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# Transposition: **Mobile introns get into line**

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**Group II introns encode highly structured, frequently self-splicing RNAs; they are also mobile genetic elements. This mobility has been found to involve DNA-primed reverse transcription, with similarities to retrotransposition and telomere maintenance.**

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The two main types of organellar intron — the group I and group II introns — are both widespread in nature, and encode RNAs that are highly structured and, in many cases, capable of self-splicing. The catalytic versatility of group I and group II introns has done much to extend our perceptions of both RNA chemistry and evolution. Remarkably, both group I and group II introns are able to spread to new locations by acting as mobile genetic elements. Evidence for intron mobility was for a long time circumstantial. A haphazard phylogenetic distribution, irregular occurrence in the genes they interrupt and trans-kingdom sequence similarities were taken as indirect signs of movement. These in turn fuelled speculations that the introns might even be able to undergo 'horizontal' transfer from one species to another. Clarification has come gradually; different (and interesting) principles have been found to be involved, depending on both intron type and whether the element is moving to a new site (transposition), or merely inserting itself into the cognate site of a gene lacking it (homing).

The most recent instalment in the story of intron mobility is reported in two recent papers by Lambowitz and colleagues [1,2]. These authors have developed an elegant *in vitro* system, in which the homing reaction of group II introns can be reconstituted. They have used this system to detect intermediates in, and products of, intron homing, and found that movement is initiated by site-specific, double-strand cleavage of target DNA, followed by primed reverse transcription of an intron-containing precursor RNA. These reactions result in co-transfer of intron and flanking sequences, by a mechanism strongly resembling that used by LINE1-like, non-LTR (long-terminal repeat) retrotransposons, and by telomerases in the reverse transcription of template RNAs carrying telomeric repeats.

All the activities required for group II intron mobility reside within a ribonucleoprotein (RNP) particle consisting of intron RNA and an intron-encoded protein, which combines DNA endonuclease, reverse transcriptase and RNA maturase activities. Remarkably, DNA cleavage occurs by two distinct mechanisms. The sense strand of the target site is cut by the intron RNA itself, in a partial reversal of a splicing reaction that uses DNA as substrate. The antisense strand is cut by the intron-encoded endonuclease, using intron RNA as cofactor. These findings make it clear that direct integration of intron RNAs into DNA is likely to be more prevalent than previously thought, and they raise interesting questions about the evolutionary origins of both LINE1-like retrotransposons and telomerases. To appreciate fully the significance of this work and its implications, a little background on group I and group II introns, and the proteins they encode, is in order.

As they occur in the mitochondrial DNA of the budding yeast, *Saccharomyces cerevisiae*, most group I introns encode a protein required for either RNA splicing or intron homing. The former are so-called RNA maturases; the latter are DNA endonucleases, highly specific for a homing site in the intronless allele of the cognate gene. Double-strand cleavage at this site by such an endonuclease is the trigger for a break–repair process that results in intron insertion and co-conversion of intron-flanking markers with almost 100 % efficiency. Interestingly, *S. cerevisiae* mitochondrial maturases and endonucleases belong to the same family of proteins, characterized by a conserved, 115-residue region flanked by repeats of the motif LAGLI–DADG. Endonucleases characterized by this LAGLI–DADG motif are not confined to yeast mitochondria; they are also encoded by (mobile) group I introns in algal chloroplasts and mitochondria, protist nuclei and archaebacterial and bacteriophage genes. In fact, the widespread distribution of sequences encoding these proteins in different intron contexts has led to suggestions that they are themselves mobile elements capable of spreading to new intron 'hosts' [3].

Group II introns are found in the genomes of mitochondria, chloroplasts, cyanobacteria and proteobacteria. They have a distinctive structure, and some members of the group are capable of self-splicing, in a reaction that displays mechanistic similarities to that catalyzed by spliceosomes in eukaryotic cell nuclei. These similarities have prompted speculation that group II introns are the evolutionary precursors of both spliceosomes and the introns they excise. Perhaps the best characterized (and most mobile) members of the group are introns 1 and 2 (aI1 and aI2) of the yeast mitochondrial gene for cytochrome c oxidase subunit I. Each of these introns encodes a protein

and previous genetic work has shown that, despite the introns' ability to self-splice *in vitro*, their excision *in vivo* is dependent on an activity provided by the intron-encoded protein. The encoded protein was designated as an RNA maturase, by analogy with group I introns which show a similar requirement.

