

MODERN THOUGHTS ON AN ANCYENT *MARINERE*: Function, Evolution, Regulation

Daniel L. Hartl¹, Allan R. Lohe², and Elena R. Lozovskaya¹

¹Department of Organismic and Evolutionary Biology, Harvard University, 16 Divinity Avenue, Cambridge, Massachusetts 02138; email: dhartl@oeb.harvard.edu;

²Molecular and Population Genetics, Research School of Biological Sciences, PO Box 475, The Australian National University, Canberra 2601 ACT, Australia

KEY WORDS: gene regulation, overproduction inhibition, *mariner*, *Tc1*, transposable element

ABSTRACT

The *mariner/Tc1* superfamily of transposable elements is one of the most diverse and widespread Class II transposable elements. Within the larger assemblage, the *mariner*-like elements (MLEs) and the *Tc1*-like elements (TLEs) are distinct families differing characteristically in the composition of the “D,D(35)E” cation-binding domain. Based on levels of sequence similarity, the elements in each family can be subdivided further into several smaller subfamilies. MLEs and TLEs both have an extraordinarily wide host range. They are abundant in insect genomes and other invertebrates and are found even in some vertebrate species including, in the case of *mariner*, humans, in which one element on chromosome 17p has been implicated as a hotspot of recombination. In spite of the extraordinary evolutionary success of the elements, virtually nothing is known about their mode of regulation within genomes. There is abundant evidence that the elements are disseminated to naive host genomes by horizontal transmission, and there is a substantial base of evidence for inference about the subsequent population dynamics. Studies of engineered *mariner* elements and induced mutations in the transposase have identified two mechanisms that may be operative in *mariner* regulation. One mechanism is overproduction inhibition, in which excessive wild-type transposase reduces the rate of excision of a target element. A second mechanism is dominant-negative complementation, in which certain mutant transposase proteins antagonize the activity of the wild-type transposase. The latter process may help explain why the vast majority of MLEs in nature undergo “vertical inactivation” by multiple mutations and, eventually, stochastic

loss. There is also evidence that *mariner/Tc1* elements can be mobilized in hybrid dysgenesis; in particular, certain dysgenic crosses in *Drosophila virilis* result in mobilization of a TLE designated *Paris* as well as the mobilization of other unrelated transposable elements.

CONTENTS

INTRODUCTION	338
RELATION TO OTHER D,D(35)E ELEMENTS	339
MECHANISM OF TRANSDUCTION AND EXCISION	340
DISTRIBUTION AMONG ORGANISMS	342
<i>Insects and Other Invertebrates</i>	343
<i>Humans and Other Vertebrates</i>	343
COPY NUMBER AND SUBFAMILY COEXISTENCE	344
VERTICAL INACTIVATION	345
STOCHASTIC LOSS	346
HORIZONTAL TRANSMISSION	347
<i>Examples from Insects and Other Invertebrates</i>	347
<i>Prevalence of Horizontal Transmission</i>	349
GERMLINE TRANSFORMATION	349
MECHANISMS OF REGULATION	350
<i>Overproduction Inhibition</i>	352
<i>Dominant-Negative Complementation</i>	352
<i>Transposase Titration</i>	353
CONCLUSIONS AND PROSPECTS	354

INTRODUCTION

This is a summary review of the *mariner* family of transposable elements: What do we know? What do we not know that we should? Why is it important that we know it? The end of the matter is that we know the most about mechanism (much of it by inference from related transposable elements). We also know quite a lot about general features of population dynamics and evolution, although we are short on the details and lack a comprehensive model. The major gap is that we know almost nothing about regulation. That the study of regulatory mechanisms has not been pursued more aggressively is quite paradoxical, because regulation is perhaps the key issue in the application of *mariner*-like elements to methods of germline transformation of insect pests and vectors of human disease. Happily, experiments carried out for other purposes have recently identified several candidate mechanisms of regulation that may, singly or in combination, mediate regulation of this important family of transposable elements.

RELATION TO OTHER D,D(35)E ELEMENTS

Members of the *mariner* family of transposable elements (MLEs), as well as members of its presumed sister group, the *Tc1* family (TLEs), are of great current interest for a number of reasons. First, they are eukaryotic members of a larger superfamily of transposable elements that includes such prokaryotic members as the bacteriophage Mu, the transposon Tn7, and many bacterial insertion sequences including the *Escherichia coli* elements IS2, IS3, IS4, and IS30 (19). The *mariner/Tc1/IS* superfamily of transposable elements is related to a still larger assemblage of sequences that includes human immunodeficiency virus (HIV) and the *copia* and *gypsy* families of long-terminal-repeat (LTR) retrotransposons (15).

What these sequences have in common is that their transposase or integrase proteins include a sequence motif called the D,D(35)E motif, which consists of two aspartic acid residues, typically separated by more than 90 amino acids, followed by a glutamic acid residue, typically 34 or 35 amino acids further toward the carboxyl end (19). Given its rather vague specification, the D,D(35)E motif is more a sort of signature than a “motif” in the usual sense of the word. Nevertheless, the signature is found in a very diverse set of proteins.

The possible evolutionary relationships between various members of the D,D(35)E superfamily have been examined by molecular phylogenetic methods (15). Even though there are not a large number of phylogenetically informative sites in the blocks of amino acids surrounding the key acidic residues of the D,D(35)E signature, the analysis does lead to three seemingly robust conclusions. First, the *mariner/Tc1* superfamily, on the one hand, and the LTR retrotransposons and retroviruses, on the other hand, are separated into two monophyletic groups. Second, the bacterial IS elements in the superfamily are located willy nilly in the tree and not in a coherent group; it is not clear whether the dispersion of the IS elements results from an inadequate number of phylogenetically informative sites or whether the IS elements are truly polyphyletic. Third, there is relatively strong bootstrap support (though still less than 80%) that groups *mariner* elements with *Tc1* elements.

The apparent common ancestry of MLEs and TLEs is all the more interesting in light of the fact that *mariner* is the only member of the extended D,D(35)E superfamily that does not have the D,D(35)E signature. Bear in mind that the D,D(35)E signature is not a consensus sequence in the usual sense of “majority rule” over a set of aligned sequences. Indeed, the three acidic residues are absolutely invariant across members of the *Tc1* family thought to be functional (65), and the last two are invariant across members of the extended superfamily, prokaryotic IS elements included (19). In contrast, the *mariner*-like elements have the signature D,D(34)D (65). Does it matter? Who would bet it does?

After all, glutamic acid and aspartic acid are both acidic residues, the pK values for the side-chain carboxyl groups are almost the same (4.3 versus 3.9), a change from one residue into the other is “conservative,” and the amino acids are often found at corresponding sites in homologous proteins. The answer, however, is that it does matter. In *mariner*, the “conservative” change from D,D(34)D to D,D(34)E completely obliterates transposase activity (40).

MECHANISM OF TRANSPOSITION AND EXCISION

Much of the work on the extended D,D(35)E superfamily concerns molecular mechanisms. What is the molecular structure of the transposase? What are its DNA-binding sites and what sequences are recognized in the transposable element that is mobilized? What is the molecular mechanism of transposition? Is the transposase alone sufficient to carry out the transposition reaction?

