Perspective

Mobile Introns and Intron-Encoded Proteins

PHILIP S. PERLMAN AND RONALD A. BUTOW*

IT IS GENERALLY BELIEVED THAT SPLIT GENES IN THE NUCLEAR genomes of eukaryotes arose by a process of exon shuffling (*1*). Once formed, the intervening sequences would be free to evolve in the absence of direct selection pressures to forms no longer recognizable (or barely so) from their progenitors. In this perspective, we review and discuss some implications of recent findings of directed insertion of a class of related intron elements. These new data raise the likelihood that intron mobility was another source of split genes.

Some Group I Introns Are Mobile

Recent findings have revealed a new class of mobile genetic elements (*2, 3*). They are group I introns, one of two classes of introns that can self-splice. Group I introns are distinguished from group II introns by characteristic secondary structural features, key elements of which are shown in Fig. 1. Moreover, group I introns splice by a transesterification mechanism first defined for the intron of the large ribosomal (rRNA) gene of *Tetrahymena* nuclear DNA (*4*), whereas group II introns splice by a reaction pathway more closely resembling nuclear pre-mRNA splicing (*5*). A special property of both introns is that they often contain a reading frame capable of encoding a protein.

Unlike most other transpositions, which involve nonhomologous donor and recipient sites, group I intron mobility is site-specific in that it is restricted largely to exchanges between alleles of genes that contain (I^+) or lack (I^-) the intron. This process, previously referred to as unidirectional intron conversion or site-specific intron transposition, is now termed "homing" (*6*). The exchanges are characteristically nonreciprocal and efficient; that is, they are unidirectional gene conversions whereby I^- alleles are depleted from a population of I^- and I^+ DNA molecules as a result of repeated rounds of intron insertion. Typical of such recombinations, sequences flanking the intron are coconverted at frequencies that decrease with increasing distance from the intron borders. A further distinction between group I intron mobility and most "conventional" transpositions is that the recipient DNA sequences that flank the inserted intron are not duplicated. In all cases, intron movement requires the activity of a site-specific double-stranded DNA endonuclease encoded by the intron whose cleavage site specificity is considerably more complex than typical restriction endonucleases.

P. S. Perlman is in the Department of Molecular Genetics, The Ohio State University, Columbus, Ohio 43210, and R. A. Butow is in the Department of Biochemistry, University of Texas Southwestern Medical Center, Dallas, Texas 75235.

*To whom correspondence should be addressed.

Given the observed high frequencies of these directional intron insertions, it is puzzling that the I^- alleles have not simply disappeared. Perhaps the I^+ alleles have appeared only recently, and with time the intron will spread inexorably to other I^- alleles in the population. Another possibility is that there could be some disadvantage, particularly in rapidly growing organisms in which most of these mobile introns were found, that selects against the intron-containing form. A third possibility is that there might be some active mechanism for removing introns once inserted into a gene. Indeed, there is evidence in yeast mitochondria that introns can be cleanly excised from genes at the DNA level (*7*); such events are, however, quite rare relative to endonuclease-mediated intron insertions.

The first mobile intron identified was ω, a 1.1-kb intron found in the large (21*S*) rRNA gene of mitochondrial DNA of some, but not all, strains of *Saccharomyces cerevisiae*. Like the other mobile introns, $\omega^+(I^+)$ is transferred nearly quantitatively to $\omega^-(I^-)$ rRNA genes when strains carrying these different alleles are mated. The basic genetic phenomenology of this process was apparent some 20 years ago (*8*), and the molecular distinction between ω^+ and ω^- alleles was reported in 1980 (*9*). Insight to the mechanism of the process was obtained by the finding that a transient double-strand break appears at the intron homing site in ω^- DNA in zygotes derived from matings between ω^+ and ω^- cells (*10*). Subsequent studies revealed that the expression of an open reading frame (ORF) located within the intron is essential for both the DNA cleavage and the intron conversion (*11*). That intron ORF product has been shown to be a site-specific DNA endonuclease, and its action appears to initiate intron conversion (*12*).

Although the full details of this conversion mechanism have not been worked out, the overall process closely resembles the double-strand break/gap repair pathway of recombination proposed in 1983 by Szostak *et al.* (*13*). Until recently, the only other clear-cut example of a unidirectional gene conversion initiated by a site-specific endonuclease was mating-type switching in the yeast nucleus (*14*), a process that requires the activity of the HO endonuclease, which is encoded by a gene unlinked to the mating-type locus (*15*). More recent studies indicate that other conversion events in the yeast nuclear genome may also be initiated by double-strand breaks at specific sites, although the relevant endonuclease has not been identified (*16*).

