Review

Technology transfer from worms and flies to vertebrates: transposition-based genome manipulations and their future perspectives

Lajos Mátés*, Zsuzsanna Izsvák*† and Zoltán Ivics*

*Max Delbrück Center for Molecular Medicine, Robert-Rössle-Str., 13092 Berlin, Germany. †Institute of Biochemistry, Biological Research Center of the Hungarian Academy of Sciences, Temesvári krt., 6726 Szeged, Hungary.

Correspondence: Zoltán Ivics. E-mail: zivics@mdc-berlin.de

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Abstract

To meet the increasing demand of linking sequence information to gene function in vertebrate models, genetic modifications must be introduced and their effects analyzed in an easy, controlled, and scalable manner. In the mouse, only about 10% (estimate) of all genes have been knocked out, despite continuous methodologic improvement and extensive effort. Moreover, a large proportion of inactivated genes exhibit no obvious phenotypic alterations. Thus, in order to facilitate analysis of gene function, new genetic tools and strategies are currently under development in these model organisms. Loss of function and gain of function mutagenesis screens based on transposable elements have numerous advantages because they can be applied *in vivo* and are therefore phenotype driven, and molecular analysis of the mutations is straightforward. At present, laboratory harnessing of transposable elements is more extensive in invertebrate models, mostly because of their earlier discovery in these organisms. Transposons have already been found to facilitate functional genetics research greatly in lower metazoan models, and have been applied most comprehensively in *Drosophila*. However, transposon based genetic strategies were recently established in vertebrates, and current progress in this field indicates that transposable elements will indeed serve as indispensable tools in the genetic toolkit for vertebrate models. In this review we provide an overview of transposon based genetic modification techniques used in higher and lower metazoan model organisms, and we highlight some of the important general considerations concerning genetic applications of transposon systems.

Introduction

Class II transposable elements (TEs) that move in the host genome via a "cut and paste" mechanism are the most useful for genetic analyses, because of their easy laboratory handling and controllable nature. A schematic outline of the transposition process of a *Tc1/mariner* TE is presented in Figure 1a. Class II TEs are simply organized; they encode a transposase protein in their simple genome flanked by the inverted terminal repeats (ITRs). The ITRs carry the transposase binding sites that are necessary for transposition (Figure 1a). The transpositional process can easily be controlled by separating the transposase source from the transposable DNA harboring the ITRs, thereby creating a non-autonomous TE. In such a two component system, the transposon can only move by *trans* supplementing the transposase protein (Figure 1a,b). Practically any sequence of interest can be positioned between the ITR elements, depending on experimental need. Transposition will result in excision of the element from the vector DNA and subsequent integration into a new sequence environment.

In this review we discuss the utility of class II TEs for various genetic modifications in metazoan model systems. We do not cover applications of class I retrotransposable elements, such

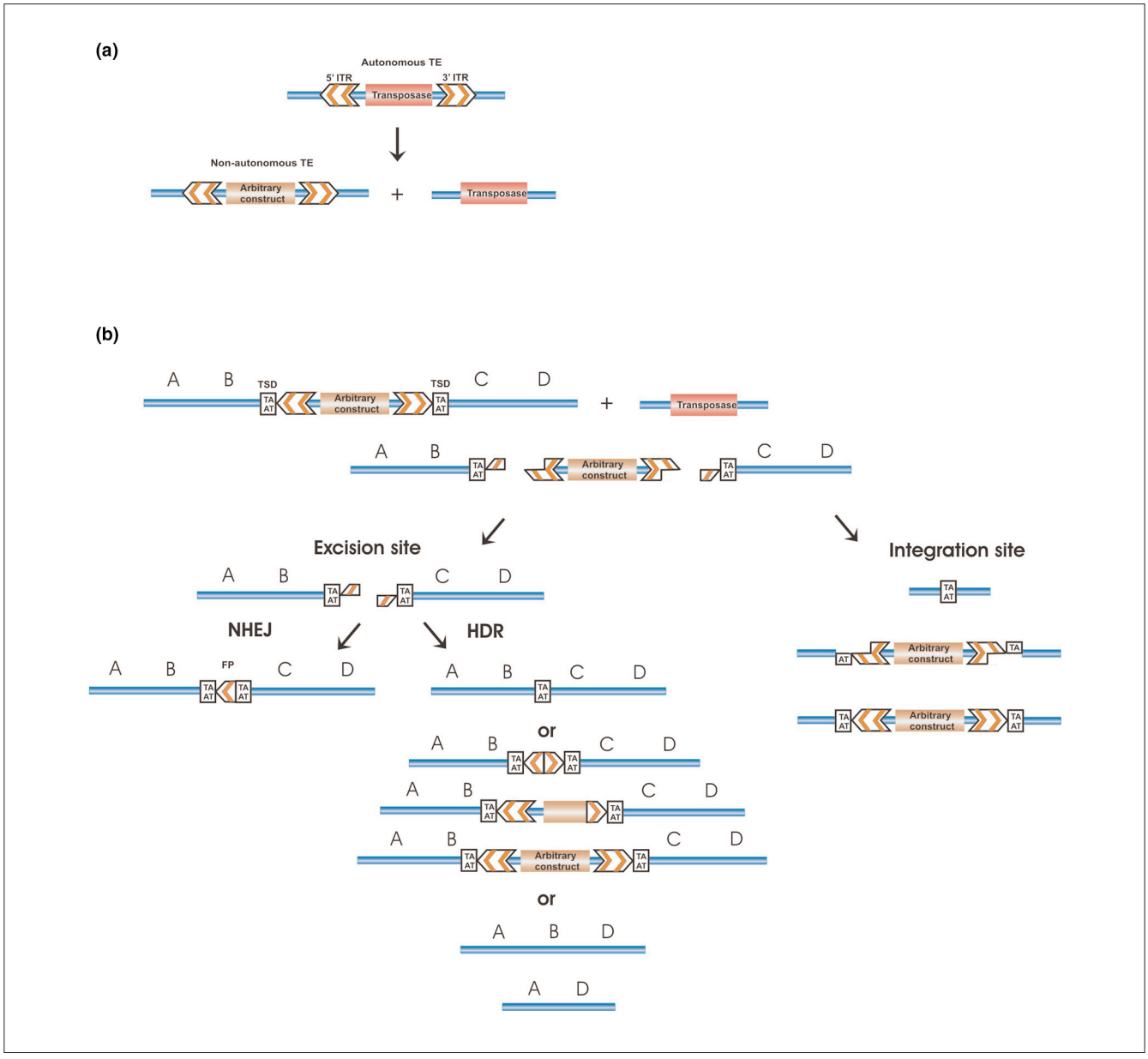

Figure 1
'Cut and paste' DNA transposition. **(a)** Scheme of a class II 'cut and paste' transposable element (TE) and that of a binary transposition system created by dissecting the transposase source from the transposon. **(b)** Outline of the mechanism of 'cut and paste' transposition and the DNA repair events that complete the transposition reaction. The model shows transposition of a *Tc1/mariner* element. The transposase introduces double strand DNA breaks at the ends of the transposon. *Tc1/mariner* elements generate 3' overhangs of varying length at the excision sites. At the excision site, nonhomologous end joining (NHEJ) typically generates a footprint (FP) that consists of the terminal base pairs of the transposon. Homology dependent DNA repair (HDR) can also contribute to repairing the transposase induced gaps. HDR can restore the wild-type sequence in cells that are heterozygous for the transposon insertion, if the homologous chromosome is available as a template. HDR can also restore either complete or partial transposon sequences at the excision site, if a homologous template containing a copy of the transposon is available. HDR may also generate deletions of flanking sequences at the excision site. The excised transposon integrates into a new TA target sequence. The single stranded gaps flanking the integrated element are repaired and give rise to target site duplications (TSD) flanking the newly integrated element. ITR, inverted terminal repeat.

as L1; for coverage of such elements, the reader is referred to another review included in this supplement [1]. We describe those features of the transposons that are important to consider for their proper use as genetic tools. Next, we provide an overview of their applications in the most prominent animal models, with a focus on *Caenorhabditis elegans* and *Drosophila*. Finally, we discuss and suggest directions for further development of transposon technology in vertebrate genetics.

Table 1

The most frequently used transposable element systems in metazoan models and some of their main characteristics

Transposon name	Transposon family	Tolerated cargo size	Target site sequence	Chromatic integration pattern
Minos (*Drosophila hydei*) [112]	*Tc1*	Possibly similar to other *Tc1/mariner* transposons	TA [113]	No preference for genes. Gene hits dominantly target introns [114]
Mos1 (*Drosophila mauritiana*) [115]	*Mariner*	Sensitive to increased cargo size [116]	TA [115]	Possibly similar to other *Tc1/mariner* transposons
P element (*Drosophila melanogaster*) [44,45]	*P*	Usually the cargo size is not limiting utility (10 to 20 kb routinely handled)	Heterogenic [117]	Bias for 5' regulatory sequences [61,62]
piggyBac (*Trichoplusia ni* cell line TN-368) [118]	*piggyBac*	Efficiency drops above 9.1 kb in pronucleus injected mice [68]	TTAA [119]	High preference for transcription units, (but the pattern is distinct from the *P* element pattern) [11,62,68]
Sleeping Beauty (salmonid fish) [67]	*Tc1*	Increased cargo size exponentially decreasing the efficiency in cultured cells [120]	TA [67]	Slight preference for genes. Gene hits dominantly target introns [12,121]
Tc1 (*Caenorhabditis elegans*) [29]	*Tc1*	Increased cargo size exponentially decreasing the efficiency in cultured cells [122]	TA [123]	Mild preference for introns in *C. elegans* [124]
Tc3 (*C. elegans*) [125]	*Tc1*	Possibly similar to *Tc1*	TA [123]	Mild preference for introns in *C. elegans* [124]
Tol2 (*Oryzias latipes* [medaka fish]) [126]	*hAT*	11.7 kb did not reduce transgenesis rates in zebrafish [127]; >10 kb transposons jump efficiently in human cells [98]	Heterogenic [72]	May prefer the 5' regions of genes [128]

kb, kilobases.

