It takes two transposons to tango
transposable-element-mediated chromosomal rearrangements

Transposable elements (TEs) promote various chromosomal rearrangements more efficiently, and often more specifically, than other cellular processes. One explanation of such events is homologous recombination between multiple copies of a TE present in a genome. Although this does occur, strong evidence from a number of TE systems in bacteria, plants and animals suggests that another mechanism - alternative transposition - induces a large proportion of TE-associated chromosomal rearrangements. This paper reviews evidence for alternative transposition from a number of unrelated but structurally similar TEs. The similarities between alternative transposition and V(D)J recombination are also discussed, as is the use of alternative transposition as a genetic tool.

Since the first description of mobile genetic elements, transposable elements (TEs) have been found to be associated with chromosomal rearrangements such as deletions, duplications, inversions, the formation ofacentric fragments and dicentric chromosomes, translocations and recombination of host genomes. This aspect of transposable element function has implications for evolution and for understanding several human genomic disorders. Because of this, the mechanisms involved in transposon-mediated chromosomal rearrangements warrant thorough investigation.

TEs are classified by their sequence structure and transposition mechanisms. Class I TEs – retrotransposons and retrotransposons – transposes by an RNA intermediate. Retrotransposons have a structure similar to mRNA; retrotransposons are structurally similar to retroviruses and are bounded by long terminal repeats (LTR). Class II TEs – insertion sequences (IS elements, Box 1) and transposons – transposes by a DNA intermediate catalysed by a transposase enzyme. IS elements and transposons are bounded by terminal inverted repeats (TIR). In addition to the TIR, additional sequences differentiate the two ends and are necessary for transposition. In prokaryotes, IS elements contain sequences encoding transposase, and transposons are TEs that contain sequences encoding other genes in addition to transposase, such as genes encoding enzymes responsible for antibiotic resistance. In eukaryotes, all TEs that transpose by a DNA intermediate are classified as transposons. Some Class II TEs, such as IS10, IS50, Ac/Ds (Box 1), Tam3, P, hobo and mariner, encode a single transposase gene. Other Class II TEs, such as Tn7, Phage Mu, M. cator and En/Spm, encode multiple proteins that catalyse and regulate transposition.

Two possible mechanisms by which TE-associated chromosomal rearrangements can occur are (i) indirectly by homologous recombination or (ii) directly by an alternative transposition process.

The indirect action of TEs promotes chromosomal rearrangements by presenting the genome with multiple similar, if not identical, sequences between which strand transfer can occur. This may occur by recombination of the homologous sequences or by faulty repair of double-strand breaks formed during transposable element excision using ectopic homologous sequences as a repair template.

Not all the rearrangements observed can be explained by homologous recombination between elements at different locations. For instance, rearrangements have been
Homologous recombination between repetitive sequences, such as TEs, can result in chromosomal rearrangement such as deletions, duplications and inversions. Each line represents a DNA double helix. The two sister chromatids of each of the homologous chromosomes are shown. Black ovals denote the centromere. TEs are represented by the thick black line bounded by open and closed arrows, indicating relative orientation of the element. The TE insertion sites are illustrated by open circles or boxes, with each shape representing distinct insertion sites on the chromosome and the equivalent sites on the homologous chromosome(s) without a TE at that site. Homologous recombination requires a minimum of two copies of the repetitive sequence, one at each breakpoint, and is denoted by an ‘X’ in this figure. (a) TEs in same relative orientation on homologous chromosomes result in the formation of chromosomes containing either a deletion or a duplication of the intervening sequence. Both rearrangements are associated with recombination between two homologues. (b) TEs in opposite relative orientation on homologous chromosomes result in the formation of a dicentric chromosome and an acentric fragment. (c) TEs in same relative orientation on one chromosome result in the formation of chromosomes containing either a deletion or a duplication of the intervening sequence. Both rearrangements are associated with recombination between two homologues. (d) TEs in opposite relative orientation on one chromosome can result in the formation of an inversion between the two TEs. If caused by homologous recombination, deletions and duplications can only be formed by TEs in the same relative orientation and inversions can only be formed by TEs in opposite relative orientation. Another mechanism must be invoked to explain inversions between TEs in the same relative orientation, deletions and duplications between TEs in opposite relative orientations, and all chromosomal rearrangements when a TE is present at only one of the rearrangement breakpoints.