There are now five other examples of mobile group I introns, each of which is known to encode an endonuclease needed for its mobility in appropriate crosses. These include introns in the nuclear rRNA gene of *Physarum* (*17*), the *td* and *sunY* genes of phage T4 of *Escherichia coli* (*18*), the cytochrome oxidase subunit I (*coxI*) gene of yeast mitochondrial DNA (*19*), and the large rRNA gene of chloroplast DNA of *Chlamydomonas eugametos* (*20*). In addition, there are eight other sequences (all probably group I introns) in the mitochondrial DNAs of *Aspergillus, Coprinus, Neurospora,* and *Chlamydomonas* that are inherited preferentially in crosses (*21*). From this rapidly growing list of examples spanning such a striking diversity of organisms and cellular compartments, it is likely that many other group I introns will prove to be mobile.

A summary of the overall process of intron homing and a list of homing sites are shown in Fig. 2. Also included are two examples of nonintron sequences involved in evidently similar processes—mating-type switching in yeast (*22*) and non-LTR retrotransposon movements in *Bombyx mori* (*23*)—that depend on the action of site-specific endonucleases. All of the endonucleases responsible for these conversion events differ from the type II restriction enzymes in that their recognition sites are longer (>8 bp) and do not contain any obvious dyad symmetry. It is likely that having such long recognition sites effectively minimizes promiscuous DNA cleavage

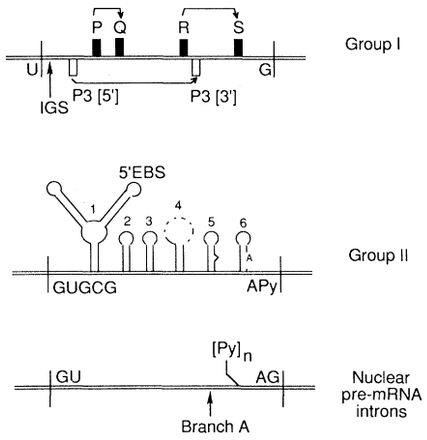

Fig. 1. Distinctions between group I, group II, and nuclear pre-mRNA introns. Group I introns contain an invariant uridine (U) residue at the 3′ end of the upstream exon and an invariant guanosine (G) at the extreme 3′ end of the intron. Four internal sequences (P, Q, R, and S) indicated by filled rectangles above the line in the figure, are highly conserved in sequence and relative position; pairs of these conserved sequences (shown by arrows) basepair to form key structural elements needed for splicing. Other structures are highly conserved, indicated as open rectangles below the line, although their primary sequences are not conserved. The internal guide sequence (IGS) forms another conserved structure by basepairing with the 3′ end of the upstream exon; each IGS is unique in primary sequence because the exons flanking group I introns do not contain more than a single conserved nucleotide noted above. Group II introns somewhat resemble those of nuclear pre-mRNA genes, having the same dinucleotide at the 5′ end of the intron, a conserved dinucleotide (APy instead of AG) at the 3′ end, and a specific adenosine residue involved in branching. Group II introns have a highly conserved secondary structure comprised of a core sequence with six radiating helices that define intron substructures or domains; only the fifth domain is highly conserved in structure and sequence. Domain 1 has the most complex set of substructures, one of which contains the 5′ exon binding site (EBS); domain 4 is the most variable in length, indicated by the dotted line (*44*). Unlike these self-splicing introns, nuclear pre-mRNA introns contain the few conserved nucleotides shown and no specific structures.

activity and helps limit DNA movements to homologous sites.

The two intron-encoded endonucleases characterized in detail to date, the ω and *coxI* intron 4α (aI4α) products of yeast mitochondrial DNA, have complex recognition sites requiring as many as 18 bp in the vicinity of the cleavage site for full activity (*19, 24, 25*). Both cleave DNA by introducing a 4-bp staggered cut, leaving 3′ OH overhangs. In these regards they resemble the endonuclease encoded by the nuclear HO gene of yeast that initiates mating-type switching (*22*). From deletions flanking each cleavage site and point mutations within and near them, it is clear that the recognition sites of these three enzymes are in the vicinity of the cleavage sites. Both intron-encoded enzymes contain short, related peptides, so-called LAGLI-DADG sequences, which are also found in most other group I intron ORFs (*26*).

