Nucleotide sequences of chloroplast 4.5 S ribosomal RNA from a leafy liverwort, *Jungermannia subulata*, and a thalloid liverwort, *Marchantia polymorpha*

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We found 4.5 S rRNAs in chloroplasts from a leafy liverwort, *Jungermannia subulata*, as well as a thalloid liverwort, *Marchantia polymorpha*, and determined their complete nucleotide sequences. That from *J. subulata* was 100 nucleotides long and that from *M. polymorpha* was 103. They were strongly homologous with those from higher plants, a fern, *Dryopteris acuminata*, and a moss, *Mnium rugicum*. This similarity of chloroplast 4.5 S rRNA sequences may be used for construction of the phylogenic tree among the green plants.

Chloroplast 4.5 S rRNA *Liverwort* *rRNA nucleotide sequence*

1. INTRODUCTION

Chloroplast ribosomes from higher plants contain 3 kinds of ribosomal RNAs: 5 S, 16 S and 23 S rRNAs [1]. Bowman and Dyer, and other investigators, independently described a unique component, 4.5 S rRNA, in chloroplast ribosomes from flowering plants [2–4], but they could not detect it in similar preparations from a cyanobacterium, a fern, liverworts, and mosses [2]. However, Takaiwa et al. [5] found 4.5 S rRNA in the chloroplasts from a fern, *Dryopteris acuminata,* and determined the nucleotide sequence. Recently Troitsky et al. [6] found and sequenced 4.5 S rRNA in a moss, *Mnium rugicum*. We have now found and sequenced 4.5 S rRNAs in two lower plants, a leafy liverwort, *Jungermannia subulata,* and a thalloid liverwort, *Marchantia polymorpha,* which differ from each other in morphology and belong below the ferns *(Pteridophyte)* in the phylogenic tree.

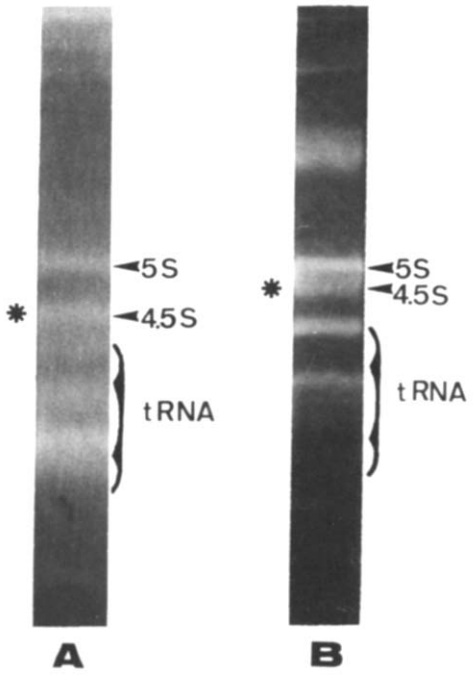

Fig.1. Electrophoretic separation of low molecular mass rRNAs on 10% polyacrylamide gels for *J. subulata* (A), and *M. polymorpha* (B).

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2. MATERIALS AND METHODS

Chloroplasts were prepared from cell suspension cultures of *J. subulata* and *M. polymorpha* by the procedure in [7]. RNA preparation, labeling of 3'- and 5'-ends with ^{32}P, and RNA sequencing were done by the procedures described [8]. The nucleotide sequence from the 3'-end using the 3'-^{32}P-

