are no Tn*7* attachment sites in the human genome, but there are a number of closely related pseudo-sites that are attractive targets for transgenic applications (Kuduvalli et al. 2005). However, progress appears to have been slow, probably because of environmental restrictions on Tn*7* transposition in the eukaryotic nucleus. None of the eukaryotic cut-and-paste transposons are known to be targeted to specific sites. However, it is possible to engineer an artificial subunit or domain, analogous to TnsD, that will target insertions to specific sites.

The modular structure of mariner transposase facilitates engineering

In general, eukaryotic proteins are more frequently organized into modular functional domains than their prokaryotic equivalents. In the cocrystal structure of prokaryotic Tn*5* transposase, for example, there is no clear physical boundary between the DNA binding, catalysis and subunit interaction domains (Davies et al. 2000). In contrast, Tc*3*, Mos*1* and *hermes* are organized as an N-terminal DNA binding domain connected to C-terminal catalytic domain by a flexible linker (Fig. 5; Plasterk et al. 1999; Watkins et al. 2004; Hickman et al. 2005; Richardson et al. 2006). Sequence alignments and secondary-structure predictions suggests that the *mariner* DNA binding domain contains a helix-turn-helix (HTH) motif that would be responsible to specifically recognize the transposon inverted repeats (Pietrokovski and Henikoff 1997; Auge-Gouillou et al. 2001).

The modular structure of the eukaryotic transposases facilitates the addition of a targeting domain, such as a transcription factor or zinc finger protein. Such targeting was achieved for the retroviruses with limited success (Bushman 1994). More recently, similar strategies have proven successful for Sleeping Beauty (Ivics et al. 2007). Disappointingly, direct fusion of a targeting domain to the transposase inhibited the transposition reaction, probably on account of solubility issues (Fig. 6a). A more successful strategy was to target the transpososome using non-covalent interactions to a third protein that acted as a bridge, or adaptor, to a site specific DNA binding domain (Fig. 6b). A third strategy that proved most successful was to use a bivalent protein in which the two moieties targeted specific DNA sequences in the transposon and the desired target site in the human genome (Fig. 6c). The success of this strategy may stem from the fact that it allows for transpososome assembly either before or after the transposon has been localized to the desired target region by the DNA binding protein.

Despite the success of these targeting strategies, they are crude compared to the elegance of natural systems such as Tn*7* where random integrations are inhibited in favor of the intended specific site. Thus, the current challenge is not to find ways to target *mariner* insertions to desired genomic locations, but to inhibit the adventitious integration at random sites that continue to represent the majority of events with the targeting constructs. This is a massive

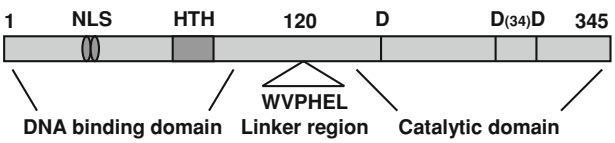

Fig. 5 Modular organization of the *mariner* transposases. The *mariner* transposases have a N-terminal DNA binding domain containing a putative bipartite nuclear localization sequence (NLS) and (at least one) helix-turn-helix (HTH) motif responsible for specifically binding the transposon end. The C-terminal domain contains the catalytic $DD_{34}D$ motif. The DNA binding and catalytic regions of *mariner* transposases fold in two independent domains and are linked by a linker region containing a highly conserved WVPHEL motif

