The nucleotide sequence of chloroplast 4.5 S rRNA from *Mnium rugicum (Bryophyta)*: mosses also possess this type of RNA

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The complete nucleotide sequence of chloroplast 4.5 S rRNA from the moss *Mnium rugicum* was determined to be $_{OH}$UAAGGUGACGGCAAGACUAGCCGUUUAUCAUCACGAUAGGUGCCAAGUGGAAGUGCAGUAAUGUAUGCAGCUGAGGCAUCCUAACAGACCGAGAGAUUUAAAC$_{OH}$. The sequence differs from that of a fern *Dryopteris acuminata* and of angiosperms 4.5 S rRNA by 8 and 9–14%, respectively. The strong conservation of 4.5 S rRNA in the course of evolution ensures its use for reconstruction of the phylogenetic relations between the higher taxa of plants.

Mnium rugicum *4.5 S rRNA* *Chloroplast* *Nucleotide sequence* *Phylogeny*

1. INTRODUCTION

Initially, a 4.5 S rRNA was considered to be a component of chloroplast ribosomes of flowering plants only [1–3]. 4.5 S rRNA genes were mapped on the circular chloroplast DNA of tobacco [4–6], spinach [2,7], maize [8,9], broad bean [10] and duckweed *Spirodela oligorhisa* [11]. A comparison of nucleotide sequences revealed a substantial degree of homology between 4.5 S rRNA and the 3′-terminal part of prokaryotic 23 S rRNA [9,12–15]. Authors in [1] were unable to detect chloroplast 4.5 S rRNA in a number of more primitive plants – ferns, liverwort and blue-green algae. However, it was recently found in a fern, *Dryopteris acuminata* [16]. Thus, the distribution of 4.5 S rRNA in the plant kingdom remained obscure.

In search of chloroplast 4.5 S rRNA in different plant taxa, we found it in several Bryophyta species and sequenced the 4.5 S rRNA of the moss *Mnium rugicum* (class *Musci*).

2. MATERIALS AND METHODS

Plants were collected in their native habitats near Moscow. For RNA isolation, plant material was frozen in liquid nitrogen, powdered in a coffee mill with pieces of solid CO_2 and suspended in a 0.05 M CH_3COONa, 0.14 M NaCl, 10 mM mercaptoethanol (pH 5.1) buffer, containing 0.3% sodium dodecyl sulphate (SDS), 0.05% polyvinylsulphate, 0.3% polyvinylpolypyrrolidone to which 1/5 vol. of bentonite suspension (0.1 mg/ml) was added; RNA was isolated by the hot phenol method [17]. After 3 deproteinizations, the aqueous phase was adjusted to 0.3 M CH_3COONa (pH 5.0) and RNA precipitated overnight at −20°C with an equal volume of isopropanol. The precipitate was washed with ethanol and reprecipitated with 3 vols of ethanol from 0.3 M CH_3COONa (pH 5.0). Total cellular RNA thus obtained was enzymatically labeled by ligation with [5′-^{32}P]pCp at the 3′-end [18]. [5′-^{32}P]pCp was prepared from [γ-^{32}P]ATP using T4 polynucleotide kinase [18]. The labeled material was electrophoresed on 8% polyacryl-

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amide gel (20 × 40 × 0.1 cm) in 0.05 M Tris–borate, 0.001 M EDTA buffer (pH 8.3) and 7 M urea. After autoradiography, the band corresponding to the 4.5 S rRNA was excised from the gel, RNA was eluted and its purity checked by re-electrophoresis.

The nucleotide sequence was determined by the chemical modification and degradation method of [19]. The sequencing gels 12–20% were 40 and 60 cm long, 0.04 cm thick. They were run hot at 1.0–3.5 kV to avoid possible zone compression. The 5′-end terminal nucleotide was identified by PEI-cellulose thin-layer chromatography in 1.2 M LiCl (pH 2.3) after complete digestion of the 4.5 S [5′-^{32}P]rRNA with P1 nuclease. For 5′-^{32}P-labeling, 4.5 S rRNA isolated by electrophoresis on polyacrylamide gel using the 4.5 S [3′-^{32}P]RNA as a marker was incubated with [γ-^{32}P]ATP and T4 polynucleotide kinase [18].

