A complex group I intron in Nectria galligena rDNA

An article by Crockard and coworkers in a recent issue of *Microbiology* reported a polymorphic insertion element of 363, 1185 or 1423 bp within the nuclear small sub-unit (SSU) rDNA of the ascomycete fungus *Nectria galligena* (3). The insertion site was mapped to position 1199 (corresponding to *Escherichia coli* SSU rRNA numbering), a site known to harbour group I introns in fungi. However, the authors failed to assign this insertion to any known class of insertion elements.

Several classes of insertions (introns and retroelements) are known to interrupt genes encoding rRNA, with the group I introns being the most frequent (10). Despite the fact that all group I introns perform the same catalytic reaction at the RNA level, resulting in intron excision and exon ligation (1), almost none of the sequence positions are universally conserved. However, the group I intron RNAs can be folded into a common well-defined secondary and three-dimensional structure (12), and 12 subgroups, based on distinct primary sequence motifs and characteristic structural features in peripheral regions, have been proposed (5, 13).

We have re-analysed the *Nectria* sequences reported by Crockard et al. (3) and discovered that the smallest insertion (the 363 intron) contains a typical group I intron structure (Fig. 1a). The intron, which belongs to subgroup IC4, is distantly related to the more common subgroup IC1 introns in nuclear rDNA (5). A homologous structure is also present at the 5’ end of the *Nectria* intermediate-sized insertion (the 1185 intron). We have previously reported functional analyses of a group IC4 intron in the SSU rDNA of the myxomycete *Didymium* (4, 11). The group IC4 intron follows the same splicing pathway as the prototype group IC1 intron from *Tetrabyinema* (1).

A fast search (GGC Computer Package) using the 363 intron sequence as a ‘probe’ identifies three related fungal group IC4 introns inserted at the SSU rDNA position 1199 (the same as the *Nectria* intron). These include two *Cryptophy nocl* introns (2) and an intron from *Tillitiopsis flava* (15). The fact that similar group IC4 introns are found in distantly related eukaryotic microorganisms suggests that the *Nectria* 363 intron, in contrast to what was suggested by Crockard et al. (3), has been gained by horizontal transfer during evolution.

Some nuclear group I introns are known to contain ORFs encoding functional endonucleases involved in intron mobility by homing at the DNA level (6, 8, 14). All these nuclear homing endonucleases contain the characteristic His-Cys box motif of a 30-aa histidine- and cysteine-rich sequence (9).

A homologous structure is also reported in homing endonucleases from the myxomycete *Physarum*, the amoebolagellate *Naegleria* and the red alga *Porphyra* (8). The *Physarum* homing endonuclease is well studied and known to be directly involved in group I intron mobility (14). The recently deduced crystal structure of this endonuclease suggests that the His-Cys box residues are directly involved in novel zinc-binding motifs and in the active site of the enzyme (7).

In *Nectria*, the His-Cys box-
containing ORF is located on the opposite, i.e. antisense, strand compared with that encoding the group IC4 structure and the rRNA. Coding of a group I intron nuclear homing endonuclease on the antisense pre-rRNA strand has also been observed in other eukaryotic microorganisms, including Porphyra (16, our unpublished results), and suggests a novel expression strategy of a protein gene located in the rDNA.

Even though the Nectria rDNA insertions contain the obvious hallmark of nuclear group I introns, several simple experiments should be performed to confirm the results reported (3). These include the following: (i) to obtain and compare homologous intron sequences from different isolates to exclude the possibility of PCR-generated errors; (ii) to perform an RT-PCR sequencing analysis containing ORF is located on the opposite, other eukaryotic micro-organisms, including Porphyra (16, our unpublished results), and suggests a novel expression strategy of a protein gene located in the rDNA.

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