The D,D(35)E motif is thought to be a key player in the reaction mechanism, part of the active site that serves as a binding domain for a divalent cation (Mg^{2+} or Mn^{2+}) necessary for catalysis (34). The unifying mechanistic feature of proteins that share this motif is the ability to execute a single-strand scission in a duplex DNA molecule that exposes a reactive 3' hydroxyl (18). A single-strand scission at each end of a mobile DNA sequence is the essential D,D(35)E contribution to the simple insertion of a retrovirus or to cointegrate formation of bacteriophage Mu (55). In each case, the reactive 3' hydroxyl groups are joined with nucleotides at displaced positions on opposite strands of a target sequence, which, when repaired by host enzymes, creates the direct duplication characteristic of transposable element insertions.

The transposase reactions of many Class II transposable elements initiate with a staggered cleavage of both strands at each end of the transposable element. The entire element is thereby released from the donor molecule and free to insert by a “cut-and-paste” mechanism into a target site, again through initial joining of the 3' overhanging ends of the transposable element (18). The 5' overhang created at the vacated target site apparently forms a short heteroduplex, which is repaired by the host mismatch repair system and results in a characteristic “footprint” after excision. First established for Tn7 (17) and Tn10 (33), the cut-and-paste mechanism is also the mode of transposition of *Tc1*-related elements. For *Tc3* and for one *mariner*-like element, the cut-and-paste mechanism has been demonstrated in vitro (35, 76, 77).

Based on general similarity with *Tc1*-related elements, the presumption is very strong that all *mariner* elements also transpose by a cut-and-paste mechanism. The strongest indirect evidence comes from the footprints left after *mariner* excision. The experimental assay is based on a two-component experimental system in *Drosophila*. One component consists of the *Mos1* (*mosaic-1*)

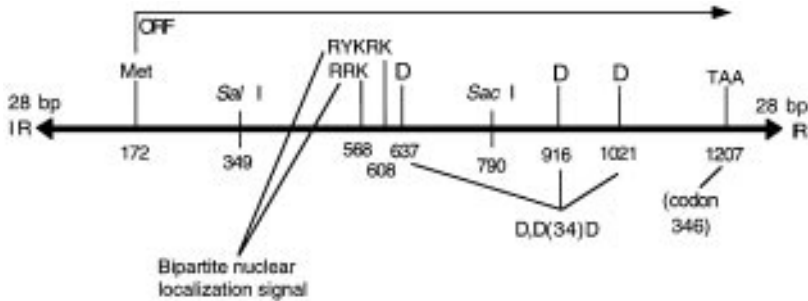


Figure 1 Sequence organization of the *Mos1* mariner element.

mariner element, which supplies the functional transposase. This element is a full-length element of 1286 base pairs (bp), including the 28-bp inverted repeats, containing an uninterrupted open reading frame that codes for a putative transposase of 345 amino acids (52, 54). The functional organization of the *Mos1* element is illustrated in Figure 1. The locations of the D,D(34)D signature residues are indicated. A bipartite nuclear localization signal has also been identified by sequence similarity to a bona fide nuclear localization signal present in certain TLEs in zebrafish (26). The middle R of the RRK region is conserved in both MLEs and TLEs (65); genetic evidence that it is essential comes from an R-to-H mutation in the *Mos1* transposase, which completely eliminates activity (40).

The second component of the transposase assay is an inactive *mariner* element, called *peach*, which is also a full-length element but one coding for an inactive transposase (22, 27). The *peach* element differs from *Mos1* at 11 nucleotide sites, including four amino acid replacements (52, 53). Presence of the *peach* element is detected through its phenotypic effects when inserted in the 5' noncoding region of the X-linked *white* gene; the *peach* insertion in the *wpch* transgene results in peach-colored eyes. Excision of the *peach* element from *wpch* by functional transposase supplied in *trans* restores wild-type expression of the *white* gene. In the soma, excision results in eye-color mosaicism (red spots on a peach background); in the germline, it results in a reverse mutation of *wpch* to wild type (9, 10).

In the *wpch* excision assay, the *Mos1* element is highly active, resulting in eye-color mosaicism in all flies that carry it (52, 54). The type of footprints remaining after *peach* excision are exactly analogous to those found with *Tc3*, except that they are of a different length (8). *Tc3* excision results in a typical footprint consisting of two base pairs derived from either the 5' or the 3' end of *Tc3* and flanked by the TA target-site duplication created in the original



Figure 2 Mechanism of *mariner* excision inferred from excision footprints expected from mismatch repair of the 3-bp heteroduplex (8). The pins denote the locations of the staggered cuts. Modeled after the mechanism of excision and mismatch repair of *Tc3* (76).

insertion event (76). The typical *Tc3* footprint is therefore either 5'-TACATA-3' or 5'-TATGTA-3'. *Mos1* excision of the peach element yields a footprint of the same type but including three nucleotides from either end of the element instead of two. The *Mos1* footprint is therefore usually either 5'-TACCATA-3' or 5'-TATGATA-3'. These are the expected result of mismatch repair of a heteroduplex formed by a double-stranded cleavage flanking the *peach* element in such a way as to leave a 3' overhang of three base pairs at each end (Figure 2). Analysis of 20 independent *mariner* excision events yielded 10 footprints of the 5'-TACCATA-3' type, 8 of the 5'-TATGATA-3' type, and 2 with more complex types of resolution (8).

DISTRIBUTION AMONG ORGANISMS

There are also wide-ranging evolutionary issues relative to MLEs and TLEs. The elements are probably the most widespread and diverse group of Class II transposable elements found in animals (65, 67). They are prevalent not only in insects and other invertebrates but also in vertebrates, including the human genome. Judging from the sequence diversity of elements present within a genome, MLEs can persist in a genome through evolutionary time, including passage through speciation events.

Insects and Other Invertebrates

The first demonstration of a wide phylogenetic distribution of MLEs came from PCR amplifications using degenerate oligonucleotide primers complementary to regions of the putative transposase found in elements from *Drosophila mauritiana* and *Hyalophora cecropia* (64). In the initial survey of 404 insect species, 63 of the genomes were found to contain MLEs (69). The insect species in which MLEs are present represent all major orders. The MLEs themselves can be grouped into at least five distinct subfamilies based on three criteria: (a) each subfamily forms a distinct group in the phylogenetic tree (but usually not with strong bootstrap support), (b) the putative transposase from each subfamily contains certain diagnostic amino acids for the subfamily, and (c) each subfamily usually has at least one shared insertion/deletion characteristic of the subfamily (69). The major subfamilies are designated *mauritiana*, *cecropia*, *mellifera*, *irritans*, and *capitata*, after the species in which the subfamily was originally identified (69). There are at least an equal number of minor subfamilies (65).