General considerations in the application of transposons as DNA delivery tools

The class II TEs most frequently used in metazoan models are listed in Table 1. Five out of the eight TEs listed belong to the *Tc1/mariner* family. The predominant application of the *Tc1/mariner* elements results mainly from their broad host range [2] as compared with other TEs (for example, the *P* element is only active in *Drosophila* flies). The basic requirements for applicability of a TE in any given model organism are that there is a sufficient level of transpositional activity in the given host and that there is a lack of endogenous copies in the targeted host genome (in order to avoid mobilization of resident copies). Studies aimed at developing hyperactive transposon mutants have established that it is possible to increase transpositional activity using various laboratory modifications [3-5]. However, host restrictions of *P* elements could not be circumvented, despite much effort [6]. Because host specificity barriers cannot easily be challenged, transposon based genetic technologies in all of the major model organisms were dependent on the discovery of TEs that are active in the species of interest (for details, see below). The use of TEs that originated from distantly related species could guarantee satisfaction of the second requirement, if they still exhibit acceptable activity in the desired host. However, cross-mobilization is not fully predictable and can be an issue, as was shown for the related *hAT* superfamily elements *Hobo* and *Hermes* [7].

Other important phenomena should also be considered when designing an individual experiment, such as cargo capacity of the TE, sensitivity to over-production inhibition (OPI), integration site preference, and transposition to linked chromosomal sites ('local hopping').

Capacity for cargo

For mutagenesis purposes, small TE vectors can be designed that retain two basic functions: the *cis* requirements for transposition and a mutagenic feature designed to disrupt normal gene function. However, size does matter for transgenesis purposes, including the generation of germline modified laboratory stocks of model species and species of biotechnologic interest, as well as for human gene therapy applications. In these instances, transgene constructs that include coding regions of genes with all of the necessary transcriptional regulatory elements can exceed several kilobases in size. The effects of increasing the size of the DNA fragments cloned between the ITRs of the different TEs are listed in Table 1. Tolerance for cargo size varies greatly between elements. Although a 100 kilobase extended *P* element that is capable of transposition has been reported

[8], members of the *Tc1/marine*r family are inhibited by increasing size. The minimal 5' and 3' terminal sequences necessary for highly active transposition are also well defined for the elements listed in Table 1.

Over-production inhibition

OPI describes a phenomenon of decreasing efficiency of transposition beyond a certain level of cellular transposase concentration. OPI has been described for a wide variety of TEs. Of the TEs listed in Table 1, it appears to affect at least the *Tc1/marine*r elements [3,9] and *piggyBac* [10] (although a recent study [11] was unable to confirm OPI of *piggyBac*). Several lines of direct and indirect evidence suggest that *Tc1/mariner* transposases act as oligomers. OPI is thought to occur when transposase monomers are present in excess concentrations; thus, in the absence of available DNA substrate, they form inactive or weakly active oligomers. Therefore, increasing the amount of transposase protein can have an inhibitory effect on transposition and, accordingly, the ideal level of transposase expression is not necessarily the highest level. In case OPI is an issue in a given experimental setup, it is advisable to test several conditions for transposase expression.

Integration site preference

Target site preference varies among transposons. For example, at the level of primary DNA sequence, the *Tol2* element does not appear to exhibit a pronounced preference for any sequence for insertion. In contrast, the *piggyBac* transposon targets the sequence TTAA, whereas all *Tc1/mariner* TEs target their integration into TA dinucleotides (Table 1). In the case of *Sleeping Beauty* (SB), this preference was studied in detail, and palindromic AT repeats were found to be preferred sites for integration [12]. Computational analyses revealed that target selection is determined primarily on the level of DNA structure, and not by specific base pair interactions. It was shown that preferred target sites have a bendable structure and increased distance between the central base pairs [12,13]. It is possible that similar structures are favored by other *Tc1/marine*r TEs. This could be interpreted as meaning that integrations will occur into any DNA available, depending solely on these preferences. This, however, is not the case. In the context of chromatin, *Tc1/mariner* elements have no or weak preference for transcription units, the 5' regulatory regions are disfavored, and most hits in genes are localized in introns (Table 1). On the contrary, the *P* element has a clear preference for the 5' regulatory regions of genes. The *P* element in this respect is similar to retroviruses, because murinie leukaemia virus prefers the 5' end of transcription units, whereas HIV exhibits strong preference for the entire length of transcription units (for review [14]). This control of integration at the chromatin level is poorly understood. One possible explanation for this can be the affinity of the transposase for unknown, chromatin associated factors. Supporting this hypothesis, a recent study showed that a host protein, lens epithelium derived growth factor, is involved in directing HIV integration into active genes [15]. Integration site preference can greatly influence the utility of transposon vectors for different applications. For example, human gene therapy protocols would require application of transposon vectors showing the least preference for genes, for obvious safety reasons. On the contrary, mutagenesis screens can capitalize on elements that exhibit a tendency to land in genes. In this respect, the utility of transposons for mutagenesis is greatly enhanced by the availability of multiple vector systems with distinct preferences for insertion.

Local hopping

'Local hopping' describes a phenomenon of chromosomal transposition in which transposons have a preference to land into *cis* linked sites in the vicinity of the donor locus. Local hopping appears to be a shared feature of cut and paste transposons. However, the actual extent of hopping to linked chromosomal sites and the interval of local transposition varies. For example, the *P* element prefers to insert within about 100 kilobases of the donor site at a rate that is about 50-fold higher than that in regions outside that interval [16]. Similarly, in germline mutagenesis screens in mice using SB, 30% to 80% of transposons have been observed to reinsert locally on either side of the transposon donor locus [17-19]. In contrast to the *P* element, SB appears to have a much larger local transposition interval between 5 and 15 megabases [18].

The local hopping feature not only differs between different transposons; a given transposon may also exhibit great variation in different hosts, and variations can be seen between different donor loci even in the same host. For example, in case of *Ac* element transposition in maize, about 50% to 60% of the reinserted elements were distributed within a 5 cM distance of the donor site [20,21], whereas the frequency of local hopping greatly varies in *Arabidopsis* and tobacco, depending on the chromosomal location of the donor site [22-24]. Moreover, local hopping of the *Ac* element in tomato appears to be overall less prevalent than in maize [25,26], and differences with regard to its tendency toward local hopping out of different transposon donor loci have been observed [27]. This variation in local hopping of the same element could possibly be explained by varying affinity of the transposase to unknown, chromatin associated factors in different hosts [28]. If that is the case, then it can be assumed that transposase mutants with altered tendency for local hopping could be created.

Local hopping can play a significant role in mutagenesis using chromosomally resident transposons. In practical terms, local hopping limits the chromosomal regions accessible to a transposon jumping out of a given chromosomal site. To circumvent this limitation, establishing numerous 'launch pads' to initiate transposition out of different loci can be a viable strategy to increase coverage of gene mutations. On the other hand, local hopping can be useful

for saturation mutagenesis within limited chromosomal regions. Even for that purpose, however, starting with more donor sites could be the most effective arrangement, because even two donor sites in closely related loci can produce strikingly different local hopping frequencies [27].

Current state of applications of transposon systems in prominent metazoan model organisms

In all model organisms described in the following sections, the TEs are used via slightly different approaches to reach similar goals. This is because of the distinct biology of the model organisms. Alterations in culturing or maintenance and other features of the organisms, and the features of the TEs alike, all influence the methodology of harnessing transposons as experimental tools in the particular model systems.

Nematodes: *Caenorhabditis elegans*

In *C. elegans*, *Tc1* and *Tc3* (see Table 1) have been widely used for insertional mutagenesis during the past 20 years. The reason for this extensive use is their early discovery in this model organism. However, their use is also limited here because of two major drawbacks. First, all known isolates of *C. elegans* contain multiple copies of *Tc1* and *Tc3*, which makes identification of the relevant mutagenic insertions difficult. The second drawback is that germline mobilization of *Tc* transposons cannot be controlled in mutator strains, in which these elements are active in the germline. However, these drawbacks can to some extent be circumvented, because the *Tc* insertion sites are well known in most of the laboratory strains, and because of differential transpositional activity in the germline of the different strains.

