Transposable elements and host genome evolution

Margaret G. Kidwell and Damon R. Lisch

Several recent reports have challenged the idea that transposable elements (TEs) are mainly 'selfish' or 'junk' DNA with little importance for host evolution. It has been proposed that TEs have the potential to provide host genomes with the ability to enhance their own evolution. They might also be a major source of genetic diversity, allowing response to environmental changes. Because the relationships between TEs and host genomes are highly variable, and because the selfish, junk and beneficial DNA hypotheses are by no means mutually exclusive, a single label for these relationships appears to be inappropriate and potentially misleading.

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Transposable elements (TEs) are mobile DNA sequences that are widely distributed in bacteria, plants and animals. At least two levels of selection can apply to TEs – selection at the DNA level and selection at the host organism level. Positive selection at the DNA level results from the ability of TE sequences to replicate faster than those of the host genome. This aspect of selection is the basis of 'the selfish DNA hypothesis' or, more accurately, 'the parasitic DNA hypothesis'. Negative selection at the host organismal level commonly results either from TE-induced insertional mutations, which are deleterious to hosts, or from ectopic recombination between homologous TE sequences located in nonhomologous regions of the genome. Neutrality of TEs at the organismal level is also common, especially for inactive TEs, which make up the vast majority of the TE complement of many eukaryotic genomes. This neutrality is the basis of 'the junk DNA hypothesis'.

Although it is accepted generally that positive selection of TEs is possible at the organismal level, currently, expert opinion is divided sharply about the frequency of the beneficial effects of TEs on host genomes. One school of thought claims that positive selection is so rare that it has virtually no impact on host evolution. A second perspective, expressed increasingly in recent months, is that TEs might provide beneficial effects to their hosts more frequently than acknowledged previously and sometimes in unexpected ways.

This review summarizes some recent ideas concerning actual and potential beneficial effects of TEs on host evolution, and focuses on evidence that TEs have the capacity to restructure genomes in interesting ways. This capacity might not have been exploited often, nor has it necessarily been subject to positive selection. However, given the opportunistic nature of evolution, the presence of such a potent agent for change at the molecular level is at least potentially important for host evolution at the phenotypic level.

TE structure and distribution

Eukaryotic TEs are divided into two main classes according to their structural organization and their mechanism of transposition. Class I elements use an RNA-mediated mode of transposition and encode a reverse transcriptase (RT). There are three distinct subclasses of RT-encoding TEs found throughout eukaryotes: the retrotransposons, the retroposons and the retrointrons[1]. The retroposons include the short interspersed nuclear elements (*SINES*) and the long interspersed nuclear elements (*LINES*). Class II elements, the transposons (*sensu strictu*), use a DNA-based mode of transposition.

Although wide variation in copy number, distribution and type exists from one species to another, TEs comprise a huge fraction of the genomes of many animals and plants; for example, the human genome consists of at least 35% TEs (Ref. 2). In addition, retrotransposons constitute more than 50% of the maize genome (Fig. 1). TE insertions have resulted in a doubling of the size of this genome within the past few million years[3]. More compact plant genomes, such as that of *Arabidopsis thaliana*, carry a smaller fraction of TEs and might have mechanisms to prevent amplification of extant element families[4].

By contrast to earlier interpretations that large genomic TE fractions provide evidence for the absence of any important role in organismal evolution, Henikoff *et al.*[5] conclude that they 'may be a manifestation of the evolutionary benefits of genomic flexibility'. However, given the huge variation in TE copy number among a wide variety of successful eukaryotic lineages, it is unclear whether increased TE copy number is itself subject to positive selection by virtue of its potential to increase genome flexibility.

Charlesworth *et al.*[6] have documented the low frequency of fixed TE sites in *Drosophila melanogaster.* They concluded that, apart from the deleterious consequences of induced mutation and recombination, these elements are of little or no importance to the evolution of their hosts. However, it has not been demonstrated whether these results are generally applicable to a wide range of species. Indeed, a large number of TE insertions are fixed within mammalian species, leaving open the possibility that a subset of insertions has been subject to positive selection[7].

