Positions of Multiple Insertions in SSU rDNA of Lichen-Forming Fungi

Andrea Gargas, Paula T. DePriest,* and John W. Taylor†*

*Department of Botany, National Museum of Natural History, Smithsonian Institution, and †Department of Plant Biology, University of California at Berkeley

Lichen-forming fungi, in symbiotic associations with algae, frequently have nuclear small subunit ribosomal DNA (SSU rDNA) longer than the 1,800 nucleotides typical for eukaryotes. The lichen-forming ascomycetous fungus *Lecanora dispersa* contains insertions at eight distinct positions of its SSU rDNA; the lichen-forming fungi *Calicium tricolor* and *Porpidia crustulata* each contain one insertion. Insertions are not limited to fungi that form lichens; the lichen ally *Mycocalicium albonigrum* also contains two insertions. Of the 11 insertion positions now reported for lichen-forming fungi and this ally, 6 positions are known only from lichen-forming fungi. Including the 4 newly reported in this study, insertions are now known from at least 17 positions among all reported SSU rDNA sequences. Insertions, most of which are Group I introns, are reported in fungal and protistan lineages and occur at corresponding positions in genomes as phylogenetically distant as the nuclei of fungi, green algae, and red algae. Many of these positions are exposed in the mature rRNA tertiary structure and may be subject to independent insertion of introns. Insertion of introns, accompanied by their sporadic loss, accounts for the scattered distribution of insertions observed within the SSU rDNA of these diverse organisms.

Introduction

The ubiquity of ribosomes for translation of RNA messages into proteins and the conservation of regions of their nucleotide sequence have fostered the comparison of the small subunit rRNA gene (SSU rDNA) as a fixture of molecular phylogenetic studies (Bruns et al. 1991; Hamby and Zimmer 1992; Wainwright et al. 1993). As a consequence, nucleotide sequences of SSU rDNA have been obtained for more than 2,000 organisms (Gutell 1993; Larsen et al. 1993; Neefs et al. 1993). Among the eukaryotes, the SSU rDNA of representative animals, plants, and the model fungi (e.g., *Saccharomyces, Neurospora, Schizosaccharomyces,* and *Aspergillus*) was sequenced initially, and these each had SSU rDNA of approximately 1,800 nucleotides in length. Length increases of ribosomal DNA were reported in some groups (Gutell 1992; Sogin et al. 1986*a*) resulting from additional nucleotides in predicted variable regions (Gray et al. 1984; Hinkle et al. 1994) or in the intergenic spacers (Rogers et al. 1986). Consequently, SSU rDNA was hypothesized to have a highly conserved sequence (Gerbi et al. 1982; Woese 1987), to have little variation in sequence within species (Hillis and Davis 1988), and to be subject to concerted evolution (Arnheim et al. 1980; Arnheim 1983).

Recently, SSU rDNA with complete lengths significantly greater than 1,800 nucleotides has been observed in less well-studied fungi and protista, including green algae, red algae, and amoebae (references in table 1). In the eubacteria similar increases in SSU rDNA length result from intervening sequences (IVS) of 194 or 235 nucleotides near *Escherichia coli* position 199 (Springer et al. 1993; Linton et al. 1994). In the eukaryotes, many SSU rDNA size increases result from Group I introns, with some as large as 1,436 nucleotides each (Johansen and Vogt 1994). Group I introns are remarkably numerous among the lichen-forming fungi in the *Cladonia chlorophaea* complex, which have ribosomal DNA of up to 1,000 nucleotides longer than the corresponding region in *Saccharomyces cerevisiae* (DePriest and Been 1992). Previous studies showed that the SSU rDNA from the lichen-forming fungus *Porpidia crustulata* (as *Lecidea crustulata)* was approximately 100 nucleotides longer than the expected size (Gargas and Taylor 1992). Other lichen-forming fungi have larger SSU rDNAs including members of the Arthoniales (M. Grube and J. Hafellner, personal communication), other Lecanorales (K. H. Beard, N. Ivanova, and J. E. Marsh, personal communication), and the Peltigerales (P. T.

Key words: ribosomal DNA evolution, 18S rDNA, Group I introns, Ascomycotina, lichen-forming fungi, Lecanorales, Caliciales, rDNA = ribosomal DNA, SSU = small subunit, LSU = large subunit.

Address for correspondence: Andrea Gargas, Department of Botany, NHB-166, National Museum of Natural History, Smithsonian Institution, Washington, D.C. 20560.

Mol. Biol. Evol. 12(2):208–218. 1995.

0737-4038/95/1202-0003$02.00

DePriest and A. Gargas, unpublished results). We predict that these longer lengths of SSU rDNA indicate the presence of insertions, perhaps Group I introns, at discrete positions, and that these positions may be shared among different lichen-forming fungi or other intron-containing organisms. Insertions at a few positions shared among diverse organisms can provide evidence of the insertions' common ancestry and reflect their phylogenetic relationships. Conversely, insertions at a large number of scattered positions in the rDNA gene will provide evidence of their insertion in independent evolutionary events. In this study we have determined the location and sequence of SSU rDNA insertions in four fungi, including three lichen-forming fungi and one ally, and compared their insertions to those reported for other organisms.

Material and Methods

Cultures of four fungal species were used for extraction of DNA: *Calicium tricolor* F. Wilson and *Mycocalicium albonigrum* (Nyl.) Tibell, polyspore cultures gift of L. Tibell, University of Uppsala (UC accession numbers 1598218 and 1598220, respectively), and *Lecanora dispersa* (Pers.) Sommerf. and *Porpidia crustulata* (Ach.) Hertel and Knoph, polyspore cultures obtained from the American Type Culture Collection, Rockville, Maryland (ATCC accession numbers 18293 and 18297, respectively). Vouchers of the cultures were placed in the University Herbarium, University of California, Berkeley (UC) under the indicated accession numbers.

Standard fungal protocols (Lee and Taylor 1990) were used to isolate DNA from the cultured fungal mycelium. The fungal DNA was amplified with the polymerase chain reaction (PCR), as described by White et al. (1990). The fungal nuclear SSU rDNA was preferentially amplified using various combinations of the primers NS17UCB-NS24UCB (Gargas and Taylor 1992); NS2-NS7, ITS2-ITS5 (White et al. 1990); MB2 (the complement of NS23UCB; M. L. Berbee, personal communication); and CNS26 (TCGAA AGTTG ATAGG GCAG; gift of B. Bowman). The PCR cycle conditions were initial denaturation for 2 min at 95°C, subsequent denaturations for 30 s at 97°C, primer annealing for 1 min at 48°C, primer extension for 45 s at 72°C with an increase of 4 s each cycle, for a total of 30 cycles. Cleaned PCR products were used for a second PCR amplification of 30 cycles (as above) to produce either single-stranded (Gyllensten and Erlich 1988) or double-stranded DNA (Kusukawa et al. 1990). Sequencing primers (as above), either external or internal, were used to sequence both the coding and the noncoding strands by the dideoxy-labeling method (TAQuence kit; US Biochemical Corp., Cleveland, Ohio). Sequences of rDNA were deposited in GenBank, accession numbers L37733, L37734, L37735, and L37736. The sequences were aligned with the Pileup computer program (Genetics Computer Group, Madison, Wisconsin), and the alignment was manually adjusted to minimize gaps and optimize the alignment of apparently homologous regions.