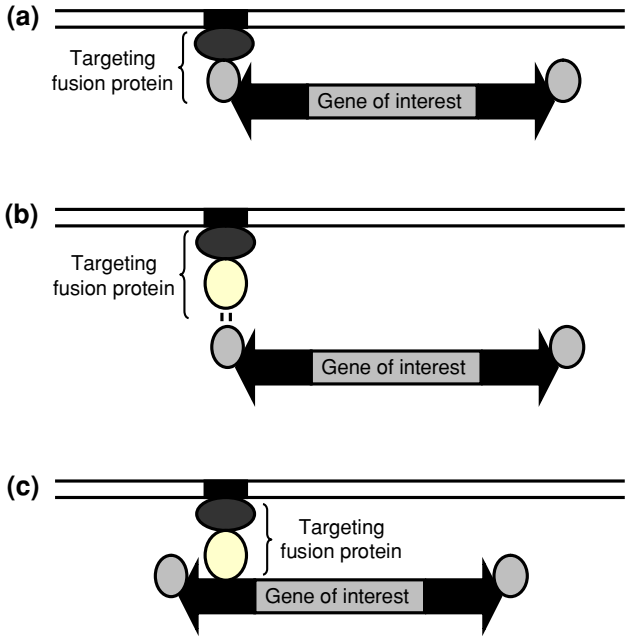

Fig. 6 Strategies for targeting transposon insertions. The gene of interest is flanked by the inverted repeats of the transposon (*arrowheads*). The transposase is represented as a *light grey oval*. Touching ovals represent fused domains. Non-covalent interactions are represented as a pair of *vertical lines*. **a** Targeting with transposase fusion protein. Targeting is achieved by fusing a specific DNA binding domain (*dark oval*) to the transposase. **b** Targeting with a bridging protein. A bridging protein (*white oval*) is used to establish a non-covalent targeting-interaction between the transposase and a site-specific DNA binding protein (*dark oval*). **c** Targeting with a bipartite DNA binding protein. Two DNA binding proteins are fused together to provide interactions that link the transposon DNA to the target DNA. Figure redrawn from (Ivics et al. 2007)

challenge that requires a better understanding of the mechanism of target capture and residues involved in non-specific DNA binding.

Conclusion

The simplicity of cut-and-paste transposition makes these systems attractive for the development of transposon-based gene delivery tools. The *mariner* elements are particularly promising because they are active in a wide variety of organisms probably because their catalysis is independent of any specific host factor. They seem to integrate preferentially into euchromatin, as required for optimal expression of the transgene, but they lack strong target site specificity. Research focusing on the engineering of chimeric transposases with novel target specificities will hopefully make it possible in the future to select a short region of the genome and specifically integrate a therapeutic transgene within this region.

Acknowledgments Work in the author's laboratory is funded by The European Commission (Project SyntheGene Delivery, No. 018716) and the Wellcome Trust. We would like to thank Dr. Yves Bigot for his help and his enthusiastic inspiration throughout this work.

References

Aldaz H, Schuster E, Baker TA (1996) The interwoven architecture of the Mu transposase couples DNA synapsis to catalysis. Cell 85:257–269

Atkinson H, Chalmers R (2009) Delivering the goods: viral and non-viral gene therapy vectors and the inherent limits on cargo DNA and internal sequences. Genetica (in press)

Auge-Gouillou C, Hamelin MH, Demattei MV, Periquet G, Bigot Y (2001) The ITR binding domain of the mariner Mos-1 transposase. Mol Genet Genomics 265:58–65

Auge-Gouillou C, Brillet B, Germon S, Hamelin MH, Bigot Y (2005a) Mariner Mos1 transposase dimerizes prior to ITR binding. J Mol Biol 351:117–130

Auge-Gouillou C, Brillet B, Hamelin MH, Bigot Y (2005b) Assembly of the mariner Mos1 synaptic complex. Mol Cell Biol 25:2861–2870

Baker TA (1993) Protein-DNA assemblies controlling lytic development of bacteriophage Mu. Curr Opin Genet Dev 3:708–712

Barabas O, Ronning DR, Guynet C, Hickman AB, Ton-Hoang B, Chandler M, Dyda F (2008) Mechanism of IS200/IS605 family DNA transposases: activation and transposon-directed target site selection. Cell 132:208–220

Bessereau JL (2006) Insertional mutagenesis in C. elegans using the Drosophila transposon Mos1: a method for the rapid identification of mutated genes. Methods Mol Biol 351:59–73

Bhasin A, Goryshin IY, Reznikoff WS (1999) Hairpin formation in Tn5 transposition. J Biol Chem 274:37021–37029

Bischerour J, Chalmers R (2007) Base-flipping dynamics in a DNA hairpin processing reaction. Nucleic Acids Res 35:2584–2595

Bischerour J, Chalmers R (2009) Base flipping in Tn10 transposition: an active flip and capture mechanism. PLoS One 4:e6201

Bolland S, Kleckner N (1996) The three chemical steps of Tn10/IS10 transposition involve repeated utilization of a single active site. Cell 84:223–233

Buisine N, Tang CM, Chalmers R (2002) Transposon-like correia elements: structure, distribution and genetic exchange between pathogenic Neisseria sp. FEBS Lett 522:52–58

Buning H, Perabo L, Coutelle O, Quadt-Humme S, Hallek M (2008) Recent developments in adeno-associated virus vector technology. J Gene Med 10:717–733

Bushman FD (1994) Tethering human immunodeficiency virus 1 integrase to a DNA site directs integration to nearby sequences. Proc Natl Acad Sci USA 91:9233–9237

Bushman FD (2007) Retroviral integration and human gene therapy. J Clin Invest 117:2083–2086

