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Short Sequence-Paper

Sequence and putative secondary structure of group I introns in the nuclear-encoded ribosomal RNA genes of the fungus *Hymenoscyphus ericae* ☆

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Abstract

Two putative group I introns in the nuclear ribosomal RNA genes of *Hymenoscyphus ericae* are described. One is in the small subunit gene about 30 nucleotides upstream of the 3′ end of the gene at a site common to several other group I introns. The other is in the large subunit gene approx. 930 bp downstream of the 5′ end of the gene. This is the only report of an intron at this location.

Keywords: Fungus; Ascomycete; Group I intron; Ribosomal RNA

The fungus *Hymenoscyphus ericae* is a common symbiotic root endophyte of plants in the family Ericaceae [1]. In a study of the relationship between *H. ericae* and other root endophytes based upon comparison of ribosomal DNA (rDNA), two insertions were discovered in the 18S and 28S ribosomal RNA genes [2]. They are putatively identified as group I introns and we report here on their structure.

Group I introns are characterized by conserved sequence elements that are involved in formation of secondary structural features essential for self-splicing from RNA transcripts [3,4]. They have been identified in the nuclear and organelle genomes of a wide variety of prokaryotes and eukaryotes, and within genes encoding messenger RNAs, ribosomal RNAs, and transfer RNAs. Their occurrence in nuclear genomes appears to be restricted to lower eukaryotes, including fungi [5–9].

Insertions in the rDNA were originally observed when restriction fragment length polymorphisms in polymerase chain reaction (PCR) amplified products were noted between different strains of *Hymenoscyphus ericae* [2]. PCR products were digested with restriction endonucleases to map the location of the insertions, then oligonucleotide primers were designed, based upon published sequences [10], to specifically amplify these regions. PCR products were sequenced by the dideoxy chain termination method using either an Applied Biosystems Model 373A automated DNA sequencer or manual sequencing protocols. Information on isolates of *H. ericae* and details of the DNA extraction, PCR, restriction analysis, and automated DNA sequencing protocols are given in Egger and Sigler [2].

Sequencing of inserts revealed features common to group I introns [11]: the conserved elements P, Q, R, and S, an Internal Guide Sequence (IGS) capable of base pairing with the 5′ and 3′ exons, the conserved nucleotides U and G at the intron 5′ and 3′ splice junctions, respectively, and a U-G pair between the exon and the IGS at the 5′ splice junction (see Fig. 1).

The proposed secondary structure for these introns is shown in Fig. 1A and B. Conserved secondary structure features include formation of stem P4 by the base pairing of P and Q elements, formation of stem P7 by the pairing of R and S, formation of stem P1 by pairing between the 5′ exon and the IGS, and the A-rich bulge and tetraloop features identified by Murphy and Cech [12].

☆ The nucleotide sequence data reported in this paper have been submitted to the EMBL/GenBank Data Libraries under the accession numbers U06868 and U06869.

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Based on primary and secondary structure, we conclude that the two elements are group I introns. The LSU intron is located in a region of highly conserved secondary structure in the 28S gene, at a position corresponding to nucleotide 929 of *Saccharomyces cerevisiae* in Guttel and Fox [10]. We know of no other reports of an intron at this location.

The SSU intron in *H. ericae* is located in the same region, stem 48 of the SSU gene [13], as group I introns in the fungi *Pneumocystis carinii* [5], *Protomyces inouyei* [7], *Cladonia* spp. [6], and *Cenococcum geophilum* [9]. Although the latter intron was originally described as an mRNA-like intron, the authors have since determined that it is has group I features and is able to self-splice (M. Shinohara, personal communication).