By contrast to *D. melanogaster*, in which TEs are distributed randomly along the distal sections of the chromosome arms[8], TEs are distributed nonrandomly in the genomes of many other species. Clustering of TEs appears to be facilitated by specific characteristics of particular regions of host genomes. For instance, the TE complement of the compact genome of *Saccharomyces cerevisiae*

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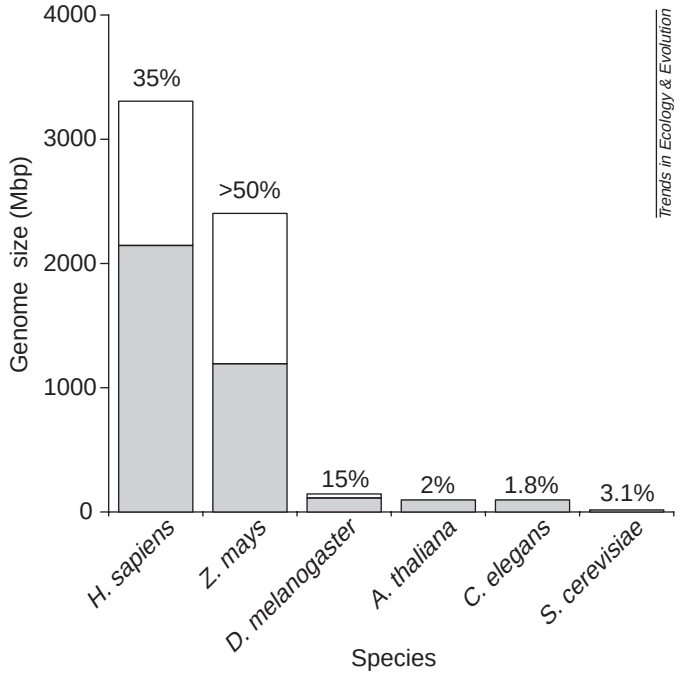

Fig. 1. A histogram showing total genome sizes and the percentage of those sizes occupied by transposable elements (TEs) (open bars) for humans and five model organisms. Other sequences are shown by shaded bars. The data were obtained from the following references: *Homo sapiens*[39], *Zea mays*[40], *Drosophila melanogaster*[41], *Arabidopsis thaliana*[4] (Stephen Wright, pers. commun.), *Caenorhabditis elegans* (Zhirong Bao, pers. commun.) and *Saccharomyces cerevisiae*[9]. Mbp, million base pairs.

is restricted to five families of retroelements[9]. In this yeast, target site preference can be altered by mutations in genes encoding proteins involved in chromatin assembly and chromatin-mediated gene silencing[10,11], and TEs are targeted to specific regions of the genome. Even in *D. melanogaster*, TEs are nonrandomly distributed within heterochromatin, with each class of TE occupying a distinct region[12].

Roles in mutation and genomic reorganization

It is possible that TEs play an especially useful role as mutators in evolution because of the broad spectrum of mutations produced by their activity[13]. It has been claimed that 'although simple DNA base substitutions are well suited for the generation, diversification and optimization of local protein space, a hierarchy of mutational events is required for the rapid generation of protein diversity in evolution'[14]. If this turns out to be true, then it should be noted that TEs and viruses are important generators of the more complex types of mutations in the mutational hierarchy.

The role of TEs as potent genomic reorganizers continues to be documented. By contrast to the high stability of the yeast genome during mitosis, chromosomal reorganization occurs with high frequency in meiosis[15]. A major factor in this instability is interchromosomal translocation between multiple copies of the *Ty1* and *Ty2* TE families. The direct implication of TEs in inversion break formation in natural populations of many species has been hypothesized for a long time. Now, the presence of a TE at the junction of the naturally occurring inversion 2Rd′ in the malaria mosquito, *Anopheles arabiensis*, has been documented[16]. This species is rich in paracentric inversions, perhaps owing to TE activity.

