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Invasive introns

The evidence that introns in fungal and plant organellar DNAs are mobile genetic elements has strengthened significantly; like Shaw's cockney heroine, Eliza Doolittle, they cannot help but betray their origins.

Ever since their discovery back at the beginning of the 1980s, evidence that the introns in genes carried by fungal and plant organellar chromosome are mobile genetic elements has grown steadily. Suspicions of mobility were aroused by the observation that the many introns in fungal mitochondrial (mt) DNAs were related in sequence [1]. These similarities pointed to the existence of intron families, the members of which appeared to have arisen from common ancestors by a process of duplication and dispersal. Genetic flux was also suggested by the wide phylogenetic distribution of the various intron families, the variable presence of introns at specific sites in different members of the same species and irregularities in the introns' distributions among different organisms [2].

Since then, other circumstantial evidence for intron mobility has accumulated. In yeast mtDNA, 'clean excision' of one or more introns is a relatively frequent event [3]. In *Euglena*, the existence of 'twintrons' — elements consisting of two introns, one of which seems to have inserted itself 'piggyback style' into the other — has been attributed to intron mobility [4]. In *Podospira*, the phenomenon of senescence is associated with the massive accumulation of a closed circular DNA, senDNA, which turns out to be an excised group II intron [5]. Suggestive though these observations are, they do not constitute proof of mobility. Ideally, one would like to catch the introns on the move to a new location, and to understand the mechanisms responsible for movement. Work carried out by several groups in the past three years, culminating in the appearance of two papers in a recent issue of *Nature* [6,7], has brought us closer to achieving both goals.

Some history

Before describing these recent findings, it is perhaps useful briefly to review salient features of organellar introns. There are two main organellar intron families, groups I and II, defined on the basis of similarity in sequence, structure and mechanism of (self) splicing [8]. Group I introns are also found in the nuclear DNAs of lower eukaryotes, and in bacteriophage and eubacterial genomes. Many group I introns possess self-splicing activity, which proceeds via coupled transesterification reactions that are triggered by binding of a guanine nucleotide in the neighbourhood of the catalytic site. Consistent with thermodynamic considerations, the splicing reaction is reversible, and the reverse reaction has been demonstrated *in vitro*. Many organellar and bacteriophage group I introns contain an open reading frame. This may encode a protein required for RNA

splicing (a maturase) or, more frequently, a sequence-specific endonuclease that acts in intron spreading via a recombinative process known as homing (see below) [2]. The most common group of endonucleases and maturases shares the decapeptide sequence motif LAGLI-DADG (using the single-letter amino-acid code and – for 'any amino acid').

Group II introns have been found in fungal and plant mitochondria, chloroplasts and, more recently, in cyanobacteria and proteobacteria [9]. They also share a distinctive structure, distinct from that of group I introns. Some members of the group are capable of self-splicing, in a reaction that displays mechanistic similarities to that catalyzed by spliceosomes in the cell nucleus. Just as with group I introns, splicing is reversible and, given appropriate conditions, can be demonstrated *in vitro*. Interestingly, a group II intron can undergo a reverse splicing reaction with a foreign RNA species, provided that this RNA contains a short sequence motif, known as exon-binding site 1 (EBS1), which is complementary to the intronic sequence UCUGUC [10]. During the forward reaction, EBS1 base-pairs with a motif of the 5' exon that is known as intron-binding site 1 (IBS1).

When group II introns contain reading frames, they also encode maturases involved in RNA splicing. These proteins have no resemblance to those encoded by the reading frames of group I introns, but they do exhibit significant sequence similarity to reverse transcriptases, in particular to those encoded by the *LINE1*-like, or non-LTR (long terminal repeat), class of retroviral elements. The sequence similarity extends across the seven conserved sequence domains present in all functional reverse transcriptases. In yeast mitochondria, reverse-transcriptase-like proteins are encoded by the group II introns a11 and a12, located within the gene for subunit I of cytochrome c oxidase, and reverse transcriptase activity that is dependent on the presence of a functional copy of one or other of these introns has recently been demonstrated biochemically [11].

Group I intron homing

It is now clear that intron movement occurs by different mechanisms, depending on intron type and on whether movement is from an interrupted gene to the equivalent, cognate site within an intronless allele of that gene ('homing'), or to a new location (intron transposition). For the group I introns, it is ironic that the first experimental evidence for homing of a yeast mitochondrial intron came from genetic analysis in 1971,

long before the existence of the intron itself had been recognized [2]. It was noted that certain genetic markers, which have since been mapped to the organellar large ribosomal (r) RNA gene, displayed non-reciprocal recombination and highly biased transmission, with the alleles of one parent being over-represented in the progeny at the expense of the other.

The explanation for this behaviour became clear with the discovery of an optional intron in the rRNA gene of parents displaying high transmission. The analysis of mtDNA in progeny arising from such crosses showed that, in all cases, high transmission of parental markers was associated with a gene conversion event that resulted in acquisition of the intron by the other, initially intronless, parental allele. Gain of the intron was accompanied by extensive co-conversion of flanking sequences. The trigger for intron transfer turned out to be a double-strand break at the exon-exon junction of the intronless gene, produced by the action of the intron-encoded endonuclease, a member of the IAGLI-DADG family mentioned above. In the meantime, several other examples of intron homing have come to light. In each case, sequence-specific cleavage by an intron-encoded endonuclease results in spread of the intron to the corresponding site in an intronless gene [12].