Results

The SSU rDNAs from the four fungi were longer than the 1,800 nucleotides predicted from the model organism *Saccharomyces cerevisiae* (Rubtsov et al. 1980; Mankin et al. 1986), an ascomycetous yeast. The estimated size for SSU rDNA from PCR products of *Lecanora dispersa* was 3,350 nucleotides, with an additional 1,550 nucleotides. The estimated sizes for SSU rDNAs of *Calicium tricolor, Mycocalicium albonigrum,* and *Porpidia crustulata* were at least 270, 650, and 70 nucleotides longer than expected, respectively. When the sequences of the PCR products from these fungi were aligned with the SSU rDNA sequence of *S. cerevisiae,* each contained all the typical rDNA conserved sequences and sequence domains. The increases in length resulted from extra segments of DNA with discrete boundaries as defined by comparison with conserved rRNA sequences. Lengths of these insertions are shown in table 1, in which we have named the positions for the 5′ flanking nucleotide in *Escherichia coli* (Gutell 1993).

Some of the insertions have tandem repeats of two to five nucleotides in their termini, often duplicating the flanking sequences and making their exact location in the surrounding conserved sequences ambiguous (positions 114 and 287). Including those previously reported for the *Cladonia chlorophaea* complex, insertions are now known from 11 positions in the SSU rDNA of lichen-forming fungi (positions 114, 287, 392, 516, 789, 943, 1046, 1199, 1210, 1389, and 1516 in table 1). Number and location of insertions varied between the organisms; *L. dispersa* contained eight insertions (positions 114, 287, 516, 789, 943, 1046, 1210, and 1516), *M. albonigrum* contained two (positions 516 and 1199), *P. crustulata* contained one insertion (position 516), and *C. tricolor* contained one insertion (position 392). The insertions were on the average 212 nucleotides in length yet ranged between 78 and 388 nucleotides (table 1). After removing the insertion sequences, we assembled the sequence encoding rDNA of *L. dispersa* into a predicted secondary structure following the model of Gutell (Gutell 1993; Gutell et al. 1994) (fig. 1).

More than one rDNA repeat type has been observed in some natural individuals and single-spore isolates of the lichen-forming *C. chlorophaea* complex (DePriest 1993; P. T. DePriest, unpublished manuscript). These repeats differed in the presence or absence of an intron

Table 1
Sites of Insertions or Group I Introns in the SSU rDNA

Position	Classification	Organism	Lifestyle	Size (nucleotides)	Insertion Type	Reference
114*	Fungi (Ascomycotina, Lecanorales)	*Lecanora dispersa*	Lichen symbiont	189	Group I intron	This study; A. Gargas and S. Damberger, unpublished manuscript
287*	Fungi (Ascomycotina, Lecanorales)	*L. dispersa*	Lichen symbiont	191	Group I intron	This study; A. Gargas and S. Damberger, unpublished manuscript
323	Green algae (Chlorophyta, Chlorococcales)	*Chlorella sorokiniana*	Autotroph	465	[Not reported]	Huss et al. 1993*a*
392	Fungi (Ascomycotina, Caliciales)	*Calicium tricolor*	Lichen symbiont	276	[Not analyzed]	This study
516	Fungi (Ascomycotina, Caliciales)	*Mycocalicium albonigrum*	Heterotroph	388	[Not analyzed]	This study
	Fungi (Ascomycotina, Lecanorales)	*Porpidia crustulata*	Lichen symbiont	78	Degenerate	This study
		L. dispersa	Lichen symbiont	298	[Not analyzed]	This study
	Green algae (Chlorophyta, Chlorococcales)	*Chlorella luteoviridis* (A & B)	Autotroph	350, 358	Group I intron	Huss et al. 1993*b*, 1993*c*
		Chlorella saccharophila	Autotroph	362	[Not reported]	Huss et al. 1993*f*
	Amoebae (Sarcomastigophora, Amoebida)	*Acanthoamoeba griffini*	Heterotroph	519	Group I intron	Gast et al. 1994
		Naegleria sp.	Incl. mammalian pathogen	1277	Group I intron	Embley et al. 1992
531	Green algae (Chlorophyta, Volvocales)	*Chlamydomonas mœwusi*	Chloroplast endosymbiont	390	Group I intron	Durocher et al. 1989
789	Fungi (Ascomycotina, Lecanorales)	*L. dispersa*	Lichen symbiont	231	[Not analyzed]	This study
943	Fungi (Ascomycotina, Lecanorales)	*L. dispersa*	Lichen symbiont	111	Degenerate	This study
	(Ascomycotina, Leotiales)	*Spathularia flavida*	Heterotroph	300	Group I intron	Landvik et al. 1993
	(Ascomycotina, Diaporthales)	*Leucostoma cincta*	Plant pathogen	411	[Not reported]	†
	Fungi (Basidiomycotina, Ustilaginales)	*Ustilago maydis*	Plant pathogen	411	Group I intron	De Wachter et al. 1992
	Fungi (undetermined)	*Protomyces inoyei*	Plant pathogen	340	Group I intron	Nishida et al. 1993
	Green algae (Chlorophyta, Chlorococcales)	*Dunaliella parva*	Autotroph	397	Group I intron	Wilcox et al. 1991
		Dunaliella salina	Autotroph	381	Group I intron	Wilcox et al. 1991
	Amoebae (Sarcomastigophora, Amoebida)	*Acanthoamoeba lenticulata*	Heterotroph	656	Group I intron	Gast et al. 1994
956	Acellular slime molds (Myxomycetes)	*Didymium iridis*	Heterotroph	1436	Group I intron	Johansen and Vogt 1994
1046	Fungi (Ascomycotina, Lecanorales)	*Cladonia chlorophaea*	Lichen symbiont	225	Group I intron	DePriest and Been 1992
		C. chlorophaea	Lichen symbiont	217	Group I intron	DePriest and Been 1992
		L. dispersa	Lichen symbiont	231	Group I intron	This study; A. Gargas and S. Damberger, unpublished manuscript