Remarkably, however, these group II intron proteins show little resemblance to those encoded by group I introns, but in several cases they exhibit significant sequence similarity to reverse transcriptases, particularly those encoded by LINE1-like, non-LTR retrotransposons [4]. This sequence similarity extends across the seven conserved sequence domains present in all functional reverse transcriptases [5]. The similarity has been gratifyingly confirmed by a biochemical demonstration of reverse transcriptase activity that is dependent on the presence of a functional group II intron [6]. Additionally, the group II intron proteins display weak sequence similarity to a retroviral protease domain, and contain a zinc-finger-like motif close to their carboxyl terminus [7]. Part of this latter element contains two conserved sequences that are characteristic of a family of DNA endonucleases [8,9]. Between the reverse transcriptase and the zinc-finger-like motif lies a region which is conserved in all group II proteins, and which in some cases is the only recognizable motif present. The conserved nature of this region, and the finding of missense mutations of it that prevent splicing, have led to the suggestion that it has an essential function in RNA maturase activity [10].

Yeast introns aI1 and aI2 are capable of both transposition and homing. Transposition of group II introns in both yeast and *Podospora anserina* mitochondria proceeds by a mechanism that requires a splicing-proficient intron, and involves reversal of the splicing reaction. An RNA possessing sequences resembling an authentic exon–exon junction is 'invaded' by the intron; invasion is then followed by reverse transcription. Subsequent recombinative integration of the resulting copy (c)DNA into the genome produces a second, ectopic copy of the intron [11,12].

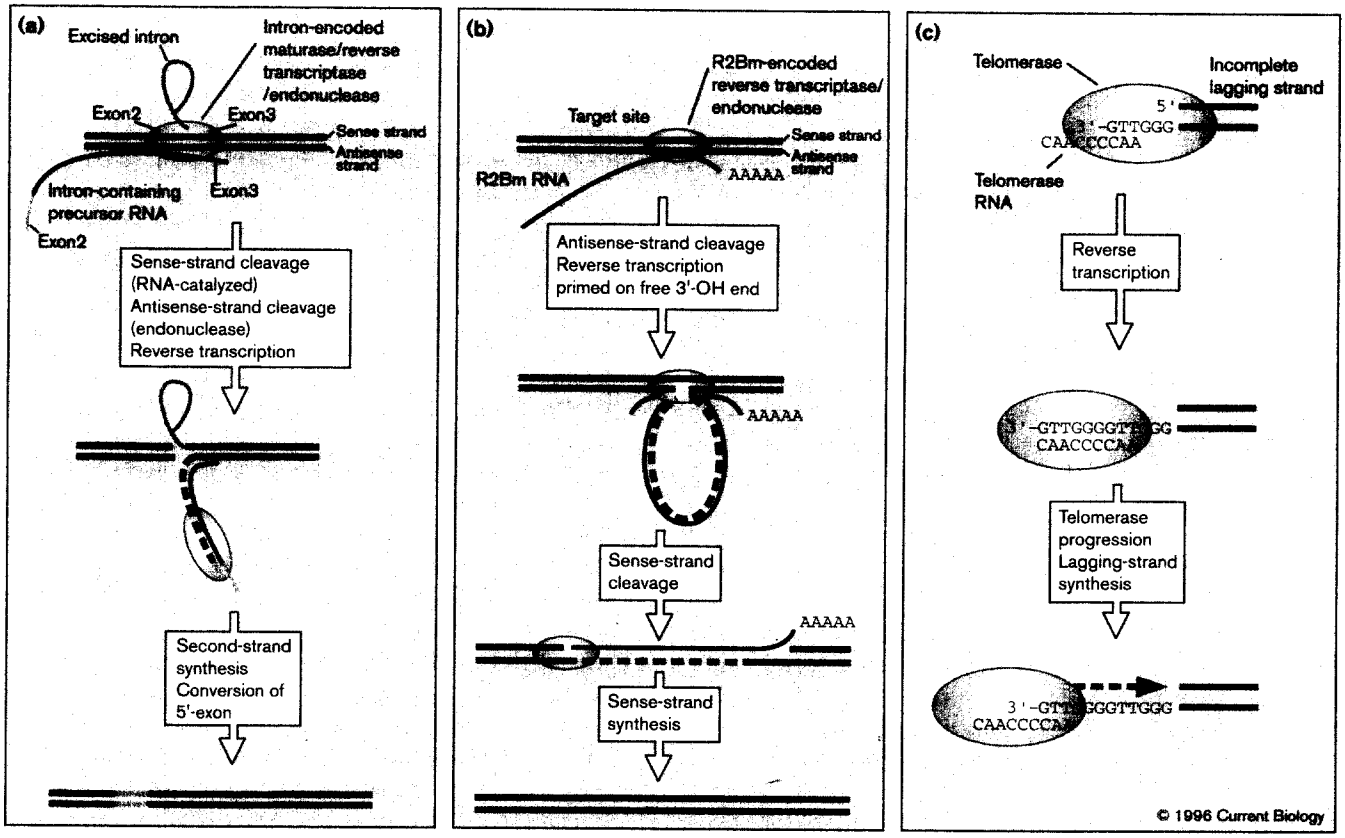
As discussed in an earlier dispatch [13], it seems unlikely that homing of group II introns occurs by a DNA-endonuclease-based mechanism like that used by group I introns. Homing of group I introns is insensitive to *cis*-acting mutations that block splicing [14], whereas group II intron homing requires splicing proficiency [15]. Homing of group I introns is accompanied by bidirectional co-conversion of sequences in flanking exons [16]. In contrast, homing of group II introns is accompanied by a unidirectional co-conversion, which proceeds in a 3′-to-5′ direction towards the upstream exon [17]. Both features strongly suggest that group II homing is an RNA-mediated process, but do not make clear what principles are involved. In particular, a simple model assuming recombination between intronless DNA and a cDNA derived from reverse transcription of an unspliced RNA accounts neither for the dependence of the process on splicing proficiency, nor for the ability of sequence changes in the recipient allele to impede intron transfer in interspecific crosses. The latter suggests involvement of a site-specific DNA endonuclease.

