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,
†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 *Myxocalicium albomigrum* 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; Soin et al. 1996) resulting from additional nucleotides in predicted variable regions (Gray et al. 1981; Hinkle et al. 1994) or in the intergenic spacers (Rogers et al. 1986a). 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 eu-

Key words: ribosomal DNA evolution, 18S rDNA, Group I introns, Ascomycotina, lichen-forming fungi, Lecanorales, Calicilales, 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.
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, polypore 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, polypore 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 NS1-UCB-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 non-coding strands by the dideoxy-labeling method (TAT-Quence 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, 1199, 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>
<thead>
<tr>
<th>Position</th>
<th>Classification</th>
<th>Organism</th>
<th>Lifestyle</th>
<th>Size (nucleotides)</th>
<th>Insertion Type</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>114*</td>
<td>Fungi (Ascomycotina, Lecanorales)</td>
<td>Lecanora dispersa</td>
<td>Lichen symbiont</td>
<td>189</td>
<td>Group I intron</td>
<td>This study; A. Gargas and S. Damberger, unpublished manuscript</td>
</tr>
<tr>
<td>287*</td>
<td>Fungi (Ascomycotina, Lecanorales)</td>
<td>L. dispersa</td>
<td>Lichen symbiont</td>
<td>191</td>
<td>Group I intron</td>
<td>This study; A. Gargas and S. Damberger, unpublished manuscript</td>
</tr>
<tr>
<td>323</td>
<td>Green algae (Chlorophyta, Chlorococcales)</td>
<td>Chlorella sorokiniana</td>
<td>Autotroph</td>
<td>465</td>
<td>[Not reported]</td>
<td>Huss et al. 1993a</td>
</tr>
<tr>
<td>392</td>
<td>Fungi (Ascomycotina, Calicculales)</td>
<td>Calicium tricolor</td>
<td>Lichen symbiont</td>
<td>276</td>
<td>[Not analyzed]</td>
<td>This study</td>
</tr>
<tr>
<td>516</td>
<td>Fungi (Ascomycotina, Calicculales)</td>
<td>Mycocalicum albonigrum</td>
<td>Heterotroph</td>
<td>388</td>
<td>[Not analyzed]</td>
<td>This study</td>
</tr>
<tr>
<td></td>
<td>Fungi (Ascomycotina, Lecanorales)</td>
<td>Porpida cristulata</td>
<td>Lichen symbiont</td>
<td>78</td>
<td>Degenerate</td>
<td>This study</td>
</tr>
<tr>
<td></td>
<td>Green algae (Chlorophyta, Chlorococcales)</td>
<td>Chlorella luteoviridis (A &amp; B)</td>
<td>Autotroph</td>
<td>350, 358</td>
<td>Group I intron</td>
<td>Huss et al. 1993b, 1993c</td>
</tr>
<tr>
<td></td>
<td>Green algae (Chlorophyta, Volvocales)</td>
<td>Chlorella saccharophila</td>
<td>Autotroph</td>
<td>362</td>
<td>[Not reported]</td>
<td>Huss et al. 1993f</td>
</tr>
<tr>
<td></td>
<td>Amoebae (Sarcomastigophora, Amoebida)</td>
<td>Acanthamoeba griffini</td>
<td>Heterotroph</td>
<td>519</td>
<td>Group I intron</td>
<td>Gast et al. 1994</td>
</tr>
<tr>
<td></td>
<td>Naegleria sp.</td>
<td>Incl. mammalian pathogen</td>
<td>7277</td>
<td>Group I intron</td>
<td>Embley et al. 1992</td>
<td></td>
</tr>
<tr>
<td>531</td>
<td>Green algae (Chlorophyta, Volvocales)</td>
<td>Chlamydomonas marcellus</td>
<td>Chloroplast endosymbiont</td>
<td>390</td>
<td>Group I intron</td>
<td>Durocher et al. 1980</td>
</tr>
<tr>
<td>789</td>
<td>Fungi (Ascomycotina, Lecanorales)</td>
<td>L. dispersa</td>
<td>Lichen symbiont</td>
<td>231</td>
<td>[Not analyzed]</td>
<td>This study</td>
</tr>
<tr>
<td>943</td>
<td>Fungi (Ascomycotina, Lecanorales)</td>