Introns in stem 48 of the SSU gene have been reported to splice at slightly different locations. The *P. carinii* and *P. inouyei* A introns splice 31 base pairs upstream of the 3′ end of the gene. Based upon the location of the conserved U-G pair at the 5′ exon splice junction, we propose that the *H. ericae* SSU intron splices at a site 1 nucleotide downstream of the *P. carinii* and *P. inouyei* A introns, and at the same location as the *Cenococcum geophilum* intron. Introns in other organisms show greater splice-site divergence. A group I intron in stem 48 of the alga *Dunaliella parva* splices 5 bp downstream of the *H. ericae* SSU position [14].

Despite the fact that these introns splice at slightly different positions, they may have had a common origin followed by migration or 'slippage' of splice sites. Al-

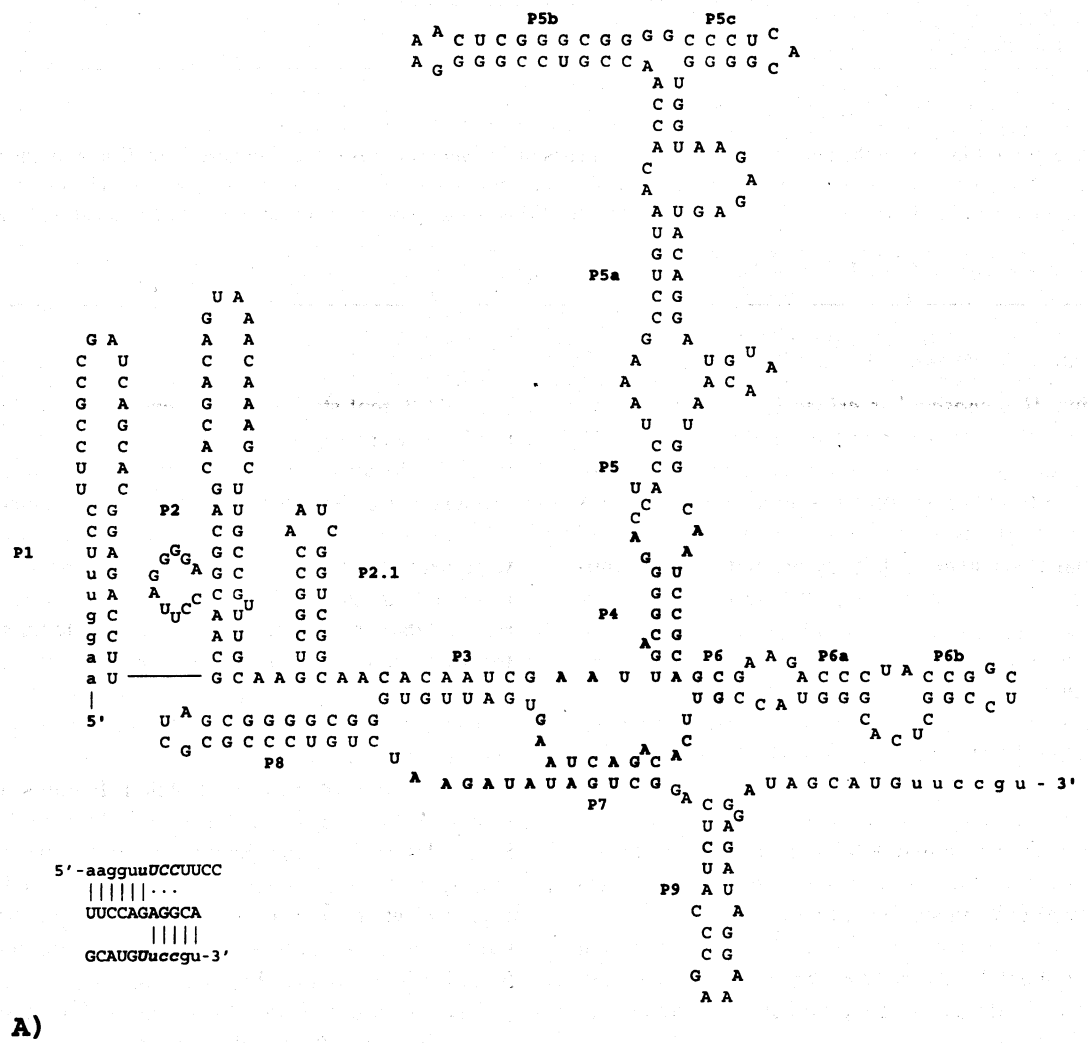

Fig. 1. Proposed secondary structure of group I introns in *Hymenoscyphus ericae*. Bold uppercase letters represent bases that form conserved elements P, Q, R, and S. Letters in lower case show the 5′ and 3′ exon splice junctions. The diagram in the lower left hand corner of the figure shows the proposed alignment of intron splice junctions with the internal guide sequence. Again, exon sequences are shown in lower case and intron sequences in upper case. The direct repeats are shown in bold italics. Pairing required for splicing at the −30 site is indicated by '|'; alternate pairing that would not alter the 18S RNA sequence is indicated by '·'. (A) Intron in the small subunit ribosomal RNA gene of strain UAMH 6562 (EMBL/GenBank accession No. U06868). (B) Intron in the large subunit ribosomal RNA gene of strain UAMH 6600 (EMBL/GenBank accession No. U06869).