TEs - a common resource for genome plasticity
In order to comprehend complex chromosomal rearrangements induced by alternative transposition of TEs, one must first understand the basics of traditional transposition. The TEs inducing rearrangements described in this review are all Class II TEs encoding a single transposase and include prokaryotic IS elements and both prokaryotic and eukaryotic transposons. Functionally, these TEs share a common conservative transposition mechanism, known as cut-and-paste, where the first step of transposition is the synthesis of complementary left- and right-TE ends, followed by excision of the ends, target site capture and strand transfer. Insertion of the TE into the target molecule can occur in either orientation relative to the original element, resulting in a simple insertion. Repair of the double-strand breaks produced during alternative transposition is analogous to V(D)J recombination and provides additional evidence supporting the theory that V(D)J recombination is derived from a so-called RAG transposon.

Chromosomal rearrangements more complex than simple insertions result from alternative transposition events where complementary ends from separate TEs synapse rather than the traditional synopsis of complementary ends from a single TE. The synopsis of TE ends from separate molecules has been demonstrated in vitro and is referred
to as bimolecular synapsis (Box 1)\textsuperscript{14,24}, Figure 2b depicts an alternative transposition event, in steps equivalent to those depicted in Figure 2a for traditional transposition.

Once the hybrid element is formed (Box 1), the chemical steps of the alternative transposition reaction are identical to those of a normal transposition reaction. Excision of the hybrid element forms two double-strand breaks. The ‘excised’ hybrid element may reinsert into the genome. The remaining double-strand breaks at the site of hybrid element excision may be repaired. Transposase is required for alternative transposition to occur. However, in contrast to the excision of an intact TE, one end of each of the TEs in a hybrid element remains covalently bound to a large chromosomal fragment.

The type of rearrangements produced by alternative transposition depends on the type of DNA molecules involved – either linear or circular – for both donor and target, and on the location of the target site relative to the ends involved in alternative synapsis. The types of rearrangements observed depend on the viability of the resulting chromosome structure in the species being examined. Detailed examples of various types of rearrangements can be found in the original publications\textsuperscript{9-11,13-24}.

Figure 2b examines an alternative transposition event where the complementary ends involved are from homologous elements on sister chromatids, with an insertion target site located on the same chromosome arm on the homologue as the element ends forming the hybrid element. This scenario results in the formation either of an acentric fragment and a dicentric chromosome or of recombinant chromosomes with recombinants containing a reciprocal deletion/duplication. Most of the reported chromosomal rearrangements (Box 1) consistent with alternative transposition are of deletions, duplications, and inversions. Such a bias in the types of observed events could be due to higher frequency of occurrence or viability.

While Figure 2b details one type of rearrangement that can be formed by alternative transposition, Figure 3 contains a schematic summary of sixteen possible classes of rearrangement caused by alternative transposition. Notably, an inversion is produced if the hybrid element inserts into one of the chromosome arms involved in formation of the hybrid element (Classes 2’ and 3’ in Fig. 3). Inversions formed by alternative transposition will contain both copies of the target site duplication on a single chromosome. One of the target site duplications is located within the inverted segment and, therefore, the duplicated target sites are in inverse complementary orientation, rather than the direct orientation found flanking normal transposon insertions. The experimental determination of a number of independent inversion events containing the predicted structure, including the target site duplication typical of TE insertion events, was instrumental in demonstrating that alternative transposition does occur in eukaryotes\textsuperscript{22}. An animated diagram of the formation of an inversion caused by alternative transposition can be found at http://www.wisc.edu/genestest/CATG/engels/Pelements/HEiliv.html.

A translocation event could result if the insertion target site is on a different chromosome from that which the TE ends forming the hybrid element originate (Fig. 4). Specifically, precise reciprocal translocations result when caused by alternative transposition. Translocations were amongst the first observed TE-mediated chromosomal