In the first of the two new papers, Zimmerley *et al.* [1] show that RNP particles isolated from mitochondria of *S. cerevisiae* intron donor strains are capable of aI2 reverse transcription *in vitro*, in a reaction that is specifically dependent on a plasmid containing an intact homing site, a splicing-proficient intron and the intron-encoded protein with functional reverse transcriptase and zinc-finger domains. The intron cDNA is primed from a site in the antisense strand, eleven nucleotides downstream of the exon 2–exon 3 junction in the cytochrome oxidase subunit I gene. Priming is preceded by a staggered double-strand cut at this site in the antisense strand and precisely at the exon–exon junction in the sense strand. Homing is thus proposed to proceed according to the model shown in Figure 1a, whereby the 3′ end of the antisense strand cleaved in exon 3 is used to prime reverse transcription of intron sequences in the donor allele. cDNA synthesis is presumed to extend into the upstream exon, thus accounting for subsequent conversion of recipient sequences.

Despite differences in detail, this mechanism shows a remarkable resemblance to that proposed for non-LTR retrotransposons (Fig. 1b; [18]). These retrotransposons, too, achieve mobility through combined reverse transcriptase and DNA endonuclease activities provided by a protein encoded by the elements themselves. There are also clear mechanistic similarities between these two reactions and that catalyzed by telomerases, the specialized reverse transcriptases that add short DNA repeats to the 3′ ends of chromosomes (Fig. 1c). From the presence in the genomes of bacteria and eukaryotic organelles of reverse-transcriptase-encoding group II introns that are likely to have evolved from endosymbiotic bacteria, Zimmerley *et al.* [1] make a strong case for view that group II introns may in fact be progenitors of non-LTR retrotransposons, and that present-day telomerases are descendants of the intronic reverse transcriptases.