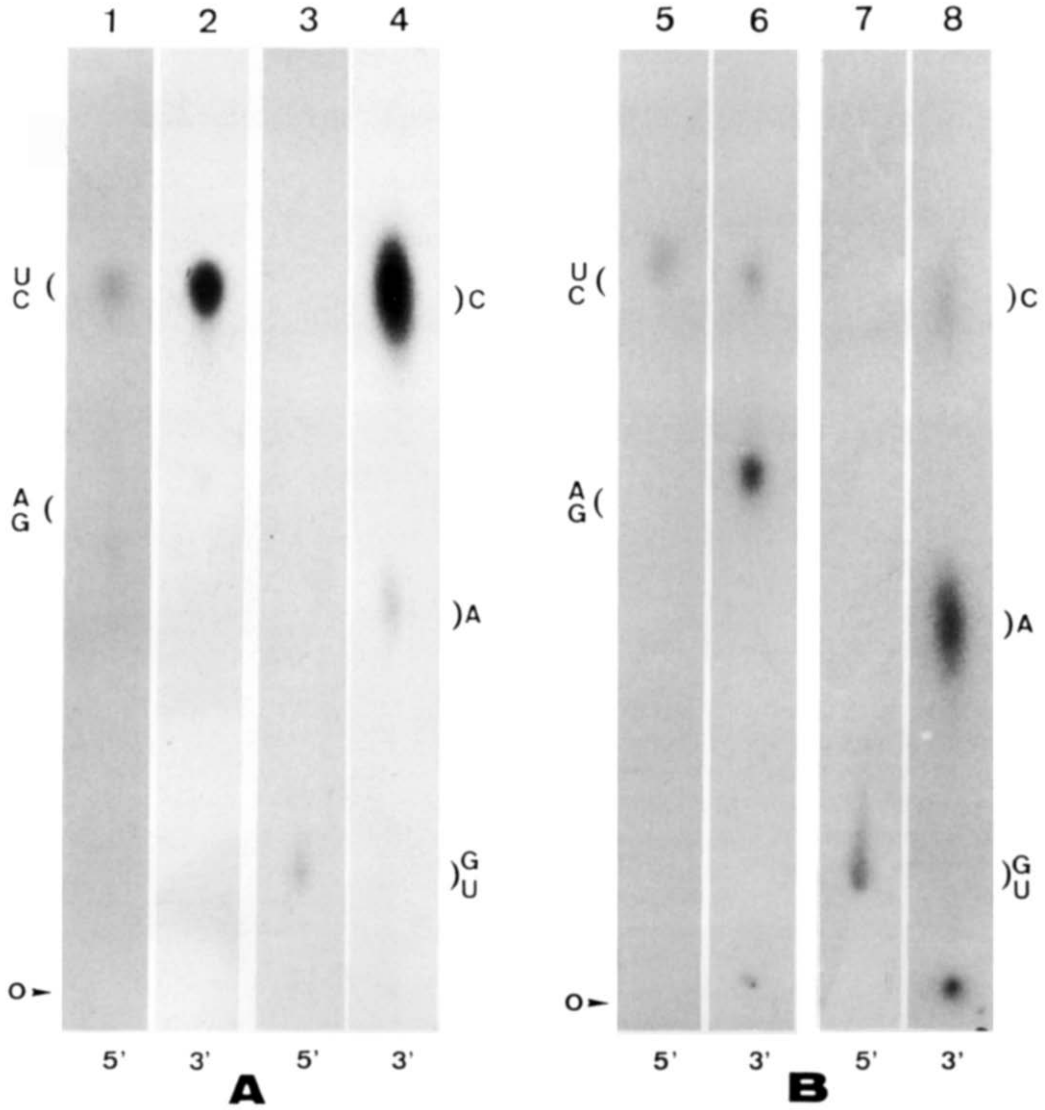

Fig.2. The 5'- and 3'-end base analyses of *J. subulata* and *M. polymorpha* 4.5 S rRNA. The bases of the (5'- and 3'-^{32}P)-labeled ends were determined by PEI-cellulose thin-layer chromatography. A complete nuclease P_1 digest of the chloroplast (5'-^{32}P)- or RNase T_2 digest of the chloroplast (3'-^{32}P)-labeled 4.5 S rRNA was developed on a PEI-cellulose plate (Merck, 20×20 cm) in 1 M LiCl (lanes 1,2,5,6). Under these conditions pyrimidine nucleotides (CMP, UMP) can migrate faster than purine nucleotides (AMP, GMP), but the pyrimidine nucleotides cannot be separated from each other, nor can the purine nucleotides. On the other hand, in 1 M acetic acid solution the nucleotides CMP and AMP migrate faster than GMP and UMP (lanes 3,4,7,8). Thus the bases of 5'- and 3'-ends of *J. subulata* were determined to be U (lanes 1,3) and C (lanes 2,4), respectively. The 5'-end base of *M. polymorpha* 4.5 S rRNA was determined to be U (lanes 5,7), but the base of 3'-end was either C or A (lanes 6,8). This caused difficulty in RNA sequencings from the 3'-end of *M. polymorpha* 4.5 S rRNA.

labeled 4.5 S rRNA, especially from *M. polymorpha* chloropasts, could not be read because the 4.5 S rRNA preparation contained 2 species of molecules different in the 3′-end. Therefore, we sequenced the DNA fragment containing the chloroplast 4.5 S rRNA gene by M13 cloning and sequencing [9].

