the spread of disease; however, this is largely offset by the possibility that isolation will result in individuals that are more susceptible to diseases, so that the potential for a catastrophic epidemic is enhanced. This is of particular concern given that diseases may invade from other species. One solution is to have several reserves, each supporting a density of at least the MVP, and each containing heterogeneous habitat which naturally divides the population into viable subpopulations.

Acknowledgements

We would like to thank D. Elam, A. Montalvo, R. Lande and two anonymous reviewers for their valuable comments on the manuscript.

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Transposable Elements as Introns: Evolutionary Connections

Michael D. Purugganan

Recent molecular genetic studies demonstrate that many transposable elements, when inserted into nuclear genes, can behave as introns and create novel intron processing patterns. These studies point to possible mechanisms by which transposable element insertions participate in the evolutionary diversification of gene structure, the rise of alternative splicing patterns and the production of novel regulatory interactions. Moreover, they provide us with fresh insights into the evolutionary dynamics of these mobile sequences.

Over the last two decades molecular geneticists have identified numerous transposable elements in eukaryotic genomes, many of which are significant sources of spontaneous mutations and genetic variability within populations[1–3]. Some biologists are intrigued by the possibility that these mobile elements may contribute to the evolutionary processes of adaptation and speciation[4,5], while others argue that elements are simply selfish genomic parasites, maintained within populations primarily by their ability to replicate[6,7].

Two element classes have received the most attention[8]. The first class contains the inverted repeat (IR) transposons, such as the *Zea mays* (maize) *Ac/Ds* and *Drosophila P* elements, which excise from their genomic location and move via a DNA intermediate. This class is named for the inverted repeat sequences found at both ends of the element. The second class contains the retrotransposons, such as the *copia* elements of *Drosophila* or maize *Stonor* elements, which transpose via an RNA intermediate[8]. Unlike IR transposons, retrotransposons do not excise from their genomic position; instead, an RNA transcript from the element is synthesized and is subsequently converted into a DNA copy that integrates elsewhere in the genome. Retrotransposons are characterized by long terminal repeat (LTR) sequences at both ends, and each LTR is bounded by short IR sequences.

The evolutionary relationship between transposable elements and introns has been the subject of

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Box 1. Introns, exons and the processing of nuclear pre-mRNA[43,44]

In the late 1970s, molecular biologists discovered that the functional segments of eukaryotic nuclear genes were not continuously organized, and that protein-coding sequences were split within the gene. Eukaryotic nuclear genes are divided into the coding sequences, called **exons**, which were separated by non-coding DNA regions called **introns**. Introns range in size from 50–60 to several thousand nucleotides in length and are bounded at both ends by splice sites. The proximal or **donor** site has the dinucleotide GT, while the distal or **acceptor** site contains the dinucleotide AG. These donor and acceptor sites play a central role in defining the intron during splicing.

Eukaryotic genes are transcribed into **precursor messenger RNA (pre-mRNA)**, which contains both exon and intron sequences. This pre-mRNA is processed in the nucleus by RNA–protein complexes which form the spliceosome. Splicing removes the intervening introns and joins adjacent exons to form a continuous stretch of coding sequence. The transcript terminus is separately processed through cleavage of the pre-mRNA end and the addition of a stretch of adenosine residues, called the **polyA+ tail**. The terminal processing of the pre-mRNA is carried out by various cellular factors, which recognizes a **cleavage/polyadenylation signal** found within the pre-mRNA. The final spliced, polyadenylated transcript represents the mature transcript, which is exported from the nucleus and translated into protein in the cytoplasm.

intense speculation. It has been suggested, for example, that introns arose as a result of element insertion into coding sequences[9–11]. The recent discovery that several transposable element insertions are spliced from pre-mRNA strengthens arguments invoking an association between mobile elements and introns. To date, splicing of insertions has been reported for members of four element families in maize and *Drosophila*[12–22]. Several more transposable elements are able to alter pre-mRNA processing patterns when inserted into a gene[23–29].

The specific mechanisms by which transposable elements are spliced from pre-mRNA are summarized in several recent reviews[30,31] and Box 1 gives a brief overview of pre-mRNA processing. In this discussion, I focus attention on how transposable element insertions may contribute to the evolutionary diversification of gene structure, as well as the origin of nuclear introns. I also outline the impact of transposable element splicing on the evolutionary fitness of both element and host organism.