	Green algae (Chlorophyta, Chlorococcales)	*Ankistrodesmus stipitatus*	Autotroph	394	Group I intron	Dávila-Aponte et al. 1991
		C. sorokiniana	Autotroph	428	[Not reported]	Huss et al. 1993*a*
1052	Green algae (Chlorophyta, Chlorococcales)	*Chlorella luteoviridis* (B)	Autotroph	421	Group I intron	Huss et al. 1993*c*
1199	Fungi (Ascomycotina, Lecanorales)	*C. chlorophaea*	Lichen symbiont	226	Group I intron	DePriest and Been 1992
	(Ascomycotina, Caliciales)	*Mycocalicium albonigrum*	Heterotroph	275	[Not analyzed]	This study
	(Ascomycotina, Diaporthales)	*Leucostoma cincta*	Plant pathogen	>250	[Not reported]	†
1210	Fungi (Ascomycotina, Lecanorales)	*C. chlorophaea*	Lichen symbiont	228	Group I intron	DePriest and Been 1992
		L. dispersa	Lichen symbiont	194	[Not analyzed]	This study
1389	Fungi (Ascomycotina, Lecanorales)	*C. chlorophaea*	Lichen symbiont	212	Group I intron	DePriest and Been 1992
1506	Fungi (undetermined)	*Pneumocystis carinii*	Mammalian pathogen	390	Group I intron	Sogin and Edman 1989
		P. inoyei	Plant pathogen	393	Group I intron	Nishida et al. 1993
	Fungi (Ascomycotina, Leotiales)	*Hymenoscyphus ericae*	Root endophyte	ca. 350	[Not reported]	Egger and Sigler 1993
	Fungi (Deuteromycetes)	*Cenococcum geophilum*‡	Mycorrhizal symbiont	459	[Not reported]	Rogers et al. 1993
		Phialophora americana§	Heterotroph	67	Degenerate	Rogers et al. 1993
	Green algae (Chlorophyta, Chlorococcales)	*Chlorella ellipsoidea*	Autotroph	441, 442	Group I intron	Huss et al. 1992; Aimi et al. 1994
		Chlorella mirabilis	Autotroph	488	Group I intron	Huss et al. 1993*d*
		Mougeotia scalaris	Autotroph	535	[Not reported]	Huss et al. 1993*e*
	(Chlorophyta, Zygnematales)	*Genicularia spirotaenia*	Autotroph	370	[Not reported]	Surek et al. 1993*a*
		Staurastrum sp.	Autotroph	406	[Not reported]	Surek et al. 1993*b*
	Red algae (Rhodophyta)	*Porphyra spiralis* var. *amplifolia*	Autotroph	743, 907, 1054	Group I intron	Oliveira and Ragan 1994
		Hildenbrandia rubra	Autotroph	501	Group I intron	Ragan et al. 1993
1512	Green algae (Chlorophyta, Chlorococcales)	*Dunaliella parva*	Autotroph	419	Group I intron	Wilcox et al. 1991
		Characium saccatum	Autotroph	447	Group I intron	Wilcox et al. 1991
	(Chlorophyta, Acrosiphoniales)	*Urospora penicilliformis*	Autotroph	452	Group I intron	Van Oppen et al. 1993
1516	Fungi (Ascomycotina, Lecanorales)	*C. chlorophaea*	Lichen symbiont	228	Group I intron	DePriest and Been 1992
		C. chlorophaea	Lichen symbiont	210	Group I intron	DePriest and Been 1992
		L. dispersa	Lichen symbiont	212	Group I intron	This study; A. Gargas and S. Damberger, unpublished manuscript

NOTE.—The position of each site is the number of the nucleotide 5′ to the insertion position in the SSU rDNA sequence of *Escherichia coli* (Gutell 1993). For each site, organisms with insertions or introns reported in that position, their taxonomic classification, lifestyle, and the insertions, size in nucleotides are noted. For insertions that have not been completely sequenced, a minimum estimate is noted as greater than (>) the number of nucleotides sequenced. Insertions reported as Group I introns are indicated. The original reference is given for each insertion.

* Ambiguous insertions positions due to tandem repeats of two to five nucleotides in the insertion terminii.

† M. L. Berbee, G. Adams, and J.W.T., unpublished data.

‡ We predict that the SSU rDNA insertion is a Group I intron located at position 1506.

§ We predict that the SSU rDNA insertion is a degenerate Group I intron, located at position 1506 or 1512.

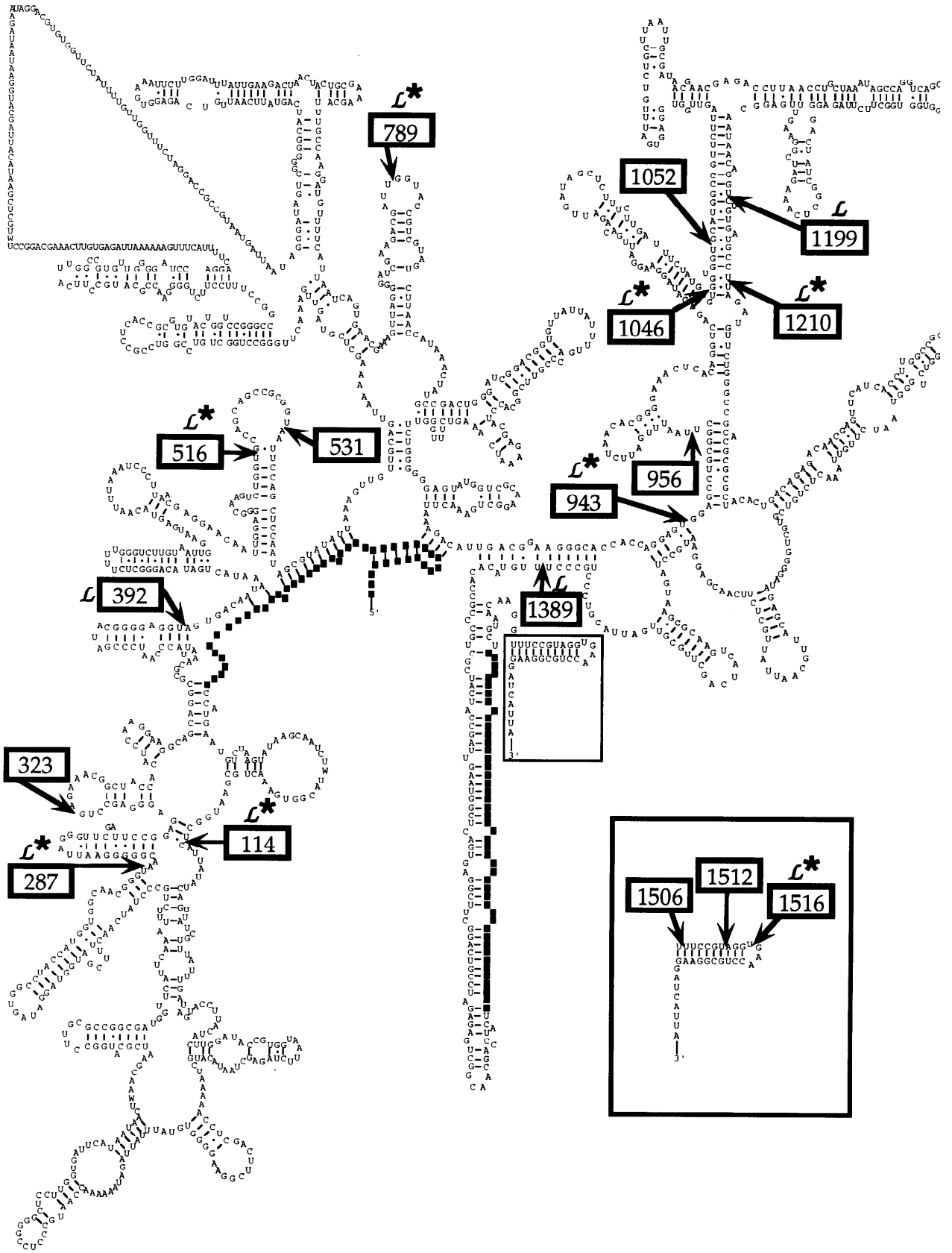

FIG. 1.—Nucleotide sequence and secondary structure model for *Lecanora dispersa* SSU rRNA. Numbered arrows indicate the positior of insertions or introns as in table 1. Canonical base pairs are indicated by dashes, and the noncanonical base pairings of G and U are indicatec by dots. Unknown nucleotides are indicated by small squares. The number next to each insertion corresponds to the position of the intron relative to the SSU rDNA sequence of *Eschericia coli* (Gutell 1993). Insertions present in lichen-forming fungi are marked with a script *L*. Insertions present in *L. dispersa* are marked with an asterisk.