At the DNA level, pairs of MLEs from different subfamilies are 40–56% identical in nucleotide sequence. When the putative transposase proteins are optimally aligned (usually after introducing small insertions or gaps in the DNA sequence to disclose an open reading frame), the transposase proteins from members of different subfamilies are 23–45% identical at the amino acid level (64). MLEs are also found in invertebrates other than insects, including the centipede *Scutigera coleoptrata* and a tipulid mite (69), the nematode *Caenorhabditis elegans* (72), and the planarian *Dugesia (Girardia) tigrina* (20,21). Among the most divergent MLEs is an element isolated from the silk moth *Bombyx mori*. This *Bmmar1* element has many of the consensus amino acids found in *Tc1*-like elements, which is attributed to shared common ancestry between *Tc1*-like elements and MLEs; but the *Bmmar1* element also has sites diagnostic of MLEs, including the residues DY at amino acid positions 315 and 316 (66).

Humans and Other Vertebrates

Perhaps the most unexpected host for MLEs is the human genome, but they are indeed present in humans and also in other vertebrates. Three subfamilies of MLEs have been identified in the human genome: *cecropia* (3, 56, 60, 70, 74), *irritans* (60), and *mellifera* (74). These elements appear to have been present for a very long time, and it is not known whether functional copies are still present (56, 70). The copy number of each MLE has been estimated at 100–300 (60).

A derivative of the human *copia*-subfamily element has been identified that consists of a pair of 37-bp inverted repeats flanking a 6-bp unique sequence (60, 74). This element, called *Mrs* (60) or *Made1* (74), is present in an estimated 1000–5000 copies (60). The inverted repeats of *Mrs* have only one mismatch

with the consensus complete MLE. It seems likely that the truncated element has proliferated in the human genome owing to *trans*-activation by the cognate MLE transposase (60).

A pair of MLEs separated by about 1.5 Mb in human chromosome 17p11.2-p12 has recently received considerable attention because of its potential role in human disease (24). These particular MLEs are part of a larger ~30-kb duplication called the *CMT1A-REP* repeats. The *CMT1A-REP* repeats are prone to undergo misalignment and unequal crossing-over in male meiosis, resulting in recombinant chromosomes that carry either a duplication of the 1.5 Mb or the complementary deletion. Within the 1.5 Mb is contained the gene for peripheral myelin protein 22 (*PMP22*), and the duplication and deletion products result in distinct hereditary neurological syndromes, namely, Charcot-Marie-Tooth disease type 1A (*CMT1A*) and hereditary neuropathy with liability to pressure palsies (*HNPP*), respectively. Analysis of the *CMT1A-REP* repeats have implicated the MLE as the hotspot of recombination (32, 63).

What do MLEs do? At one level, they may represent “selfish DNA” that invade the genome and are able to persist through time by virtue of their ability to replicate and transpose. The elements are maintained presumably against negative selection pressure resulting from slightly detrimental changes in gene expression due to insertion. (Favorable mutations are possible but much less likely.) *Mariner* elements are active in somatic cells, and it has been demonstrated that somatic activity can result in a shortened life span and other unfavorable effects on fitness in *Drosophila* (57). A second type of host effect may derive from the ability of the elements to serve as targets for DNA cleavage, as exemplified by the MLE implicated as a hotspot of recombination in human 17p.

COPY NUMBER AND SUBFAMILY COEXISTENCE

The copy number of MLEs varies tremendously from one species to the next. At the low end of the scale are species that have a very small number of copies, such as *Drosophila sechellia*, which has just two copies of an MLE closely related to *Mos1* (14). *D. mauritiana*, from which *Mos1* was derived, has ~30 copies of this subfamily. It is not unusual to find species with ~100 copies of any MLE subfamily, such as *D. erecta*, which has approximately this abundance of an element in the *mellifera* subfamily (45). Next on the scale are MLEs present in ~1000 copies per genome, for example, the *cecropia* MLE found in *Hyalophora cecropia* (38). The planarian *Dugesia tigrina* contains ~8000 copies of a *cecropia*-subfamily MLE (20). However, the current record for copy number is held by an *irritans* MLE in the horn fly *Haematobia irritans*,

which is found in approximately 17,000 copies and accounts for 1 percent of the total genome (68).

What accounts for the differences in copy number is not known. Each subfamily of MLE apparently represents a different lineage. There may be differences in promoter strength (perhaps depending in part on the host species), differences in selection against MLE insertions (population size is certainly one factor to consider), differences in regulatory mechanisms among species (or in the relative preponderance of MLEs that have regulatory capabilities), or differences in interactions among MLE subfamilies.

Not only do copy numbers vary among species, multiple MLE subfamilies can exist in the same genome. There are many examples in a variety of organisms (64, 69), including human beings (70). The *mellifera* element found in *D. erecta* is also present in *D. yakuba* and *D. teissieri* (45), and these latter species also contain MLEs from the *mauritiana* subfamily (51). The greatest number of coexistent subfamilies thus far identified is in the Mediterranean fruit fly *Ceratitis capitata*, whose genome contains MLEs from at least four of the major subfamilies (69).

Do the MLE subfamilies in a genome interact? Do they cross-regulate one another? Are there transposases that can bind to elements in more than one subfamily? Can any of the subfamilies cross-mobilize? Do they compete for target sites? Do they act independently in their effects on fitness? Are the different subfamilies functionally divergent with respect to their ability to transpose and self-regulate in different groups of organisms? Interesting questions, all, but none of them resolved.

VERTICAL INACTIVATION

Although MLEs are abundant in many animal genomes, the majority of MLEs present in natural populations are nonfunctional pseudogenes. Many are inactive because they contain multiple chain-termination, deletion, or frameshift mutations that disrupt the open reading frame (64, 69). Others have an open reading frame with only missense replacements but produce an inactive transposase; examples include the *mauritiana* elements *peach*, *Ma310*, *Ma311*, and *Ma331* from *D. mauritiana* (52). Still other MLEs with an open reading frame produce a transposase with very limited activity (13, 52). Granted that not many MLEs have actually been tested for activity *in vivo*, but *Mos1* and closely related *mauritiana* MLEs are the only elements so far demonstrated to be functional in an intact organism. Indeed, active *Mos1*-related elements are active in natural populations of *D. simulans* (11, 13, 16). Oddly, an element from the *irritans* subfamily that has been reported to be functional *in vitro* does not appear to

function when present in *D. melanogaster* (35). For that matter, neither does *Tc1* appear to work in *D. melanogaster* (75).

Why are most MLEs inactive? The simplest model is mutation pressure. If an MLE is not selected for function, then mutations may accumulate by chance alone. Since most insertions of transposable elements are probably short-lived owing to slightly detrimental effects on fitness (28), the ability of MLEs to be mobilized by transposase supplied in *trans* would seem to be critical. Transposable elements that insert in heterochromatin, such as the *mellifera* subfamily MLE in *D. erecta*, which is concentrated in heterochromatin (45), may have less of an effect on fitness and persist longer, thereby allowing more time for mutations to accumulate. In a euchromatic location, once an MLE loses the ability to move, it seems virtually doomed to extinction. It is also possible that transposition itself is mutagenic, although transposition by a cut-and-paste mechanism of a sequence that has been replicated by the host machinery renders this possibility unlikely except for regions at the ends near the cleavage sites.