### FIGURE 2. Traditional versus alternative transposition

The basic steps of transposition are shown as discrete steps for illustration purposes. Each line represents a DNA double helix. The two sister chromatids of each of the homologous chromosomes are shown. Black ovals denote the centromere. Complementary left- and right-ends of the TE are shown as open or closed triangles, respectively. The original target site duplications are shown as open circles. The new target site duplications are shown as open boxes. Asterisks denote the double-strand breaks that are repaired and can result in formation of an excision footprint, regeneration of the TE using the sister chromatid as a template, gene conversion or recombination. (a) Traditional cut-and-paste transposition – complementary TE ends from an intact element synapse, excise and reinsert into a new target site. The TE can insert in either of two orientations relative to the directionality of the original insertion. In the case of traditional transposition, either insertion orientation results in a simple insertion. (b) Alternative transposition – the first step in alternative transposition is the synthesis of complementary TE ends from separate TEs to form a hybrid element. In the case illustrated here, the complementary TE ends are derived from homologous elements on sister chromatids. Once bimolecular synapsis occurs, excision, insertion of the hybrid element into the new target site and repair of the double-strand breaks occurs by the same mechanisms as in traditional transposition. Because the hybrid element remains covalently bound to the chromosome, different insertion orientations result in different types of chromosomal rearrangements. In the example shown here, one insertion orientation results in formation of an acentric fragment and a dicentric chromosome, while the other insertion orientation results in the formation of recombinant chromosomes. Note that the recombinant chromosomes in this example also contain a reciprocal deletion or duplication of the genomic segment between the original and new target sites. All chromosomal rearrangements resulting from alternative transposition have two distinctive structures consistent with a TE insertion event. First, one of the breakpoints in the rearrangement should be at the terminus of a functional TE end. Second, a target site duplication should be produced. The two copies of the target site duplication will be situated on two different chromosomes in many of the resulting rearrangements.
Alternative transposition results in sixteen classes of rearrangement when the insertion target site is on the same chromosome arm as the TEs involved in forming the hybrid element. The diversity of possible rearrangements formed by alternative transposition is in contrast to the specificity of rearrangements formed by homologous recombination (Fig. 1). The basic steps, (i)–(vi), of traditional and alternative transposition as shown in Figure 2 are repeated here illustrating the process and outcomes of alternative transposition for target sites in the eight zones possible relative to the TEs involved in forming the hybrid element. Each line represents a DNA double helix. The two sister chromatids of each of the homologous chromosomes are shown. Black ovals denote the centromere. Complementary left- and right-ends of the TE are shown as open or closed triangles, respectively. The original target site duplications are shown as open circles. The new target site is shown as open boxes. Asterisks denote the double-strand breaks that are repaired and can result in formation of an excision footprint, regeneration of the TE using the sister chromatid as a template, gene conversion, or recombination. Symbols: Δ = deletion, Σ = duplication.

**FIGURE 3.** Many types of chromosomal rearrangement can result from a single TE insertion.
alternative transposition. The only difference between traditional and alternative transposition is the choice of TE ends to synapse.

Bimolecular synapsis cannot explain all TE-mediated rearrangements not explained by homologous recombination. True one-ended transpositions and adjacent inverted duplications reported in snapdragon and Drosophila are just some of the rearrangements fitting this description. The purpose of this review is to demonstrate the ubiquity of alternative transposition in a number of TE systems, without diminishing the role other mechanisms in TE-mediated genome rearrangement.

**Regulation of alternative transposition**

In addition to similarities in structure and transposition mechanism, described above, the IS10, hAT elements – such as Ac/Ds and Tam3 – and P elements show other similarities that must be considered in the context of their effects on alternative transposition, including regulation mechanisms, choice of insertion site and complexity of TE structure.

Regulation of transposition is similar in P elements, the hAT superfamily (Box 1) and IS10, although not all regulatory mechanisms have been demonstrated for all the elements examined. Relevant to alternative transposition is the regulation of transposition by the methylation state of the element. Both traditional and alternative transpositions of IS10 and Ac/Ds elements have been shown to be regulated by methylation. Only hemi-methylated sequences at the transposase binding sites are recognized by transposase, thus restricting transposition to immediately after passage of a DNA replication fork and providing an additional regulation mechanism in the recognition of TE end complementarity. D. melanogaster does not display obvious differential methylation and this regulation mechanism is therefore unlikely to affect P or other elements in Drosophila.

Another important factor affecting the types of chromosome rearrangements caused by alternative transposition is the location of hybrid element insertion. Here, the issue of physical constraint of the hybrid element should be considered. Deletions extending over at least 100 kb have been described. It is therefore reasonable to expect that duplications or inversions of over 100 kb are also formed. No absolute limit has yet been established for the distance between the locations of hybrid element excision and insertion, so the possibility remains that alternative transposition is not physically constrained by the covalent tether between the hybrid element and the remaining chromosome arms. This supports the possibility of hybrid element insertion into a separate chromosome, resulting in the formation of exact reciprocal translocations (Fig. 4).

In terms of preference of the insertion distance, both P and Ac/Ds elements have been shown to transpose to closely linked sites and to sites in close proximity of other P and Ac/Ds elements more often than if insertion sites were chosen at random. The preference for insertion into nearby target sites would decrease the possibility of large deletions and duplications, as well as translocations – an aspect that may be crucial to the viability of alternative transpositions. Also, the choice of a target site close to an existing element may explain the number of rearranged chromosomes derived from progenitor chromosomes containing elements at both rearrangement breakpoints.