With respect to *Tc1* copy numbers, the strains can be divided to low copy strains such as Bristol N2 (containing approximately 30 copies) and high copy strains such as Bergerac (containing >500 copies per haploid genome) [29-31]. *Tc1* is active in somatic cells in all genetic backgrounds examined, but its germline activity is undetectable in Bristol and 1,000-fold lower as compared with somatic cells in Bergerac. Mutants have been isolated that exhibit increased *Tc1* (and occasionally other TE) activity in the germline, and their certain derivatives have low *Tc1* copy numbers. These mutant strains with elevated germline transposition rates are the so-called mutator strains. Generally, the mutations in the mutators affect the RNA interference and transgene co-suppression pathways [32]. The *mut-2* mutator exhibits the highest frequency of transposition, at about 40-fold above Bergerac [33]. Some of its derivatives, in which the original *mut-2* strain is back-crossed into the Bristol background, harbor fewer *Tc1* copies. Intensive germline transposition in the mutator strains as well as stabilization of the new insertions by repeated back-crosses to low copy nonmutator strains can be achieved. The *C. elegans* model has significant advantages in supporting rapid crossing and selection procedures needed for mutagenesis by transposons.

More recently, use of the heterologous transposon *Mos1* (Table 1) has also been established in *C. elegans* [34,35]. The *Mos1* elements are absent from the *C. elegans* genome; therefore, different experimental strategies involving this transposon could also be developed in *C. elegans* (see below).

Transposon-mediated reverse genetic approaches

Transformation of *C. elegans* is traditionally carried out by microinjection of DNA into the cytoplasm of the syncytial part of the gonad [36]. This predominantly results in formation of long, extrachromosomal arrays (a special characteristic of *C. elegans*) that can pass through the germline. Infrequently, chromosomal integrations can also occur, depending on the injection conditions. Because this method is efficient, and the extrachromosomal arrays can be maintained in the transgenic strains for a long period of time, the transposon systems have not typically been utilized for the purpose of simple transgenesis.

Reverse genetics in *C. elegans* has been hampered by the practical inability to target specific mutations to a selected gene via homologous recombination based gene targeting. Developing this technology in *C. elegans* has been unsuccessful for a long time, because of the inefficient homologous recombination and chromosomal transformation in this model organism. Recently, these difficulties were addressed by the introduction of DNA into the worms using novel methodology, which involved more efficient harvesting of chromosomal integrations and use of the new selection marker *unc-119* [37]. This method is not yet in routine use, however.

TE based mutagenesis is suitable for the purpose of target selected gene modifications in *C. elegans*. This approach relies on screening mutant libraries created by transposon insertions. In *C. elegans* it is relatively easy to isolate a specific transposon insertion in any genomic region of interest, using a polymerase chain reaction (PCR) based sib-selection method [38]. One difficulty in generating loss of function phenotypes with transposon insertions is that by using endogenous, unmodified *Tc1*-like elements, the chance for insertions creating null alleles is very low. For example, it was shown that animals homozygous for transposon insertions in the myosin light chain 2 (*mlc-2*) gene are phenotypically wild type. Interestingly, not even insertions in exons caused loss of function effects, because different atypical splicing events removed the *Tc1* part out of the *mlc-2* pre-mRNA, leaving only small, in-frame deletions and insertions [39]. Analysis of five more *Tc1* insertions in two other genes revealed that each mutant expressed substantial quantities of mature mRNA from which most or all of the *Tc1* sequences have been removed.

To circumvent the above problem and to obtain knockouts via TE insertions, a strategy of searching for chromosomal

deletions following TE excision can be used. The following mechanism is suggested to explain transposition associated deletions. TEs generally create target site duplications upon insertion and leave footprints upon excision. These footprints are generated when the double strand break (DSB), created by the transposase at the excision site, is repaired by the nonhomologous end joining DNA repair pathway (Figure 1b). Alternatively, the DSB can undergo homology dependent DNA repair (HDR). This may not result in any change to the original sequence, if the wild-type homologous chromosome is available as template, and it is used for the repair. HDR can also fill back transposon sequences to different extents, in case a homologous template containing the transposon is available for repair (Figure 1b). However, one of the free DNA ends can scan the DNA at the other end for a short homology; when this is encountered, DNA repair reactions ligate the two ends, thereby deleting the intervening DNA. In the study conducted by Zwaal and coworkers [40], gene specific PCR primer pairs were used to detect *Tc1* insertions in five genes, and subsequently to detect the DNA repair mediated partial deletions of these genes following transposon excision. Such deletion derivatives were detected at a surprisingly high frequency ($>10^{-3}$). This approach is definitely useful in a model organism such as *C. elegans* that has short generation time and easy culturing properties.

However, the transposon insertion-deletion method described above has limitations as compared with homologous recombination based approaches. Namely, it is limited to isolating only those deletions that are of a size that is detectable by PCR and that occur between the two predetermined gene specific primers; therefore, it is not useful for creating gene mutations other than null alleles. To circumvent these limitations, a method based on transgene directed, transposon mediated gene conversion was recently introduced [41]. This approach capitalizes on the observation that an episomal transgene construct can also serve as a template for the repair after the excision of the TE. In this protocol, a plasmid carrying an engineered gene modification and some regions homologous to the targeted locus is introduced into a mutator *C. elegans* strain harboring the gene specific *Tc1* insertion. This strain is then expanded into independent populations, in some of which the DSBs generated by transposon excision are repaired from this extrachromosomal template. These populations are subsequently tested for the presence of the desired mutation. The efficiency of this method seems to be about tenfold higher than that of the standard insertion-deletion approach mentioned above. Recently, the protocol was adapted to the use of *Mos1* transposition in worms [42]. The advantage of using a heterologous TE is that background transposition events can practically be ruled out.

Transposon mediated forward genetic approaches

Mutagenic insertions for forward genetics involve molecular tags that are used to clone the mutated gene rapidly. Therefore, the above strategy utilizing the *Tc1* transposon is rather cumbersome for use in forward genetics research in *C. elegans*, because the *Tc1* insertions do not provide unique tags, and so it is difficult to identify the relevant insertion responsible for a given phenotypic change. Moreover, the *Tc1* elements are active in all *C. elegans* strains, at least in their soma. Therefore, a phenotype driven insertional mutagenesis screen is more promising by using the heterologous transposon *Mos1*, which expedites the process of identifying the mutated genes [35,43]. It has been shown that mobilization of *Mos1* can also be carried out via the generally used binary system of a non-autonomous transposon and a separate transposase source (Figure 1a). These two components are maintained as extrachromosomal arrays in separate strains, and expression of the transposase protein is controlled by a heat shock promoter [34]. The mutagenicity of the *Mos1* system is in the range of that of *Tc* elements in mutator strains [43]. Therefore, the newly developed *Mos1* system is a promising tool for use in insertional mutagenesis in *C. elegans*.

Insects: *Drosophila melanogaster*

The predominantly used transposable elements in *Drosophila* are the *P* element, *piggyBac*, *Mos1*, and *Minos* (Table 1). The two closely related *hAT* elements *Hobo* and *Hermes* are also used to some extent, essentially for simple transgenic purposes. The most prominently used transposon in *Drosophila* has been the *P* element. *P* elements are currently active resident TEs in *Drosophila*. They have a very narrow host spectrum, because they are not active outside the *Drosophila* genus. *P* elements are thought to be very recent invaders of the *D. melanogaster* genome, because they were present in recently wild caught strains but not in laboratory stocks established during the first half of the past century. The source of this horizontal transfer was presumably another *Drosophila* sp. The early discovery of the *P* element, its high transpositional activity, and the *P*-element-free nature of the *D. melanogaster* laboratory strains have made this transposable element the workhorse of *Drosophila* genetics research. Early mutagenesis schemes, similar to some extent to the *C. elegans* mutator strain system, were based on crossing two strains to mobilize *P* elements in the genome, exploiting the phenomenon of P-M hybrid dysgenesis [44,45]. However, use of hybrid dysgenesis was soon exchanged for the easily controllable binary system mentioned earlier. In contrast to *Tc1* in *C. elegans*, building of this system in *Drosophila* was facilitated by the fact that the *P* element has no resident copies in the *D. melanogaster* laboratory strains. This allowed the generation of transgenic stocks each containing a separate component of the binary transposon system in its genome: one element, encoding the *P* element transposase, is carried by the 'jump starter' strain, which, upon inter-crossing, efficiently mobilizes the second, non-autonomous transposon in the genome of the 'mutator' strain [46]. This system is extremely suitable for forward genetics applications.

Transgenesis

Genetic transformation in *Drosophila* is traditionally done by the injection of preblastoderm embryos [47]. Enhancement of this transformation procedure via TE transposition into the genome is a widespread technique. Practically, all TEs listed above are successfully used for this purpose in *Drosophila*. The injection of *in vitro* synthesized mRNA as a transposase source can further enhance the efficiency of this technique because of the more rapid availability of the transposase, resulting in reduced transgene mosaicism in the embryo and therefore elevated germline transmission rates. For example, in the case of *Minos* based transformations, such methods have been shown to yield germline transgenesis rates about tenfold higher as compared with straight plasmid injections [48]. Moreover, the application of insulator sequences in combination with TEs can facilitate reliable transgene expression, and may protect endogenous genes from the effect of enhancer elements carried by the transposon construct. Indeed, a recent study demonstrated the usefulness of scs/scs', *gypsy*, and β-globin HS4 insulators to minimize position effects influencing the expression of transgenes delivered by the *piggyBac* transposon in *Drosophila* [49].