Transposable elements as introns

Transposable element insertions affect pre-mRNA splicing in two distinct ways: first, element insertions within exons are capable of functioning as new introns, and secondly, insertions in both introns and exons can transform constitutive splicing patterns by altering pre-mRNA processing signals. These two insertion classes comprise a group which are designated as transposable element introns. A total of 26 transposable element intron alleles have been reported in maize, *Drosophila*, *Homo sapiens* and *Mus* and the list is steadily growing (see Table 1). The extensive number of transposable element intron insertions suggests that interaction between mobile element insertions and the cellular pre-mRNA processing machinery is an integral part of transposable element biology.

Insertions of the maize *Ds* and *dSpm* and the *Drosophila 412* and *P* elements were among the first examples of new intron formation by transposable element insertions. Element insertions within these genes are processed from pre-mRNA using splice signals encoded within the element (see Fig. 1). Cryptic splice sites within the host gene are also activated by mobile element insertions and used in pre-mRNA processing. These cryptic sites are nucleotide sequences that are similar in structure to wild-type splice sites but are not part of the intron termini; these cryptic sites normally go unrecognized by the splicing machinery in normal wild-type splicing.

Splicing removes most, if not all, of the insertion sequence from the final transcript. However, this does not necessarily lead to the restoration of the wild-type gene sequence. Transposon splice signals are not located at precisely the termini of the element, resulting in the imprecise splicing of the insertion and alterations in the final encoded message. The nature, extent and impact of these sequence changes depend both on the identity of the element and its site of insertion within the host gene. In some cases, splicing of the element insertion is sufficient to permit expression of the mutant gene. For instance, the maize allele *a2-m1* (*Class II*) appears phenotypically wild-type in certain genetic backgrounds despite the presence of a 1.3 kb *dSpm* insertion within the *a2* coding region[16]. For other alleles, processing may result in the restoration of low levels of gene expression.

The second broad class of transposable element introns consists of insertions that result in the transformation of pre-mRNA processing signals within the host gene. Unlike the *de novo* intron formation described above, this class of element-induced processing can significantly alter transcript structure. Element insertions, for example, may result in the recognition of nonconsecutive splice sites within a gene, creating exon-skipping patterns which lead to the deletion of exons in the final transcript (see Fig. 2a). The maize *waxy* retrotransposon insertion alleles[25], as well as a human $NF1^{DD}$ *Alu* insertion allele responsible for the genetic disorder neurofibromastosis[26], display this type of transposable element induced exon skipping. Transposable elements may also lead to the joining of host gene splice sites with cryptic sites within or adjacent to the element insertion, as in the maize *adh-2F11 Ds* (Ref. 14) or the *bz-m13 dSpm* alleles[15]. Finally, use of transposable element encoded RNA processing signals can lead to the incorporation of large tracts of element sequences into the final transcript[24].

Together, these molecular genetic mechanisms point to possible pathways by which transposable element introns can contribute to the evolutionary diversification of genes and gene products. Aside from the imprecise splicing of insertions, which results in localized sequence alterations, transposable elements can also condition a variety of alternative pre-mRNA processing patterns. Moreover, transposable element introns are able to impose novel regulatory signals on a transcription unit.

Alternative splicing of transposable element introns

Alternative pre-mRNA processing is a mechanism by which multiple transcripts are produced from a single transcription unit. Many

eukaryotic and viral gene transcripts are alternatively processed to yield multiple products[32]; intriguingly, these processing patterns are reminiscent of those described for many transposable element intron alleles.

In the maize *Ds* (*wx-m1*, *wx-m9*), *dSpm* (*bz-m13*, *a2-m1*) and *Drosophila 412* (v^k) and *P* (y^{76d28}) element alleles, various donor and acceptor sites within the transposon or host gene are utilized in diverse combinations. The *Ds1* insertion in the *wx-m1* allele, for example, creates as many as five distinct mRNA transcripts by splicing three alternative donor sites to three different acceptor sites[12]. Exon skipping patterns described for the *waxy* retrotransposon alleles are another example of transposable element induced alternative splicing events.

Aside from splicing, other pre-mRNA processing events are also affected. For instance, inserted elements within introns may contain cleavage/polyadenylation signals which compete with the splicing of host introns, as in the *copia* insertion allele *white-apricot* [w^a] (see Fig. 2b)[23].