The massive amplification of retroelements in a single generation in wallaby (Macropodidae) species hybrids[17] provides an impressive example of the capacity of TEs to change the structural architecture of host genomes without disrupting the normal function of somatic cells. In this study, genome-wide undermethylation of retroelement sequences was demonstrated in a species hybrid, in contrast to the normal methylation of nonhybrid parents. Because DNA methylation is thought to play a role in transposon regulation, it is hypothesized that hypomethylation in the hybrids leads to rapid transposon amplification. Similar observations of deficient methylation and spontaneous chromosome changes were reported in other mammalian hybrids. In these cases, the hybrids are sterile; however, other hybrids are fertile and such changes have the potential to facilitate rapid karyotypic evolution. The potential for fertile female hybrids to produce new species remains speculative. This area of research appears to be ripe for further investigation.

Although basal genome sizes (C values) of Archea and Eubacteria vary by only about one order of magnitude, those of eukaryotes vary greater than 80 000-fold. The mechanisms responsible for the variation in the C value among eukaryotes are poorly understood, but it seems that, along with other mechanisms such as polyploidization, the amplification of TEs is a major contributing mechanism. There is now increasing evidence that shifts both in intron length and in the amounts of other noncoding DNA are driven by genome-wide changes in deletion and insertion frequencies[18,19]. Eukaryotic genomes might exist in a state of dynamic equilibrium, with the potential for massive changes in genome size being balanced by the presence of host-encoded mechanisms, such as DNA methylation, and by self-regulation by transposons themselves.

Epigenetic aspects

Previously, it had been assumed that both nucleosomal chromatin formation and cytosine methylation of DNA evolved to regulate host gene expression. For example, because cytosine methylation of DNA is associated with variations in gene expression, it has been hypothesized to be a major component of gene regulation in mammals. However, this view has been challenged recently. It has been argued that instead of being a means by which host genes are regulated, the primary role of methylation in mammals is to regulate transposon activity[20]. Because the mammalian genome is rich in TE sequences, Yoder *et al.*[20] claimed that previous evidence for fluctuations in overall levels of methylation during development actually represents changes in the methylation status of transposons. Indeed, the wallaby study mentioned previously might provide an example of the disruption of normal regulatory pathways by hybridization[17]. Be that as it may, the lethality of a mutant in the only known methyl transferase in mice[21] and the physical abnormality of *Arabidopsis* plants carrying an antisense version of the methyltransferase gene *MET1* suggest that methylation is required for normal development[22]. A recent report on the invertebrate chordate *Ciona intestinalis*[23] further complicates the matter. In this species, active genes are predominantly methylated and transposons are hypomethylated[23]. Whether the primary role of methylation is to regulate transposons or to regulate host gene expression, it seems likely that methylation plays an important role in both host regulation and TE gene regulation.

One regulatory pathway that has a clear link to TE activity is homology-dependent gene silencing, in which an endogenous copy of a gene is silenced following the introduction of an additional copy of that gene. Homology-dependent gene silencing has been associated with plant transgenes for a long time, but evidence for a similar phenomenon in animals has been accumulating only recently. In *D. melanogaster* it has been demonstrated[24] that the introduction of a hybrid white-*Adh* gene construct induced silencing of the endogenous *Adh* gene. Interestingly, these authors also show that silencing requires *Polycomb* group genes[24], suggesting a link, in animals, between mechanisms for developmental differentiation and for the regulation of repetitive DNA.

In both animals and plants (reviewed by Matzke and Matzke[25]) homology-dependent gene silencing is related to the defense of the host genome against repetitive or foreign nucleic acids (DNA and RNA). More specifically, post-transcriptional gene silencing is a cellular defense against RNA viruses, and transcriptional gene silencing, associated with methylation, reflects a genomic defense against TEs (Ref. 26). Given the links between homology-dependent gene silencing and other epigenetic phenomena involving the regulation of host genes, it appears that transposons might have profoundly influenced the evolution of a remarkably complex system of gene regulation in both animals and plants.