Transposon-mediated reverse genetic approaches

Homologous recombination is fairly inefficient in flies. Thus, classical gene targeting, as applied in mouse embryonic stem (ES) cells, has not been successfully established in *Drosophila*. However, induced DSBs can enhance recombination to some extent, and can trigger HDR at the breakpoints; therefore, the excision of TEs can efficiently facilitate gene conversion events. Utilizing this phenomenon, targeted gene replacement in *Drosophila* via *P* element induced gap repair has already been reported (some 15 years ago) [50]. *P* elements have also been used for the isolation of rare deletion derivatives after element excision from a gene of interest, to obtain null alleles [51], long before such a strategy was applied in *C. elegans*.

Transposon-mediated forward genetic approaches

Insertional mutagenesis using engineered TEs has proven to be one of the most productive and versatile approaches to disrupting and manipulating *Drosophila* genes on a genome-wide scale, and by far it leads the field among metazoan model systems. The experimental strategies generally utilize the 'jump starter/mutator' experimental setup, as described above. The usefulness of these screens is strongly promoted by the highly developed classical genetic tools and methods in *Drosophila*.

The *P* element has been the most widely used vehicle for these purposes. The mutagenicity of *P* element insertions is higher than that of *Tc1/mariner* elements (Table 1). Moreover, *P* elements appear to transpose efficiently with large cargo sequences inserted within the transposon (Table 1). The early mutagenesis screens carried out in *Drosophila* utilized vectors that harbor marker genes that are easy to screen such as *white*, and functional bacterial components (antibiotic resistance genes, origins of replication) that aid molecular analysis of the transposon insertion sites. Vectors of later generations were equipped with gene trapping features, representing an improvement to the basic design. The basic strategies employed to enhance the mutagenicity as well as reporting capabilities of insertional vectors by trapping transcription units are shown in Figure 2. Moreover, elements of binary systems for controlled gene expression such as the GAL4 DNA binding transcription factor (GAL4)/GAL4-upstream activator sequence (UAS) system, or for site-directed recombination such as the flip recombinase (FLP)/FLP recombinase target (*FRT*) system have also been incorporated into advanced vectors. Thus, a range of versatile experimental designs using *P* elements for insertional mutagenesis has been developed.

Enhancer trap screens are designed for the identification of enhancers by activating a reporter gene within the *P* element (Figure 2b). In early enhancer trap versions, the transposase promoter itself was utilized as a weak promoter to capture the enhancers in the genome [52]. Gene trapping is based on the activation of a promoterless reporter gene that is dependent on splicing between the exons of the trapped gene and a splice acceptor site carried by the transposon (Figure 2c). Thus, gene trap vectors both report the insertion of the transposon into an expressed gene, and have a mutagenic effect by truncating the transcript through imposed splicing.

Both enhancer and gene trap vectors have been combined with the yeast GAL4/UAS transcription activation system (Figure 2). For example, enhancer detection vectors that direct the expression of GAL4 in a genomic integration site dependent manner have been developed [53]. Therefore, such vectors report the expression pattern of trapped enhancer elements, and can also activate a cloned, UAS driven gene of interest in a tissue specific manner (specifically, in those cells in which GAL4 is expressed). The system proved to be highly useful for identifying genes that are involved in a variety of biological processes, and many GAL4 driver lines have been created and made available to the research community [54].

More sophisticated vectors that contain a polyA trap cassette that reports insertion into a Pol II transcription unit have also been developed (Figure 2d). Because polyA trap cassettes have their own promoters, they can report insertion into genes irrespective of their expression status in a given cell type. Importantly, polyA trapping is not expected to be mutagenic, because the vector is not designed to express the downstream exons of the targeted gene at the protein level. Therefore, polyA trap insertions are unlikely to cause dominant effects. Dual tagging systems that combine both gene trap and polyA trap elements (Figure 2e) have been used both in mouse [55] and in *Drosophila* [56].

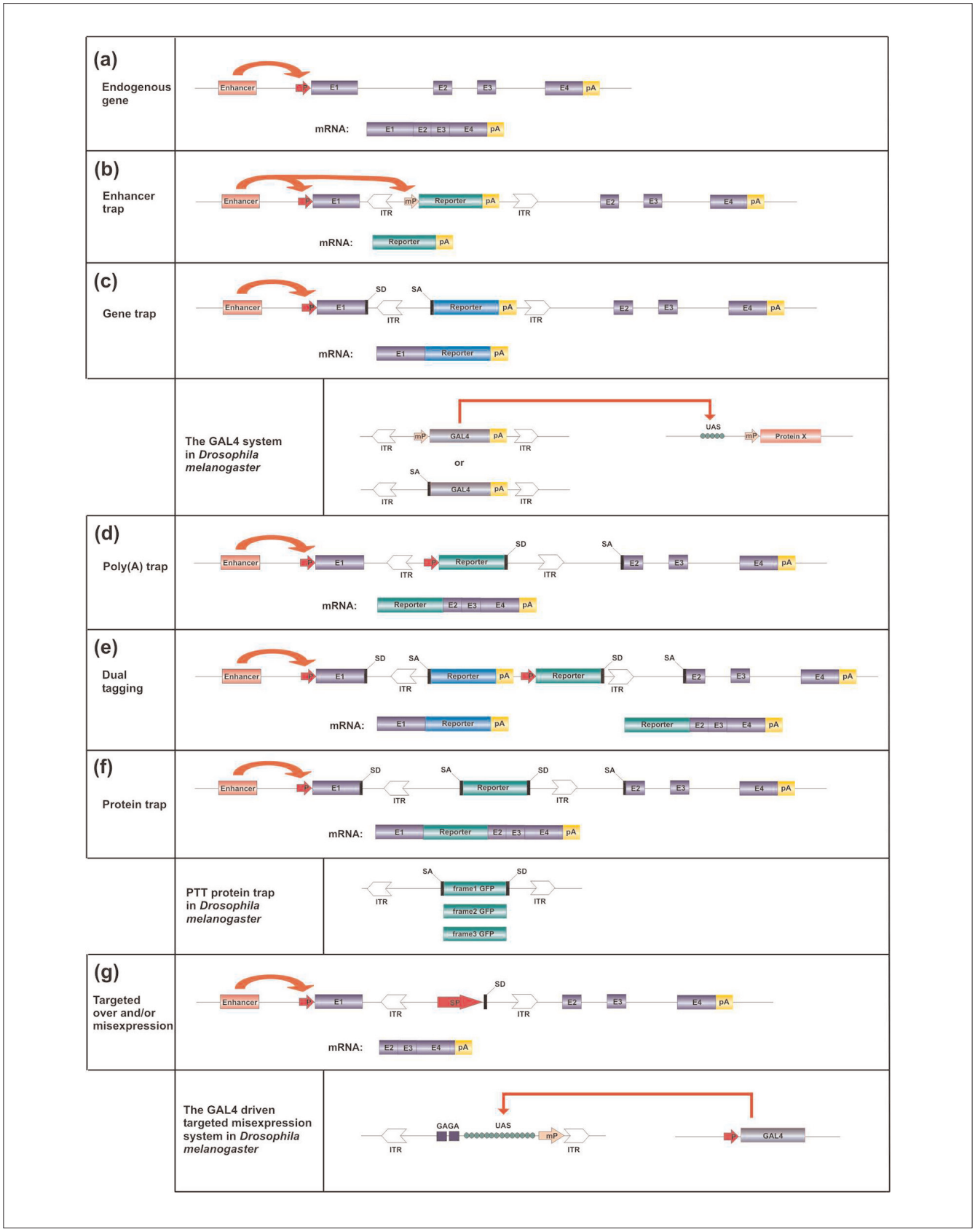

Figure 2 ***(for legend see following page)***

Figure 2 ***(see previous page)***
Summary of the basic gene trapping strategies. Genomic integration of the gene trap markers is facilitated by transposition. **(a)** Structure of a putative endogenous target gene. **(b)** The enhancer traps incorporate a reporter expression cassette driven by a minimal promoter (mP) that only results in reporter gene expression when it is affected by a genomic enhancer element, for example by transposition into a gene. **(c)** The conventional gene trapping cassettes contain a splice acceptor (SA) followed by a reporter gene and a polyadenylation signal (pA). The reporter is only expressed when transcription starts from the promoter of an endogenous transcription unit. Thus, the expression of the reporter follows the expression pattern of the trapped gene. The GAL4 system is a particularly interesting version of gene or enhancer trapping in *Drosophila*. Here, GAL4 expression is driven by the trapped regulatory regions of endogenous genes in GAL4 driver lines. Using these driver lines, any protein of interest can be over-expressed or mis-expressed by crossing these lines with others carrying the protein of interest expressed from GAL4 controlled promoter (upstream activator sequence [UAS]). **(d)** Polyadenylation (poly(A)) traps contain a promoter followed by a reporter gene and a splice donor (SD) site, but they lack a poly(A) signal. Therefore, reporter gene expression depends on splicing to downstream exon(s) of a Pol II transcription unit containing a poly(A) signal. **(e)** The 'dual tagging' vectors are based on both gene and poly(A) trapping of a targeted transcription unit. **(f)** The protein trap strategy inserts an artificial exon encoding a reporter into a gene, where the reporter is designed to be incorporated at the protein level into the endogenous gene product. The *P* element based protein trap (PTT) vector set has been created to tag proteins in all three reading frames with green fluorescent protein (GFP) in *Drosophila.* **(g)** Targeted over-expression/mis-expression is a version of the poly(A) trap strategy. Here, a strong promoter (sP) oriented toward the outside of the element is directly followed by a splice donor site. This strategy allows over-expression/mis-expression of truncated or full length endogenous proteins, depending on the site of vector integration. An improved version of this approach is the so-called modular mis-expression system in *Drosophila.* Here, a GAL4 controlled promoter (UAS) is inserted by the *P* element into an endogenous transcription unit. This arrangement allows expression of the trapped gene in any arbitrary manner of interest by crossing the carrier line with a GAL4 driver line. E1 to E4, exons 1 to 4; GAGA, GAGA transcription factor (GAF) binding site; ITR, inverted terminal repeat; P, promoter; pA, poly(A).

