the origin of replication of the symbiosis plasmid, DnaB is a possible y4dM target in *Rhizobium*.

This is the first evidence that a plasmid stabilization system may exist in *Rhizobium* and suggests a mechanism by which such a system might operate to retain certain symbiosis plasmids.

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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 subunit (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 nuclear 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 *Tetrahymena* (1).

A FASTA search (GCG 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 *Cryphonectria* introns (2) and an intron from *Tilletiopsis flava* (15). The fact that similar group IC4 introns are found in distantly related eukaryotic micro-organisms 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

(a) Group IC4 intron

(b) His-Cys box

Nec. **SHLCGMPLCLEPANIHVEPKTANEARKGC**
Phy. **SHLCNTRCHNPLMLCWESLDDNKGRNWC**
Nae. **SHLCGMGGCARPGMLRIKKSVNDETRHC**
Por. **SHTCNMAKGVNKAHLTLESGLNKSRIYC**

Fig. 1. Hallmarks of nuclear group I introns within the rDNA insertions of *Nectria galligena*. (a) Secondary structure model of the group IC4 intron present in both the 363 and 1185 introns. Paired segments (P) are indicated. The 5' exon sequence is represented in lower case letters. The sequence of the 363 intron is shown. (b) The His-Cys box motif from *Nectria galligena* (*Nec.*) 1185 and 1423 introns aligned to the corresponding motifs (9) present in nuclear group I introns from *Physarum polycephalum* (*Phy.*), *Naegleria jamiesoni* (*Nae.*) and *Porphyra spiralis* (*Por.*).

histidine- and cysteine-rich sequence (9). An identical His-Cys box is shared by the 1185 intron and in the large *Nectria* insertion (the 1423 intron), and the motif in *Nectria* (Fig. 1b) is very similar to those previously reported in homing endonucleases from the myxomycete *Physarum*, the amoeboblastellate *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 micro-organisms, 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 of the ligated SSU rRNA exons to confirm that the introns are in fact spliced out *in vivo* and that splicing generates the correct ligated exons; and (iii) to establish an *in vitro* assay for G-labelling experiments specific for group I intron splicing. It is remarkable that none of the *Nectria* intron versions ends in a G residue (3), a universal feature among the group I introns (1), and that the 1423 intron is able to splice out from its precursor without a recognizable group I ribozyme structure.

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