J.Mol.Evol.5,307-313 (1975)

Dinoflagellate Ribosomal RNA; An Evolutionary Relic?

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Received March 18, 1975

Summary. The apparent molecular weights of the rRNA subunits of the dinoflagellate *Peridinium cinctum* fa. *westii* were determined by polyacrylamide gel electrophoresis to obtain an indication of its evolutionary status. The apparent molecular weights of the *Peridinium* light and heavy rRNA subunits were 0.7 and 1.23 x 10^6 respectively. The light rRNA is therefore typically eukaryotic but the heavy rRNA has a significantly lower MW than previously reported for higher eukaryotes which range from 1.3 to 1.75 x 10^6 depending on their evolutionary position. Thus by this criterion *Peridinium* is similar to red algae and may represent a eukaryotic stage, lower than fungi or green plants.

Key words: rRNA Evolution - Dinoflagellate Phylogeny - Dinoflagellate rRNA.

INTRODUCTION

The phylogenetic relationships between presently recognized algal classes and the sequence of their evolution are not clearly defined. However, it is generally accepted that chromosomes of the *Dinophyceae* lack the typical histones and have a primitive form of nuclear organization and division (Dodge, 1966; Kubai & Ris, 1969), although their chromatin does contain chromosomal proteins (Rizzo & Nooden, 1974). On the basis of these characteristics, Rae (1970) described the dinoflagellate nucleus as a possible "contemporary manifestation of the primeval eukaryotic nucleus", but was unable, using sucrose gradient centrifugation, to find any differences between the rRNA of the dinoflagellate *Gyrodinium cohnii* and that of eukaryotes. Loening (1968) has compared

some prokaryotic and eukaryotic rRNAs by polyacrylamide gel electrophoresis, a technique with higher resolving power (Loening, 1967). We thus used this method to demonstrate the unusual molecular weight of the heavy rRNA subunit of *Peridinium westii*, a thecate freshwater dinoflagellate which predominates in the phytoplankton of Lake Kinneret (Sea of Galilee), Israel (Berman & Pollingher, 1974). Similar methods were used by Howland & Ramus (1971) who found a similar anomalous molecular weight for the heavy subunit of rRNA in two species of red algae (*Porphyridium aerogineum* and *Griffithsia pacifica*).

MATERIAL AND METHODS

Monoalgal cultures of *Peridinium cinctum* f. *westii* Lemm. (Strain K1) were isolated from L. Kinneret and grown in 80% filtered Kinneret water and 20% medium 1336 (Carefoot, 1968) with the addition of micronutrients and vitamins. Axenic cultures were obtained by several transfers through growth medium containing 20 µg/ml Gentamycin (Schering) and 25 units/ml Mycostatin (Squibb). The cultures were grown at 20 °C under continuous light (Growlux bulbs, 4-4.5 watt/m^2). For incorporation, 1 mCi/l of ^{32}P carrier-free, was added to exponentially growing axenic cultures of *Peridinium* , which were incubated for a further week prior to the extraction of RNA.

RNA extraction: *Peridinium* cells proved to be very resistant to standard extraction procedures. We therefore modified the method of Loening (1969) as outlined below and suggest that this technique may be useful for extracting RNA from other recalcitrant cells.

The algae were collected on 25 µ mesh Nitex netting and washed with sterile water. They were dispersed in homogenization buffer (2% Na tri-isopropylnaphthalene sulfonate 1% NaCl and 50 mM Tris-HCl, pH 7.6 at 0 °C), which directly lyses many bacteria, but had no visible effect on *Peridinium*. Glass beads (0.2 mm diameter) were added and the mixture was vibrated at top speed in a Braun Cell Homogenizer for 2' at near 0 °C. The cells remained unbroken when examined microscopically even after repeated treatments. To overcome this difficulty, an equal volume of phenol: cresol mixture (1 kg phenol, 140 g m-cresol and 1 g hydroxyquinoline, saturated with water) was added. Further homogenization resulted in complete lysis of the cells (as viewed microscopically). In later experiments all reagents were added together and the material was vibrated for 5' at top speed.