<td>L. dispersa</td>
<td>Lichen symbiont</td>
<td>111</td>
<td>Degenerate</td>
<td>This study</td>
</tr>
<tr>
<td></td>
<td>(Ascomycotina, Leotiales)</td>
<td>Spathularia flavida</td>
<td>Heterotroph</td>
<td>300</td>
<td>Group I intron</td>
<td>Landvik et al. 1993</td>
</tr>
<tr>
<td></td>
<td>(Ascomycotina, Diaportheles)</td>
<td>Lencostoma cincta</td>
<td>Plant pathogen</td>
<td>411</td>
<td>[Not reported]</td>
<td>De Wachter et al. 1991</td>
</tr>
<tr>
<td></td>
<td>Fungi (Basidionymycetes, Ustilaginales)</td>
<td>Ustilago maydis</td>
<td>Plant pathogen</td>
<td>411</td>
<td>Group I intron</td>
<td>De Wachter et al. 1992</td>
</tr>
<tr>
<td></td>
<td>Fungi (undetermined)</td>
<td>Protomyces inoyei</td>
<td>Plant pathogen</td>
<td>340</td>
<td>Group I intron</td>
<td>Nishida et al. 1993</td>
</tr>
<tr>
<td></td>
<td>Green algae (Chlorophyta, Chlorococcales)</td>
<td>Dunaliella parva</td>
<td>Autotroph</td>
<td>397</td>
<td>Group I intron</td>
<td>Wilcox et al. 1991</td>
</tr>
<tr>
<td></td>
<td>Amoebae (Sarcomastigophora, Amoebida)</td>
<td>Dunaliella salina</td>
<td>Autotroph</td>
<td>381</td>
<td>Group I intron</td>
<td>Wilcox et al. 1991</td>
</tr>
<tr>
<td></td>
<td>Acanthamoeba lentisculata</td>
<td>Heterotroph</td>
<td>656</td>
<td>Group I intron</td>
<td>Gast et al. 1994</td>
<td></td>
</tr>
<tr>
<td>956</td>
<td>Acellular slime molds (Myxomycetes)</td>
<td>Didymium iridis</td>
<td>Heterotroph</td>
<td>1436</td>
<td>Group I intron</td>
<td>Johansen and Vogt 1994</td>
</tr>
<tr>
<td>1046</td>
<td>Fungi (Ascomycotina, Lecanorales)</td>
<td>Cladonia chlorophaea</td>
<td>Lichen symbiont</td>
<td>225</td>
<td>Group I intron</td>
<td>DePriest and Been 1992</td>
</tr>
<tr>
<td></td>
<td>C. chlorophaea</td>
<td>Lichen symbiont</td>
<td>217</td>
<td>Group I intron</td>
<td>DePriest and Been 1992</td>
<td></td>
</tr>
<tr>
<td></td>
<td>L. dispersa</td>
<td>Lichen symbiont</td>
<td>231</td>
<td>Group I intron</td>
<td>This study; A. Gargas and S. Damberger, unpublished manuscript</td>
<td></td>
</tr>
<tr>
<td>Position</td>
<td>Organism Type</td>
<td>Organism</td>
<td>Lifestyle</td>
<td>Intron Type</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>----------</td>
<td>--------------</td>
<td>----------</td>
<td>-----------</td>
<td>-------------</td>
<td>-----------</td>
<td></td>
</tr>
<tr>
<td>1052</td>
<td>Green algae</td>
<td>Ankistrodesmus stipitatus</td>
<td>Autotroph</td>
<td>394</td>
<td>Group I intron</td>
<td>Dávila-Aponte et al. 1991</td>
</tr>
<tr>
<td>1199</td>
<td>Fungi</td>
<td>C. sorokiniana</td>
<td>Autotroph</td>
<td>428</td>
<td>[Not reported]</td>
<td>Huss et al. 1993a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chlorella luteoviridis (B)</td>
<td>Autotroph</td>
<td>421</td>
<td>Group I intron</td>
<td>Huss et al. 1993c</td>
</tr>
<tr>
<td>1210</td>
<td>Fungi</td>
<td>C. chlorophaea</td>
<td>Lichen symbiont</td>
<td>266</td>
<td>Group I intron</td>
<td>DePriest and Been 1992</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Leuconostoma cinerea</td>
<td>Plant pathogen</td>
<td>&gt;250</td>
<td>[Not analyzed]</td>
<td>This study</td>
</tr>
<tr>
<td>1389</td>
<td>Fungi</td>
<td>L. dispersa</td>
<td>Lichen symbiont</td>
<td>212</td>
<td>Group I intron</td>
<td>DePriest and Been 1992</td>
</tr>
<tr>
<td>1506</td>
<td>Fungi</td>
<td>P. inovei</td>
<td>Plant pathogen</td>
<td>390</td>
<td>Group I intron</td>
<td>Sogin and Edman 1989</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hymenoscyphus ericae</td>
<td>Root endophyte</td>
<td>ca. 350</td>