**FIGURE 4. Formation of precise reciprocal translocations**

Insertion of a hybrid element into a separate chromosome results in the formation of exact reciprocal translocations, regardless of the target site. A viable zygote could result if the reciprocal products of hybrid-element-mediated translocation segregated into the same germ cell and no deleterious gene interruption occurred at the breakpoints.

In terms of TE structures that promote alternative transposition, an inverse correlation between the complexity of element structure and the formation of chromosomal rearrangements has been established for Ac/Ds and P elements. Ac elements and State-II Ds elements, which are simple deletion derivatives of Ac, produce high rates of transposition. Conversely, State-I Ds elements have complicated structures and produce high levels of chromosome breakage with very little transposition. Chromosomal rearrangements associated with alternative transposition of P elements have also been shown to be more likely to occur with more complex presentation of functional element ends. One explanation is that, unlike intact elements that can undergo either traditional or alternative transposition, disjointed element ends may be recognized by transposase only as part of a hybrid element.

Clearly, intact TEs undergo traditional transposition more frequently than alternative transposition. However, intact TEs do participate in the formation of hybrid elements that then undergo alternative transposition. The relative frequency of traditional versus alternative transposition can be determined by examining systems in which
**Ends from two copies of a TE in the same chromosome arm can associate to form a compound transposon (e.g. with IS10/Tn10). Similar structures have been called macrotransposons when Ac/Ds elements are involved. The resulting structure allows intervening ectopic sequences to be placed in a new genomic context. This figure illustrates that transposition of the macrotransposon to the homologous chromosome can result in duplication of the sequences between the two TEs. In the case of Tn10, a tetracycline resistance gene is contained in the ectopic sequence. Similar structures have been constructed using mariner elements with the inner inverted repeats mutated. These ‘mariner sandwiches’ have been shown to excise and transpose (E. Lozovskaya and D. Hartl, pers. commun.).**

**Ac/Ds – family of transposons first described in the 1940s by Barbara McClintock in maize. Originally described as two separate elements, Activator (Ac) and Dissociation (Ds), molecular analysis has subsequently revealed that Ds elements are, in fact, derivatives of Ac elements.**

**Bimolecular synopsis – synopsis of complementary TE ends from separate molecules.**

**Chromosomal rearrangement – rearrangement of the linear sequence of chromosomes including transposition, duplication, deletion, inversion or translocation of nucleic acid segments.**

**hAT superfamily – group of eukaryotic transposons that have related transposase genes. The superfamily name is derived from the hobo, Ac/Ds and Tam3 elements, which were the first ‘members’ of this superfamily recognized to have similar transposases. The level of protein similarity ranges between 20 and 60%. The superfamily now includes a number of other transposases, including Ascot-1 (from the fungus Ascochloris immersus) and Ac (first described in the housefly Musca domestica), hermit (first described in Queensland fruit fly, Bactrocera tryoni), hopper (from the oriental fruit fly, Bactrocera dorsalis), restless (first described in fungus Tolypocladium inflatum) and Tfo1 (from the fungus Fusarium oxysporum).**

**Hybrid element – the unit of DNA consisting of complementary TE ends from separate elements that have synapsed and can undergo excision, target site capture and insertion by the same mechanism as normal transposition.**

**Insertion sequence (IS) – prokaryotic TEs that transpose by a DNA intermediate and contain only sequences necessary for transposition (termini and transposase gene). Some IS elements can form the termini of prokaryotic transposons, such as IS10 forming the ends of Tn10 or IS50 forming the ends of Tn5.**

**mariner – transposon first isolated from Drosophila and since shown to exist in a number of species, including humans. All mariner elements duplicate the 2bp sequence, TA, upon insertion and contain a D,D, (35) D catalytic triad, rather than the D, D, (35) E motif shared by most other transposases. Several subfamilies of mariner have been described, with each subfamily containing elements with highly conserved transposase proteins.**

**RAG transposon – proposed structure from which originated the signal ends and RAG1/RAG2 proteins involved in V(D)J recombination. For further discussion on the model for evolution of V(D)J recombination from a transposon insertion event, see Reference 40.**

**V(D)J recombination – the process by which V, D and J coding segments are spliced together in somatic cells of the immune system to produce a diverse range of antibodies.**