at a particular position. The variable presence of insertions may produce anomalous PCR amplification products. Initially SSU rDNA primers were designed for sequence regions of rDNA conserved among model eukaryotes. Many of the conserved areas used for design of primer sequences also contain insertions. The primers that anneal across an insertion position will not amplify or sequence templates which contain this insertion. The annealing regions of universal primers NS2/3, NS6/7, and NS8/ITS1 (White et al. 1990) span insertion positions 516, 1199, and 1210, and 1516 respectively. These primers will amplify only those templates which lack the associated insertion. For example, *L. dispersa,* which contains an insertion at position 1516, cannot be amplified with ITS1, and *M. albonigrum,* which contains an insertion at position 516, cannot be amplified with NS2 or NS3. If rDNA repeats in a tandem array differ in the presence or absence of insertions, a primer annealing across any of these positions will preferentially amplify the rDNA repeats lacking the insertion. Therefore, PCR products and sequences from some primers may not sample all the rDNA repeat types present in an organism as indicated by sequencing anomalies in *L. dispersa* with primers NS6 and NS7. This suggests that more than one rDNA repeat type is present in this culture, which differ in the presence of the intron at position 1210.

Discussion

The lichen-forming fungi and their ally examined in this study have unusually long rDNAs, for example, *Lecanora dispersa,* at 3,350 nucleotides. This length increase results from the presence of eight insertions in the SSU rDNA. Insertions are abundant throughout the SSU rDNA of lichen-forming fungi and are now known from 11 positions. Five of these positions were originally identified by DePriest and Been (1992) in a 500-nucleotide region of SSU rDNA, which suggested that insertions were abundant in other regions of the gene. The additional six positions identified here for the remaining 1 kb of SSU rDNA confirm that numerous insertions are typical for the SSU rDNA of lichen-forming fungi. The insertions in *Cladonia* were reported to be Group I introns (DePriest and Been 1992), and the insertions reported here are likely to be Group I introns as well (A. Gargas and S. Damberger, unpublished manuscript). Group I introns are abundant in mitochondrial and chloroplast genomes (Dujon 1989; Dujon and Belcour 1989; Michel and Westhof 1990; Turmel et al. 1993) but in nuclear genomes are known only from ribosomal DNA (Cech 1988; Dujon 1989; Michel and Westhof 1990). In the large subunit (LSU) rDNA Turmel et al. (1993) reported 12 Group I intron positions among the chloroplasts from 17 taxa of *Chlamydomonas.* Given the abundance of insertions in the SSU rDNA of lichen-forming fungi, the nuclear LSU rDNAs from these fungi may have numerous Group I introns as well. Even though numerous Group I introns are reported from the nuclear rDNA of fungi and protista (Dujon 1989; Dujon and Belcour 1989; Michel and Westhof 1990), none have been reported from plants or animals.

These insertions or introns occur repeatedly in the same sequence positions of the SSU rDNA, even among representatives of divergent lineages (table 1, fig. 2). Including the four positions (positions 114, 287, 392, and 789) newly reported here, insertions are now known from 17 positions for all SSU rDNA sequences. Most of the insertions reported from conserved regions of the rDNA, encoding either the SSU or LSU rRNA, have the characteristic secondary structures and conserved sequence elements of Group I introns (Cech 1989; Davies et al. 1982; Michel et al. 1982). In the SSU rDNA, insertions at 15 of the 17 positions have been reported to be Group I introns (see table 1), and insertions in the remaining positions appear to be this type of intron as well (A. Gargas, unpublished results; A. Gargas and S. Damberger, unpublished manuscript). Insertions at most positions were present in two or more species. We predict that insertions will be found at these locations in other organisms as more taxa are examined. Four of the insertion positions (positions 516, 943, 1046, and 1506) are shared among species of lichen-forming fungi and green algae (table 1)—members of the same group as some algae in lichen associations. To our knowledge, no insertions have been reported from the SSU rDNA of lichen-forming green algae, yet few have been examined with molecular tools (Kantz et al. 1990; Zechman 1990). Insertions also occur at position 516 and position 943 in amoebae and at position 1506 in red algae. Although one insertion has been reported from chloroplasts of the green alga *Chlamydomonas mœwussi* (Durocher et al. 1989), insertions have not been reported from this position in any nuclear SSU rDNA.

One challenge is to understand the evolution and phylogenetic distribution of these introns. The pattern of introns suggests that they were repeatedly and independently inserted into the SSU rDNA. Insertions at different positions in the SSU rDNA are not recognizably similar in sequence, suggesting that they have not recently transposed among the positions, as was concluded for insertions in the green algae (Van Oppen et al. 1993). Even insertions at the same position in divergent organisms are often not recognizably similar in sequence or are significantly different in size and are difficult to compare. For example, insertions of less than 120 nucleotides in the fungi *Porpidia crustulata, Lecanora dispersa,* and *Phialophora americana* may represent degenerate Group