<td>Group I intron</td>
<td>Nishida et al. 1993</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cenococcum geophilum</td>
<td>Mycorrhizal symbiont</td>
<td>459</td>
<td>Group I intron</td>
<td>Rogers et al. 1993</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Phialophora americana</td>
<td>Heterotroph</td>
<td>67</td>
<td>Degenerate</td>
<td>Rogers et al. 1993</td>
</tr>
<tr>
<td></td>
<td>Green algae</td>
<td>Chlorella ellipsoidea</td>
<td>Autotroph</td>
<td>441.442</td>
<td>Group I intron</td>
<td>Huss et al. 1992; Aimi et al. 1994</td>
</tr>
<tr>
<td>1512</td>
<td>Green algae</td>
<td>Chlorella mirabilis</td>
<td>Autotroph</td>
<td>488</td>
<td>Group I intron</td>
<td>Huss et al. 1993d</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mougeotia scalaris</td>
<td>Autotroph</td>
<td>535</td>
<td>[Not reported]</td>
<td>Huss et al. 1993e</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Geniculatella sprotaeana</td>
<td>Autotroph</td>
<td>370</td>
<td>[Not reported]</td>
<td>Surek et al. 1993a</td>
</tr>
<tr>
<td></td>
<td>Red algae</td>
<td>Porphyrula spiralis var. amplifolia</td>
<td>Autotroph</td>
<td>406</td>
<td>[Not reported]</td>
<td>Surek et al. 1993b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dunaliella parva</td>
<td>Autotroph</td>
<td>419</td>
<td>Group I intron</td>
<td>Ragan et al. 1993</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Characium saccatum</td>
<td>Autotroph</td>
<td>447</td>
<td>Group I intron</td>
<td>Wilcox et al. 1991</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Urospora penicilliformis</td>
<td>Autotroph</td>
<td>452</td>
<td>Group I intron</td>
<td>Van Oppen et al. 1993</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C. chlorophaea</td>
<td>Lichen symbiont</td>
<td>228</td>
<td>Group I intron</td>
<td>DePriest and Been 1992</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C. chlorophaea</td>
<td>Lichen symbiont</td>
<td>210</td>
<td>Group I intron</td>
<td>DePriest and Been 1992</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L. dispersa</td>
<td>Lichen symbiont</td>
<td>212</td>
<td>Group I intron</td>
<td>This study; A. Gargas and S. Damberger, unpublished manuscript</td>
</tr>
</tbody>
</table>

**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 termini.
‡ 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 position 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 indicated 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 increases 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; Zeichman 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 marussia (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
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...
A similar process of reverse transcription and homologous recombination with an intron-lacking sequence may lead to precise deletion of introns (Dujon 1989; Levra-Julet 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. 1986b; 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 (Arnhem et al. 1980; Arnhem 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


Literature Cited


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


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

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


SOGIN, M. L., A. INGOLD, M. KARLOK, H. NIELSEN, and J. ENGBERG. 1986b. 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.


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


MITCHELL SOGIN, reviewing editor

Received May 31, 1994

Accepted September 9, 1994