The *td* and *sunY* intron-encoded endonucleases of T-even bacteriophages lack the LAGLI-DADG motif and share short peptides in common with each other and with a minority of intron ORFs of mitochondrial DNA of filamentous fungi (*27*). A further distinction between these endonucleases and those in yeast is that the bacteriophage intron-encoded enzymes cleave at a distance (up to 25 bp) from the intron insertion site (*28*). Thus, it appears that at least two families of intron-encoded endonucleases exist. Remarkably, however, a comparison of the homing sites for the *td* and aI4α introns shows a 9 out of 14 nucleotide identity (Fig. 2) (*28*).

The process of group I intron mobility described thus far is highly efficient. Woodson and Cech (*29*) showed that intron movement can also occur at the RNA level in vitro by reversal of the self-splicing reaction. They found that the excised *Tetrahymena* intron RNA can reinsert precisely into its original site in the ligated exons. They further observed that intron insertion can occur into an unrelated RNA if that RNA contains a short sequence complementary to the internal guide sequence. Intron transpositions could thus be achieved by reverse transcription of the RNA product containing the inserted intron, followed by recombination to form a genomic copy of the intron. These observations show a potential for intron mobility that would depend on an intron-encoded endonuclease. The overall process would be much less efficient, however, than the endonuclease-dependent intron homing described here, but could nevertheless be significant on an evolutionary time scale.

Multifunctional Intron-Encoded Proteins

More than 43 group I intron sequences have been published (*30*). Most of these introns contain an ORF capable of encoding a protein. As shown in Fig. 3, three different modes of expressing group I intron ORFs are known. The most typical arrangement, exemplified by aI4α of yeast mitochondrial DNA, is where the intron ORF is in-frame with the preceding exon. In such cases the mRNA for the intron-encoded protein is the RNA species not yet spliced for that intron. A significant minority of intron ORFs are free-standing, that is, contain both translational initiation and termination signals. For some of these intron ORFs (for example, ω, Fig. 3) it appears that the mRNA for the intron-encoded protein is either the excised intron or an alternatively processed form of the precursor (unspliced) RNA. Other free-standing intron ORFs, such as those in bacteriophage T4, are expressed from promoters internal to the intron (*31*).