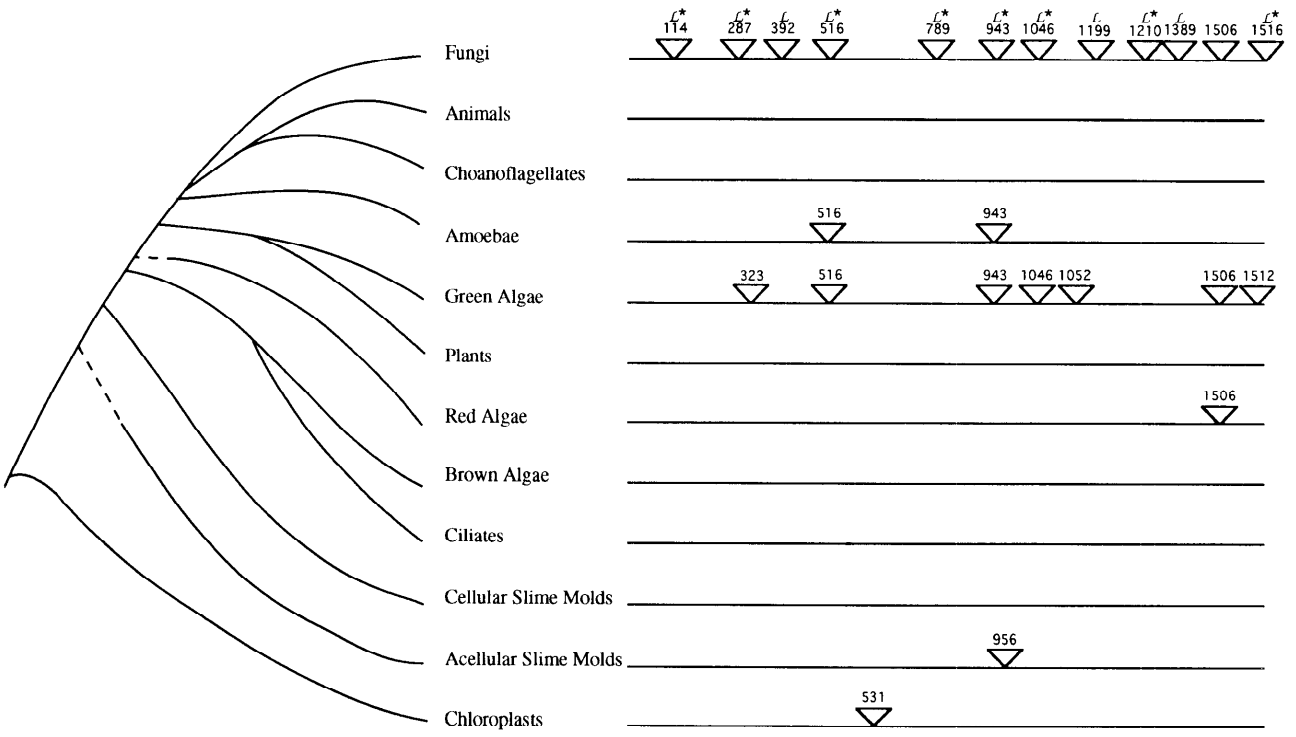

FIG. 2.—A phylogenetic scheme for representative eukaryotic lineages adapted from Wainwright et al. (1993). The chloroplast genome is shown as an outgroup to the nuclear lineages. For each of the lineages, a line represents the small subunit ribosomal gene. The presence of Group I introns or insertions in the SSU rDNA of at least one taxon of a lineage is denoted by an inverted triangle. The number above each triangle corresponds to the position of the intron relative to the SSU rDNA sequence of *Escherichia coli* (Gutell 1993). Insertions present in lichen-forming fungi are marked with a script *L*. Insertions present in *Lecanora dispersa* are marked with an asterisk.

I introns. These small insertions lack the conserved core sequences of Group I introns and may be too streamlined to fold into the characteristic Group I intron secondary structure. Therefore, a simple phenetic comparison of the sequence among divergent organisms cannot clearly identify the degenerate introns as homologous with other insertions at the same site. Instead, homology must be ascertained over a range of organisms and relationships using methods of phylogenetic reconstruction. Presence of SSU rDNA Group I introns is indicated on a phylogenetic scheme for eukaryotic groups based on SSU rDNA sequence data (adapted from Wainwright et al. 1993) (fig. 2). Insertions are present within SSU rDNA of a diversity of phylogenetic clades, mainly in the "crown" of eukaryotes (Knoll 1992). At present, Group I introns have not been reported from the nuclei of terrestrial plants or multicellular animals, despite their sister-taxa relationship to insertion-containing lineages. If these introns were present before the divergence of fungi and animals (Belfort 1991), then there is a staggering number of intron losses to explain. If they appeared after the divergence of these groups (Palmer and Logsdon 1991), then a staggering number of insertion events is required. Both insertion and deletion are required to account for the modern distribution of introns, with insertion of introns at positions such as 516, 943, 1046, and 1506 representing relatively ancient events compared to the insertion of the four introns currently known only from lichen-forming fungi.

It seems plausible that insertions and Group I introns have been recently mobile. Some Group I introns are considered autonomous sequence elements (Dujon 1989; Lambowitz 1989) that are mobile by intron insertion (Jaquier and Dujon 1985; Woodson and Cech 1989; Mohr and Lambowitz 1991), deletion (Levra-Jullet et al. 1989), or, possibly, horizontal transposition (Dover and Coen 1981; Lambowitz and Perlman 1990). Some introns are known, genetically and experimentally, to be inserted or "home" into specific target positions that are recognized by an endonuclease encoded by the same intron (Jaquier and Dujon 1985; Belfort 1991). With intron homing, an intron could be inserted into an intron-lacking rDNA repeat as a result of interaction with an intron-containing rDNA repeat from another nucleus, perhaps during sexual reproduction. Also, Group I introns may be inserted or transposed by reversal of the splicing reaction and subsequent reverse transcription of the intron-lacking sequence and its incorporation into the rDNA by homologous recombination (Woodson and Cech 1989; Mohr and Lambowitz

1991; Thompson and Herrin 1994). A similar process of reverse transcription and homologous recombination with an intron-lacking sequence may lead to precise deletion of introns (Dujon 1989; Levra-Jullet et al. 1989) and produce the variation in intron presence observed among closely related organisms. The presence of similar Group I introns in different genes, genomes, and organisms provides indirect evidence that introns are transposed horizontally even among divergent organisms (Sogin et al. 1986*b*; Dujon 1989; Lambowitz 1989; Woodson and Cech 1989; Mohr and Lambowitz 1991). The intimate contact of symbiotic or parasitic associations provides an opportunity for horizontal intron transfer among organisms. A search for intron transposition in the algal symbiont of lichen-forming fungi known to harbor introns may be more worthwhile than similar searches in host plants and animals whose nuclei lack Group I introns.

Group I introns are occasionally clustered in regions separated by only a few nucleotides—six pairs of insertions are separated by fewer than 15 nucleotides (table 1). Turmel et al. (1993) suggested that introns in distinct but spatially close positions of the *Chlamydomonas* chloroplast LSU rDNA represent independent insertions into regions that are exposed in the subunit's tertiary structure. In the SSU rRNA of these fungi, multiple insertions into exposed regions would explain the observed clustering of intron positions on some helices (positions 1046, 1052, 1199, and 1210 and positions 1506, 1512, and 1516). Some insertion pairs distant in primary sequence are close in their predicted secondary structure (positions 114 and 287, positions 1046 and 1210, and positions 1052 and 1199) and tertiary structure (positions 516, 531, and 1389) (Noller 1991). Seven of the insertion positions (positions 516, 531, 789, 1389, 1506, 1512, and 1516) are in regions identified by DNA-hybridization electron microscopy as accessible on the exterior surface of the subunit tertiary structure (Oakes and Lake 1990; Oakes et al. 1990). Six of the remaining positions (positions 1046, 1052, 1199, and 1210 and positions 943 and 956) are in or adjacent to the presumed positions, A and P, respectively, where mRNA enters the ribosomal complex for translation (Dahlberg 1989). At present, three insertion positions (positions 114, 287, and 392) reported here for fungi have no mapped ribosomal functions. However, the clustering of three insertions in one secondary structure region suggests that the region is exposed in the rRNA tertiary structure. Presumably these exposed rRNA regions were subject to reinsertion of spliced introns (Woodson and Cech 1989; Mohr and Lambowitz 1991), followed by reverse transcription and homologous recombination.