Exciting as these findings are, the twist in the tale is provided by the exploration in the second new paper [2] of issues not fully resolved in the first, namely the mechanism of target-DNA cleavage and the dependence of this process on RNA. In the case of the LINE1-like retrotransposon R2Bm, cleavage of the sense strand occurs only after reverse transcription, primed by the antisense strand, has been completed. This is not the case for intron aI2 mobility, where cDNA synthesis occurs predominantly at a double-strand break, and time-course experiments

**Figure 1**




**(a)** Homing of the aI2 group II intron is mediated by an RNP particle containing intron RNA and the intron-encoded protein. The exon–exon junction at the target site is cut in the sense strand by intron RNA, in a partial reversal of the splicing reaction. Cutting of the antisense strand in the downstream exon, and subsequent reverse transcription, is catalyzed by the intron protein using excised intron RNA and/or intron-containing precursor RNA as a template. Extension of reverse transcription into the upstream exon results in a cDNA which can direct both intron insertion and co-conversion of 5′ flanking sequence into DNA. **(b)** DNA-primed reverse transcription, carried out by a combined endonuclease/reverse transcriptase, is also a feature of the transposition mechanism used by non-LTR retrotransposons, such as R2Bm in *Drosophila*. **(c)** The action of the reverse transcriptases encoded by both aI2 and R2Bm is reminiscent of the way in which telomerases use DNA-primed reverse transcription to add short repeats to the 3′ ends of eukaryotic chromosomes. An intriguing possibility is that present day telomerases are descendents of a reverse transcriptase originally associated with a mobile group II intron.

indicate that sense-strand cleavage even precedes that of the antisense strand! Despite this difference, both systems have in common the fact that DNA cutting depends on the presence of RNA. For the R2Bm retrotransposon, this dependence seems to be limited to the sense strand; for the aI2 intron, RNA is clearly required for sense-strand cleavage, and may also be required for antisense-strand cleavage.

As mentioned above, involvement of RNA in intron mobility had already been suggested by the immobility of splicing-defective mutant RNAs. That this involvement is at the level of target-site cutting is strongly implied by three additional observations. First, yeast mutants lacking an intact aI2 domain V, which is required for ribozyme activity of aI2-derived RNA, express an active reverse transcriptase and continue to synthesize aI2 cDNA, but are completely deficient in aI2 mobility [19]. Second, the endonuclease activity of yeast mitochondrial RNP particles is highly sensitive to treatment with ribonuclease A. Finally, the endonuclease activity is dependent on an intact domain V in intron RNA, as well as on the zinc-finger domain in the intron-encoded protein.

Now, closer examination of events at each of the cuts shows that cleavage of the sense strand at the exon–exon junction is accompanied by covalent attachment of RNA to DNA, in a reaction which closely resembles the first step in ribozyme-catalyzed reverse splicing. That a group II ribozyme is capable of cleaving single-stranded DNA has been known for some time [20]. The novel aspect here is the cleavage of double-stranded DNA, a reaction which Zimmerley *et al.* [2] show to be dependent on two separate domains in the intron-encoded protein: the maturase and zinc-finger domains. Based on the lack of reaction with DNA in the absence of protein, and clear differences in

ionic requirement of reactions with RNA and DNA, the authors suggest that the aI2-encoded protein may serve both to stabilize a catalytically active RNA structure and to modify its substrate specificity.

RNA is also required for antisense-strand cleavage, but as yet it is not clear whether this requirement stems merely from a need for prior cleavage of the sense strand by RNA, or, more interestingly, from a role for the RNA as a cofactor for the intron-encoded DNA endonuclease. A role as catalytic component of the endonuclease seems unlikely, as antisense-strand cleavage occurs ten nucleotides downstream of the sense-strand cut, at a site lacking resemblance to any other substrate for group II ribozyme activity. Moreover, the cleavage products do not contain covalently bound RNA. Studies with mutants constructed by site-directed mutagenesis and mitochondrial transformation do make clear, however, that the nuclease-like motifs in the zinc-finger domain of the intron-encoded endonuclease are necessary for antisense-strand cleavage and, furthermore, that the motifs most important for this activity are located in the strongly conserved carboxy-terminal region of this domain.

Where do these findings leave us? As pointed out by Zimmerley *et al.* [2], the ability of intron RNA to insert directly into DNA is relevant to both intron homing and transposition. Different and interesting scenarios can be envisaged, dependent on the site attacked and whether initial attack in the sense strand is followed by completion of reverse splicing followed by reverse transcription of the integrated RNA, or partial reverse splicing followed by reverse transcription on an intron-containing precursor RNA. Finally, given the possibility that group II protein-coding sequences, like their group I counterparts, may themselves represent mobile genetic elements that were acquired in evolution by pre-existing self-splicing introns [3], the extent of cooperation observed between present-day introns, or derived retrotransposons, and their associated proteins in promoting each other's dispersal is remarkable indeed.

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