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Comparative study on the evolution of chloroplast ribosomal 5 S RNA of a living fossil plant, *Cycas revoluta* Thunb

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The complete nucleotide sequence of *Cycas revoluta* Thunb chloroplast 5 S rRNA was determined. It consists of 122 nucleotides. This is the only known 5 S rRNA sequence in Gymnospermae. It is highly homologous with chloroplast 5 S rRNA of higher plants (92–97%), but less homologous (about 54%) with those of lower plants. There is however 67% homology between *Cycas* and a procaryote *A. nidulans*. The chloroplast 5 S rRNAs of Angiospermae are nearly identical with each other (95–97%). *S. oligorhize* and *L. minor* have 100% homology among themselves. We have constructed a phylogenic tree of 5 S rRNA sequences from fifteen plant chloroplasts. The result suggests that the emergence of algae occurred at an early stage of plant chloroplast evolution and that green plants originated from green algae. This is in agreement with the classical view and other theories of molecular evolution. However there is no common ancestor in the case of Bryophyta and ferns. Among the Angiospermae, a precise evolutionary process cannot be deduced because the K_{nuc} values among the species are very close to each other.

Chloroplast 5S rRNA sequence; Phylogenic tree

1. INTRODUCTION

The ribosomal 5 S RNA is a stable component which is highly conserved in primary and secondary structures of the large ribosomal subunit. Comparison of their sequences has proven to be a useful tool in the study of evolutionary relationship among species [1,2]. The first attempt to use 5 S rRNA as a phylogenic marker was made in 1975 by Hori [3]. The principle of constructing the 5 S rRNA phylogenic tree is the alignment of 5 S rRNA sequences of different species according to the highest homology. The evolutionary distances (K_{nuc}) between two sequences were compared and calculated. Using K_{nuc} values the branching order and the relative evolutionary distance can be determined [4,5]. The 5 S rRNA phylogenic tree of eubacteria [6,7], fungi [8], metazoa [9,10] and plant cytoplasts [11] have been reported. However, the 5 S rRNA phylogenic tree of plant chloroplasts still awaits construction. In this paper, we report on a *Cycas* chloroplast 5 S rRNA sequence and the construction of a phylogenic tree for fifteen plant chloroplast 5 S rRNAs.

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2. MATERIALS AND METHODS

The chloroplast 5 S rRNA of *Cycas revoluta* Thunb was isolated by the phenol method and purified by polyacrylamide gel electrophoresis [12,13]. The *Cycas* chloroplast 5 S rRNA sequence was determined both by the chemical method of Peattie [14] and the enzymatic method of Donis-Keller et al. [15,16]. After aligning 5 S rRNA sequences by Erdmann and Wolters' method [17], the sequence homology among *Cycas* chloroplast 5 S rRNA, other plant chloroplast 5 S rRNAs and cyanobacterial 5 S rRNA was studied. The K_{nuc} values between two sequences were calculated by the Osawa and Hori [4] equation, $K_{nuc} = -3/4 \ln(1-4/3\lambda)$, where λ is the fraction of sites which differ from each other. The K_{nuc} values obtained can be

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used for the determination of the branching order and the relative evolutionary distance in the construction of a phylogenic tree. Using 1/2 K_{nuc} values, we constructed a phylogenic tree for fifteen plant chloroplast 5 S rRNAs.