Co-option of TEs for host functions

In the course of their evolution, TEs accumulate various features that presumably enhance their ability to increase their copy number. Some of these features might be co-opted by their hosts. For instance, TEs often carry regulatory signals such as enhancer elements; these have the potential to 'rewire' regulatory networks and to modulate host gene expression. Britten[27] has described several examples of the long-term evolutionary effects of insertion sequence elements from a wide variety of organisms. Using stringent criteria, Britten concluded that TEs provide a significant source of regulatory variation in evolution. A recent example is provided by the *apolipoprotein(a)* [*apo(a)*] gene in humans[28]. An enhancer of this gene, located 20 kilobases upstream of the start of transcription, is situated entirely within a *LINE* element (Fig. 2); thus, a TE has become an integral part of the regulation of a host gene.

A common feature of 5′, intron and 3′ non-coding regions of host plant genes is the miniature inverted repeat transposons (*MITES*). Recently, similar elements have been found in species as diverse as *Xenopus*[29], Zebra fish[29], *Aedes aegypti*[30], *A. thaliana*[31] and *Homo sapiens*[2]. The propensity of these elements to insert into (and in some cases even make up) the regulatory regions of genes in all of these species suggests that they have an important role in host regulatory evolution. In rice (*Oryza sativa*) and sorghum (*Sorghum bicolor*), matrix attachment regions (MARs) were found to co-localize with *MITES*, thus suggesting that *MITES* preferentially insert near MARs and/or that they can serve as MARs (Ref. 32).

Mechanisms to maintain telomere length are tied intimately to the evolution of retrotransposons. In most species that have been examined, telomerase is the primary means by which telomeres are maintained. However, in *Drosophila*, telomeres are hypothesized to be maintained via serial transposition of the non-LTR retroelements *HetA* and *TART* (Fig. 3)[33]. In the silkworm *Bombyx mori*[34], similar retroelements target subtelomeric repeats, and are thus unlikely to be required for telomere maintenance; presumably,

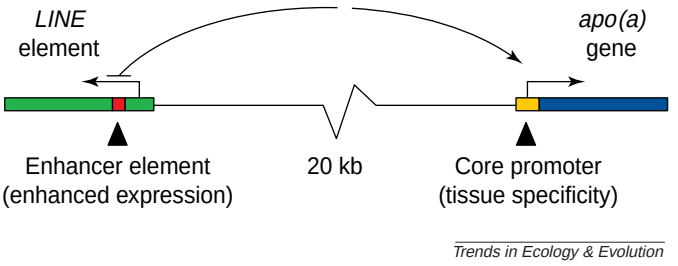

Fig. 2. Relative positions of the *apolipoprotein(a)* [*apo(a)*] gene and its enhancer element. The long interspersed nuclear element (*LINE*) (green) is located approximately 20 kilobases upstream from the start of transcription of the *apo(a)* gene (blue). The enhancer element (red) is located entirely within the *LINE* element and is competent to enhance expression levels of *apo(a)*. Tissue specificity is provided by the core promoter element (yellow). *Modified, with permission, from Ref. 28.*

B. mori retains 'normal' telomerase function. By contrast, telomerase function in *Drosophila* might have become unnecessary when efficient telomere targeting of several classes of elements made it redundant.

Recent evidence suggests that telomerase itself either evolved from or has given rise to many families of retroelements[35,36]; thus, retroelements have taken on a basic role in cell division. Also, because of its basic capacity to produce DNA from RNA, telomerase might represent a continuing source of new TEs – the cost of this capacity might have been the risk of spawning new families of parasitic elements.