Following centrifugation, the supernatant was brought to 0.5 N NaCl and re-extracted first with the phenol-cresol mixture and then with phenol-cresol:chloroform (1:1). The supernatant was brought to 67% ethanol. The resulting precipitate was dissolved in acetate SLS (0.15 M Na acetate, (pH 6) 0.5% sodium lauryl sulfate) and then made up to 67% ethanol. The precipitated pellet was washed with 70% ethanol containing 0.1 NaCl. This pellet, which contained nucleic acids as well as polysaccharides, and probably other compounds, was dispersed (only partially dissolved) in sterile MES buffer (0.05 M 1-9 N morpholino - ethane sulfonic acid, 2.5 mM Mg acetate, pH 7.0 with NaOH). DNase I (Sigma-electrophoretically purified) dissolved in MES buffer containing 10% dimethyl sulfoxide was then added to give 20 µg/ml and incubated for 20 min at 0^{o}. This treatment somewhat cleared the solution which was further mixed by Vortex stirring prior to adding DNase again at the same concentration for another 20 minutes. After dilution with 4 volumes of acetate SLS the turbid solution was centrifuged briefly and the clear supernatant brought to 67% ethanol. The resultant precipitate, which now contained RNA free of proteins, DNA and many other contaminants, was collected by centrifugation, and dissolved in a small volume of half strength electrophoresis buffer. Portions of the RNA preparation were analysed by electrophoresis on 9.5 cm long, 2.4% polyacrylamide gels for 5 hrs at 50 v (Loening, 1967 for preparation; Loening, 1968 for running buffer), with or without the addition of RNA prepared from whole fronds of axenically cultured duckweed (*Spirodela oligorhiza*) (see Rosner et al., 1973 for procedures). Gels were scanned, sliced and counted as outlined in Rosner et al. (1973).

Base ratio analysis: Gels were sliced into 0.75 mm pieces. Two slices were placed per scintillation vial in 1 ml of water and the ^{32}P was estimated by Cerenkov counting. In order to extract the RNA, the vials were placed on a shaker overnight. From each peak of radioactivity the liquid in the middle 3 vials was bulked and dried in vacuo. The residue was dissolved in 50 µl 0.3 N KOH and hydrolyzed overnight at 37 ^{o}. Duplicate spots were made on Whatman No. 1 paper without further treatment. Salts were removed by beginning electrophoresis with a low voltage flux (500 V, 30') and then the bases were separated at 3000 V for 110' using 0.5% pyridine 5% acetic acid buffer (pH 3.5). After the locations of the bases on the paper were found with ultraviolet light, the paper strips were cut into 1 cm pieces and counted in a toluene based scintillation fluid.

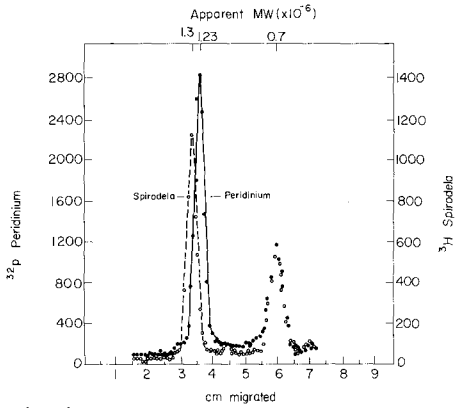

Fig.1

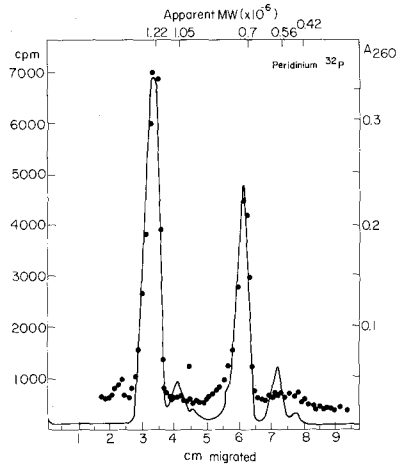

Fig.2

Fig.1. Co electrophoresis of ^{3}H labelled *Spirodella* --o-- and ^{32}P labelled *Peridinium* RNA —●— from axenic cultures. The *Spirodela* RNA was of a much higher specific activity and did not show up in the ultraviolet scans. The ultraviolet scan of the *Peridinium* rRNA was coincident with the ^{32}P and thus was not shown

Fig.2. Axenic ^{32}P-*Peridinium* RNA extracted using unlabelled non axenic *Peridinium* as a carrier for extraction. Solid line: $A_{260\ nm}$ scan, dots: ^{32}P *Peridinium*

RESULTS AND DISCUSSION

We carried out a preliminary experiment in which a nucleic acid extract prepared from a monoalgal culture of *Peridinium* was mixed with ^{32}P labelled higher plant RNA (from *Spirodela*) of sufficient specific activity to give insignificant U.V. absorbance. After electrophoresis, the U.V. absorbancy peak of the lighter *Peridinium* rRNA was coincident with the 0.7×10^6 MW plant rRNA. The heavy rRNA of *Peridinium*, however moved differently from that of *Spirodela* and had an 'unusual' apparent molecular weight of 1.22×10^6.