Chalmers RM, Kleckner N (1996) IS10/Tn10 transposition efficiently accommodates diverse transposon end configurations. Embo J 15:5112–5122

Chalmers R, Guhathakurta A, Benjamin H, Kleckner N (1998) IHF modulation of Tn10 transposition: sensory transduction of supercoiling status via a proposed protein/DNA molecular spring. Cell 93:897–908

Coen ES, Robbins TP, Almeida J, Hudson A, Carpenter R (1989) Consequences and mechanisms of transposition in *Antirrhinum majus*. Mobile DNA, Washington, DC, pp 413–436

Crellin P, Sewitz S, Chalmers R (2004) DNA looping and catalysis; the IHF-folded arm of Tn10 promotes conformational changes and hairpin resolution. Mol Cell 13:537–547

Crenes G, Ivo D, Herisson J, Dion S, Renault S, Bigot Y, Petit A (2009) The bacterial Tn9 chloramphenicol resistance gene: an attractive DNA segment for Mos1 mariner insertions. Mol Genet Genomics 281:315–328

Curcio MJ, Derbyshire KM (2003) The outs and ins of transposition: from mu to kangaroo. Nat Rev Mol Cell Biol 4:865–877

Davies DR, Goryshin IY, Reznikoff WS, Rayment I (2000) Three-dimensional structure of the Tn5 synaptic complex transposition intermediate. Science 289:77–85

Dawson A, Finnegan DJ (2003) Excision of the Drosophila mariner transposon mos1. Comparison with bacterial transposition and v(d)j recombination. Mol Cell 11:225–235

Duval-Valentin G, Marty-Cointin B, Chandler M (2004) Requirement of IS911 replication before integration defines a new bacterial transposition pathway. EMBO J 23:3897–3906

Edelstein ML, Abedi MR, Wixon J (2007) Gene therapy clinical trials worldwide to 2007—an update. J Gene Med 9:833–842

Emmons SW, Yesner L, Ruan KS, Katzenberk D (1983) Evidence for a transposon in Caenorhabditis elegans. Cell 32:55–65

Fernando S, Fletcher BS (2006) Sleeping beauty transposon-mediated nonviral gene therapy. BioDrugs 20:219–229

Fu J, Wenzel SC, Perlova O, Wang J, Gross F, Tang Z, Yin Y, Stewart AF, Muller R, Zhang Y (2008) Efficient transfer of two large secondary metabolite pathway gene clusters into heterologous hosts by transposition. Nucleic Acids Res 36:e113

Geurts AM, Hackett CS, Bell JB, Bergemann TL, Collier LS, Carlson CM, Largaespada DA, Hackett PB (2006) Structure-based prediction of insertion-site preferences of transposons into chromosomes. Nucleic Acids Res 34:2803–2811

Gorman C, Bullock C (2000) Site-specific gene targeting for gene expression in eukaryotes. Curr Opin Biotechnol 11:455–460

Grindley ND, Whiteson KL, Rice PA (2006) Mechanisms of site-specific recombination. Annu Rev Biochem 75:567–605

Groth AC, Calos MP (2004) Phage integrases: biology and applications. J Mol Biol 335:667–678

Gueguen E, Rousseau P, Duval-Valentin G, Chandler M (2005) The transpososome: control of transposition at the level of catalysis. Trends Microbiol 13:543–549

Guynet C, Hickman AB, Barabas O, Dyda F, Chandler M, Ton-Hoang B (2008) In vitro reconstitution of a single-stranded transposition mechanism of IS608. Mol Cell 29:302–312

Haren L, Ton-Hoang B, Chandler M (1999) Integrating DNA: transposases and retroviral integrases. Annu Rev Microbiol 53: 245–281

Hartl D (2001) Discovery of the transposable element mariner. Genetics 157:471–476

Hickman AB, Li Y, Mathew SV, May EW, Craig NL, Dyda F (2000) Unexpected structural diversity in DNA recombination: the restriction endonuclease connection. Mol Cell 5:1025–1034

Hickman AB, Perez ZN, Zhou L, Musingarimi P, Ghirlando R, Hinshaw JE, Craig NL, Dyda F (2005) Molecular architecture of a eukaryotic DNA transposase. Nat Struct Mol Biol 12:715–721

Ivics Z, Izsvak Z (2006) Transposons for gene therapy!. Curr Gene Ther 6:593–607

Ivics Z, Katzer A, Stuwe EE, Fiedler D, Knespel S, Izsvak Z (2007) Targeted sleeping beauty transposition in human cells. Mol Ther 15:1137–1144

Jacobson JW, Medhora MM, Hartl DL (1986) Molecular structure of a somatically unstable transposable element in Drosophila. Proc Natl Acad Sci USA 83:8684–8688