3. RESULTS AND DISCUSSION

In all, 14 chloroplast 5 S rRNA sequences have been reported in the literature [17–19,22]. Fig.1A shows the secondary structure of the *Cycas* chloroplast 5 S rRNA containing 122 nucleotides. Like many chloroplast 5 S rRNAs, the *Cycas* chloroplast 5 S rRNA has structural properties common to the procaryotic 5 S rRNAs and is different from that of its cytoplast partner. For example, the chain length between the universal positions U41–G45 and G70–A77 are three and six nucleotides, respectively. Between positions 42–48, it has the converse sequence PyCGAAC and a hexanucleotide palindromic sequence (A84GGGGA) both of which are the same as procaryotic 5 S rRNAs. The length of L4 is two base pairs and there is no loopout position in the helix L5 (fig.1A). In the *Cycas* cytoplast 5 S rRNA reported in the literature [11,13], the length of L4 is 7 base pairs and there is a loopout position (U) in helix L5 (fig.1B). The *Cycas* chloroplast 5 S rRNA has the unique structural feature common to green plant chloroplast 5 S rRNAs in having more than four contiguous base pairs in helix L3 proximal to the hairpin loop I3 bounded by the helix (fig.1A). Using Erdmann and Wolters' method, we aligned fifteen 5 S rRNA sequences of chloroplasts and the procaryotic cyanobacterium *A. nidulans* (fig.2). The *Cycas* chloroplast 5 S rRNA is the only 5 S rRNA determined in Gymnospermae. 5 S rRNA sequence homology is compared by alignment. The advantage of Erdmann and Wolters' method is the universal comparability of 5 S rRNA sequences. Table 1 compares Cycas chloroplast 5 S rRNA sequences with those of cyanobacteria and other plant chloroplasts. The result suggests that *Cycas* is highly homologous in 5 S rRNA sequences with chloroplasts of higher plants (Angiospermae, 92–97%), less homologous (50–58%) with those of lower plants (Euglenophyta and Chlorophyta). However, there is 67% homology between *Cycas* and cyanobacteria (e.g. *A. nidulans)* which must for the present remain unexplained. Chloroplast 5 S rRNAs of Angiospermae are nearly identical (92–97%), while some, such as *S. oligorhize* and *L. minor* have even 100% homology between themselves.

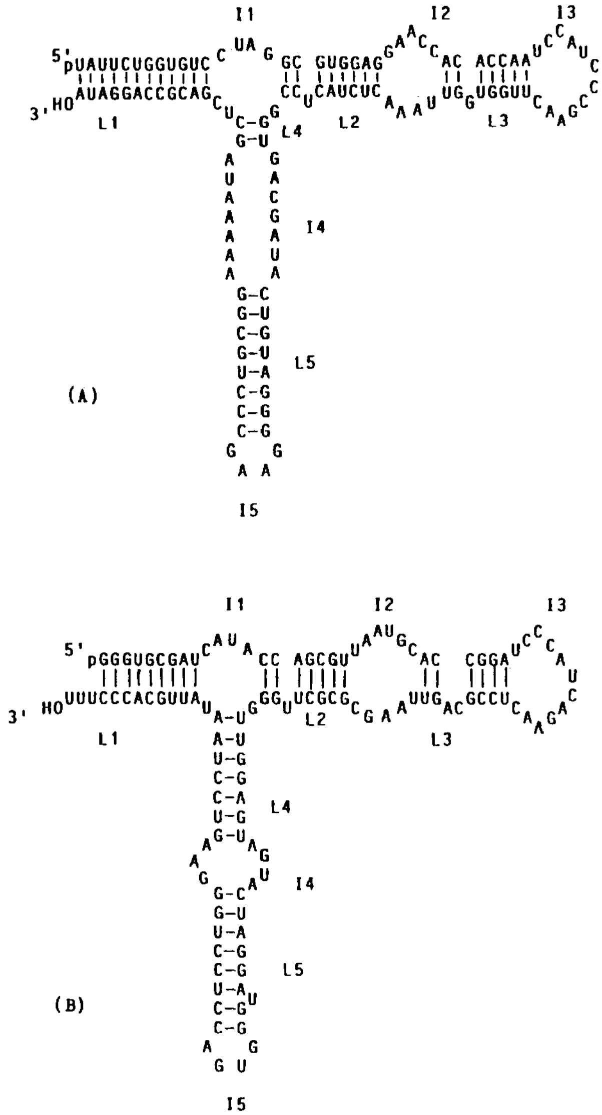

Fig.1. (A) Secondary structure of the chloroplast 5 S rRNA from *Cycas revoluta* Thunb; (B) secondary structure of the cytoplastic 5 S rRNA from *Cycas revoluta* Thunb. L1–L5, five helices; I1, multibranched loop; I2 and I4, interior loop; I3 and I5, hairpin loop.