In order to confirm this result, we attempted to obtain radioactively labelled *Peridinium* RNA. Under our conditions bacteria-free cultures of *Peridinium* did not incorporate ^{3}H-adenosine or ^{3}H-uridine. However, the organisms did take up ^{32}P which was partially incorporated into rRNA with apparent molecular weights of 1.23 and 0.70×10^6 (as extrapolated from higher plant rRNA in a double label experiment, Fig.1) or when compared to the bacterial (or chloroplast) contamination of the *Peridinium* carrier RNA when run alone (Fig.2). With axenic *Peridinium* cultures we found no incor-

Table 1. Base analysis of Dinoflagellate and Spirodela rRNAs

Species	CMP	AMP	GMP	UMP	G+C	Reference
Heavy rRNA (%)						
Peridinium westii	20.3	23.6	29.3	26.7	49.6	-
Gyrodinium cohnii	18.5	25.1	28.0	28.4	46.5	Rae (1970)
Spirodela oligorhiza	24.4	20.1	33.6	25.5	58.0	Rosner (unpubl.)
Light rRNA (%)						
Peridinium westii	17.9	24.6	28.7	28.6	46.6	-
Gyrodinium cohnii	19.5	26.8	26.5	27.2	46.0	Rae (1970)
Spirodela oligorhiza	23.4	22.5	30.9	23.3	54.3	Rosner (unpubl.)

poration of ^{32}P into rRNA with molecular weights corresponding to bacterial rRNA (0.56 and 1.05 x 10^6 MW). In seven runs from three preparations of *Peridinium* RNA, the heavy rRNA had an average molecular weight of 1.227 (S.D. ± 0.019). In these runs, the absorbancy of *Peridinium* RNA was compared to labelled *Spirodela* RNA (not shown); or unlabelled bacterial or chloroplast RNA (Fig.2); or, as stated above, ^{32}P labelled *Peridinium* was compared to labelled higher plant rRNA (Fig.1). The ratio of the heavy to light rRNA subunits, determined from both the ^{32}P incorporation and an integration of the ultraviolet scan, was a 'normal' 1.94.

Large differences of base composition can affect the apparent molecular weights of RNA (Edelman et al., 1969). When G + C content is high, the RNA has a more compact conformation and is more mobile on gels (i.e. has lower apparent molecular weight). We therefore analyzed the base ratios of the major rRNA peaks (Table 1). Even though the *Spirodela* heavy rRNA had a somewhat high G + C it ran with an apparent molecular weight of 1.3 when fractionated with *E.coli* rRNA (Rosner & Gressel, unpublished). At most, the high G + C of *Spirodela* would imply a slightly lower apparent molecular weight for *Peridinium* rRNA. It is also noteworthy that the rRNA base ratios of the two dinoflagellates are similar but contrast with those of the higher plant (*Spirodela*). This would be expected from the evolutionary differences in base ratios summarized by Lava-Sanchez et al. (1972).

Loening (1968) extensively studied and tabulated rRNA fractionated by polyacrylamide electrophoresis from various organisms along the phylogenetic scale. The apparent molecular weight of the light rRNA for all prokaryotes studied,

including bacteria and blue green algae, as well as that of organelle rRNA, was 0.56 x 10^6. The only eukaryotes having apparent molecular weights differing from about 0.7 x 10^6 for the light rRNA were *Amoebae* and *Euglena* (Loening, 1968). Thus, in respect to the light rRNA component, *Peridinium* is clearly similar to eukaryotes. Cytoplasmic heavy rRNA of prokaryotes has a molecular weight of 1.05 - 1.1 x 10^6 whereas that of eukaryotes varies. Fungi, green plants and some protozoa have apparent molecular weights of 1.3 x 10^6, crustaceans and insects 1.4 x 10^6, birds 1.5 x 10^6 and mammals 1.75 x 10^6 (Loening, 1968). Thus, using these molecular weights as a measure of evolution, without considering all other factors, one might consider *Peridinium* to be a eukaryote of a lower evolutionary status than plants and fungi. We suggest that Rae (1970) did not discern the anomalous size of this fraction of dinoflagellate rRNA because of the lower resolution of the sucrose gradient technique, or perhaps there is a real difference between the unrelated dinoflagellate genera of *Peridinium* and *Gyrodinium*.

Recently, endosymbiotic cryptomonad type inclusions which could affect RNA determinations have been reported by Thomas and Cox (1973) in *P.balticum*. These inclusions have not been observed in *P.westii*, although they were specifically sought out (Pollingher, personal communication).

In this work, we assume that our extraction procedures for RNA have not caused a specific breakage of the heavy rRNA. The cells were extracted in the presence of a strong detergent as well as phenol and cresol and no RNA cleavage was previously noted with this technique. Our results are similar to those of Howland & Ramus (1971) with red algae using quite different extraction techniques. Furthermore, we obtained almost identical apparent molecular weights for the *Peridinium* heavy rRNA extracted from three separate cultures. It is not clear why there was no ^{32}P labelling of *Peridinium* plastid rRNAs.

Paleological evidence indicates that *Peridinium* species existed perhaps as early as Lower Jurassic times or even before (Wall & Dale, 1968). Morphological features of the dinoflagellate nucleus (e.g.Dodge, 1966; Kubai & Ris, 1969) also point to the ancient lineage of these organisms. A similar value for the heavy rRNA subunit of red algae was reported by Howland & Ramus (1971). It would appear that the dinoflagellates and red algae share a similar form of heavy rRNA of molecular weight 1.2 x 10^6 which may indeed be an archaic intermediate in the evolutionary pathway from prokaryotic to higher eukaryotic rRNA.

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