In accounting for the prevalence of inactive elements, a more interesting possibility is that there is positive selection pressure for them. Inactive elements may participate in the regulation of transposition in at least two ways. First, in serving as substrates for transposition, they titrate the functional transposase, yet their multiplication does not increase the total amount of transposase produced. A prevalence of inactive elements would therefore tend to ameliorate the dysgenic effects of excessive transposase production and transposition. A second mechanism of regulation offered by MLEs with inactive open reading frames is that of direct interference with functional transposase, either through competition for transposase binding sites or through poisoning the transposase with inactive subunits. Both of these mechanisms have been described (40, 46).

STOCHASTIC LOSS

In species whose phylogeny is known and in which MLEs have been studied, it is often found that a species whose genome almost certainly contained an MLE at the time of speciation no longer harbors the element. The subfamily of MLEs is said to have undergone stochastic loss (45), which means that, for a long enough time, the rate of loss of elements by random genetic drift exceeds the rate of gain by transposition until, eventually, no elements remain in the genome. (Excessive sequence divergence is also a mechanism of "stochastic loss" because the elements can no longer be identified by PCR or DNA hybridization.) Among MLEs, the best known examples of stochastic loss are in the subgroup of species related to *D. melanogaster*. In this case, sequence

comparisons indicate that the *mauritiana* subfamily of elements was present in the common ancestor of the subgroup but lost in the lineage leading to *D. erecta* and *D. orena*, on the one hand, and in the lineage leading to *D. melanogaster*, on the other hand (12, 51). Studies of *D. sechellia* identify what appears to be a stage just short of stochastic loss. The genome of this species contains two elements, each of which is fixed in the population (14). One element is inactive owing to the presence of three deletions, the largest of which eliminates almost the entire 3' half of the element; the other contains an open reading frame that appears to code for a transposase with very low or no activity (14). Given this situation, there must have been a time when the presently fixed insertion sites were both polymorphic, and an alternative course of evolution would have been fixation of the noninsertions rather than the insertions, yielding stochastic loss. Even though the MLEs are still present in *D. sechellia*, it seems inevitable that, barring reinvasion by horizontal transmission or interspecific hybridization, the MLEs will eventually be lost either by deletion, which happens at a high rate in *Drosophila* (61), or by sequence divergence.

HORIZONTAL TRANSMISSION

Soon after the discovery of the *Mos1* element, MLEs capable of hybridization with it were found in distantly related species (50). A case in point is the isolation of an MLE from *Zaprionus tuberculatus* that was 97% identical in sequence with the *Mos1* element from *D. mauritiana*, whereas a *mauritiana*-subfamily element from a species in the same subgenus, namely, *D. tsacasi*, was only 92% identical with *Mos1* (50). On the face of it, this sort of evidence suggests horizontal transmission, because the MLE sequences are much too similar given the phylogenetic distance between the species. Even within smaller assemblages of the family Drosophilidae, the distribution of MLEs is often nonuniform and inconsistent with the phylogeny of the host species (7).

Examples from Insects and Other Invertebrates

One example of horizontal transmission across orders of insects is summarized in the data in Table 1. The comparisons are between an MLE in the *mellifera*

Table 1 Evidence for horizontal transmission

Sequence comparison	Identical nucleotide	Different nucleotide	Percent identity
<i>Demar12</i> vs <i>Cfmar10.6</i>	140	6	95.9
<i>Demar19</i> vs <i>Cfmar10.6</i>	148	1	99.3
Na ⁺ -K ⁺ pump gene	403	631	39.0

subfamily isolated from either *D. erecta* (*Demar12* and *Demar19*) or from the cat flea *Ctenocephalides felis* (*Cfmar10.6*). Across about 150 codons, the identity of the nucleotides in the third position was 96–99%. The contrast with the chromosomal gene for the sodium-potassium transmembrane pump is striking: only 39% identity in this case. (The comparison here is between the genes in *D. melanogaster* and *C. felis*, but the divergence times must be the same as that for *D. erecta* and *C. felis*.)

A strong similarity between MLEs in very distantly related species is taken as evidence for horizontal transmission (64). Many other examples based on the same type of evidence have also been found. For example, the major type of MLE clones from the horn fly *Haematobia irritans* is >90% identical in nucleotide sequence with that from *Anopheles gambiae*, whereas these species diverged at least 200 Mya; similarly, the major type of MLE clone from the earwig *Forficula auricularia* is >90% identical with that from the honey bee, although the divergence time between the species is greater than 265 My (64). Interordinal horizontal transmission is also indicated by the observation that an *irritans* subfamily element is >88% identical in nucleotide sequence between *Drosophila ananassae*, *Anopheles gambiae*, *Haematobia irritans*, and the green lacewing *Chrysoperla plorabunda* (68). Interphylum horizontal transmission has also been reported in the case of a *cecropia* element in the planarian *Dugesia tigrina*, which is not found in other species of planaria and is >70% identical at the nucleotide level with a *cecropia* element from an ant (20).

The horizontal transmission of a *cecropia* MLE into *Dugesia tigrina* is especially interesting because of the high copy number of this element and the apparently quite recent invasion (20, 21). It suggests that, at least in some cases, newly introduced MLEs can undergo an explosive increase in copy number. An early explosive increase after horizontal transmission also seems to have taken place in horn fly *Haematobia irritans* (68), but it is not inevitable, as the related MLE invader in the genome of *Drosophila ananassae* is present in only three copies (68).

Although horizontal transmission is strongly suggested by compelling sequence similarity between MLEs in highly divergent species, a truly rigorous proof of horizontal transmission requires more than excessive sequence similarity. In the *Zaprionus* example, the gene for alcohol dehydrogenase (*Adh*) was sequenced and analyzed in comparison with homologous sequences from various *Drosophila* species. The inferred molecular phylogeny of the *Adh* gene was found to be significantly different from that of the MLE sequences. In particular, the MLE from *Zaprionus* grouped closely with the MLEs isolated from the *melanogaster* species subgroup, whereas the *Adh* gene from *Zaprionus* fell as an outgroup (50). A nonparametric approach to this type of analysis is given in Reference 37.

Prevalence of Horizontal Transmission

Whereas the occurrence of horizontal transmission of MLEs can be regarded as well established, the rate at which horizontal transmission takes place is unknown. Depending on the opportunities for horizontal transmission, the rate may vary substantially through time and in different groups. It may be asking too much to expect a rate per unit time. Perhaps a useful measure is the relative rate, relative to, say, the rate of speciation. In the *D. melanogaster* subgroup, the rate of horizontal transmission of MLEs seems to be on the same order of magnitude as the rate of speciation (45, 51). This subgroup has undergone seven episodes of speciation, and there are two verified horizontal transmissions (45, 50). (The evidence does not indicate the direction of the transmission, but for estimating rates the direction does not matter.) Whatever the actual rate of horizontal transmission, the process is apparently critical to the long-term survival of MLEs because, otherwise, vertical inactivation and stochastic loss would ultimately render each lineage extinct (45, 68).