Plant chloroplast 5 S rRNAs have an ancestor in common with procaryotic cyanobacteria and an evolutionary process different from plant cytoplastic 5 S rRNAs [11]. This result is in agreement with the endosymbiotic theory for chloroplast origin. In fig.3, we constructed a

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                  10         20         30          40         50         60
(1)     UCCUGGUG UCUAU-GGCGG UAUGGAACCA CUCUGACCCCA UCCCGAACUC AGUUGUGAAA
(2)     UUAGGGUG CUCUU-GUCUU UGUGGAUCCA C-UUAAA-ACA UUUCGAACUU GCAAGUUAAA
(3)       AGGGUG CUCUU-GUCUU UAUGGAUCCA C-UUAAA-ACA UUUCGAACUU GCAAGUUAAA
(4)       AGGGUG CUCUU-GUCUU UAUGGAUCCA C-UUAAA-ACA UUUCGAACUU GCAAGUUAAA
(5)      CUUGGUG CUCUUUGCUCA GUUGGACCCA CACCAAU-CCA UCCCGAACUU GGUUGUGAAA
(6)    AUCCUGGUG UUCUU-GUUUU UAUGGAACCA CGCUAAC-CCA UCUCGAACUU AGUUGUGAAA
(7)     UUCUGGUG UCUCA-GGCGU GGAGGAACCA CACCAAU-CCA UCCCGAACUU GGUGGUGAAA
(8)     UUCUGGUG UCUUA-GGCGU AGAGGAACCA CACCAAU-CCA UCCCGAACUU GGUGGUGAAA
(9)   UAUUCUGGUG UCCCA-GGCGU AGAGGAACCA CACCGAU-CCA UCUCGAACUU GGUGGUGAAA
(10)  UAUUCUGGUG UCCUA-GGCGU GGAGGAACCA CACCAAU-CCA UCCCGAACUU GGUGGUUAAA
(11)  UAUUCUGGUG UCCUA-GGCGU AGAGGAACCA CACCAAU-CCA UCCCGAACUU GGUGGUUAAA
(12)  UAUUCUGGUG UCCUA-GGCGU AGAGGAACCA CACCAAU-CCA UCCCGAACUU GGUGGUUAAA
(13)  UAUUCUGGUG UCCUA-GGCGU AGAGGAACCA CACCAAU-CCA UCCCGAAUUU GGUGGUUAAA
(14)  UAUUCUGGUG UCCUA-GGCGU AGAGGAACCA CACCAAU-CCA UCCCGAACUU GGUGGUUAAA
(15)  UAUUCUGGUG UCCUA-GGCGU AGAGGAACCA CACCAAU-CCA UCCCGAACUU GGUGGUUAAA
(16)  UAUUCUGGUGCUCCUA-GGCGU AGAGGAACCA AACCAAU-CCA UCCCGAACUU GGUGGUUAAA