Jain C, Kleckner N (1993) Preferential cis action of IS10 transposase depends upon its mode of synthesis. Mol Microbiol 9:249–260

Jones JM, Gellert M (2004) The taming of a transposon: V(D)J recombination and the immune system. Immunol Rev 200:233–248

Kapitonov VV, Jurka J (2001) Rolling-circle transposons in eukaryotes. Proc Natl Acad Sci USA 98:8714–8719

Kapitonov VV, Jurka J (2005) RAG1 core and V(D)J recombination signal sequences were derived from transib transposons. PLoS Biol 3:e181

Kennedy AK, Guhathakurta A, Kleckner N, Haniford DB (1998) Tn10 transposition via a DNA hairpin intermediate. Cell 95:125–134

Kim DR, Dai Y, Mundy CL, Yang W, Oettinger MA (1999) Mutations of acidic residues in RAG1 define the active site of the V(D)J recombinase. Genes Dev 13:3070–3080

Kuduvalli PN, Mitra R, Craig NL (2005) Site-specific Tn7 transposition into the human genome. Nucleic Acids Res 33:857–863

Lampe DJ, Churchill ME, Robertson HM (1996) A purified mariner transposase is sufficient to mediate transposition in vitro. Embo J 15:5470–5479

Lampe DJ, Grant TE, Robertson HM (1998) Factors affecting transposition of the Himar1 mariner transposon in vitro. Genetics 149:179–187

Laufs S, Nagy KZ, Giordano FA, Hotz-Wagenblatt A, Zeller WJ, Fruehauf S (2004) Insertion of retroviral vectors in NOD/SCID repopulating human peripheral blood progenitor cells occurs preferentially in the vicinity of transcription start regions and in introns. Mol Ther 10:874–881

Lavoie BD, Chaconas G (1996) Transposition of phage Mu DNA. Curr Top Microbiol Immunol 204:83–102

Lipkow K, Buisine N, Chalmers R (2004a) Promiscuous target interactions in the mariner transposon Himar1. J Biol Chem 279: 48569–48575

Lipkow K, Buisine N, Lampe DJ, Chalmers R (2004b) Early intermediates of mariner transposition: catalysis without synapsis of the transposon ends suggests a novel architecture of the synaptic complex. Mol Cell Biol 24:8301–8311

Liu D, Bischerour J, Siddique A, Buisine N, Bigot Y, Chalmers R (2007) The human SETMAR protein preserves most of the activities of the ancestral Hsmar1 transposase. Mol Cell Biol 27: 1125–1132

Lohe AR, Hartl DL (1996) Autoregulation of mariner transposase activity by overproduction and dominant-negative complementation. Mol Biol Evol 13:549–555

Lohe AR, De Aguiar D, Hartl DL (1997) Mutations in the mariner transposase: the D, D(35)E consensus sequence is nonfunctional. Proc Natl Acad Sci USA 94:1293–1297

Lohe AR, Timmons C, Beerman I, Lozovskaya ER, Hartl DL (2000) Self-inflicted wounds, template-directed gap repair and a recombination hotspot. Effects of the mariner transposase. Genetics 154:647–656

McCaffrey AP, Fawcett P, Nakai H, McCaffrey RL, Ehrhardt A, Pham TT, Pandey K, Xu H, Feuss S, Storm TA, Kay MA (2008) The host response to adenovirus, helper-dependent adenovirus, and adeno-associated virus in mouse liver. Mol Ther 16:931–941

Mhashilkar A, Chada S, Roth JA, Ramesh R (2001) Gene therapy. Therapeutic approaches and implications. Biotechnol Adv 19: 279–297

Mitra R, Fain-Thornton J, Craig NL (2008) Piggybac can bypass DNA synthesis during cut and paste transposition. EMBO J 27:1097–1109

Mizuuchi K (1992) Transpositional recombination: mechanistic insights from studies of mu and other elements. Annu Rev Biochem 61: 1011–1051

Munoz-Lopez M, Siddique A, Bischerour J, Lorite P, Chalmers R, Palomeque T (2008) Transposition of Mboumar-9: identification of a new naturally active mariner-family transposon. J Mol Biol 382: 567–572

Nowotny M, Gaidamakov SA, Crouch RJ, Yang W (2005) Crystal structures of RNase H bound to an RNA/DNA hybrid: substrate specificity and metal-dependent catalysis. Cell 121:1005–1016

Peters JE, Craig NL (2001) Tn7: smarter than we thought. Nat Rev Mol Cell Biol 2:806–814