Transposable element intron insertions provide a unique mutational mechanism that could lead to the evolution of alternative RNA processing and the diversification of gene products. The maize *wx-m9* allele illustrates how alternative splicing of transposable element introns can result in a single allele encoding multiple protein isoforms. Alternative splicing of the *Ds1* insertion in *wx-m9* produces two electrophoretically distinct Wx proteins[13]. One of these isoforms remains enzymatically active, resulting in the low-level Wx activity displayed by this allele. Use of alternative *Ds1* splice sites, however, results in a presumably inactive Wx isoform that encodes an additional 24 amino acids at the site of the *Ds1* intron insertion.

Table 1. List of transposable element intron alleles

Allele	Element	Size (kb)	Location	Phenotype	Reference
Zea mays					
wx-m1	*Ds1*	0.4	exon 9	null	12
wx-m9	*Ds*	4.4	exon 10	partial	13
wx-B4	*Ds2*	1.5	exon 13	null	20
wx-Stonor	*Stonor*	4.5	intron 6/exon 7[a]	partial	25
wx-G	*G/B5*	5.0	intron 8	partial	25
wx-B5	*G/B5*	6.1	intron 2	partial	25
adh1-2F11	*Ds2*	1.3	exon 4	null	14
adh1-Fm335	*Ds1*	0.4	exon 4	partial	21
adh1-S3034	*Mu1*	1.1	intron 1	partial	24
adh1-S4477	*Mu1*	1.1	intron 1	partial	24
bz-m13	*dSpm*	2.2	exon 2	partial	15
bz-m13CS9	*dSpm*	1.3	exon 2	wild-type	15
bz-m13CS3	*dSpm*	1.8	exon 2	partial	15
bz-m13CS64	*dSpm*	2.2	exon 2	null	19
a2-m1	*dSpm*	2.2	n.a.[b]	partial	16
a2-m1 (II)	*dSpm*	1.3	n.a.[b]	wild-type	16
Drosophila					
v^k	*412*	7.5	exon 1	partial	17
v^{+37}	*412/B104*	11.1	exon 1	partial	22
y^{76d28}	*P*	1.1	exon 1	partial	18
y^{13-11}	*P*	0.4	exon 1	wild-type	18
w^a	*copia*	5.1	intron 2	partial	23
f^1	*gypsy*	7.5	intron 2	partial[c]	27
f^k	*gypsy*	7.5	intron 2	partial[c]	27
Others					
$NF1^{DD}$	*Alu*	0.3	intron 5	null	26
adr	*ETn*	5.5	intron[d]	null	28
T^{Wis}	*ETn*	5.5	intron[d]	null[e]	29

[a] *Stonor* is inserted in the intron/exon junction.
[b] Wild-type *A2* gene is intronless.
[c] The phenotype of the f^1 and f^k alleles is regulated by the *Su(Hw)* gene.
[d] Intron position not reported.
[e] T^{Wis} is a gain-of-function allele.

Transposable element introns and regulatory evolution

Transposable element insertions not only alter gene structure, but can also alter a gene's responsiveness to *trans*-acting modifier loci. These *trans*-acting modifier loci interact with mutant genes, resulting in either the enhancement or suppression of a mutant phenotype. Many of the known modifier loci interact specifically with transposable element induced mutations, leading to suggestions that these systems may be involved in regulatory evolution[4]. Most of the attention in the past focused on the mechanisms by which elements impose novel transcriptional regulatory signals on a host gene. However, recent molecular genetic studies have made it clear that transposable elements can alter gene control at the level of pre-mRNA processing as well as position regulatory signals within intron sequences.

Trans-acting modifier loci may act at the level of transcription termination, as illustrated by the maize *dSpm* alleles *bz-m13* and *a2-m1* and the *gypsy* retrotransposon *forked* alleles f^1 and f^k of *Drosophila*. In both *bz-m13* and *a2-m1*, binding of the *Spm*-encoded TNPA protein to the inserted *dSpm* element blocks transcription of the gene. For the f^1 and f^k alleles, the protein involved in premature transcription termination is encoded by the *suppressor-of-Hairy-wing* gene[27].