              70         80         90         100        110        120
(1)  CAUACCUGCG GCAACGAUAG CUCCCGGGUA GCCGGUCGCU AAAAUAGC-UC GACGCCAGGUC
(2)  CAUAAAGGGU AAAUAGAUAC UUGAAAGGUU ACUUUCCGGG AAAAGAUU-UU AGUGCCCUUAU
(3)  CAUAAAGGGU AAAUAGAUAC UUGAAAGGUU ACUUUCCGGG AAAAGAUU-UU AGUGCCCUUAU
(4)  CAUAAAGGGU AAAUGGAUAC UUGGAAGGUU GCUUUCUGGG AAAAGCUU-UU AGUGCCCUUAU
(5)  AAGCUGAGGGGACUGAAGAAC UUUACGGGUC GCCGUCUGGA AUCUCAGUUCU AGUGCUAGGGU
(6)  CGGUAAAAAG AGUGAAAAUA CUUAAGCCGU GGGCUUUGGA AAGAUAAC-UU AAUGUCAGGAU
(7)  CUCUAUUGCG GUGACGAUAC UGUAGGGGAA GCCCGAUGGA AAAAUAGC-UC GACGCCAGGAU
(8)  CUCUAUUGCG GUGACAAUAC UUUAGGGGAA GCCCUAUGGA AAAAUAGC-UC GACGCCAGGAU
(9)  CUCUGCCGCG GUAACCAAUA CUCGGGGGGG GCCCUGCGGA AAAAUAGC-UC GAUGCCAGGAUA
(10) CUCUACUGCG GUGACGAUAC UGUAGGGGAA GCCCUGCGGA AAAAUAGC-UC GACGCCAGGAUA
(11) CUCUACUGCG GUGACGAUAC UGUAGGGGAG GUCCUGCGGA AAAAUAGC-UC GACGCCAGAAU
(12) CUCUACUGCG GUGACGAUAC UGUAGGGGAG GUCCUGCGGA AAAAUAGC-UC GACGCCAGAAU
(13) CUCUACUGCG GUGACGAUAC UGUAGGGGAG GUCCUGCGGC AAAAUAGC-UC GAUGCCAGAAU
(14) CUCUACUGCG GUGACGAUAC UGUAGGGGAG GUCCUGCGGA AAAAUAGC-UC GACGCCAGGAUG
(15) CUCUACUGCG GUGACGAUAC UGUAGGGGAG GUCCUGCGGA AAAAUAGC-UC GACGCCAGGAU
(16) CACUACUGCG GUGACAAUAC UGUAGGGGAG GUCCUGCGGA AAAAUAGC-UC GGCGCCAGAAU
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Fig.2. Sequence alignment of fifteen plant chloroplast 5 S rRNAs and the 5 S rRNA of the Cyanobacterium *A. nidulans.* (1) *A. nidulans* [17]; (2) *E. gracilis* 1 [17]; (3) *E. gracilis* 2 [17]; (4) *E. gracilis* 3 [17]; (5) *C. reinhardii* [17]; (6) *C. ellipsoidea* [22]; (7) *J. subulata* [17]; (8) *M. polymorpha* [17]; (9) *D. acuminata* [17]; (10) *C. revoluta* [13]; (11) *S. oligorhiza* [18]; (12) *L. minor* [17]; (13) *Zea mays* [17]; (14) *S. oleracer* [17]; (15) *N. tabacum* [17]; (16) *V. faba* [19].

phylogenic tree of fifteen plant chloroplast 5 S rRNAs with cyanobacteria as reference. The result suggests that on the chloroplast evolutionary branch, emergence of algae occurred earliest, first Euglenophyta (e.g. *E. gracilis)* appeared and next chlorophyta (e.g. *C. reinhardii).* Chloroplasts of Bryophyta, Pteridophyta, Spermatophyta all originated from green algae. This is in agreement with the classical view and other theories of molecular evolution.

Bryophyta and Pteridophyta are all higher plants but they present two parallel developmental lines in the evolution of higher plants. In plant phylogeny, Bryophyta is a branch degenerated from ferns. However, from fig.3, it can be seen that Bryophyta and ferns do not have a common ancestor. Chloroplasts appear in ferns *(D. acuminata)* earlier than Bryophyta *(M. polymorpha* and *J. subulata).* However, the *Cycas* chloroplast 5 S rRNA is highly homologous with Bryophyta (92%) and less homologous with ferns (84%). This is different from the classical view and from the plant cytoplastic 5 S rRNA phylogenic tree. The phenomenon can be explained in several ways: (i) The data from fifteen plant chloroplast 5 S rRNAs may not be sufficient for construction of an extensive phylogenic tree for this group. (ii) Cytoplastic and chloroplast 5 S rRNAs may follow different evolutionary processes, giving rise to results which appear to be directly opposite. (iii) The classical classification of plants chiefly based on phenotypic manifestations may not be quite ac-

Table 1

Sequence homology among 5 S rRNAs of *Cycas* chloroplast, cyanobacterium and other plant chloroplasts

	Species	Difference in nucleotide numbers	Homology (%)
Cyanobacteria	*A. nidulans*	40	67
Euglenophyta	*E. gracilis* B(rrnB)	56	54
	E. gracilis Z (rrnA or B)	59	52
	E. gracilis Z (rrnC)	61	50
Chlorophyta	*C. reinhardii*	56	54
	C. ellipsoidea	51	58
Bryophyta	*J. subulata*	10	92
	M. polymorpha	10	92
Pteridophyta	*D. acuminata*	19	84
Gymnospermae	*C. revoluta*	0	100
Angiospermae	*S. oligorhize*	5	96
	L. minor	5	96
	Zea mays	8	93
	S. oleracer	4	97
	N. tabacum	4	97
	V. faba	10	92