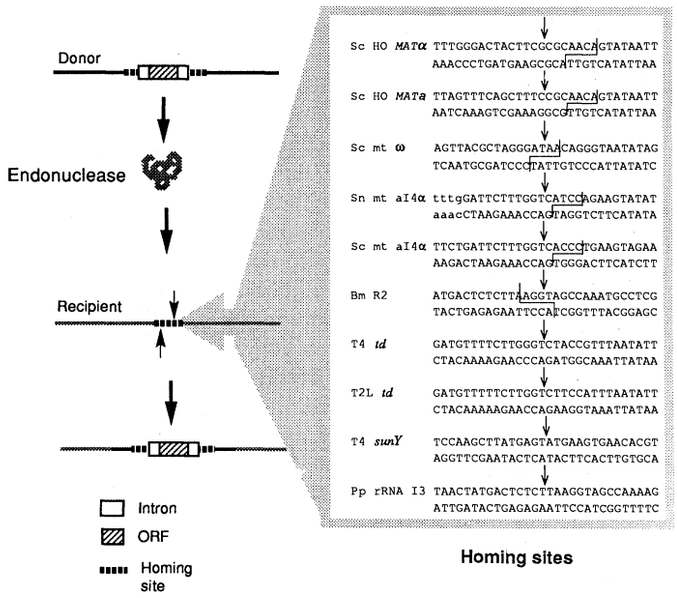

Fig. 2. Intron homing mediated by intron-encoded, site-specific endonucleases. The diagram shows a generalized version of intron conversion in which a site-specific endonuclease encoded by a reading frame within an intron of a donor molecule introduces a staggered double-strand break, indicated by the small arrows on the left-hand side of the figure, at or near the homing site of an I^- recipient allele. The homing site in the I^+ allele is interrupted by the intron, and is thus not a substrate for cleavage. The process of conversion also leads to coconversion of sequences flanking the intron insertion site in the recipient molecule. The box at the right gives the sequences in the vicinity of the intron insertion site (downward arrow) and DNA cleavage sites (lines), where known. The intron homing sites are given for the ω intron in the large rRNA gene in mitochondria (mt) of *Saccharomyces cerevisiae* (Sc), the aI4α intron in Sc and *S. norbensis* (Sn), the *td* and *sunY* introns of T-even phage, and the I3 intron in the nuclear rRNA gene of *Physarum* (Pp). Shown for comparison are the yeast nuclear MATa and MATα insertion and HO endonuclease cleavage sites and the R2 non–long terminal repeat retrotransposon insertion site in the nuclear rRNA gene of *B. mori* (Bm).

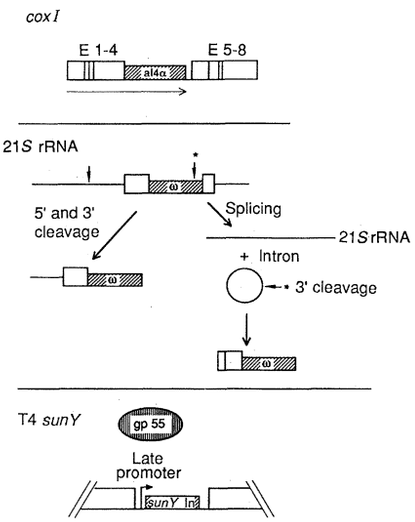

Fig. 3. Different ways of expressing intron reading frames. Most group I and group II intron ORFs are extensions of the upstream exon. For *coxI* intron 4α, shown here, and probably most others of this type, the mRNA for the intron-encoded protein is the precursor spliced for all upstream introns; the primary translation product, indicated by the horizontal arrow, is a chimeric protein, partly encoded by the exons and partly by the intron. The endonuclease activity of aI4α is probably a proteolytic product of that precursor, containing residues completely encoded by the intron (*25*). The pathway of processing of the ω^+ form of the 21*S* rRNA gene has been adapted from Zhu *et al.* (*48*). Although both 5′ exon and 3′ intron processing events are known to occur, in addition to intron excision, the actual mRNA species for the ω intron-encoded endonuclease is not known. Processing at a conserved 12 nucleotide sequence is indicated by the arrows with the asterisk. The free-standing ORF in the intron of the coliphage T4 *sunY* gene is translated from a discrete transcript initiated at a late promoter within the intron (and regulated by gp55) (*37*).

Previous genetic studies of yeast mitochondrial introns established that three group I intron ORFs encode essential splicing functions, so-called maturases (*32*). Two maturases are intron-specific, participating in splicing only the intron that encodes it; the third, encoded by cytochrome b intron 4(bI4), is required for splicing both bI4 and aI4α (*32*). In other words, although in wild-type cells aI4α encodes a potent endonuclease required for intron mobility, that protein is not involved in splicing. [The bI4-encoded maturase does not appear to have any endonuclease activity, even though it is about 70% identical to the aI4α-encoded protein (*34*); also, the ω endonuclease is not required for splicing (*35*).] Under two different in vivo conditions, however, a latent maturase activity of the aI4α-encoded protein is activated, allowing it to substitute for the bI4 maturase (*36*). The aI4α product is thus the first example of a bifunctional intron-encoded protein: it has a potent DNA cleaving activity and a latent RNA splicing activity. Both of these functions could enhance the survival of the intron: the maturase activity ensures that the presence of the intron can be tolerated by the organism, and the endonuclease activity helps ensure the persistence of the intron in populations by making it infectious. Because identifying maturase functions is currently limited to the few organisms that permit genetic studies of group I introns, it is unknown whether the other group I intron-encoded endonucleases have maturase function.

The possibility that other intron-encoded proteins may be multifunctional is hinted at by data on group II introns of yeast mitochondrial DNA. Like group I introns, some, and quite possibly all, group II introns may also depend on proteins for splicing in vivo (*37*). Although only a minority of the group II introns have ORFs, almost all of these reading frames encode proteins with significant primary sequence conservation. Group II intron-encoded proteins are larger than those of group I, and nearly all of them have regions of homology to reverse transcriptase scattered over a roughly 250-amino acid stretch in the middle of each intron ORF (*38, 39*). At their extreme COOH-terminal regions, those proteins have another lengthy conserved amino acid sequence that contain two zinc finger motifs (*40*). Although direct genetic or biochemical evidence for reverse transcriptase activity associated with group II intron-encoded proteins is lacking, two of these proteins in yeast mitochondrial DNA, the products of introns 1 and 2 of the *coxI* gene, are maturases (*37*). Thus group II–encoded proteins have the potential for being multifunctional and may also fit the suggestion that the presence of possibly unrelated biochemical activities of these intron-encoded proteins—maturase, endonuclease, and reverse transcriptase—would offer distinct and immediate advantages for the survival of the DNA sequences that encode them.