Dramatic evidence has been provided that V(D)J recombination in vertebrate lymphocytes is the product of 'domesticated' TE activity[37,38]. V(D)J recombination is a site-specific recombination reaction that occurs specifically in developing oocytes of vertebrates and plays a part in generating their vast repertoire of immunoglobulins and T-cell receptors. In this reaction the V (variable), J (joining) and D (diversity) gene segments that encode the variable portion of the T-cell antigen receptors are joined together to form the exon that encodes the antigen-binding

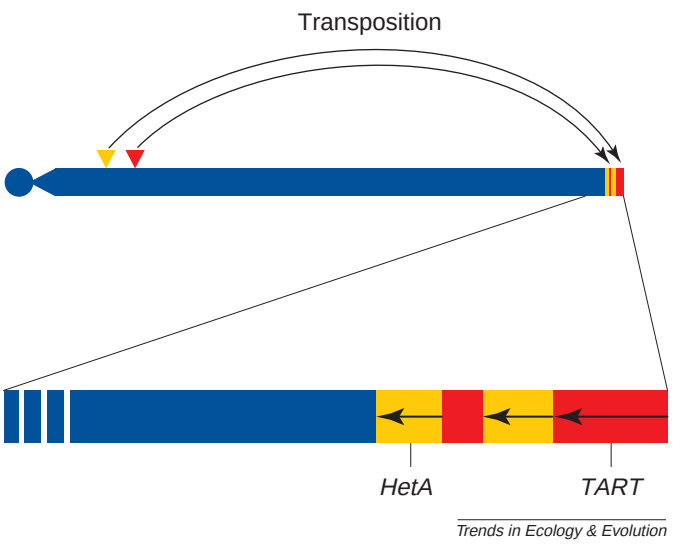

Fig. 3. *HetA* (yellow) and *TART* (red) elements transpose specifically to the ends of *D. melanogaster* chromosomes. Previously inserted elements serve as targets for subsequent insertions. The net effect of this activity is to prevent the loss of telomeres following chromosome replication. *Modified, with permission, from Ref. 33.*

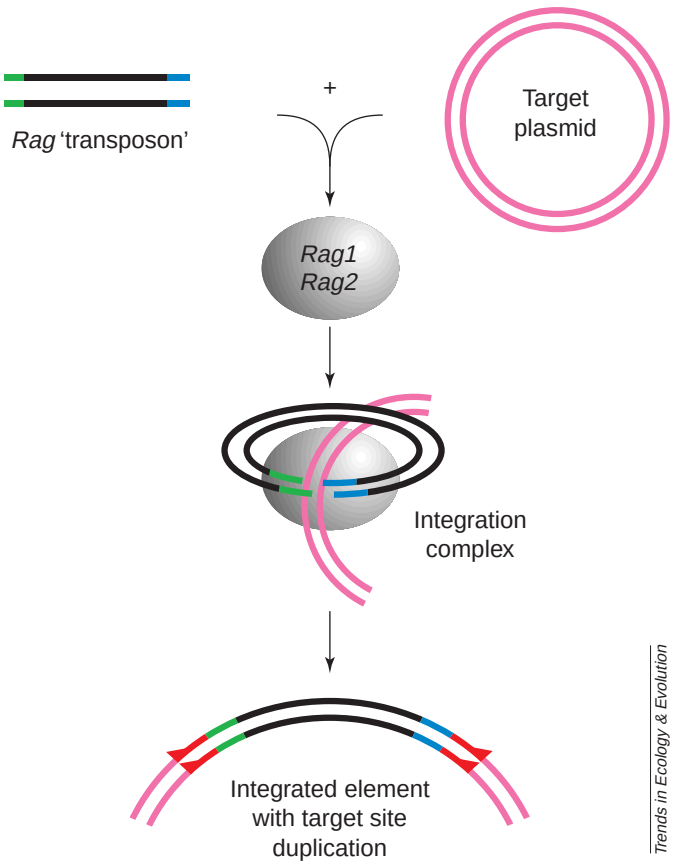

Fig. 4. An *in vitro* demonstration of *Rag1* and *Rag2* catalysed transposition. Signal sequences (blue and green) that are recognized by *Rag1* and *Rag2* surround an antibiotic resistance gene. The target plasmid (purple) contains an origin of replication and a second resistance gene. When the artificial *Rag* 'transposon' is combined with *Rag1* and *Rag2* and the target plasmid, the *Rag* transposon integrates into the plasmid in a manner similar to other transposable elements. The result of this reaction is a new plasmid carrying both resistance markers. The integrated sequence is flanked by a direct duplication, a characteristic feature of inserted transposons. *Modified, with permission, from Ref. 37.*