Protein trapping (Figure 2f), similar to the trapping systems described above, is also based on hybrid splicing events between an endogenous gene and the transgene cassette carried by the transposon; however, the vector is designed to ensure that the inserted reporter manifests at the protein level. A protein trap strategy to detect GFP tagged proteins expressed from their endogenous loci has been developed by Morin and coworkers [57]. The *P* element based PTT vector was constructed to tag proteins randomly with an enhanced GFP, without disrupting their subcellular localization. PTT carries an artificial exon that encodes GFP and is deprived of initiation and stop codons but flanked by splice acceptor and donor sequences. To enhance protein tagging efficiency, a series of constructs were created to allow reporter gene activation in each of the three reading frames (Figure 2). GFP chimeras typically retain the localization properties of the trapped proteins, except when GFP disrupts a domain necessary for subcellular targeting. Thus, transgenic lines exhibiting tissue specific GFP expression and targeting the GFP signal to virtually any compartment of the cell can be recovered. A disadvantage of the system is that it relies on the relatively rare intronic insertions of the *P* element. Other transposons such as *piggyBac* may produce more intronic insertions, which may be better suited to the requirements of such a screen (Table 1; also see below).

Another way to manipulate a trapped transcription unit that has already been proven to be useful is the targeted over-expression and/or mis-expression system (Figure 2g). Using this method, one can bring about over-expression of the full length or truncated protein product (depending on the position of transposon insertion) of the targeted gene, thereby producing dominant phenotypes by overdosing the affected gene product. An improved version of this method is the modular mis-expression system developed by Rorth [58]. It allows directed mis-expression of *P* element targeted genes in any temporal or spatial pattern. It is practically the reversal of the GAL4/UAS system. This modular mis-expression system benefits from the insertional preference of *P* element; specifically, it tends to insert upstream of the ATG codon of transcription units [58]. Therefore, the integrated transposon carrying UAS enhancer and promoter elements can frequently over-express the native full length protein. Such insertion screens can identify genes that, when over-expressed or mis-expressed in a pattern of interest, give a specific phenotype or modulate an existing mutant phenotype. For example, in the first screen conducted by Rorth [58], when activated in the developing eye, 4% of insertions gave a dominant phenotype. The next study by Rorth and coworkers [59] demonstrated the usefulness of the system in genetic interaction screens. They identified many known and new genes involved in the migration of border cells in the ovary, by suppressing the cell migration defect on a mutant background.

An important consideration for the mutagenicity of any transposon is its insertional preferences. The insertion pattern of *P* elements (and that of all other TEs) is non-random (Table 1). Moreover, *P* element insertions have numerous 'hotspots' and 'cold regions' on a genome wide scale. Investigators at the Berkeley Drosophila Genome Project have created and analyzed large collections of insertions utilizing some of the vectors mentioned above [60]. They found that, interestingly, the observed hotspots can be divided to 'common hotspots' and 'screen specific hotspots'. The first class is commonly hit by all *P* element vectors, whereas the second is preferentially hit only in a particular mutator strain [60]. Hotspots seen in local hopping are excluded from the second class. The existence of screen specific hotspots suggests that the specific parameters of the screen, such as the structure and location of the mutator transposon, can affect the spectrum of hotspots and the diversity of the targeted genes. This phenomenon is poorly characterized, and the underlying cellular mechanisms remain unclear.

Common hotspots represent the main obstacle to full genome coverage with *P* element vectors. It is estimated that about 150,000 *P* element insertions might need to be screened to obtain 87% saturation of the estimated 13,500 *Drosophila* genes [61]. To complement the use of *P* elements, alternative transposon systems have successfully been contributing to large scale insertional mutagenesis of the *Drosophila* genome. For example, investigators at the Berkeley Drosophila Genome Project have already included *piggyBac* and *Minos* element based screens in the collection. A large scale comparison of the insertion patterns of *piggyBac* and *P* element vectors has already been reported [62]. It has been found that *piggyBac* exhibits an insertional preference that is distinct from that of *P* elements. It does not share chromosomal hotspots that are associated with *P* elements and, although *piggyBac* favors genes as targets, it lacks the bias for 5' regulatory sequences [62]. The target preference of *Minos* also differs from both *P* elements and *piggyBac*, and appears to be similar to that of SB (Table 1). Therefore, these TEs are used as complementary tools for *Drosophila* mutagenesis.

The combined use of different TEs is common in *Drosophila*. Practically all of the insertional mutagenesis screens have been established by the creation of 'jump starter' and 'mutator' *P* element lines, whose generation is facilitated by use of other TEs, generally the *Hobo* or *Hermes* elements. For some more sophisticated screening strategies, it is also important to have alternative TEs for effective insertional mutagenesis on a background already carrying *P* elements and possibly also *Hobo* or *Hermes*. Hacker and coworkers [63] used *piggyBac* for insertional mutagenesis on a chromosome harboring *P* element inserted *FRT* sites for the generation of mitotic recombinants. The *FRT* sites remained stably integrated, demonstrating that the two systems are compatible.

Chromosome rearrangements

Chromosomal deletions in *Drosophila* are indispensable, classic genetic tools for mapping mutations, characterizing alleles, and identifying interacting loci. The *P* element based methods for creating deletions are extremely useful because the end-points of the deletions are molecularly marked. Furthermore, their positions can be designed, which is of particular importance in avoiding haplolethal or haplosterile loci. Two *P* element based methods are in predominant use to create chromosomal deletions [64]. One is based on the observation that *P* transposase often induces chromosomal aberrations that involve the sites of two distinct *P* element insertions. The process behind this is called 'hybrid element transposition', in which the TE ends come from separate elements rather than a single element (the 5' end of one element pairs with the 3' end of another one at a different location) [65]. This results in different chromosomal rearrangements from which the deletions can be selected. An improved version of the transposon based method for creating deletions makes use of the FLP/*FRT* site specific recombination system (Figure 3a). Transposon vectors, usually the *P* element, are used to facilitate single copy chromosomal integration of *FRT* sites. FLP mediated recombination between two of these *P* elements can result in chromosomal rearrangements. With a sufficiently high density of starting insertions, even single gene deletions can be created. Progress is currently being made toward covering the genome of *Drosophila* with characterized deletions [64].

This technique can also create predesigned chromosomal translocations between the chromatids in flies with transgenic *FRT* sites at identical positions on the homologous chromosomes [66]. Thus, FLP mediated recombination can be used to generate mitotic clones (Figure 3b), in which only clones of the cells of interest are homozygous for the studied mutation, whereas the rest of the organism is heterozygous. Spatially or temporally controlled expression of FLP recombinase can create homozygous clones in any tissue or developmental stage of interest.

Chromosomal inversions are also powerful tools in classical *Drosophila* genetics. They have been applied in the majority of genetic screens in *Drosophila* as balancer chromosomes, because they can block recombination within the region of the inversion. For practical reasons, the balancers carry visible markers, and are also designed to be recessive lethal. Most of them were isolated as spontaneous or induced chromosomal rearrangements having break points in vital genes. TEs have not been typically used to engineer balancers. The balancers greatly help to map and maintain mutations in the *Drosophila* genome.

Vertebrates

Transposons have successfully been used in lower metazoan and plants for transgenesis and insertional mutagenesis, but until the reactivation of the SB transposon system in 1997 [67] there was no indication that any DNA based transposons in vertebrates were sufficiently active for these purposes. Subsequently, other elements were shown to catalyze efficient transposition in vertebrate model organisms. For example, the insect TEs *piggyBac* and *Minos* have proven to be useful in germline mutagenesis in vertebrates [68,69]. Moreover, the reconstructed endogenous amphibian element *Frog Prince* [70], the reconstructed human *Hsmar1* element [71], and the *Tol2* element isolated from the medaka fish [72] have been found to be active in vertebrates. We do not extensively review current transposon applications in vertebrate models here (other reviews in this supplement provide coverage of these areas). However, we do discuss the general approaches that have been taken, and suggest further avenues for exploiting TEs in applications in vertebrates.