Dujon (1989) suggested that the number of introns in a genome represents an equilibrium between the processes of intron insertion and deletion. The numerous Group I introns in the SSU rDNA of some lichen-forming fungi suggest that the equilibrium has shifted toward intron insertion, at least in the recent past. Sequences and restriction-site patterns from insertions at the same position in closely related taxa are similar (Wilcox et al. 1991; De Jonckheere 1992; DePriest and Been 1992; DePriest 1993; Nishida et al. 1993), suggesting that they are homologous. For example, the insertions at the same position in two representatives of the *Cladonia chlorophaea* complex are more than 90% similar in sequence (DePriest and Been 1992). Intron variability within species and individuals of lichen-forming fungi is indirect evidence that intron insertion and deletion may have occurred recently, even within existing species and populations. This variation in rDNA repeat types observed within individuals or single-spore isolates of *Cladonia chlorophaea* (DePriest 1993; P. T. DePriest, unpublished manuscript) and *Lecanora dispersa,* perhaps even within their rDNA tandem arrays, provides empirical evidence that concerted evolution (Arnheim et al. 1980; Arnheim 1983) is not effective in this situation. Relatively rapid intron mobility may counteract the processes of concerted evolution that fix a single rDNA type within the tandem array.

Conclusion

The routinely studied organisms, animals, and higher plants, which lack nuclear Group I introns, provide a distorted view of rDNA as conserved in sequence and size during its evolution. This solid foundation for phylogenetic comparison appears now to be punctuated with variable introns, which are capable of profound evolutionary changes, even between generations. As more organisms are sequenced, more insertions will be found in the positions summarized here. New positions, even in phylogenetically distant organisms, will likely be close to known positions that are exposed in the rRNA. Although most of these insertions are Group I introns, the reports of intervening sequences in the SSU rDNA of eubacteria (Springer et al. 1993; Linton et al. 1994) suggest that similar processes may affect evolution of rDNA in a diversity of organisms. In the lichen-forming fungi, Group I introns may be present and variable in other positions in the rDNA and in other nuclear and mitochondrial genes. Studies on the distribution of these insertions at different taxonomic levels, as well as comparison of intron sequences will elucidate the evolutionary processes modifying rDNA. Additionally, introns provide a means for detection of intron-containing organisms such as *Pneumocystis carinii* (Edman et al. 1988) within the tissues of their hosts. The abundance of introns in the lichen-forming fungi, organisms resistant to laboratory studies and overlooked even by my-

cologists, is a reminder that a thorough understanding of molecular genetics requires comparative biology in addition to investigations of model systems.

Acknowledgments

We thank R. R. Gutell and S. Damberger for carefully examining the rRNA secondary structure, suggesting improved foldings and nomenclature for the positions, and examining the insertions as Group I introns; M. D. Been and R. R. Gutell for careful review of the manuscript; two anonymous reviewers for their comments; K. H. Beard, M. Grube, N. Ivanova, and J. Marsh for unpublished results; and J. Norris for discussions of red algal relationships. A.G. thanks L. Tibell for his generous gift of fungal cultures; and M. L. Berbee, S. B. Lee, T. Bruns, and B. Bowman for discussions of fungal systematics. P.T.D. thanks M. D. Been, M. Grube, R. R. Gutell, and R. Vilgalys for discussions of Group I introns in lichens. This research was partially supported by National Science Foundation (NSF) Dissertation Improvement Grant (BSR 89 14636) and Scholarly Studies Award from the Smithsonian Institution to P.T.D. and National Institutes of Health Grant (RO1 AI 28545) and NSF Grant (BSR 90007141) to J.W.T.

LITERATURE CITED

AIMI, T., T. YAMADA, and Y. MUROOKA. 1994. A group I self-splicing intron in the nuclear small subunit rRNA-encoding gene of the green alga, *Chlorella ellipsoidea* C-87. Gene **139**:64–71.

ARNHEIM, N. 1983. Concerted evolution of multigene families. Pp. 38–61 *in* M. NEI and R. K. KOEHN, eds. Evolution of genes and proteins. Sinauer, Sunderland, Mass.

ARNHEIM, N., M. KRYSTAL, R. SCHMICKEL, G. WILSON, O. RYDER, and E. ZIMMER. 1980. Molecular evidence for genetic exchanges among ribosomal genes on nonhomologous chromosomes in man and apes. Proc. Natl. Acad. Sci. USA **77**:7323–7327.

BELFORT, M. 1991. Self-splicing introns in prokaryotes: migrant fossils? Cell **64**:9–11.

BRUNS, T. D., T. J. WHITE, and J. W. TAYLOR. 1991. Fungal molecular systematics. Annu. Rev. Ecol. Syst. **22**:525–564.

CECH, T. R. 1988. Conserved sequences and structures of group I introns: building an active site for RNA catalysis—a review. Gene **73**:259–271.

DAHLBERG, A. E. 1989. The functional role of ribosomal RNA protein synthesis. Cell **57**:525–529.

DAVIES, R. W., R. B. WARING, J. A. RAY, T. A. BROWN, and C. SCAZZOCCHIO. 1982. Making ends meet: a model for RNA splicing in fungal mitochondria. Nature **300**:719–728.

DÁVILA-APONTE, J. A., V. A. R. HUSS, M. L. SOGIN, and T. R. CECH. 1991. A self-splicing group I intron in the nuclear pre-rRNA of the green alga *Ankistrodesmus stipitatus*. Nucleic Acids Res. **19**:4429–4436.

DEJONCKHEERE, J. F. 1993. A group I intron in the SSU rDNA of some *Naegleria* spp. demonstrated by polymerase chain reaction amplification. J. Eukaryot. Microbiol. **40**:179–187.

DEPRIEST, P. T. 1993. Small subunit rDNA variation in a population of lichen fungi due to optional group I introns. Gene **134**:67–71.

DEPRIEST, P. T., and M. BEEN. 1992. Numerous group I introns in the ribosomal DNA of a lichen fungus. J. Mol. Biol. **228**:315–321.

DE WATCHER, R., J.-M. NEEFS, A. GORIS, and Y. VAN DE PEER. 1992. The gene coding for small ribosomal subunit RNA in the basidiomycete *Ustilago maydis* contains a group I intron. Nucleic Acids Res. **20**:1251–1257.

DOVER, G., and E. COEN. 1981. Spring cleaning ribosomal DNA: a model for multigene evolution? Nature **290**:731–732.

DUJON, B. 1989. Group I introns as mobile genetic elements: facts and mechanistic speculation—a review. Gene **82**:91–114.

DUJON, B., and L. BELCOUR. 1989. Mitochondria DNA instabilities and rearrangements in yeasts and fungi. Pp. 861–878 *in* D. E. BERG and M. M. HOWE, eds. Mobile DNA. American Society for Microbiology, Washington, D.C.

DUROCHER, V., A. GAUTHIER, G. BELLEMARE, and C. LEMIEUX. 1989. An optional group I intron between the chloroplast small subunit rRNA genes of *Chlamydomonas moewusii* and *C. eugmetos*. Curr. Genet. **15**:277–282.