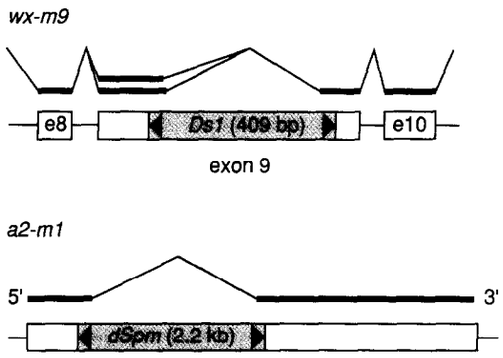

Fig. 1. The splicing of *Ds1* and *dSpm* transposable element insertions from the maize alleles *wx-m9* and *a2-m1*. Open boxes represent exons and fine lines connecting them are introns in the wild-type gene; the *Ds1* and *dSpm* insertions are indicated by the shaded boxes, with the terminal inverted repeats designated as arrows. Transcripts are drawn above these boxes, with heavy horizontal lines indicating exons while connecting diagonal lines indicate spliced introns. For *Ds1* splicing in *wx-m9*, one acceptor site is alternatively spliced to two donor sites within the *Ds1* intron. Both donor and acceptor sites are also located within the inserted *dSpm* in *a2-m1*. The figures are not drawn to scale, and depict only some of the pre-mRNA splicing events which occur[13,16].

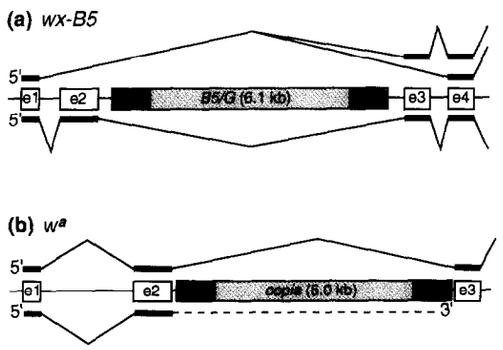

Fig. 2. Alteration of pre-mRNA processing patterns in transposable element insertion alleles. The figures are not drawn to scale, and depict only some of the pre-mRNA splicing events which occur. (a) Alternative splicing in maize *wx-B5*. The shaded boxes indicate the position of the *G/B5* insertion within intron 2 of the *wx-B5* allele; the long terminal repeat (LTR) sequences are depicted as darker boxes at the termini of the retrotransposon. The arrows within the LTRs are inverted repeat sequences. The insertion conditions wild-type splicing as well as exon skipping in this allele[25]. (b) Alternative processing in *Drosophila white-apricot* (w^a). The shaded box indicates the position of *copia* retrotransposon insertion in w^a intron 2. The dashed line in the w^a pre-mRNA depicted below the genomic structure represents transcribed intron 2/*copia* sequence that terminates in the 3' *copia* LTR. In w^a, the *copia* insertion results in either transcription readthrough followed by splicing of intron 2, or premature transcription termination within the element[23].

Host-encoded suppressor genes may also affect pre-mRNA processing control. Several of these genes have been isolated in *Drosophila*. These genes include *suppressor-of-sable*, which modulates the expression of element-induced mutant alleles in the *vermillion, yellow* and *singed* loci[17,33]. The *suppressor-of-white-apricot* and *Enhancer-of-white-apricot* genes have also been identified, both of which regulate processing of the *copia*-containing *white-apricot* allele[34].

Splicing and the population dynamics of transposable elements

Unrestricted transposition of elements within a genome promotes the spread of mobile sequences and could also elevate the incidence of deleterious host mutations. The population dynamics of transposable elements reflect the balance between the fitness of the transposable element and that of the host organism[5,35,36]. Both the element and the host are likely to evolve mechanisms that reduce the negative fitness impacts of transpositional insertions[10,37]. Charlesworth and co-workers, for example, suggest that elements evolve self-regulated transpositional mechanisms to control element copy number within genomes[35].

The splicing of transposable elements from pre-mRNA represents one mechanism by which the deleterious effects of element insertions into exons are minimized. Even before the discovery of transposable element introns, Crick speculated that the splicing machinery may have evolved as a cellular defense against transposons within the genome[9]. It is more probable that transposable elements use the splicing machinery to enhance element survival[10]. Splicing may afford at least some level of phenotypic expression, and partially shield the element from negative selection. In effect, this renders some transposable element insertion mutations neutral or effectively neutral, and permits the element to escape selective surveillance[37]. This element-encoded defense mechanism could delay the extinction of an insertion allele long enough for the element to transpose to another location.

The data on *Ds*, *dSpm*, *P* and *412* insertions demonstrate that splicing of these transposable element insertions does allow for partial to full restoration of gene expression. Of the 16 reported alleles exhibiting splicing of transposable elements from exon sequences, nine display partial function while three show wild-type expression. The number of spliced transposable elements have probably been underestimated, since wild-type insertion alleles will remain invisible in most genetic screens.