Transposon based experimental strategies in vertebrates all utilize the two component, binary approach, in which transposition is controlled by *trans* supplementation of the

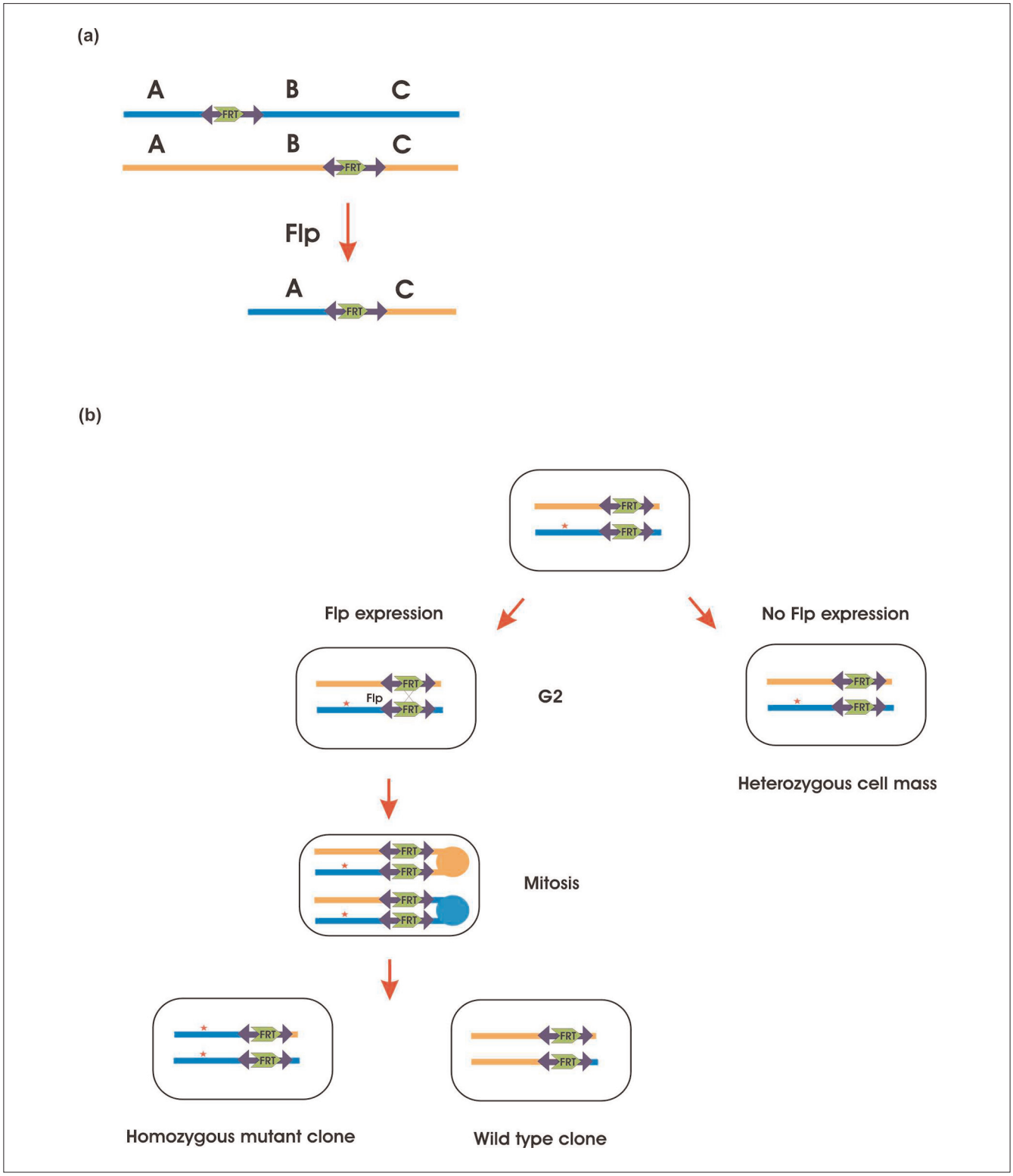

Figure 3
FLP/*FRT* site-specific recombination-mediated chromosomal rearrangements in *Drosophila.* The *FRT* sites are introduced into the genome via *P* element transposition. **(a)** Creation of deletions with well defined end-points. Two fly lines bearing *FRT* sites within *P* elements localized at different positions on the same chromosome are crossed. Subsequently, FLP creates the desired deletion. **(b)** Generation of mitotic clones in *Drosophila.* In this arrangement, the *FRT* sites are in identical positions on the homologous chromosomes of which one parental carries the mutation of interest. FLP, flip recombinase; *FRT*, FLP recombinase target.

transposase proteins, owing to the fact that the majority of these TEs are not endogenous in the most important model organisms.

Transgenesis

Classic ways to induce expression of foreign genes in vertebrates rely on microinjection of nucleic acids into oocytes or fertilized eggs. Two main drawbacks of these approaches are the low rates of genomic integration and that the injected DNA generally integrates as a concatemer. Both drawbacks can be circumvented by utilizing transposition mediated gene delivery, because it can increase the efficiency of chromosomal integration and facilitates single copy insertion events. Single units of expression cassettes are presumably less prone to transgene silencing than are the concatemeric insertions created using classical methods. Retroviral vectors are also useful tools for the same purpose, but their integration pattern is potentially more mutagenic because of their preference for the 5' end of transcription units (for review [14]). In case of transgenesis, a single copy insertion away from endogenous genes is clearly desired. The insertional spectrum of *Tc1/mariner* elements satisfies this need the best (Table 1), because these elements integrate randomly at the genome level, and do not exhibit pronounced bias for integration into genes. Another particular problem concerning transgenesis is that founders that develop from the injected oocytes or eggs are predominantly mosaic for the transgene, because integration generally occurs relatively late during embryonic development. Therefore, in order to promote successful transmission of the transgene through the germline to the next generation, it is necessary to shift the window of integration events to as early as possible. This can be facilitated by co-injection of engineered transposons with transposase mRNA. This method has been employed to generate transgenic zebrafish with *Tc3* [73], *Mos1* [74], *Tol2* [72] and SB [75]; transgenic *Xenopus* with SB [76] and *Tol2* [77]; and transgenic mice with SB [78-80].

Transposon mediated forward genetic approaches

To carry out phenotype-driven forward genetic screens in vertebrates, one must achieve efficient germline mutagenesis. To date, the most efficient mutagen in the mouse germline is the chemical *N*-ethyl-*N*-nitrosourea (ENU). Mutagenesis rates using ENU are two to three orders of magnitude higher than those with insertional mutagens [81]. However, ENU is an ethylating agent that generally causes single base pair mutations. Therefore, the average phenotypic effect of an insertional vector can be more dramatic, and the total number of insertions required to reach the same mutagenic effect is expected to be lower. Moreover, identification of the point mutations causing a phenotype requires positional cloning, which is time consuming and laborious work. Therefore, insertional mutagenesis is an attractive alternative to ENU screens.

Both TEs and retroviral vectors can successfully be applied to insertional mutagenesis. Cultured cells can be efficiently infected with viruses [82]. A pseudotyped retrovirus has also been applied to generate insertions in zebrafish via microinjection of virus particles into blastula stage embryos [83]. In this approach, however, the transgenic founder fish are mosaic and must be out-crossed to establish F_1 fish with retrovirus insertions. Therefore, one drawback of the application of retroviruses is that the convenient 'jump starter' and 'mutator' method is not applicable with these vectors. Moreover, handling of such viruses may bring up safety issues.

Using TEs as tools for insertional mutagenesis is simpler, and the 'jump starter' and 'mutator' scheme can be applied. In *Drosophila*, transposon mediated insertional mutagenesis is predominantly carried out using such a scheme. This is also the preferred method in vertebrates, and it has successfully been adopted for germline mutagenesis in the mouse, but not in zebrafish, in which the screens are currently performed by co-injection of the transposon DNA and the transposase mRNA.

In zebrafish, SB and *Tol2* were shown to be useful for insertional mutagenesis in co-injection experiments [84-88]. In the mouse, it has been demonstrated that SB, *Minos*, and *piggyBac* transposases can function in transgenic animals [17,18,68,78,89-92]. Recently, SB based insertional mutagenesis was also established in the rat [93,94]. In these experiments, chromosomally resident transposon vectors were mobilized in transgenic animals that either ubiquitously expressed the transposase or expressed the transposase in the male germline using the protamine 1 (*Prm1*) promoter. SB based insertional mutagenesis has successfully been applied in mice to recover a range of mutant phenotypes in a crossing scheme applying a balancer chromosome [95]. This and other studies [96] demonstrated that local saturation mutagenesis of a genomic region is a realistic goal using the SB transposon system with a chromosomally resident transposon donor site. The *Minos* transposase has also been shown to mobilize non-autonomous *Minos* elements in mice by transposase expression in the oocytes using ZP3 [69] and in the lymphocytes using CD2 promoters [91]. *PiggyBac* has also been used in co-injection experiments in mice [68]. The activity of *Tol2* element has already been demonstrated in mouse ES cells [97] and *in vivo* in the mouse liver [98].