Although group II introns also exhibit homing behaviour, in some cases with efficiencies approaching 100% [13], the mechanism described above is unlikely to apply, because these introns lack sequences encoding an endonuclease. In addition, the mechanism fails to account for efficient movement of either type of intron to new locations as, depending on the endonuclease, the target sequence cleaved can be anything from 14 to 40 base pairs (bp) long and the number of potential new sites is thus probably extremely limited. An effective means for dispersal of both types of intron is, however, offered by a mechanism involving reversal of the splicing reaction, with insertion of intron sequences at either cognate or new sites followed by reverse transcription and recombinative integration of the resulting cDNA into the genome (Fig. 1). This possibility has now been directly confirmed by Mueller *et al.* [6] and Sellem *et al.* [7].

Reverse splicing

The work of Mueller *et al.* [6] provides elegant proof that a yeast mitochondrial group II intron can invade a new site, provided that the intron is capable of splicing and the target site is equipped with intron-binding sequence motifs. These authors noted that a certain class of site-specific deletions in yeast mtDNA often involve splice-site sequences of the group II intron a11. These deletions extend downstream from the authentic 3'-splice-sites to locations preceded by intron-binding sequences similar to those present in the 5' exon A1. Perhaps coincidentally, three sites examined turned out to lie in group I introns. Reasoning that such deletions might be the result of recombination between two copies of the a11 intron, one of which had been

Reversal of the normal splicing reaction. An intron lariat can react with an RNA molecule containing sequences resembling the intron-binding site (IBS1), normally present at an authentic splice junction, leading to insertion of a new intron copy at an ectopic site.

Reverse transcription, followed by integrative recombination, introduces the new intron copy into the genome

Recombination across intron repeats eliminates sequences lying between the two copies

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Fig. 1. A mechanism for movement of a group II intron to a new site, triggering a genomic rearrangement. Evidence in support of such a mechanism has recently been reported [6,7].

generated by intron invasion at a new site, the authors set out to detect transposition intermediates, using the polymerase chain reaction (PCR) in combination with the appropriate primers.

The results were clear-cut: the junction fragments expected if intron a11 had inserted at all three sites were present, and molecules were found that contained the intron at both donor and recipient sites. Strikingly, sequence analysis of the junction fragments revealed that transposition is conservative with respect to sequences flanking the integration site (an important difference with respect to homing of group I introns). Finally, transposition was found to be dependent on splicing. *Cis*-acting mutations that interfere with intron folding, and *trans*-acting mutations that block maturase function, both prevent transposition. Interestingly, Meunier *et al.* [13] reported some years ago that the same mutations block homing of the same intron, thus suggesting that group II homing and transposition involve a common mechanism.

Sellem *et al.* [7] reached similar conclusions starting from quite a different angle. Working with the filamentous fungus *Podospora anserina*, these authors questioned whether the instability and rearrangements of mtDNA that are associated with the degenerative syndromes of senescence and premature death in this species could in fact be related to intron mobility. *P. anserina* senescence is accompanied by extra-chromosomal amplification, as 'senDNA', of the group II intron- α , the first intron in the gene, *cox1*, that encodes subunit I of cytochrome c oxidase [5]. The premature death syndrome, under control of two nuclear genes, is characterized by a site-specific deletion extending from the 5'-end of intron- α to an upstream site located between genes for isoleucine and serine tRNAs, some 38000 base pairs away [14]. PCR analysis gave results completely in accordance with a scenario of intron transposition to a new site, followed by recombination between the repeated introns. As in the case of the yeast mitochondrial intron studied by Mueller *et al.* [6], the new site was found to be preceded by an intron-binding sequence, and ectopic integration was not observed in a mutant strain containing a truncated, splicing-defective version of intron- α .

Where do these findings leave us? In both organisms, puzzles remain. First of all, there is the question of what drives reverse splicing. *In vitro*, a combination of the high concentration of intron 'lariat' splicing intermediates with circumstances that slow the forward reaction will yield small amounts of intron-containing RNAs by reverse splicing. These conditions may be approached in *Podospora*, in which high concentrations of both excised intron and transcripts of the targeted tRNA region are present. They are unlikely to hold for yeast, however, in which the insertion sites lie in group I introns, which are present only in partially spliced precursor RNAs. As a possible solution to this paradox, Mueller *et al.* [6] suggest that insertion may also occur at DNA replication forks, into either RNA primers

or single-stranded DNA — for both groups of introns, the first step in splicing reversal has been observed with a DNA substrate containing the splice-junction sequence [15,16]).

Then there is the process of reverse transcription. Sequence analysis carried out by both groups revealed invasion by a complete intron, implying that reverse transcription was initiated downstream of the insertion site, in regions lacking any obvious similarity to the downstream exons. This would seem remarkably promiscuous behaviour, even for an enzyme that may have a wider than normal priming specificity [11]. Finally, the insertions described here have led to massive rearrangements of the genome. What is required to stabilize the intron at a new site, allowing its independent evolution in the absence of such deletions?

On the bright side, however, the findings do provide us with satisfying proof that group II introns really do move, and they show us how they do it. Importantly, the mechanism is a general one, applicable equally well to the homing and transposition of group II introns and to the transposition of group I introns. In addition, the results further stress the importance of mitochondrial reverse transcriptase activity as a force in organellar genome evolution. This activity has previously been implicated in the process of intron loss in yeast mitochondria, which is dependent on the presence of reverse transcriptase-encoding group II introns a11/a12 [17], the generation of sub-genic modules of rRNA genes in *Chlamydomonas* mitochondria [18], and even the transfer of plant mitochondrial genes to the nucleus [19]. As a bonus, they give us new insight into the molecular mechanisms responsible for instability and the generation of rearrangements within organellar genomes. Such instability is common in the mtDNAs of both fungi and plants, and in both the resultant effects on mitochondrial function have important consequences for cell function and viability. Finally, further study of *Podospora*, in which intron movement is determined by the activity of nuclear genes, should give us important clues as to how the invasive tendencies of these introns can be controlled.

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