The primary vectors for horizontal transmission of transposable elements are still matters of speculation. Candidate vectors have been discussed (29, 30), and a mite has been suggested as a specific vector for transmission of MLEs between species of *Drosophila* (25). There are many other possibilities: external parasites, infectious agents, intracellular parasites and symbionts (especially those in the germline), DNA viruses, RNA viruses, retroviruses, even hitchhiking in other transposable elements. Considering the great diversity of animal groups into which MLEs have invaded, it is possible that many different kinds of vectors can be used.

GERMLINE TRANSFORMATION

The utility of *P*-element transformation in *Drosophila* has been a strong incentive to the development of germline transformation in other insects, especially agricultural pests and insect vectors of human disease. A variety of transposable elements are candidates for such systems (23, 49, 58, 59, 79).

The MLEs and TLEs are also of great interest as potential vectors for germline transformation because of their wide taxonomic distribution and their apparent indifference to host factors (31, 78). Gene transfer into the Medfly *Ceratitis capitata* with a TLE from *Drosophila hydei* has already been demonstrated (47, 48, 71) and made a great stir (2). Among MLEs, the *Mos1* system has been shown to be capable of supporting germline transformation in various species of *Drosophila* (39, 42, 43).

The autonomous *Mos1* element includes several convenient restriction sites for the insertion of exogenous DNA, including a *SalI* site at 349 and a *SacI* site at 790 (Figure 1). Initially, studies were carried out with a vector, called

MlwB, having a 11.9-kb cassette inserted into the *SacI* site; the cassette included an *hsp70:mini-white* gene as well as an *E. coli* β -galactosidase reporter gene and plasmid sequences for transformation rescue. When coinjected with *Mos1* helper plasmid, the rate of transformation was 0.7%, or about 20-fold less than that of *Mos1* alone (39). The transformants were found to be remarkably stable in the presence of transposase (44). To assess whether size alone was an important factor in the relative stability of the *MlwB* transformants, another vector was constructed with only a 4.5-kb *hsp70:mini-white* cassette inserted into the *SacI* site. This also proved to be quite stable in the presence of *hsp70:Mos1* transposase (44). Subsequent studies have shown that both constructs can be mobilized at a low frequency; both excision and transposition were observed and showed typical DNA-sequence characteristics of *mariner* transposase activity (43).

The two variables in these types of experiments are size of insert and choice of insertion site. Insertion at any position in a transposable element necessarily disrupts sequences that could, in principle, be important for DNA binding and transposition (43). To separate size from site, we have created a composite vector consisting of two directly repeated copies of the *peach* element flanking an *hsp70:mini-white* cassette (ER Lozovskaya, DI Nurminsky & DL Hartl, unpublished). To prevent the element at each end from serving as a transposition or excision substrate, the two base pairs of the IR immediately adjacent to the *hsp70:mini-white* were mutated. When introduced into the genome of *D. melanogaster*, this composite construct does respond to *hsp70:Mos1* transposase. In particular, about 11% of the flies are somatic eye-color mosaics, approximately half of the flies yielding one or more white sectors (expected from somatic excision) and the other half with one or more dark red spots (expected from somatic transposition). The 89% of the flies that are not obviously mosaic for eye color are also somatic mosaics, because a PCR product diagnostic of excision can be amplified. Length alone, then, does not seem to dramatically impair transposition or excision, as the composite construct is ~7.1 kb. Why is the rate of somatic excision not greater than 11%? Perhaps the extra pair of inverted repeats, although mutated, nevertheless serve as transposase binding sites that interfere with transposase utilization of the extreme outer ends.

Thus, the simplest explanation of the relative stability of the *SacI* constructs is that an insertion in the *SacI* site disrupts a region necessary for transposase recognition. This hypothesis would also explain why the region around the *SacI* site is quite strongly conserved in amino acid sequence (64).

MECHANISMS OF REGULATION

Although germline transformation is not the main focus of this review, germline transformation is relevant because it motivates perhaps the largest unanswered

biological question regarding MLEs and TLEs: How are they regulated? Unless one knows something about the regulation of these elements, how is one to devise a suitable strategy for germline transformation of diverse insects and, perhaps, other organisms? To illustrate the conundrum, suppose one has a pest insect, say *Mandibulatis gigantica* (not a real species), which contains three major subfamilies of MLE in its genome. Should we isolate these MLEs and engineer them into transformation vectors for the species? The logic seems sound. If they are present in the species, then they certainly can function in the species, and they are therefore ideal candidates as transformation vectors. But wait. What if they are regulated? What if their transposition is largely repressed? In that case, using MLEs isolated from the target species is the worst possible strategy. The presence in the species does not mean that they are active now, and continuously; it means only that they were active at one time.

Are there mechanisms of MLE regulation? In principle, there need not be any specific mechanisms. An MLE newly introduced into the germline of a species may at first transpose without let or hindrance, increasing explosively in copy number until, finally, there is so much genetic damage that heavily hit chromosomes are eliminated by natural selection against their carriers. The role of natural selection is probably minimal at first but increases gradually in intensity. Eventually, an equilibrium is reached at which the gain of new elements by transposition is offset by their loss through natural selection and, through time, vertical inactivation and stochastic loss begin to take their toll on the number of functional elements. But if this is a correct scenario, then what accounts for the sometimes very large difference (1–2 orders of magnitude) in the number of MLEs in closely related species? Chance, perhaps, but in the absence of a comprehensive mathematical and computational theory, one does not know what to expect.

On the other hand, regulatory mechanisms governing many other types of transposable elements are well known, both in eukaryotes and prokaryotes (4). Some of these mechanisms are exquisitely adapted to the host, such as the germline-specific splice of the *P*-element transcript in *Drosophila* (36). The evolution of a regulatory mechanism for a transposable element implies, first, that sufficient mutational genetic variation exists in both the transposable element and the host to fashion such a mechanism and, second, that the force of natural selection is sufficiently strong to incorporate the mechanism into the transposable element as well as into the host genome. There is also a sort of group-selectionist argument favoring the evolution of regulation, not unlike the argument for the evolution of reduced virulence in pathogens, which is that transposable elements that regulate their own activity, perhaps in concert with host factors, will persist longer in lineages owing to reduced natural selection for their elimination. In any event, the apparent ubiquitousness of regulatory

mechanisms makes it seem unlikely that MLEs and TLEs have remained unaffected by the mutational and evolutionary forces that have shaped the regulation of other types of transposable elements.

There is also experimental support, not only for the existence of regulatory mechanisms for MLEs, but also indicating what these mechanisms might be. Let it be emphasized at the beginning that the experiments involve engineered laboratory constructs and induced mutations, so it has yet to be ascertained whether these mechanisms are operative in natural populations. Nevertheless, the regulatory effects are manifest.

Overproduction Inhibition

One type of regulatory mechanism is observed when the *Mos1* transposase is produced at a high level. This effect was observed initially with constructs consisting of a *hsp70* promoter fused to part of the *Mos1* promoter and driving transcription of the *Mos1* transposase reading frame (41). The assay for transposase activity was germline excision of the *peach* element from the *wpch* transgene, resulting in revertants with wild-type eyes. The regulatory phenomenon is called "overproduction inhibition." Increasing the number of copies of the *Mos1* construct decreases the rate of germline excision by 25% at 25°C and by 45% with heat shock; heat shock alone decreases the rate of germline excision by 13% with one copy of the *Mos1* construct and by 37% with two copies (41, 46). The mechanism of overproduction inhibition is still under investigation.