All of the vectors used in vertebrate insertional mutagenesis to date are versions of gene trapping insertional mutagenic constructs (Figure 2), equipped with elevated mutagenicity and other useful properties. The mutagenicity of gene trap vectors is higher than that of simple insertional vectors, and they enable easy identification of the mutagenized gene by reverse transcription PCR of composite transcripts composed of sequences of the insertional vector and the endogenous gene. In cell culture, drug resistance markers are generally in

use, whereas in animal systems other reporters such as GFP are sufficient for this purpose. Similar to the GAL4/UAS system in *Drosophila*, a conditional, tetracycline regulated system has been shown to be applicable to TE mediated insertional mutagenesis in mice [99].

As an alternative to the loss of function approaches, targeted over-expression and/or mis-expression has been shown to be efficient in somatic tissues of mice using SB. Viral enhancer promoter elements incorporated into SB vectors were shown to be useful in inducing cancer in experimental animals [100,101]. These screens can also capitalize on TEs with an intronic preference for insertion, such as members of the *Tc1* family. In order to devise customized screens for cancer development, a current approach is focusing on establishing mouse lines that conditionally express the transposase [102]. One approach is to express the transposase from tissue specific promoters. The second is to generate a Cre recombinase inducible transposase allele, and to take advantage of the many existing Cre strains to induce mutagenesis in specific tissues in mice [102].

Perspectives

Insertional mutagenesis

The parallel development and application of alternative transposon systems will be beneficial for insertional mutagenesis in vertebrates. As described above, *P* elements, *piggyBac*, and *Minos* elements are already in use as complementary transposon tools for large scale mutagenesis in *Drosophila* [60,62]. The combined use of these TE systems makes full genome coverage with TE based insertional mutagenesis a realistic goal. Work to create comprehensive mutant collections is already underway in mice by the Knockout Mouse Project (National Institutes of Health, US) and by the European Conditional Mouse Mutagenesis project. The goal of these initiatives is to knock out every single gene in mouse ES cells using conditional gene trapping and gene targeting approaches. It is therefore likely that collections of transposon insertional mutants for reverse genetic purposes will prove even more useful in species that lack efficient homologous recombination based gene targeting techniques and ES cells, such as zebrafish, *Xenopus tropicalis*, and rats.

The 'jump starter' and 'mutator' experimental scheme is widely used in both *Drosophila* and mice. Even though microinjection does not appear to be a bottleneck in transposition based transgenesis in zebrafish, establishing a mutagenesis set-up based on breeding could be beneficial. With this method, the numerous injections of fertilized eggs could be eliminated, and transposition events segregated into offspring by simple crossing of founders. Moreover, local saturation mutagenesis would also be possible in this way in zebrafish. In mice, the existence of the mutator lines and the local hopping feature of the TE systems offers the possibility of using local saturation mutagenesis to screen through chromosomal regions harboring as yet unidentified genes in which disease causing mutations occur.

Insertional mutagenesis with SB in both germline and somatic tissues of the mouse has been approached with mutator lines harboring transposon donor loci containing many copies of the transposon vector in the form of concatemeric arrays. The reason for this is that transposition rates out of multicopy concatemers is far more efficient than out of single copy donor sites. Consequently, Dupuy and coworkers [100] used founder lines with 148, 214, and 358 transposon copies in concatemeric donor sites, whereas Collier and colleagues [101] worked with animals containing approximately 25 copies of the mutagenic transposon in somatic mutagenesis studies. Similarly, a concatemeric donor site of approximately 30 transposon copies was used in a gene trap germline screen in mice [95]. However, transposition out of multicopy donor loci may complicate assignment of a phenotype to a particular transposon insertion in at least two ways. First, a phenotype may be associated with multiple insertion events, and segregation of the insertions may lead to loss or change in the phenotype. Second, recombination between newly transposed transposon copies and the donor concatemer could lead to unwanted genomic rearrangements, as was observed by Geurts and coworkers [95]. The most likely explanation for the rearrangements is that transposition out of a concatemer generates new transposase binding sites linked in *cis* (in case of local hops) or on other chromosomes. However, because transposon copies remain at the original donor locus, transposase can recombine chromosomal sequences that are located between the transposase binding sites by hybrid element transposition (described in the section on *Drosophila*, above), leading to deletions and translocations. Alternatively, hybrid element transposition involving transposon copies in the donor concatemer could result in similar genomic rearrangements. Such chromosomal rearrangements are unlikely to occur (or would occur at a much reduced frequency) if a single copy donor was used. Thus, there is a great need for transposon systems that are sufficiently active for efficient transposition out of single copy donors in animal breeding schemes. Ongoing work in our laboratory aiming at the isolation of hyperactive transposases could potentially eliminate the need for concatemeric donor sites.

The use of single-copy donors would also enable the application of dosage-dependent color markers such as tyrosinase and agouti for phenotypic marking of transgenic animals. These markers could be exploited to confer a light brown or a much darker color to an albino coat, depending on whether they are present as single- or double-copy transgenes in the mouse genome, respectively. This would be helpful to identify homozygous transposon insertions.

The approach used for the creation of the PTT vectors (Figure 2) in *Drosophila* would possibly enhance the utility

of protein over-expression studies in mice as well. In two somatic mutagenesis studies in mice, the trapped genes were over-expressed from a viral long terminal repeat carried by the SB transposon [100,101]. In these experiments, transcription started from the viral enhancer promoter and the resulting pre-mRNA was spliced between a splice donor site carried by the transposon vector and the downstream exon of the trapped gene (Figure 2). However, translation of the hybrid mRNA (and generation of a sense protein product) is dependent on the fortuitous occurrence of an in-frame ATG codon in the downstream exons. This can clearly be a limitation to successful over-expression events. To circumvent this problem, we propose that, similar to the PTT vectors applied in *Drosophila* (Figure 2), splice donor sites set to phases 0, 1, and 2 (according to the three possible reading frames) and built in behind a strong mammalian translation initiation consensus sequence could be used in over-expression studies. Because the intron phase distribution of mouse genes is unequal [103], using only the phase 0 construct could result in a success rate of about 50% in terms of trapping potential.

In the over-expression experiments mentioned above, the transposase was continuously expressed in the soma. This could result in multiple rounds of transposition events of the same transposon copy. Therefore, for these and possibly other experimental set-ups, it would be beneficial to limit secondary jumps by transposon immobilization after transposition [104]. One possibility in this regard would be to use recombinase systems, where *FRT* or *loxP* sites could be inserted within the ITRs. This way, Cre or Flp mediated recombination would result in the partial loss of the ITR and a part of the internal transposon sequences, and so the resulting defective transposon would not be able to move again. The caveat of this strategy is that insertion of *FRT* or *loxP* sites could negatively affect transposition of the modified element.

Chromosomal rearrangements

The most effective agent in terms of mutagenicity is the widely used chemical mutagen ENU. The application of ENU is an efficient method in mice, but the precise identification of the mutations in a screen requires a laborious positional cloning approach. Classical genetic tools similar to those extensively used in *Drosophila* could facilitate this identification process. In this respect, both nested deletion sets and balancers over different loci could be especially useful in mouse forward genetics screens. One particularly interesting transposon based experimental system for insertional mutagenesis and chromosomal rearrangements, developed by Osborne and coworkers in *Arabidopsis* [105], could facilitate the creation of these genetic tools. Briefly, in this arrangement, the advantages of *Ac*/*Ds* transposition and the Cre-*lox* system were combined in *Arabidopsis*. The Ds*lox* transposon, carrying the phosphonothricin (*Ppt*) resistance gene and a *loxP* site just in front of a promoterless gentamicin resistance (*Gn*) gene, jumps out of a donor locus harboring the chlorsulfuron (*Cs*) resistance gene and another *loxP* site between a strong plant promoter and the *Cs* coding sequence (Figure 4a). The excision and re-integration of the Ds*lox* transposon may result in double Ppt^R and Cs^R phenotypes. Subsequent Cre mediated recombination leads to two types of chromosomal rearrangement; deletions are associated with Gn^R phenotype and clearance of Ppt^R and Cs^R phenotypes (Figure 4b), whereas inversions are associated with a Gn^R phenotype and silencing of the *Cs* resistance gene (Cs^S; Figure 4c). The other important key features of the system are the single copy donor sites at different chromosomal positions that make herbicide selection for coupled excision and re-insertion possible, and the local hopping feature of *Ac* transposition that generates the dominantly useful arrangement of two *loxP* sites for inversions and deletions of intervening DNA of different length. The system results in inversions, deletions, and smaller numbers of translocations, creating different resistance palettes and different phenotypes and viability. This system can be used to map loci with nested deletions and to establish balancer chromosomes from the inversions [106].

In case of vertebrates, composite arrangements of transposons carrying a *loxP* site embedded in another TE that carries the other *loxP* site could facilitate the generation of allelic series of local hops out of single copy donor sites. The *Tol2* and *piggyBac* systems, having larger cargo tolerance, appear to be suitable for the delivery of such a complex arrangement. Transposition of the inner element out of the donor locus would move one *loxP* away, whereas the other *loxP* would stay at the original site. This system, based on the subsequent Cre-mediated chromosomal engineering between the transposed *loxP* sites, would greatly facilitate the creation of both of the two experimental tools in mice described above: viable balancer chromosomes and chromosomes bearing large deletions for establishing segmental haploidy.