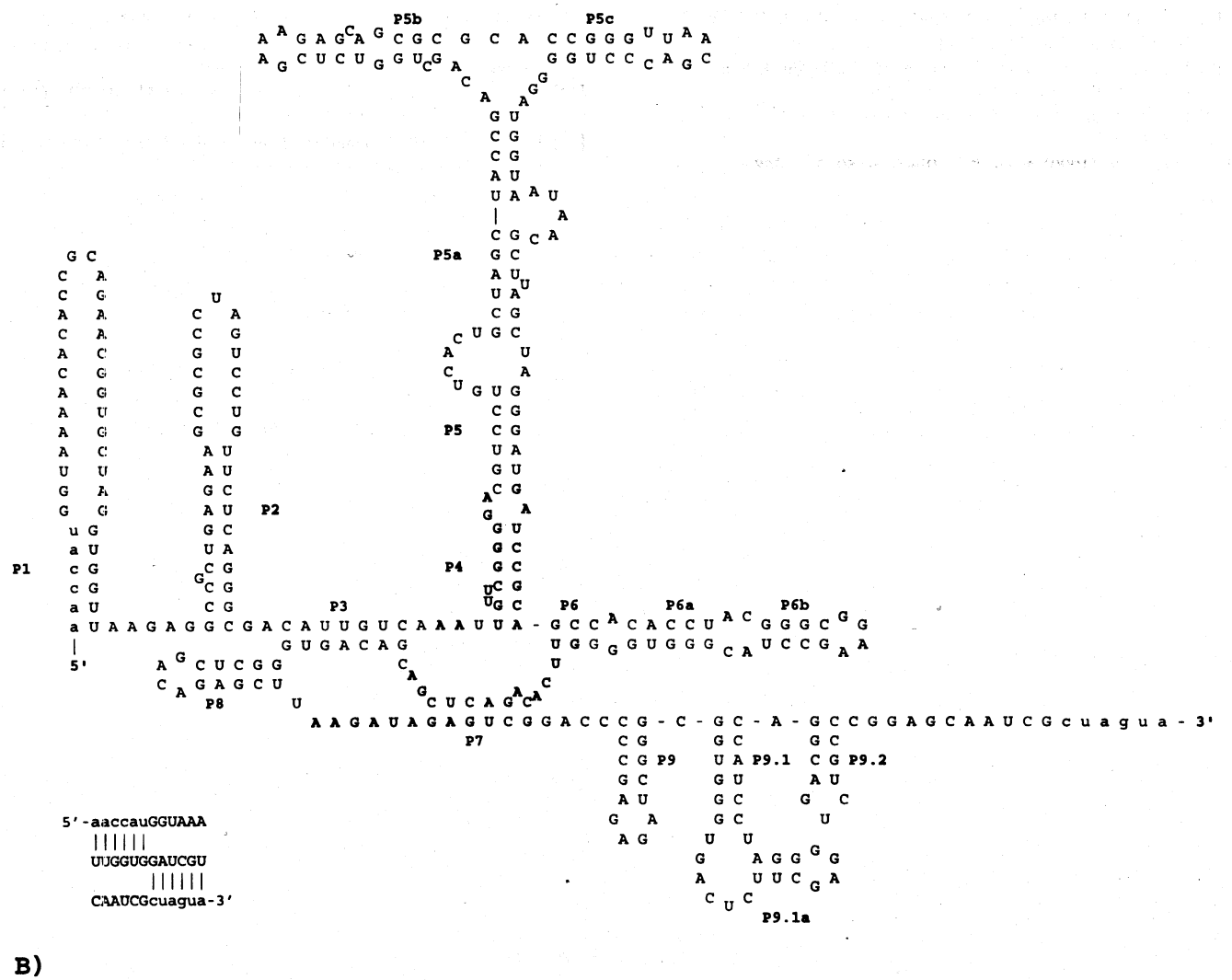

Fig. 1 (continued).

though intron slippage has been proposed for mRNA introns [15], this phenomenon has not been discussed in relation to group I introns. A mechanism for group I intron slippage is suggested by the presence of direct repeats of the 5′ exon splice junction at the 3′ end of the intron. The *H. ericae* SSU intron contains a 4 nucleotide direct repeat, and the *Cenococcum* intron contains an 8 nucleotide direct repeat [9]. The presence of direct repeats near the splice junction permits ambiguity in the splice junction alignment with the internal guide sequence of the intron (see Fig. 1A). Mutations that generate new U-G pairs between the direct repeats and the IGS, possibly accompanied by changes in secondary structure of the intron, could alter the splice site without altering the sequence of the 18S RNA, thereby permitting splice site slippage to occur. Despite the occurrence of group I introns in highly conserved regions of the rDNA, splice site slippage may be tolerated when direct repeats are present, because they would maintain the sequence integrity of the RNA.

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References