**an intact TE is also known to participate in the formation of a hybrid element. The IS10/Tn10 elements constitute one convenient system for this analysis. An IS10 element transposes about once per 10¹ cell generations. Rearrangements associated with alternative association of the ends from the two IS10 elements that form the Tn10 termini¹³ occur about once per 10⁵ cell generations³⁵. When compared with IS10 transposition and complex rearrangements consistent with alternative transposition, Tn10 transposition, which occurs about once every 10² cell generations⁵⁵, may be viewed as a specific result of aberrant synopsis of IS10 ends. Because Tn10 contains sequences conferring antibiotic resistance between the two IS10 elements, Tn10 also confers an evolutionary advantage to the bacterial cell in which it is located.**

An extended superfamily of TEs consisting of elements that transpose by a cut-and-paste mechanism, and which may also undergo alternative transposition-induced rearrangements, could be an appropriate category for TEs such as IS1 and IS50. These TEs have demonstrated cut-and-paste transposition as well as cointegrate formation³⁶–³⁸ that can now be understood as aberrant transposition events rather than a true cointegrate. In fact, an inversion resulting in replication of the 9bp IS1 target site and IS1 elements at the rearrangement breakpoints⁹ corresponds exactly with the sequence structure predicted by the alternative transposition model.
Aberrant transposition and evolution

Many TEs were discovered because of the mutations they cause. In some cases this was due to simple insertion or excision of the element. In other cases complex traits were observed, such as chromosome breakage-fusion-bridge cycles due to Ac/Ds elements in maize and hybrid dysgenesis due to P elements in D. melanogaster, which can now be explained by the alternative transposition mechanism reviewed here.

The deleterious effects of alternative transposition are balanced by the evolutionary advantages conferred by the ability to rearrange genomic information. Ability of TEs is thought to increase in times of environmental and genomic stress. Increases in traditional transposition are likely to be accompanied by increases in alternative transposition, as occurs during P-M hybrid dysgenesis. Although the majority of chromosomal rearrangements would be deleterious, occasional genome shuffling may result in increased fitness. Could the increased capacity for genome evolution constitute a selective advantage allowing TEs to persist? Such a process may be particularly relevant when the survival of a species is challenged, and could be the evolutionary basis for increased TE mobility under stressful conditions.

Similarities with V(D)J recombination

Several parallels have been observed between transposition and V(D)J recombination. The coding joints formed between V, D and J segments have structures similar to the footprints found at TE excision repair sites. Also, the finding that the signal end fragment can transpose and create a 5bp target site duplication upon inser-

tion and V(D)J recombination. The advantage of this method is that it constitutes a selective advantage allowing TEs to persist? Such a process may be particularly relevant when the survival of a species is challenged, and could be the evolutionary basis for increased TE mobility under stressful conditions.

Alternative transposition as a genetic tool

Several genetic tools have been developed using TEs, including transposon tagging and transposon-mediated transformation. Here, alternative transposition is proposed as an additional method in the repertoire of TE-based genetic manipulation.

Alternative transposition can be used to delete regions of chromatin adjacent to the TE both in vitro and in vivo (Fig. 2b). In prokaryotes, TE-induced deletions are a well-established technique. Recently, the EZ::TN™, KAN-2 insertion kit has been commercialized by Epicentre Technologies. This kit is based upon in vitro transposition of Tn5 elements (with IS50 ends) and can be used to create deletions and inversions adjacent to the Tn5 element.

In eukaryotes, deletions have been induced adjacent to intact Tam3 elements in snapdragon and P elements in Drosophila in vivo. The advantage of this method is that it constitutes a selective advantage allowing TEs to persist? Such a process may be particularly relevant when the survival of a species is challenged, and could be the evolutionary basis for increased TE mobility under stressful conditions.

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References

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Antibacterial responses in Drosophila are the focus of several recent studies. The caspase encoding gene dredd, functions in an antibacterial pathway probably with imm and relish. This conclusion is supported by results from Stöven et al., who show that Relish processing and activation require a functional dredd gene. Two members of a Drosophila IκB kinase complex, the kinase DmIKKβ and the structural factor DmIKKγ, are required for antibacterial gene induction by LPS, regulate Relish phosphorylation and processing but are not required for Toll-mediated antifungal gene expression. Mutations in the DmIKKγ gene block Relish-dependent immune induction of the genes encoding antibacterial peptides after infection. Dredd, DmIKKβ, DmIKKγ, Imm and Relish may define a pathway that mediates Drosophila antibacterial responses. Finally, recent results show that the Jak-Stat signalling cascade regulates the expression of complement-like proteins in the Drosophila fat body after infection.

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