EDMAN, J. C., J. A. KOVACS, H. MASUR, D. V. SANTI, H. J. ELWOOD, and M. L. SOGIN. 1988. Ribosomal RNA sequence shows *Pneumocystis carinii* to be a member of the fungi. Nature **334**:519–522.

EGGER, K. N., and L. SIGLER. 1993. Relatedness of the ericoid endophytes *Scytalidium vaccinii* and *Hymenoscyphus ericae* inferred from analysis of ribosomal DNA. Mycologia **85**: 219–230.

EMBLEY, T. M., P. L. DYAL, and S. KILVINGTON. 1992. A group I intron in the small subunit ribosomal RNA gene from *Naegleria andersoni* ssp. *andersoni* strain PPMFB. Nucleic Acids Res. **20**:6411.

GARGAS, A., and J. W. TAYLOR. 1992. Polymerase chain reaction (PCR) primers for amplifying and sequencing nuclear 18S rDNA from lichenized fungi. Mycologia **84**:589–592.

GAST, R. J., P. A. FUERST, and T. J. BYERS. 1994. Discovery of group I introns in the nuclear small subunit ribosomal RNA genes of *Acanthoamoeba*. Nucleic Acids Res. **22**:592–596.

GERBI, S. A., R. L. GOURSE, and C. G. CLARK. 1982. Conserved regions within the ribosomal DNA: Locations and some possible functions. Pp. 351–386 *in* H. BUSCH and L. ROTHBLUM, eds. The cell nucleus. Vol. X. rDNA Part A. Academic Press, New York.

GRAY, M. W., D. SANKOFF, and R. J. CEDERGREN. 1984. On the evolutionary descent of organisms and organelles: a global phylogeny based on a highly conserved structural core in small subunit ribosomal RNA. Nucleic Acids Res. **12**:5837–5852.

GUTELL, R. R. 1992. Evolutionary characteristics of 16S and 23S rRNA structures. Pp. 243–309 *in* H. HARTMAN and

K. MATSUNO, eds. The origin and evolution of the cell. World Scientific, River Edge, N.J.

———. 1993. Collection of small subunit (16S- and 16S-like) ribosomal RNA structures. Nucleic Acids Res. **21**:3051–3054.

GUTELL, R. R., N. LARSEN, and C. R. WOESE. 1994. Lessons from an evolving rRNA: 16S and 23S rRNA structures from a comparative perspective. Microbiol. Rev. **58**:10–26.

GYLLENSTEN, U. B., and H. A. ERLICH. 1988. Generation of single-stranded DNA by the polymerase chain reaction and its application to direct sequencing of the HLA-DLQ locus. Proc. Natl. Acad. Sci. USA **85**:7652–7656.

HAMBY, R. K., and E. A. ZIMMER. 1992. Ribosomal RNA as a phylogenetic tool in plant systematics. Pp. 50–91 *in* P. SOLTIS, D. SOLTIS, and J. DOYLE, eds. Molecular systematics of plants. Chapman & Hall, New York.

HILLIS, D. M., and S. K. DAVIS. 1988. Ribosomal DNA: intraspecific polymorphism, concerted evolution, and phylogeny reconstruction. Syst. Zool. **37**:63–66.

HINKLE, G., D. D. LEIPE, T. A. NERAD, and M. L. SOGIN. 1994. The unusually long small subunit ribosomal RNA of *Phreatamoeba balamuthi*. Nucleic Acids Res. **22**:465–469.

HUSS, V. A. R., C. FRANK, and E. KESSLER. 1993*a*. *C. sorokiniana* gene for 18S small subunit rRNA. GenBank accession X73993.

HUSS, V. A. R., M. HIRMER, and E. KESSLER. 1993*b*. *C. luteoviridis* (A) gene for 18S small subunit rRNA. GenBank accession X73997.

———. 1993*c*. *C. luteoviridis* (B) gene for 18S small subunit rRNA. GenBank accession X73998.

———. 1993*d*. *C. mirabilis* gene for 18S small subunit rRNA. GenBank accession X74000.

HUSS, V. A. R., B. SEIDEL, and E. KESSLER. 1992. *C. ellipsoidea* small subunit ribosomal RNA. GenBank accession X63520.

HUSS, V. A. R., M. L. SIEGLER, H. D. KRANZ. 1993*e*. *M. scalaris* small subunit ribosomal RNA. GenBank accession X70705.

HUSS, V. A. R., P. WENZELER, and E. KESSLER. 1993*f*. *C. saccharophila* gene for 18S small subunit rRNA. GenBank accession X73991.

JAQUIER, A., and B. DUJON. 1985. An intron-encoded protein is active in a gene conversion process that spreads an intron into a mitochondrial gene. Cell **41**:383–394.

JOHANSEN, S., and V. M. VOGT. 1994. An intron in the nuclear ribosomal DNA of *Didymium iridis* codes for a group I ribozyme and a novel ribozyme that cooperate in splicing. Cell **76**:725–734.

KANTZ, T. S., E. C. THERIOT, E. A. ZIMMER, and R. L. CHAPMAN. 1990. The Pleurastrophyceae and Micromonadophyceae: a cladistic analysis of nuclear rRNA sequence data. J. Phycol. **26**:711–721.

KNOLL, A. H. 1992. The early evolution of eukaryotes: a geological perspective. Science **256**:622–627.

KUSUKAWA, N., T. UEMORI, K. ASADA, and I. KATO. 1990. Rapid reliable protocol for direct sequencing for material amplified by the PCR. Biotechniques **9**:66–72.

LAMBOWITZ, A. M. 1989. Infectious introns. Cell **56**:323–326.

LAMBOWITZ, A. M., and P. S. PERLMAN. 1990. Involvement of aminoacyl-transfer RNA-synthetases and other proteins in group-I and group-II intron splicing. Trends Biochem. Sci. **15**:440–444.

LANDVIK, S., O. ERIKSSON, A. GARGAS, and P. GUSTAFSSON. 1993. Relationships of the genus *Neolecta* (Neolectales ordo nov. Ascomycotina), inferred from 18S rDNA sequences. Syst. Ascomycetum **11**:107–115.

LARSEN, N., G. J. OLSEN, B. L. MAIDAK, M. J. MCCAUGHERY, R. OVERBEEK, T. J. MACKE, T. L. MARSH, and C. R. WOESE. 1993. The ribosomal database project. Nucleic Acids Res. **8**:3021–3023.

LEE, S. B., and J. W. TAYLOR. 1990. Isolation of DNA from fungal mycelia and single spores. Pp. 282–287 *in* M. A. INNIS, D. H. GELFAND, J. J. SNINSKY, and T. J. WHITE, eds. PCR protocols. Academic Press, San Diego.

LEVRA-JULLET, E., A. BOULET, B. SERAPHIN, M. SIMON, and G. FAYE. 1989. Mitochondrial introns al1 and/or al2 are needed for the *in vivo* deletion of intervening sequences. Mol. Gen. Genet. **217**:168–171.

LINTON, D., J. P. CLEWLEY, A. BURNENS, R. J. OWEN, and J. STANLEY. 1994. An intervening sequence in the 16S rRNA gene of the eubacterium *Helicobacter canis*. Nucleic Acids Res. **22**:1954–1958.