3. RESULTS AND DISCUSSION

Fig.1 shows the separation of the 3′-end labeled RNAs from *M. rugicum* after electrophoresis on 8% polyacrylamide gel. Since the reaction with [5′-^{32}P]pCp was performed without preliminary dephosphorylation, only RNAs with free 3′-OH groups are detected by autoradiography. One component, marked by an asterisk, migrated between 5 S rRNA and tRNA. Electrophoretic analysis of samples of total RNA of a moss *Sphagnum sp.* belonging to the same class *Musci* and *Marchantia polymorpha* from another class of *Bryophyta* (*Hepaticae*) revealed components with the same mobility equal to the mobility of chloroplast 4.5 S rRNA from dicotyledon plants (not shown).

Some of the autoradiographs illustrating the determination of the *M. rugicum* 4.5 S RNA sequence are shown in fig.2,3. The complete nucleotide sequence of the electrophoretically purified *M. rugicum* 4.5 S rRNA consists of 103 residues (table 1). Comparison of the sequence established with that already known for chloroplast 4.5 S rRNA revealed a very high degree of homology. This result allows us to conclude that 4.5 S RNA *M. rugicum* originates from chloroplast ribosomes.

A difference matrix of the 4.5 S rRNA sequences is given in table 2 (a deletion of any region

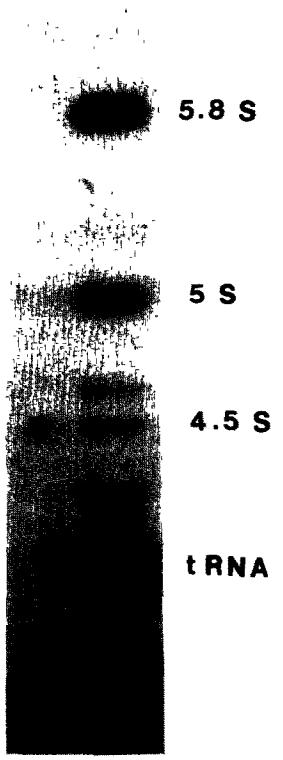

Fig.1. Electrophoretic separation on 8% polyacrylamide gel of 3′-end-labeled RNAs from *M. rugicum*.

independently of the number of nucleotides in it is equalized to one nucleotide substitution). Evidently, the structure of these molecules is highly conservative: *Bryophyta* is one of the oldest taxa of land plants, at least of siluric origin [24]. Although the pattern of evolution of gametophytic and sporophytic plants differed drastically and corresponding evolutionary lineages diverged several hundred million years ago, the nucleotide sequences of 4.5 S rRNA from *M. rugicum* and angiosperms are 86–91% homologous.

Chloroplast 4.5 S rRNA was found in all higher plants investigated so far – dicotyledon and monocotyledon angiosperms, ferns, mosses, liverworts, club-mosses (not shown). It is absent in *Chlamydomonas reinhardii* [25] and *Euglena gracilis* [26]. So, it seems that chloroplast 4.5 S rRNA is an innovation characteristic of all major phyla of land plants. Taking into account the pronounced evolutionary stability of its structure,

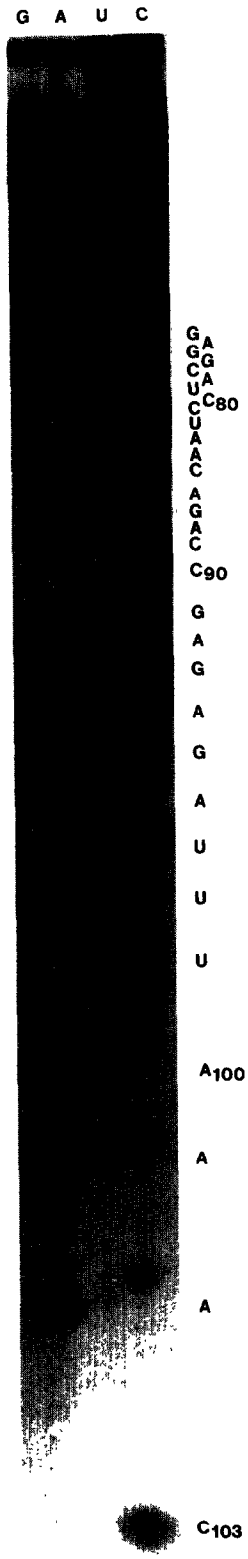

Fig.2. A 20% sequencing gel of 3′-end-labeled *M. rugicum* 4.5 S RNA.