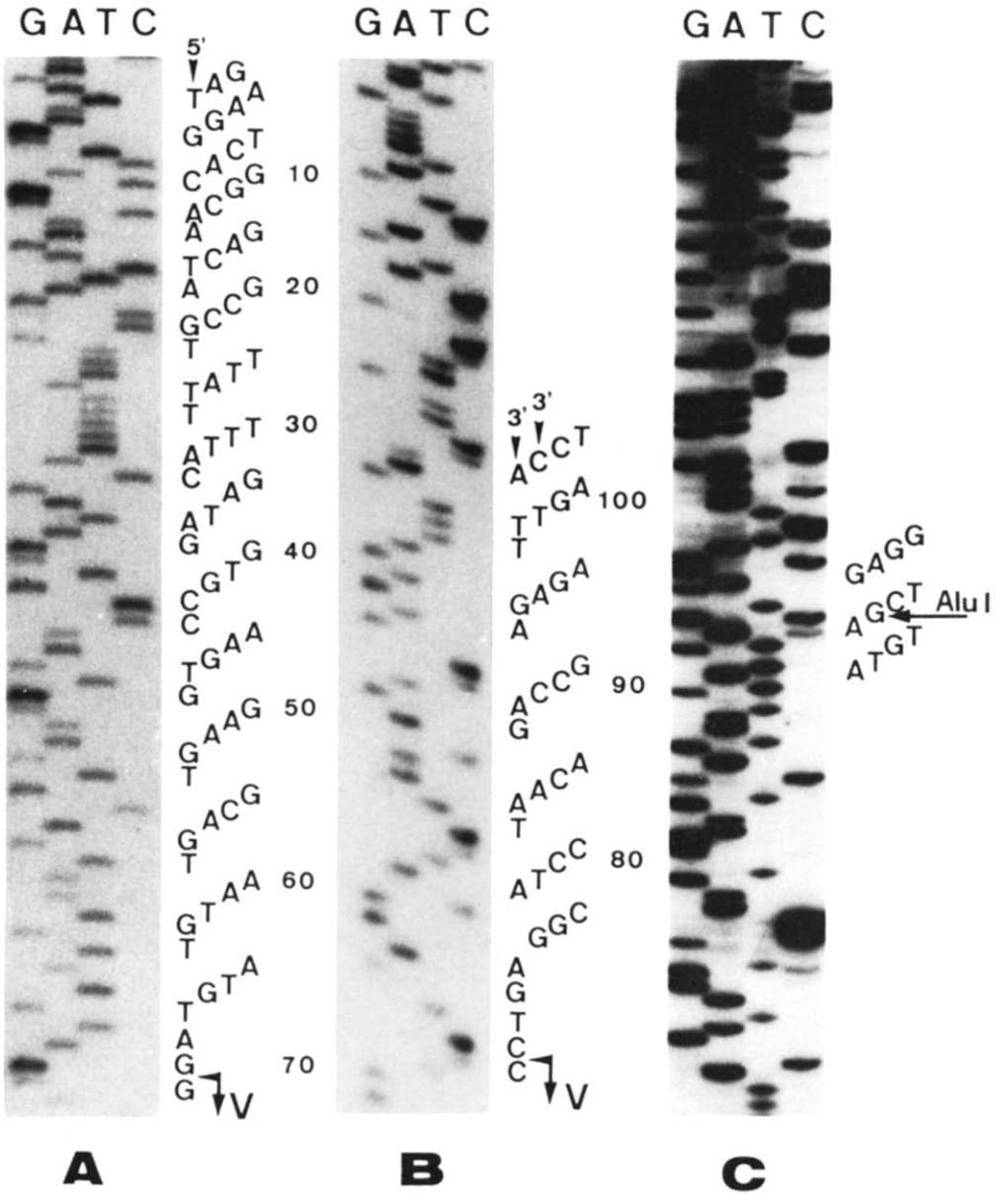

Fig.3. Nucleotide sequences of *M. polymorpha* chloroplast 4.5 S rRNA gene. The site of the 4.5 S rRNA gene was determined by Southern hybridization to be on the 2 *Alu*I-digested restriction fragments derived from a clone containing the 4.5 S rRNA gene. (A) DNA sequence from the *Alu*I site to the 5′-end of the 4.5 S rRNA gene. V, M13 cloning vector DNA sequence. (B) DNA sequence from the *Alu*I site to the 3′-end of the 4.5 S rRNA gene. (C) DNA sequence of the *Hin*fI restriction fragment which includes the *Alu*I site in 4.5 S rRNA gene. This sequence shows the continuity of two *Alu*I fragments determined in A and B.

3. RESULTS AND DISCUSSION

Nucleotide sequences of chloroplast 4.5 S rRNA are known for the higher plants wheat [4], maize [10], tobacco [11], spinach [12], duckweed [13], a fern, *D. acuminata* [5], and a moss, *M. rugicum* [6]. Chloroplast 4.5 S rRNA had not been reported in liverworts, but we found and completely sequenced 4.5 S rRNAs in the chloroplasts from both a leafy liverwort, *J. subulata,* and a thalloid liverwort, *M. polymorpha* (fig.1). In combination with the results of chemical digestions, the complete nucleotide sequence of the 4.5 S rRNA from *J. subulata* chloroplasts was determined by partial digestions with base-specific RNases and alkali. The 4.5 S rRNA has 100 nucleotides and its 5′-end is not phosphorylated. The nucleotide sequencing of the chloroplasts 4.5 S rRNA by the method of chemical digestions from *M. polymorpha* has not been successful because of the presence of 2 species of molecules different at the 3′-end (fig.2). The physical maps of the chloroplast DNA from *M. polymorpha* have been constructed [14]. The site of the 4.5 S rRNA gene was determined by Southern hybridization using ^{32}P-labeled 4.5 S rRNA from *M. polymorpha* chloroplasts as the probe. The DNA fragment containing the 4.5 S rRNA gene was sequenced by M13 cloning and sequencing (fig.3).