Pietrokovski S, Henikoff S (1997) A helix-turn-helix DNA-binding motif predicted for transposases of DNA transposons. Mol Gen Genet 254:689–695

Plasterk RH, Izsvak Z, Ivics Z (1999) Resident aliens: the Tc1/mariner superfamily of transposable elements. Trends Genet 15: 326–332

Reznikoff WS, Goryshin IY, Jendrisak JJ (2004) Tn5 as a molecular genetics tool: In vitro transposition and the coupling of in vitro technologies with in vivo transposition. Methods Mol Biol 260:83–96

Richardson JM, Dawson A, O'Hagan N, Taylor P, Finnegan DJ, Walkinshaw MD (2006) Mechanism of Mos1 transposition: insights from structural analysis. Embo J 25:1324–1334

Rio DC (1990) Molecular mechanisms regulating Drosophila P element transposition. Annu Rev Genet 24:543–578

Robertson HM (1993) The mariner transposable element is widespread in insects. Nature 362:241–245

Rodgers KK, Bu Z, Fleming KG, Schatz DG, Engelman DM, Coleman JE (1996) A zinc-binding domain involved in the dimerization of RAG1. J Mol Biol 260:70–84

Ronning DR, Guynet C, Ton-Hoang B, Perez ZN, Ghirlando R, Chandler M, Dyda F (2005) Active site sharing and subterminal hairpin recognition in a new class of DNA transposases. Mol Cell 20:143–154

Rubin EJ, Akerley BJ, Novik VN, Lampe DJ, Husson RN, Mekalanos JJ (1999) In vivo transposition of mariner-based elements in enteric bacteria and mycobacteria. Proc Natl Acad Sci USA 96:1645–1650

Sakai J, Chalmers RM, Kleckner N (1995) Identification and characterization of a pre-cleavage synaptic complex that is an early intermediate in Tn10 transposition. Embo J 14:4374–4383

Savilahti H, Mizuuchi K (1996) Mu transpositional recombination: donor DNA cleavage and strand transfer in trans by the Mu transposase. Cell 85:271–280

Schaffer DV, Koerber JT, Lim KI (2008) Molecular engineering of viral gene delivery vehicles. Annu Rev Biomed Eng 10:169–194

Shapiro JA (1979) Molecular model for the transposition and replication of bacteriophage Mu and other transposable elements. Proc Natl Acad Sci USA 76:1933–1937

Singh RK, Liburd J, Wardle SJ, Haniford DB (2008) The nucleoid binding protein H-NS acts as an anti-channeling factor to favor intermolecular Tn10 transposition and dissemination. J Mol Biol 376:950–962

Tosi LR, Beverley SM (2000) Cis and trans factors affecting Mos1 mariner evolution and transposition in vitro, and its potential for functional genomics. Nucleic Acids Res 28:784–790

Vigdal TJ, Kaufman CD, Izsvak Z, Voytas DF, Ivics Z (2002) Common physical properties of DNA affecting target site selection of sleeping beauty and other Tc1/mariner transposable elements. J Mol Biol 323:441–452

Watkins S, van Pouderoyen G, Sixma TK (2004) Structural analysis of the bipartite DNA-binding domain of Tc3 transposase bound to transposon DNA. Nucleic Acids Res 32:4306–4312

West RB, Lieber MR (1998) The RAG-HMG1 complex enforces the 12/23 rule of V(D)J recombination specifically at the double-hairpin formation step. Mol Cell Biol 18:6408–6415

Whitfield CR, Wardle SJ, Haniford DB (2009) The global bacterial regulator H-NS promotes transpososome formation and transposition in the Tn5 system. Nucleic Acids Res 37:309–321

Yang W, Lee JY, Nowontny M (2006) Making and breaking nucleic acids: two-Mg^{2+}-ion catalysis and substrate specificity. Mol Cell 22:5–13

Young LS, Searle PF, Onion D, Mautner V (2006) Viral gene therapy strategies: from basic science to clinical application. J Pathol 208:299–318

Yu K, Lieber MR (2000) The nicking step in V(D)J recombination is independent of synapsis: implications for the immune repertoire. Mol Cell Biol 20:7914–7921

Zayed H, Izsvak Z, Khare D, Heinemann U, Ivics Z (2003) The DNA-bending protein HMGB1 is a cellular cofactor of sleeping beauty transposition. Nucleic Acids Res 31:2313–2322

Zhou L, Mitra R, Atkinson PW, Hickman AB, Dyda F, Craig NL (2004) Transposition of hAT elements links transposable elements and V(D)J recombination. Nature 432:995–1001