MANKIN, A. S., K. G. SKRYABIN, and P. M. RUBTSOV. 1986. Identification of ten additional nucleotides in the primary structure of yeast 18S rRNA. Gene **44**:143.

MICHEL, F., J. A. JACQUIER, and B. DUJON. 1982. Comparison of fungal mitochondrial introns reveals extensive homologies in RNA secondary structure. Biochimie **64**:867–881.

MICHEL, F., and E. WESTHOF. 1990. Modelling of the three-dimensional architecture of group I catalytic introns based on comparative sequence analysis. J. Mol. Biol. **216**:585–610.

MOHR, G., and A. M. LAMBOWITZ. 1991. Integration of a group I intron into a ribosomal RNA sequence promoted by a tyrosyl-tRNA synthetase. Nature **354**:164–167.

NEEFS, J.-M., Y. VAN DE PEER, P. DE RIJK, A. GORIS, and R. DE WACHTER. 1993. Compilation of small ribosomal subunit RNA structures. Nucleic Acids Res. Suppl. **21**:3025–3049.

NISHIDA, H., P. A. BLANZ, and J. SUGIYAMA. 1993. The higher fungus *Protomyces inouyei* has two group I introns in the 18S rRNA gene. J. Mol. Evol. **37**:25–28.

NOLLER, H. F. 1991. Ribosomal RNA and translation. Annu. Rev. Biochem. **60**:191–227.

OAKES, M. I., L. KAHAN, and J. A. LAKE. 1990. DNA-hybridization electron microscopy tertiary structure of 16S rRNA. J. Mol. Biol. **211**:907–918.

OAKES, M. I., and J. A. LAKE. 1990. DNA-hybridization electron microscopy: localization of five regions of 16S rRNA on the surface of 30S ribosomal subunits. J. Mol. Biol. **211**: 897–906.

OLIVEIRA, M. C., and M. A. RAGAN. 1994. Variant forms of a group I intron in nuclear small subunit rRNA genes of the marine red alga *Porphyra spiralis* var. *amplifolia*. Mol. Biol. Evol. **11**:195–207.

PALMER, J. D., and J. M. LOGSDON. 1991. The recent origin of introns. Curr. Opin. Genet. Dev. **1**:470–477.

RAGAN, M. A., C. J. BIRD, E. L. RICE, and R. K. SINGH. 1993. The nuclear ribsosomal RNA gene of the red alga *Hildenbrandia rubra* contains a group I intron. Nucleic Acids Res. **21**:3898.

ROGERS, S. O., S. HONDA, and N. J. BENDICH. 1986. Variation in the ribosomal RNA genes among individuals of *Vicia faba*. Plant Mol. Biol. **6**:339–345.

ROGERS, S. O., Z. H. YAN, M. SHINOHARA, K. F. LOBUGLIO, and C. J. K. WANG. 1993. Messenger RNA intron in the nuclear 18S ribosomal RNA gene of deuteromycetes. Curr. Genet. **23**:338–342.

RUBTSOV, P. M., M. M. MUSAKHANOV, V. M. ZAKHARYEV, A. S. KRAYEV, K. G. SKRYABIN, and A. A. BAYEV. 1980. The structure of the yeast ribosomal RNA genes. I. The complete nucleotide sequence of the 18S ribosomal RNA gene from *Saccharomyces cerevisiae*. Nucleic Acids Res. **8**: 5779–5794.

SOGIN, M. L., and J. D. EDMAN. 1989. A self-splicing intron in the small subunit rRNA gene of *Pneumocystis carinii*. Nucleic Acids Res. **17**:5349–5359.

SOGIN, M. L., H. J. ELWOOD, and J. H. GUNDERSON. 1986*a*. Evolutionary diversity of eukaryotic small-subunit rRNA genes. Proc. Natl. Acad. Sci. USA **83**:1383–1387.

SOGIN, M. L., A. INGOLD, M. KARLOK, H. NIELSEN, and J. ENGBERG. 1986*b*. Phylogenetic evidence for the acquisition of ribosomal RNA introns subsequent to the divergence of some of the major *Tetrahymena* groups. EMBO J. **5**:3625–3630.

SPRINGER, N., W. LUDWIG, R. AMANN, H. J. SCHMIDT, H.-D. GÖRTZ, and K.-H. SCHLEIFER. 1993. Occurrence of fragmented 16S rRNA in an obligate bacterial endosymbiont of *Paramecium caudatum*. Proc. Natl. Acad. Sci. USA **90**:9892–9895.

SUREK, B., U. BEEMELMANNS, M. MELKONIAN, and D. BHATTACHARYA. 1993*a*. *G. spirotaenia* 16S-like small subunit ribosomal RNA. GenBank accession X74753.

———. 1993*b*. *Staurastrum* sp. 16S-like small subunit ribosomal RNA. GenBank accession X74752.

THOMPSON, A. J., and D. L. HERRIN. 1994. A chloroplast group I intron undergoes the first step of reverse splicing into host cytoplasmic 5.8S rRNA. J. Mol. Biol. **236**:4[illegible]468.

TURMEL, M. R., R. GUTELL, J.-P. MERCIER, C. OTIS, and LEMIEUX. 1993. Analysis of the chloroplast large subunit ribosomal RNA gene from 17 *Chlamydomonas* taxa. J. M[illegible] Biol. **232**:446–467.

VAN OPPEN, M. J. H., J. L. OLSEN, and W. T. STAM. 19[illegible] Evidence for independent acquisition of group I intron green algae. Mol. Biol. Evol. **10**:1317–1326.

WAINRIGHT, P. O., G. HINKLE, M. L. SOGIN, and S. STICKEL. 1993. Monophyletic origins of the Metazoa: evolutionary link with fungi. Science **260**:340–342.

WHITE, T. J., T. BRUNS, S. B. LEE, and J. W. TAYLOR. 19[illegible] Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pp. 315–322 *in* M. A. IN[illegible] D. H. GELFAND, J. J. SNINSKY, and T. J. WHITE, eds. P[illegible] protocols. Academic Press, San Diego.

WILCOX, L. W., L. A. LEWIS, P. A. FUERST, and G. L. FLO[illegible] 1991. Group I introns within the nuclear-encoded small subunit rRNA gene of three green algae. Mol. Biol. E[illegible] **9**:1103–1118.

WOESE, C. R. 1987. Bacterial evolution. Microbiol. Rev. [illegible]221–271.

WOODSON, S. A., and T. R. CECH. 1989. Reverse self-splicing of the *Tetrahymena* group I intron: implication for the[illegible]rectionality of splicing and for intron transposition. [illegible] **57**:335–345.

ZECHMAN, F. W., E. C. THERIOT, E. A. ZIMMER, R. L. CH[illegible]MAN. 1990. Phylogeny of the Ulvophyceae (Chlorophy[illegible] cladistic analysis of nuclear-encoded rRNA sequence d[illegible] J. Phycol. **26**:700–710.

MITCHELL SOGIN, reviewing editor

Received May 31, 1994

Accepted September 9, 1994