Dominant-Negative Complementation

A second type of regulatory mechanism is observed in certain missense mutations in the *Mos1* transposase (41, 46). In the presence of these mutations, the overall activity of the wild-type transposase is decreased in the *wpch* germline excision assay. This effect is not observed with all missense mutations. Among 18 missense mutations in the transposase, 7 had no effect on wild-type transposase as assayed by the rate of *wpch* excision (40). This is the result expected of classical amorphic or loss-of-function mutations, and included in this class is a knockout of the initiator methionine codon. Another eight of the mutations did have an effect on the activity of wild-type transposase; in the presence of the mutation, the rate of *wpch* excision decreased significantly from wild-type to a level approximately equal to half that of wild-type. The greatest effect was observed in three missense mutations that inhibited the activity of wild-type transposase significantly more than twofold. Interestingly, all three were located near the D(34)D motif, thought to be part of the active site, and one of the mutations was a targeted D-to-E replacement at position 284 creating a D(34)E signature (40). This effect was attributed to dominant negative complementation from "poison subunits" that combine with wild-type monomers

to create oligomers with impaired activity. In any case, the data from induced mutations suggest that some of the missense mutations of MLEs present in natural populations may be positively selected and maintained by virtue of their inhibitory effects on the wild-type transposase. This hypothesis lends itself to straightforward experimental validation.

Transposase Titration

Defective MLEs in natural populations that retain their transposase binding sites may play a role in regulation through “titration” of the active transposase. Titration effects have also been suggested for defective *P* elements (73). This mechanism might explain why certain deletions have virtually replaced all functional MLEs in a few species. MLEs with large internal deletions are not commonly found, but one particular deletion of a *Mos1*-like element is found at an extremely high frequency in *D. ercepeae*. This deletion spans nucleotides 66–788 and does not have an intact open reading frame (P Capy, personal communication). Although other explanations for the high frequency of this deletion are possible, positive selection for transposase titration is a plausible hypothesis.

A different deletion is especially common in *D. teissieri* (6, 51). This deletion spans nucleotides 544–1260 and its length and position are consistent with its originating through homologous recombination (or replication slippage) between direct repeats of a 7-bp sequence in a *Mos1*-like element (6); therefore, all deleted copies are not necessarily identical by descent. Nevertheless, many natural populations of *D. teissieri* contain no active copies of *mariner* but 5–10 copies of the deleted element per diploid genome (6). Positive selection for transposase titration may not be the only factor operating in this case, however. The deleted element retains an open reading frame coding for the amino-terminal portion of the transposase, and it has been suggested that the truncated transposase might serve a regulatory role (6), perhaps analogous to that of the *KP* deletion of the *P* element in *D. melanogaster* (1, 5).

There is yet another potential level of regulation of MLEs and TLEs that should be mentioned, although its molecular basis is not yet understood. There is a type of hybrid dysgenesis in *D. virilis* in which at least four unrelated transposable elements are all mobilized following a dysgenic cross (62). One of the elements that is mobilized is a TLE designated *Paris*. This element is very asymmetrically distributed between the parental dysgenic strains (euchromatic sites of hybridization, 29 versus 1), and therefore its mobilization may reflect a system of regulation specific to *Paris* that is disrupted in the dysgenic cross, leading to derepression of transposition. Alternatively, the data are also consistent with a mechanism in which mobilization of a single element triggers that of others, perhaps through chromosome breakage. Whatever the explanation,

the phenomenon itself implies the existence of at least one other mechanism of regulation of MLEs and TLEs that has yet to be characterized.

CONCLUSIONS AND PROSPECTS

The *mariner* family of transposable elements (MLEs) is unique among Class II transposable elements because of its extraordinarily widespread distribution in animals, from invertebrates to vertebrates including humans. The mechanism of excision and transposition is via a cut-and-paste process initiated with a staggered cut at each end of the element (not necessarily made simultaneously) and insertion into a staggered cut flanking a TA dinucleotide in the target site. The nucleotides flanking the excision form a heteroduplex that is mismatch repaired, resulting in a characteristic footprint. Much still remains to be learned about the details of target-site selection, the interaction of the transposase with the terminal inverted repeats (and possibly internal sites in the element), the cleavage and exchange reactions, and especially the molecular structure of the transposition complex.

The phylogenetically wide distribution of MLEs suggests minimal use of host factors, except possibly factors that are highly conserved in evolution. This promiscuity has probably contributed to the ability of MLEs to persist by occasional horizontal transmission. In the long run, the horizontal transmission into new lineages balances the loss by mutational inactivation and stochastic loss within lineages. The ability of MLEs to function in a wide variety of animal genomes strongly suggests that these elements may be put to practical use in the germline transformation of insect pests and vectors of human disease. Because many potential target genomes already contain MLEs from one or more subfamilies, it is important to define possible interactions between elements in different subfamilies, especially regulatory interactions.

The functional status of MLEs in the human genome is still unclear. Three distinct subfamilies of MLEs have been reported, but in no case has a copy with an open reading frame been discovered. The association of an MLE with a hotspot of unequal recombination in chromosome 17p, leading to the CMT1A duplication and the reciprocal HNLPP deficiency, suggests that an active transposase might be present, at least in some germline cells. It is not yet known whether MLEs are associated with hotspots of recombination in other organisms.

The greatest unsolved mystery about MLEs (and also about *Tc1*-like elements) is how these elements restrict their activity in the genome. Mechanisms that restrict transposable element activity are critical for the survival of both a transposable element and its host organism, because increased frequencies of transposition result in reduced viability, reduced fertility, and increased

frequencies of mutation. Restriction of activity of MLEs and *TcI*-like elements is even more critical than for most transposable elements, because these families of elements are active not only in the germline but also in the soma. Knowledge of regulation is essential to understanding both the molecular genetics and evolutionary biology of these elements. It is also of practical importance in learning how best to use these elements in the germline transformation of insect pests and disease vectors. Three potential mechanisms of regulation have been described: overproduction inhibition, dominant-negative complementation, and titration. Defining the molecular basis of overproduction inhibition is a priority because this mechanism is potentially of great importance in restricting the level of activity of *mariner* elements and establishing the equilibrium copy number.

ACKNOWLEDGMENTS

We are grateful for continuing support of this research through grant number GM33741 from the National Institutes of Health. The archaic spelling in the title of this paper is that used in the original (1798) version of the poem *The Rime of the Ancient Mariner* by Samuel Taylor Coleridge.

Visit the *Annual Reviews* home page at
<http://www.annurev.org>.