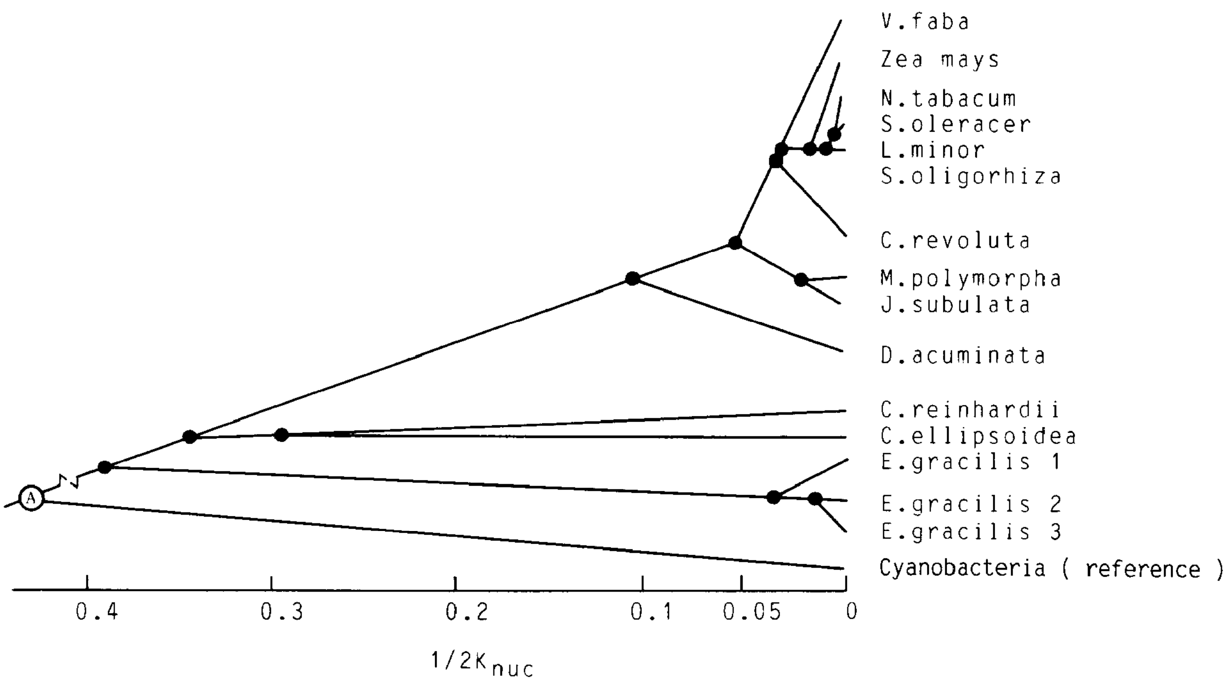

Fig.3. Phylogenic tree of fifteen plant chloroplast 5 S rRNAs.

curate from the molecular viewpoint. (iv) The sequence of 5 S rRNA is highly conserved; it is thus only a poor evolutionary marker and offers only macroscopic information.

In conclusion, the chloroplasts 5 S rRNA phylogenic tree in general agrees with the classical view and also with the molecular evolution theory.

The Spermatophyta belongs to the Tracheophyta. There are two major hypotheses for its evolutionary process [20,21]. The major difference between the two hypotheses lies in the evolutionary positions of *Cycas,* which belongs either to Gymnospermae or Angiospermae. It was clearly shown that *Cycas* is closely related to Gymnospermae from examination of the plant cytoplastic 5 S rRNA phylogenic tree [11]. From fig.3 we see that *Cycas* occurred earlier than Angiospermae, but the times of occurrence are very close to one another. The sequence determined for the *Cycas* chloroplast 5 S rRNA is the only one representing Gymnospermae. Cycasopside, ginkgopside and coniferopside, etc. are all antique plants or 'living fossils'. So it is understandable that they should be closely related. The sequence homology among Angiospermae chloroplasts is very high, the precise evolutionary process cannot be deduced because the K_{nuc} values among these species are very close.

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