Balancer chromosomes usually contain one or more inverted segments, and they suppress recombination within those segments. They are used as genetic tools to maintain recessive lethal mutations, providing an easy selection system for the heterozygous carrier animals. Balancer chromosomes are extensively used in *Drosophila* and also proved to be very useful in mouse genetics [106,107]. In *Drosophila*, because many animals are allowed to mate randomly, and several generations can be maintained in one tube, the balancer must contain a homozygous embryonic lethal mutation. These recessive lethal mutations are generally defined by the end-points of the inversion, in which a gene necessary for embryonic development is broken. In contrast, mice are maintained as single mating pairs, and the offspring can easily be selected and separated. This means that the recessive embryonic lethal mutation on the balancer chromosome could be replaced

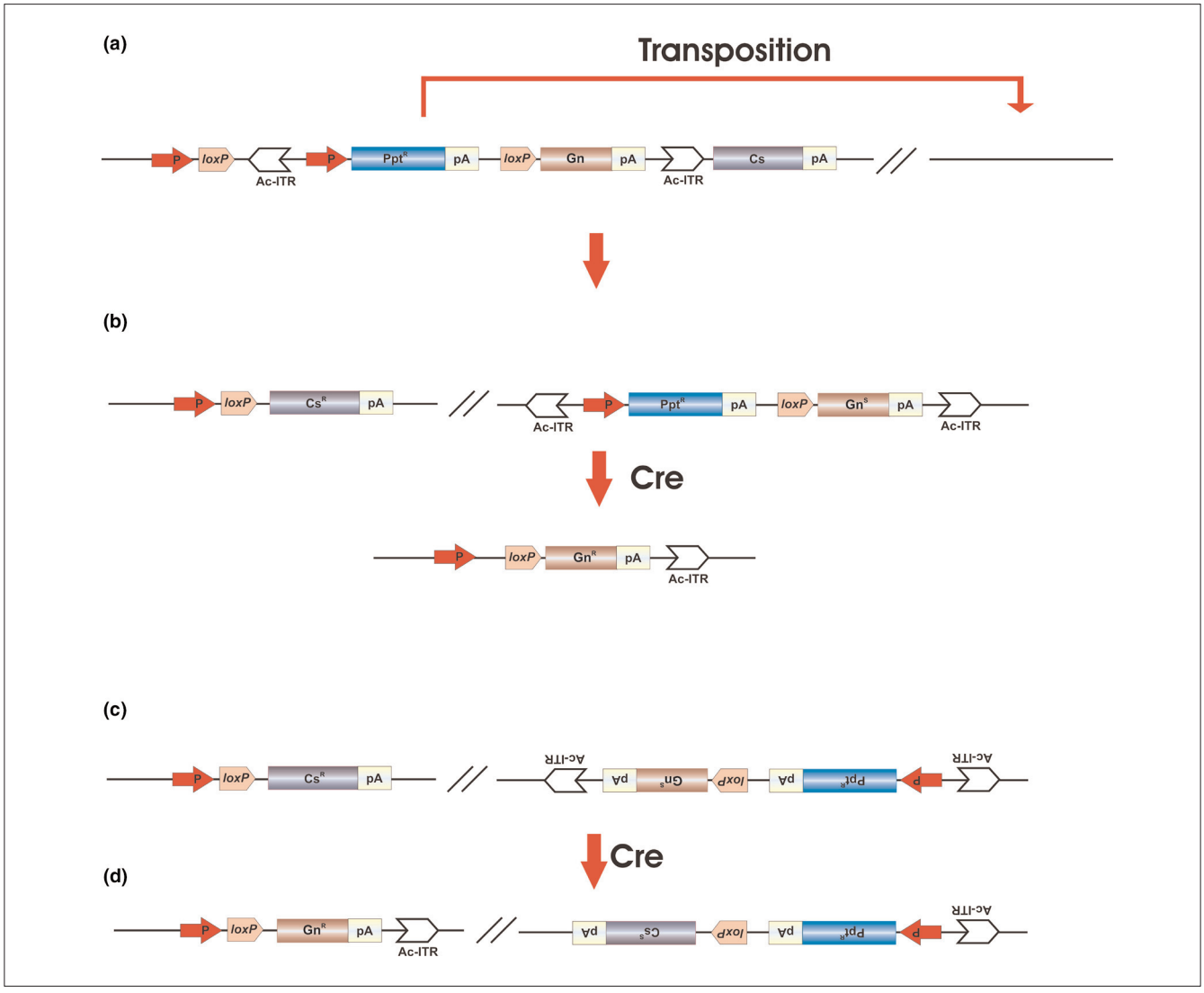

Figure 4
A transposition mediated system for creating chromosomal rearrangements in *Arabidopsis*. **(a)** Structure of the donor locus. **(b)** Creation and detection of chromosomal deletions. **(c)** Creation and detection of chromosomal inversions (for details, see text). Ac-ITR, *Ac* element inverted terminal repeat; Cs^R and Cs^S, chlorsulfuron resistance gene (active and silenced, respectively); Gn^R and Gn^S, gentamicin resistance gene (active and silenced, respectively); *loxP*, recognition site of the Cre recombinase; P, promoter; pA, poly(A); Ppt^R and Ppt^S, phosphonothricin resistance gene (active and silenced, respectively).

with a visible marker, allowing the discrimination of animals that are heterozygous or homozygous for that marker [106]. This improvement permits discrimination of the embryonic lethal mutations generated in a screen from the mutation carried by the balancer, and it makes the creation of useful balancers without pre-defined end points possible. Such an improved mouse balancer chromosome has been reported by Nishijima and coworkers [108]. They used two coat color markers, tyrosinase and K14-agouti, and therefore the dosage of the inversion chromosome could be visually recognized.

Because of their usefulness, a current goal of the mouse community is to establish balancers that cover the entire mouse genome. Technologies based on transposition could be developed to create a series of viable inversions bridging over a genomic locus using an experimental scheme similar to the one developed by Osborne and coworkers [105] in *Arabidopsis*. The donor chromosome is generally favored for insertion over the other chromosomes in SB transposition about 60% of the time, with about 40% occurring in a 5 to 15 Mbp local hopping window and about 20% outside the typical local hopping range, but still chromosomally linked

to the donor locus [95,96]. Thus, a large portion of transposition events could yield potentially useful chromosomal rearrangements in mice.

Chromosomes harboring segmental haploidy can be used for deletion mapping of loci. An effective approach for mapping novel recessive mutations is based on a nested chromosomal deletion set, in which the deletions vary in size and have different end-points, but they partially overlap across the locus suspected of carrying the candidate gene affected by the mutation. After crossing these mice with a mouse strain carrying a novel recessive mutation, the recessive phenotype is observed when the deletion spans over the mutated gene on the other allele, and therefore no wild-type gene product is present.

The classical gene targeting approach to create these deletions would require two targeting steps for each deletion end-point (for review [109]). It is a laborious work, especially in the case of nested deletion sets, despite the fact that one end-point could be common for all of the deletions. Moreover, it is difficult to predict the viability of a given chromosomal rearrangement. To circumvent these difficulties, a deletion set has been made where the second end-points of the deletions were defined by a randomly integrating recombinant retrovirus that carried the second *loxP* site necessary for Cre mediated rearrangements [110]. Recently, this method has been further developed by delivering both of the *loxP* sites into the genome of mouse ES cells via retroviral gene transfer [111]. In this study, the authors created two recombinant retroviruses, one of which, referred to as 'anchor virus', introduced the first *loxP* site, whereas the other, referred to as 'saturating virus', introduced the second *loxP* site into the genome of ES cells. The drug selection system applied by these authors was fairly similar to the one used by Osborne and coworkers in *Arabidopsis* [105]. Although these methods based on the application of replication-defective retroviruses proved to be useful, one can predict that a transposon-based system that exploits local hopping of transposons, and guarantees single-copy insertion of the second *loxP* site, would represent an efficient and elegant approach to the creation of nested chromosomal deletion sets in mice.

Conclusion

Significant progress has recently been made toward the development of improved transposon based systems for genome manipulations in vertebrate model organisms, including transgenesis and insertional mutagenesis in both germline and somatic tissues. These efforts begin to pay dividends to the research community, as we witness an increasing interest in applying transposon tools for applications ranging from simple tissue culture setups to generate transgenic cell clones to experimental systems aiming to unravel genetic networks cooperating in tumorigenesis using transposon mutagenesis in living animals. Nevertheless, applications of transposons for vertebrate genetics are still lagging somewhat behind the sophisticated, transposon-based technology platform that has been established in invertebrate model systems, especially in *Drosophila*. We highlighted in this article possible improvements, and new avenues for the use of transposons in vertebrates, based on the lessons that have been learned in invertebrates. This 'technology transfer' offers a possibility to expoit transposable elements as gene delivery agents to their full potential.

Competing interests

The authors declare that they have no competing interests.

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