There are several lines of evidence which suggest that splice site signals within element termini evolved to permit the removal of transposable element insertions from exons[31]. First, most splice donor and acceptor sites within transposable elements are positioned very close to the ends of the element. Elements are apparently also able to activate host cryptic splice signals in close proximity to the insertion. This ensures that splicing removes most of the insertion sequence from pre-mRNA and increases the likelihood that the mutant gene is able to at least partially function. Second, the capability to be spliced and/or to alter pre-mRNA processing is widely distributed among transposable elements. Even within a heterogenous element family, internal splice signals are apparently conserved. For example, the maize *Ds* elements are grouped into several subfamilies which share little internal sequence similarity. Terminal regions required for transposition, however, are similar between *Ds* elements; the conserved sequences include the splice donor sites necessary for element splicing. The conservation of these splice signal sequences suggest that they are important for *Ds* splicing as well as mobility[12,31].

Both transposition and splicing are characteristics that promote transposable element survival and spread within populations. The importance of splicing in enhancing element fitness and regulating copy number requires further investigation, and should be incorporated into theoretical models of transposable element population dynamics[35]. Since most splice signals of transposable element introns are located within element sequences important for transposition, it would be interesting to discover whether there exists a trade-off between element mobility and intron function. Theoretical and empirical studies can also resolve the question of whether a transposable element with intron-like characteristics can compete effectively within the genome with elements that do not possess the ability to be spliced.

The question of intron origins

Molecular biologists have long been fascinated by the question of intron origins[11,30,38–40]. Molecular evolutionists have debated whether introns were originally present when primordial genes were assembled, or whether they arrived after gene formation. The discovery of mobile group II self-splicing introns in fungal mitochondria provides some foundation for the assertion that introns were inserted late in evolution[41].

The transposable element introns discussed here represent the first examples of contemporary intron insertion events into nuclear genes. The possibility that transposable elements are responsible for intron insertions was previously considered[11]. This view, however, was discarded when it was pointed out that splice signals were absent at the ends of transposable elements; thus, element insertions could not be precisely spliced from pre-mRNA[38]. The maize and *Drosophila* transposable element introns, however, function as introns despite the lack of terminal splice sites. Instead, internal splice sites adjacent to the end, as well as nearby cryptic sites, are used. As noted earlier, however, this imprecise positioning of splice signals does lead to changes in the final transcript sequence.

The numerous examples of transposable element splicing indicate that evolutionarily recent intron insertions, which occurred after the formation of the primordial genes, are quite feasible. Most nuclear introns, however, do not appear to share the structural features of transposable elements. There may be several reasons for the difficulty in identifying transposable elements that have been incorporated into genes as nuclear introns. Only a fraction of nuclear introns may have their origins in a transposable ancestor; most introns may have arisen by quite different evolutionary pathways[42]. It is possible that a few nuclear introns are indeed relic transposons, but that mutations have eroded the nucleotide sequence beyond recognition. The mobility of transposable elements may also have precluded their stable fixation as introns, and their intron-like features are nothing more than a short-term survival mechanism.

Nevertheless, as we continue to isolate and study the structure of various genes, we may be able to catch a transposable element insertion in the act of transforming itself into a nuclear intron. We may already have such an example in *a2-m1* and its derivative, the *a2-m1 (Class II)* allele. The phenotypically wild-type *class II* allele has sustained a deletion within the inserted *dSpm* that increases splicing efficiency while simultaneously rendering the element insertion immobile[16]. This deletion has resulted in the creation of a stable, wild-type intron within the previously intronless maize *A2* gene, and vividly illustrates how a transposable element insertion can evolve into a stable intron.

Conclusion

As we learn more about the biology of transposable elements, we begin to understand the precise molecular mechanisms by which these genomic entities generate genetic variability. The splicing of transposable element insertions points to an intimate connection between mobile sequences and nuclear introns, and suggests that some introns may have had a transposable ancestor. We are also beginning to realize that transposable element insertions condition a wide array of pre-mRNA processing mutations, some of which may be involved in the structural diversification that accompanies gene evolution. More importantly, the ability of mobile elements to interact with the cellular RNA processing apparatus reveals mechanisms by which elements are able to shield themselves from negative phenotypic selection, a trait that may have contributed to their survival within eukaryotic genomes.

Acknowledgements

I wish to thank S.R. Wessler and J.F. McDonald for stimulating discussions, encouragement and support.

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