Literature Cited

1. Andrews JD, Gloor GB. 1995. A role for the *KP* leucine zipper in regulating *P* element transposition in *Drosophila melanogaster*. *Genetics* 141:587–94
2. Ashburner M. 1995. Medfly transformed—official. *Science* 270:1941–42
3. Auge-Gouillou C, Bigot Y, Pollet N, Hamelin MH, Meunierrotival M, Periquet G. 1995. Human and other mammalian genomes contain transposons of the *mariner* family. *FEBS Letters* 368:541–46
4. Berg DE, Howe MM, eds. 1989. *Mobile DNA*. Washington, DC: Am. Soc. Microbiol.
5. Black DM, Jackson MS, Kidwell MG, Dover GA. 1987. *KP* elements repress *P*-induced hybrid dysgenesis in *D. melanogaster*. *EMBO J.* 6:4125–35
6. Brunet F, Godin F, Bazin C, David JR, Capy P. 1996. The *mariner* transposable element in natural populations of *Drosophila teissieri*. *J. Mol. Evol.* 42:669–75
7. Brunet F, Godin F, David JR, Capy P. 1994. The *mariner* transposable element in the *Drosophilidae* family. *Heredity* 73:377–85
8. Bryan G, Garza D, Hartl DL. 1990. Insertion and excision of the transposable element *mariner* in *Drosophila*. *Genetics* 125:103–14
9. Bryan GJ, Hartl DL. 1988. Maternally inherited transposon excision in *Drosophila simulans*. *Science* 240:215–17
10. Bryan GJ, Jacobson JW, Hartl DL. 1987. Heritable somatic excision of a *Drosophila* transposon. *Science* 235:1636–38
11. Capy P, Chakrani F, Lemeunier F, Hartl DL, David JR. 1990. Active *mariner* transposable elements are widespread in natural populations of *Drosophila simulans*. *Proc. R. Soc. London Ser. B* 242:57–60
12. Capy P, David JR, Hartl DL. 1992. Evolution of the transposable element *mariner* in the *Drosophila melanogaster* species subgroup. *Genetica* 86:37–46

13. Capy P, Koga A, David JR, Hartl DL. 1992. Sequence analysis of active *mariner* elements in natural populations of *Drosophila simulans*. *Genetics* 130:499–506
14. Capy P, Maruyama K, David JR, Hartl DL. 1991. Insertion sites of the transposable element *mariner* are fixed in the genome of *Drosophila sechellia*. *J. Mol. Evol.* 33:450–56
15. Capy P, Vitalis R, Langin T, Higuert D, Bazin C. 1996. Relationships between transposable elements based upon the integrase-transcriptase domains: Is there a common ancestor? *J. Mol. Evol.* 42:359–68
16. Chakrani F, Capy P, David JR. 1993. Developmental temperature and somatic excision rate of *mariner* transposable element in three natural populations of *Drosophila simulans*. *Genet. Sel. Evol.* 25:121–32
17. Craig NL. 1989. Transposon Tn7. See Ref. 4, pp. 211–25
18. Craig NL. 1995. Unity in transposition reactions. *Science* 270:253–54
19. Doak TG, Doerder FP, Jahn CL, Herrick G. 1994. A proposed superfamily of transposase genes: transposon-like elements in ciliated protozoa and a common “D35E” motif. *Proc. Natl. Acad. Sci. USA* 91:942–46
20. Garcia-Fernández J, Bayascas-Ramírez JR, Marfany G, Muñoz-Mármol AM, Casali A, et al. 1995. High copy number of highly similar *mariner*-like transposons in planarian (Platyhelminthe): evidence for a trans-phyla horizontal transfer. *Mol. Biol. Evol.* 12:421–31
21. Garcia-Fernández J, Marfany G, Baguña J, Saló E. 1993. Infiltration of *mariner* elements. *Nature* 364:109
22. Garza D, Medhora M, Koga A, Hartl DL. 1991. Introduction of the transposable element *mariner* into the germline of *Drosophila melanogaster*. *Genetics* 128:303–10
23. Handler AM, Gomez SP. 1995. The *hobo* transposable element has transposase-dependent and -independent excision activity in drosophilid species. *Mol. Gen. Genet.* 247:399–408
24. Hartl DL. 1996. The most unkindest cut of all. *Nat. Genet.* 12:227–29
25. Houck MA, Clark JB, Peterson KR, Kidwell MG. 1991. Possible horizontal transfer of *Drosophila* genes by the mite *Proctolaelaps regalis*. *Science* 253:1125–29
26. Ivics Z, Izsvak Z, Minter A, Hackett PB. 1996. Identification of functional domains and evolution of *Tc1*-like transposable elements. *Proc. Natl. Acad. Sci. USA* 93:5008–13
27. Jacobson JW, Medhora MM, Hartl DL. 1986. Molecular structure of a somatically unstable transposable element in *Drosophila*. *Proc. Natl. Acad. Sci. USA* 83:8684–88
28. Kaplan N, Darden T, Langley CH. 1985. Evolution and extinction of transposable elements in Mendelian populations. *Genetics* 109:459–80
29. Kidwell MG. 1992. Horizontal transfer. *Curr. Opin. Genet. Dev.* 2:868–73
30. Kidwell MG. 1992. Horizontal transfer of *P* elements and other short inverted repeat transposons. *Genetica* 86:275–86
31. Kidwell MG. 1993. Voyage of an ancient *mariner*. *Nature* 362:202
32. Kiyosawa H, Chance PF. 1996. Primate origin of the CMT1A-REP repeat and analysis of a putative transposon-associated recombinational hotspot. *Hum. Mol. Genet.* 5:745–53
33. Kleckner N. 1989. Transposon Tn10. See Ref. 4, pp. 227–70
34. Kulkosky J, Jones KS, Katz RA, Mack JPG, Skalka AM. 1992. Residues critical for retroviral integrative recombination in a region that is highly conserved among retroviral/retrotransposon integrases and bacterial insertion sequence transposases. *Mol. Cell. Biol.* 12:2331–38
35. Lampe DJ, Churchill MEA, Robertson HM. 1996. Purified *mariner* transposase is sufficient to mediate transposition *in vitro*. *EMBO J.* 15:5470–79
36. Laski FA, Rio DC, Rubin GM. 1986. Tissue specificity of *Drosophila P* element transposition is regulated at the level of mRNA splicing. *Cell* 44:7–19
37. Lawrence JG, Hartl DL. 1992. Inference of horizontal genetic transfer from molecular data: an approach using the bootstrap. *Genetics* 131:753–60
38. Lidholm D-A, Gudmundsson GH, Boman HG. 1991. A highly repetitive, *mariner*-like element in the genome of *Hyalophora cecropia*. *J. Biol. Chem.* 266:11518–21
39. Lidholm D-A, Lohe AR, Hartl DL. 1993. The transposable element *mariner* mediates germline transformation in *Drosophila melanogaster*. *Genetics* 134:859–68
40. Lohe AR, De Aguiar D, Hartl DL. 1997. Mutations in the *mariner* transposase: The “D,D(35)E” consensus sequence is nonfunctional. *Proc. Natl. Acad. Sci. USA* 94:1293–97