A comparison of the nucleotide sequences of chloroplast 4.5 S rRNA from liverworts, a moss [6], a fern [5], tobacco [11], spinach [12], duckweed [13], wheat [4] and maize [10] is shown in fig.4. As a unique feature, 3 nucleotides are missing between positions 31 and 33 in 4.5 S rRNA from the leafy liverwort, *J. subulata.* The nucleotide sequences of chloroplast 4.5 S rRNA from a leafy liverwort, *J. subulata,* and a thalloid liverwort, *M. polymorpha,* were highly homologous to each other (similarity of 95 nucleotides out of 103 nucleotides; a deletion of any nucleotide is taken into account for one nucleotide substitution) as well as to those from a moss, *M. rugicum* (91/100 and 97/103 similarity, respectively), a fern (90/100

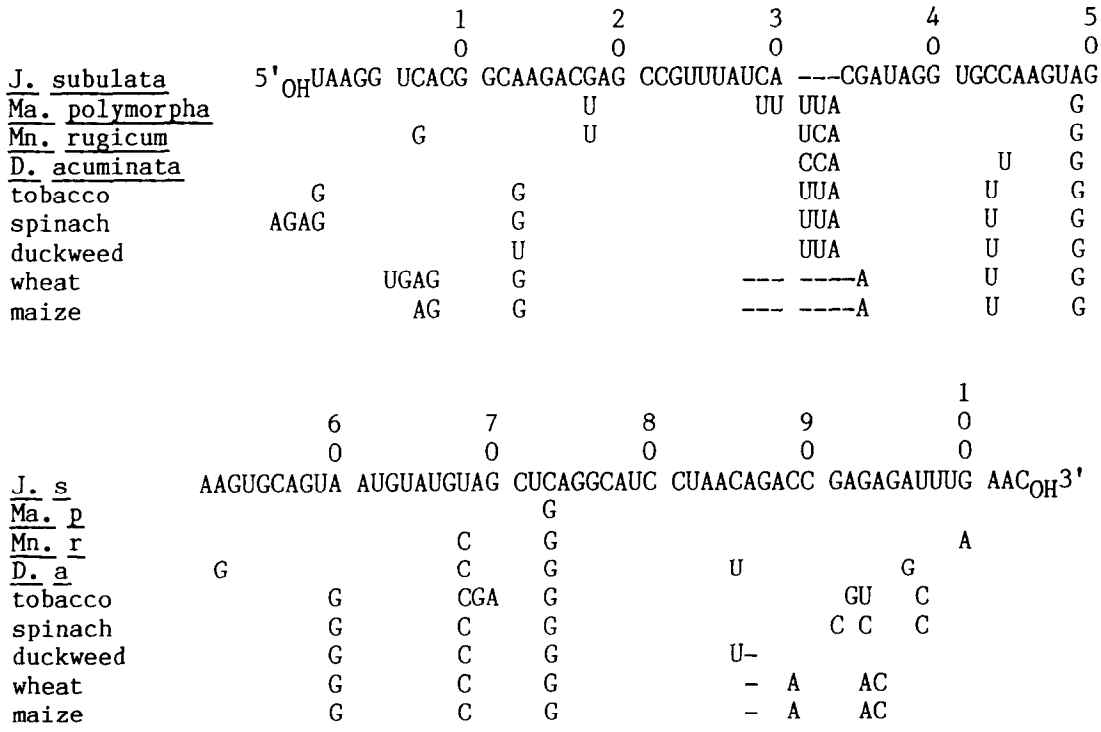

Fig.4. The nucleotide sequences of chloroplasts 4.5 S rRNA of liverworts, a moss [6], a fern [5], tobacco [11], spinach [12], duckweed [13], wheat [4], and maize [10]. Nucleotides identical to those found in the chloroplast 4.5 S rRNA sequence from a liverwort, *J. subulata,* were not typed. Bars indicate missing nucleotides.

and 93/103 similarity, respectively), and a higher plant, for example, tobacco (85/100 and 90/103, respectively). These results may give some information on chloroplast divergence in green plants. Although *Chlamydomonas reinhardii* [15] and *Euglena gracilis* [16] do not have 4.5 S rRNA in their chloroplasts, additional information on chloroplast 4.5 S rRNA sequences from a variety of green plants may facilitate the investigation on the molecular evolution and the divergence of chloroplasts.

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