[1] Read, D.J. (1983) Canad. J. Bot. 61, 985–1004.
[2] Egger, K.N. and Sigler, L. (1993) Mycologia 85, 219–230.
[3] Burke, J.M. (1988) Gene 73, 273–294.
[4] Michel, F. and Westhof, E. (1990) J. Mol. Biol. 216, 585–610.
[5] Sogin, M.L. and Edman, J.C. (1989) Nucleic Acids Res. 17, 5349–5359.
[6] DePriest, P.T. and Been, M.D. (1992) J. Mol. Biol. 228, 315–321.
[7] Nishida, H., Blanz, P.A. and Sugiyama, J. (1993) J. Mol. Evol. 37, 25–28.

[8] DeWachter, R., Neefs, J.-M., Goris, A. and Van de Peer, Y. (1992) Nucleic Acids Res. 20, 1251–1257.
[9] Rogers, S.O., Yan, Z.H., Shinohara, M., LoBuglio, K.F. and Wang, C.J.K. (1993) Curr. Genet. 23, 338–342.
[10] Gutell, R. and Fox, G.E. (1988) Nucleic Acids Res. 16 (Suppl.), 175–269.
[11] Cech, T.R. (1990) Annu. Rev. Biochem. 59, 543–568.
[12] Murphy, R.L. and Cech, T.R. (1994) J. Mol. Biol. 236, 49–63.
[13] DeRijk, P., Neefs, J.-M., Van de Peer, Y. and DeWachter, R. (1992) Nucleic Acids Res. 20 (Suppl.), 2075–2089.
[14] Wilcox, L.W., Lewis, L.A., Fuerst, P.A. and Floyd, G.L. (1992) Mol. Biol. Evol. 9, 1103–1118.
[15] Kersanach, R., Brinkmann, H., Liaud, M.-F., Zhang, D.-X., Martin, W. and Cerff, R. (1994) Nature 367, 387–389.