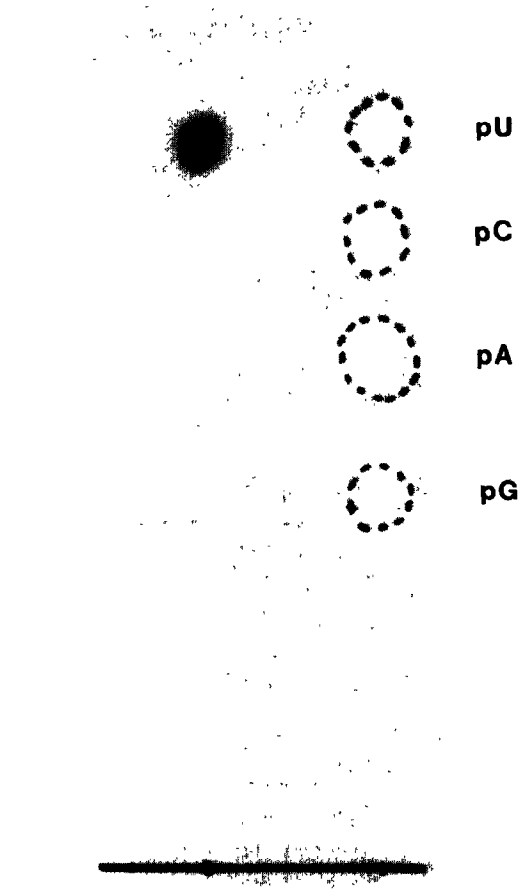

Fig.3. Identification of the 5′-terminal nucleotide of *M. rugicum* 4.5 S RNA by PEI-cellulose thin layer chromatography in 1.2 M LiCl (pH 2.3) of complete P1 nuclease digest of 4.5 S [5′-^{32}P]RNA.

chloroplast 4.5 S rRNA may be a good tool for deducing phylogenetic relations between higher taxa of land plants.

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Table 1

Alignment of chloroplast 4.5 S rRNA sequences from *Mnium rugicum* (this work), *Dryopteris acuminata* [10], *Spinacia oleracea* [20], *Nicotiana tabacum* [6,21], *Spirodela oligorhisa* [22], *Triticum aestivum* [23] and *Zea mays* [8,9]

	10	20	30	40	50
Mnium	UAAGGU–GACGGCAAGACUAGCCGUUUAUCAUCACGAUAGGUGCCAAGUGG				
Dryopteris	C	G	C	U	
Spinacia	AGAG C	G G	U	U	
Nicotiana	G C	G G	U	U	
Spirodela	C	U G	U	U	
Triticum	GAG	G G	-------A	U	
Zea	AG	G G	-------A	U	

	60	70	80	90	100	
Mn.	AAGUGCAGUAAUGUAUGCAGCUGAGGCAUCCUAACAGACCGAGAGAUUUAAAC					3′
Dr.	G		U	G	G	
Sn.	G			C C	C	G
Nc.	G			GU	C	G
Sr.	G		U–			G
Tr.	G		– A	AC		G
Zea	G		– A	AC		G

Bars denote gaps

Table 2

Matrix of nucleotide differences between chloroplast 4.5 S rRNA sequences (in %)

	Mn.	*Dr.*	*Sn.*	*Nc.*	*Sr.*	*Tr.*	*Zea*
Mnium rugicum		8	12	11	9	14	13
Dryopteris acuminata	8		14	13	9	16	14
Spinacia oleracea	12	14		4	8	13	12
Nicotiana tabacum	11	13	4		7	12	11
Spirodela oligorhisa	9	9	8	7		10	9
Triticum aestivum	14	16	13	12	10		1
Zea mays	13	14	12	11	9	1	

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