41. Lohe AR, Hartl DL. 1996. Autoregulation of *mariner* transposase activity by overproduction and dominant-negative complementation. *Mol. Biol. Evol.* 13:549–55
42. Lohe AR, Hartl DL. 1996. Germline transformation of *Drosophila virilis* with the transposable element *mariner*. *Genetics* 143:365–74
43. Lohe AR, Hartl DL. 1996. Reduced germline mobility of a *mariner* vector containing exogenous DNA: effect of size or site? *Genetics* 143:1299–306
44. Lohe AR, Lidholm D-A, Hartl DL. 1995. Genotypic effects, maternal effects and grand-maternal effects of immobilized derivatives of the transposable element *mariner*. *Genetics* 140:183–92
45. Lohe AR, Moriyama EN, Lidholm D-A, Hartl DL. 1995. Horizontal transmission, vertical inactivation, and stochastic loss of *mariner*-like transposable elements. *Mol. Biol. Evol.* 12:62–72
46. Lohe AR, Sullivan DT, Hartl DL. 1996. Genetic evidence for subunit interactions in the transposase of the transposable element *mariner*. *Genetics* 144:1087–95
47. Loukeris TG, Arcà B, Livadaras I, Dialrtaki G, Savakis C. 1995. Introduction of the transposable element *Minos* into the germ line of *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. USA* 92:9485–89
48. Loukeris TG, Livadaras I, Arcà B, Zabalou S, Savakis C. 1995. Gene transfer into the Medfly, *Ceratitis capitata*, with a *Drosophila hydei* transposable element. *Science* 270:2002–5
49. Lozovskaya ER, Nurminsky DI, Hartl DL, Sullivan DT. 1996. Germline transformation of *Drosophila virilis* mediated by the transposable element *hobo*. *Genetics* 142:173–77
50. Maruyama K, Hartl DL. 1991. Evidence for interspecific transfer of the transposable element *mariner* between *Drosophila* and *Zaprionus*. *J. Mol. Evol.* 33:514–24
51. Maruyama K, Hartl DL. 1991. Evolution of the transposable element *mariner* in *Drosophila* species. *Genetics* 128:319–29
52. Maruyama K, Schoor KD, Hartl DL. 1991. Identification of nucleotide substitutions necessary for trans-activation of *mariner* transposable elements in *Drosophila*: analysis of naturally occurring elements. *Genetics* 128:777–84
53. Medhora M, Maruyama K, Hartl DL. 1991. Molecular and functional analysis of the *mariner* mutator element *Mos1* in *Drosophila*. *Genetics* 128:311–18
54. Medhora MM, MacPeck AH, Hartl DL. 1988. Excision of the *Drosophila* transposable element *mariner*: identification and characterization of the *Mos* factor. *EMBO J.* 7:2185–89
55. Mizuuchi K. 1992. Transpositional recombination: mechanistic insights from studies of Mu and other elements. *Annu. Rev. Biochem.* 61:1011–51
56. Morgan GT. 1995. Identification in the human genome of mobile elements spread by DNA-mediated transposition. *J. Mol. Biol.* 254:1–5
57. Nikitin AG, Woodruff RC. 1995. Somatic movement of the *mariner* transposable element and lifespan of *Drosophila* species. *Mutat. Res.* 338:43–49
58. O'Brochta DA, Handler AM. 1988. Mobility of *P* elements in drosophilids and nondrosophilids. *Proc. Natl. Acad. Sci. USA* 85:6052–56
59. O'Brochta DA, Warren WD, Saville KJ, Atkinson PW. 1996. *Hermes*, a functional non-drosophilid insect gene vector from *Musca domestica*. *Genetics* 142:907–14
60. Oosumi T, Belknap WR, Garlick B. 1995. *mariner* transposons in humans. *Nature* 378:672
61. Petrov DA, Lozovskaya ER, Hartl DL. 1996. High intrinsic rate of DNA loss in *Drosophila*. *Nature* 384:346–49
62. Petrov DA, Schutzman JL, Hartl DL, Lozovskaya ER. 1995. Diverse transposable elements are mobilized in hybrid dysgenesis in *Drosophila virilis*. *Proc. Natl. Acad. Sci. USA* 92:8050–54
63. Reiter LT, Marukami T, Koeth T, Pentao L, Muzny DM, et al. 1996. A recombination hotspot responsible for two inherited peripheral neuropathies is located near a *mariner* transposon-like element. *Nat. Genet.* 12:288–97
64. Robertson HM. 1993. The *mariner* transposable element is widespread in insects. *Nature* 362:241–45
65. Robertson HM. 1995. The *Tc1-mariner* superfamily of transposons in animals. *J. Insect Physiol.* 41:99–105
66. Robertson HM, Asplund ML. 1996. *Bm-mar1*: a basal lineage of the *mariner* family of transposable elements in the silkworm moth, *Bombyx mori*. *Insect Biochem. Mol. Biol.* 26:945–54
67. Robertson HM, Lampe DJ. 1995. Distribution of transposable elements in arthropods. *Annu. Rev. Entomol.* 40:333–57

68. Robertson HM, Lampe DJ. 1995. Recent horizontal transfer of a *mariner* transposable element among and between Diptera and Neuroptera. *Mol. Biol. Evol.* 12:850–62
69. Robertson HM, MacLeod EG. 1993. Five major subfamilies of *mariner* transposable elements in insects, including the Mediterranean fruit fly, and related arthropods. *Insect Mol. Biol.* 2:125–39
70. Robertson HM, Zumpano KL, Lohe AR, Hartl DL. 1996. Reconstructing the ancient *mariners* of humans. *Nat. Genet.* 12:360–61
71. Savakis C, Arcà B, Loukeris TG, Zabalou S. 1995. Transposition of the *Minos* element in *Drosophila melanogaster*. *Abstr. 36 Annu. Drosophila Res. Conf.* 208
72. Sedensky MM, Hudson SJ, Everson B, Morgan PG. 1994. Identification of a *mariner*-like repetitive sequence in *C. elegans*. *Nucleic Acids Res.* 22:1719–23
73. Simmons MJ, Bucholz LM. 1985. Transposase titration in *Drosophila melanogaster*: a model of cytotype in the P-M system of hybrid dysgenesis. *Proc. Natl. Acad. Sci. USA* 82:8119–23
74. Smit AFA, Riggs AD. 1996. Tiggers and other DNA transposon fossils in the human genome. *Proc. Natl. Acad. Sci. USA* 93:1443–48
75. Szekely AA, Woodruff RC, Mahendran R. 1993. P element mediated germ line transformation of *Drosophila melanogaster* with the *Tc1* transposable DNA element from *Caenorhabditis elegans*. *Genome* 37:356–66
76. van Luenen HGAM, Colloms SD, Plasterk RHA. 1994. The mechanism of transposition of *Tc3* in *C. elegans*. *Cell* 79:293–301
77. Vos JC, De Baere I, Plasterk RHA. 1996. Transposase is the only nematode protein required for in vitro transposition of *Tc1*. *Genes Dev.* 10:755–61
78. Warren AM, Crampton JM. 1994. *mariner*: its prospects as a DNA vector for the genetic manipulation of medically important insects. *Parasitol. Today* 10:58–63
79. Warren WD, Atkinson PW, O'Brochta DA. 1994. The *Hermes* transposable element from the house fly, *Musca domestica*, is a short inverted repeat-type element of the *hobo*, *Ac*, and *Tam3* (*hAT*) family. *Genet. Res.* 64:87–97