Synergism Among Introns?

It is possible that intron insertions and deletions are processes that together lead to the persistence of some group I and II introns within groups of relatively closely related organisms. Could that interaction benefit the organism? One possibility is that because of intron conversions, coconversion of flanking exon sequences could help maintain exon sequence homogenity, and that would be of considerable benefit to a multicopy genetic system. Intron excision could arise by recombinational replacement of I^+ alleles from reverse-transcribed cDNAs of processed RNAs. If the reverse transcriptase activity is supplied by group II introns, the interplay between group I and group II introns would be beneficial by providing, through repeated cycles of intron insertions and deletions, a mechanism to maintain flanking sequence homogeneity. In this connection, conversion processes have been suggested to play an important role in homogenization of sequences in multigene families (*41*).

Independent Mobility of Intron and Intron-Like Reading Frames

Features previously thought to be uniquely associated with group I or group II introns have recently been found in new combinations with other DNA sequences. For example, some *Neurospora* species harbor mitochondrial plasmids that closely resemble group I introns (*42*), yet encode a protein with reverse transcriptase activity (*43*). A similar protein, encoded by a non-LTR retrotransposon in *Bombyx*, is related to group II intron-encoded proteins (*39*). Mobility of that retroelement appears to be site-specific, and it encodes an endonuclease that is probably involved in the homing site selection (within the *Bombyx* nuclear large rRNA gene) (*23*).

It is likely that the intron reading frames are or were mobile elements independent of their intron host. In both intron groups most (and in some cases all) of the ORF is sequestered in a single intron substructure (*44*). These findings suggest that various regions of group I and group II introns could act as sinks for a variety of additional sequences without any effect on splicing. The acquisition of sequence information that confers mobility or assistance in splicing functions would provide distinct advantages for intron survival. However, other sequences irrelevant to either intron survival or to functions directly related to the product of the gene harboring the particular intron could be acquired as well. Perhaps the intron within the large rRNA gene of *Neurospora* mitochondria, which encodes a protein associated with the small mitochondrial ribosomal subunit, is such an example (*45*). Different ORFs can reside within the same intron among different isolates of the same species, as is the case for an intron in the ND1 gene of *N. crassa* mitochondria (*46*). These examples support the concept that some introns can accept a variety of sequences as long as splicing functions are not impaired.

Concluding Remarks

Studies on a new class of mobile genetic elements, the group I introns, and other DNA sequences associated with unidirectional gene conversion events, such as mating-type switching in yeast and retroelements in insects that lack the long terminal repeat have also uncovered an unusual class of site-specific DNA endonucleases. These enzymes display recognition sites that appear to be more complex than most restriction endonucleases. Besides the intrinsic biological interest in understanding how these endonucleases work, there are some immediate considerations for the utility of these enzymes. Recent interest in genomic mapping and sequencing has generated a pressing need for enzymes with relatively long recognition sites. The restriction enzyme Not I (8-bp site) has been valuable for such studies; however, its sites contain only G-C base pairs and, in the human genome, they tend to be highly clustered, so that genomic regions of more typical base composition are not cleaved. It will be important to evaluate whether the endonucleases described here (plus the less well-characterized enzymes encoded by other group I introns) can become useful reagents in the mapping enterprise. Even if they prove to recognize long sequences (more than 12 bp) that may be present as infrequently as once per 10,000 kb, they will still be useful. For example, their cleavage sites can be included in a variety of vectors used for random integration in transformation experiments, and their precise integration sites can be defined in double digests with an enzyme such as Not I by using pulsed-field electrophoresis or related methods. Given the recent successes of directed integration of DNA sequences into animal cell genomes (*47*), it is also reasonable to consider site-directed introduction of cleavage sites at appropriate regions of chromosomes, further enhancing the potential utility of these novel endonucleases.

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