portion of the polypeptide. Experiments using the products of the *Rag1* and *Rag2* genes demonstrate that these enzymes can catalyse transposition events *in vitro*, therefore supporting earlier speculation that these genes were carried by a TE originally (Fig. 4). These results suggest that a fundamental component of the vertebrate immune system probably evolved from a transposon, whose capacity for DNA rearrangement was exploited to produce rapid somatic variability in specific host cells.

Conclusions

In addition to their so-called parasitic characteristics, TEs might have played an important role in enhancing the evolutionary potential of their hosts. The properties that lead TEs to be labeled 'junk DNA' might have enabled TEs to provide genomes with the plasticity to evolve new tools for generating diversity. Thus, a balance between fidelity and exploration might have evolved through the operation of natural selection and chance on the products of ancient interactions between hosts and transposable elements.

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Evolutionary consequences of dating the Yixian Formation

Paul M. Barrett

The Yixian Formation of northeastern China has yielded important new fossils that are fuelling debates on the origin of angiosperms, on the early radiation of birds and of mammals, and on the origin of feathers. Although these fossils provide a wealth of detailed anatomical information, knowledge of the absolute age of the Yixian Formation is crucial if we are to understand their true evolutionary significance. The age of the Yixian Formation has been disputed, but recent evidence provides strong support for an Early Cretaceous rather than a Late Jurassic age.

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The Yixian Formation, a series of intercalated lacustrine and volcanic deposits, outcrops around the cities of Chaoyang and Beipiao in Liaoning Province in the northeast corner of the People's Republic of China. Since the 1980s, the remnants of this ancient lake system have yielded the spectacular remains of a rich and varied terrestrial biota[1], which includes plants (ginkgophytes, conifers, cycadophytes, pteridosperms and angiosperms)[2–5], insects[6,7], diverse freshwater invertebrates[5], fish[8,9], amphibians[1], small reptiles[1,10], mammals[11,12], dinosaurs[1,13–17], pterosaurs[18,19] and birds[20–25]. Fossils from these deposits are exceptionally well preserved; skeletons are often near-complete and found in articulation[10–16,20,22], and soft-tissue impressions are known from several taxa, most famously from several theropod dinosaur skeletons, which possess the remnants of feathers and possible 'proto-feathers'[13–16]. The specimens recovered from these remarkable strata have the potential to increase greatly our knowledge of Mesozoic terrestrial ecosystems and to shed light on the early evolution of clades as divergent as birds and angiosperms.

Establishing the age of the Yixian Formation

One crucial piece of information on the Yixian biota is missing: an accurate assessment of its age. Estimates of the age of the Yixian Formation have ranged from the latest Jurassic (in the Tithonian stage; Fig. 1) to the late Early Cretaceous (in the Aptian stage). A 25-million-year difference might not seem important given the immensity of geological time, but pinning down the exact age of these deposits is of great importance to evolutionary biologists and to palaeontologists. Several major biotic changes, such as the origin of angiosperms and the early radiation of birds, occurred in terrestrial ecosystems sometime during this time interval, and the evolutionary significance of the Yixian biota cannot be assessed properly until its age is known more precisely.

If the Yixian Formation is Late Jurassic in age it provides us with the earliest known records of several clades. Perhaps the most notable first appearance would be the angiosperms, several taxa of which (*Archaefructus* and *Chaoyangia*, etc.) have been described from these deposits[2–4]. Currently, most palaeobotanists accept an Early

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