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Post-Maturation of the Plastid Ribosomal RNA in the Plant Kingdom

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Summary. The in vivo fragmentation of the plastid rRNA from plants situated at different places in the evolutionary scale, with the exception of *Algae*, was analysed by electrophoresis using fully denaturing conditions. This fragmentation corresponds to an in vivo post-maturation. It exists only in some bacteria and is not random. Five main groups of fragments with the following real molecular weights (*Mr*) are found in 23 S: *ca* 0.9×10^6; 0.7×10^6; 0.45×10^6; 0.35×10^6 and 0.15×10^6. The existence of a large fragment ($Mr = 0.9 \times 10^6$) corresponds to a primitive type of fragmentation found in some archaic plants. Dicotyledons and several other groups have the same pattern of 23 S fragmentation, often comprising all the fragments mentioned above, whilst *Graminaceae* (Monocotyledons) constitute a special group with a very predominant 0.35×10^6 dalton fragment and the absence of the 0.45×10^6 dalton fragment. The plastid 16 S rRNA in all plants studied here has a *Mr* of 0.54×10^6 which is smaller than the 16 S of *Escherichia coli* taken as reference (0.56×10^6 dalton).

Key-words: Plant ribosomal RNA/23 S post-maturation – Formamide gels

Introduction

The ribosomal RNA from green plants is composed of four main species of high molecular weight. The 25 S and 18 S originate from the cytoplasm and the 23 S and 16 S come from plastids. The smaller rRNA species 18 S and 16 S are stable (Leaver, 1973) and the 25 S species is always composed of one large (*ca* 20 S) and one small (5.8 S) polynucleotide chain (Payne and Dyer, 1972). The 23 S species is known to be unstable. The newly formed 23 S rRNA detected by labelling (mature 23 S) is more stable than the 'old' postmature 23 S (Ingle, 1968). The fragmentation of the 23 S rRNA is known to occur in vivo in the ribosomes (Munsche and Wollgiehn, 1974; Mache et al., 1978). The fragmentation of the old 23 S rRNA is general in plants (Loening and Ingle, 1967; Ingle, 1968; Leaver, 1973; Grierson, 1974; Munsche and Wollgiehn, 1973, 1974) and exists only sometimes in bacteria (Marrs and Kaplan, 1970, Schuch

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and Loening, 1975; Meier and Brownstein, 1976). We have found no fragmentation of the 23 S rRNA in *Escherichia coli.* Leaver and Ingle (1971) found that the stability of the 23 S rRNA, the size and number of fragments produced vary with the different plant species. They noticed that the cleavage occurs in three particular regions of the 23 S.

We present a study of the rRNA of some lower plants, Dicotyledons and Monocotyledons, using electrophoresis in fully denaturing conditions, to detect whether the in vivo fragmentation of the 23 S rRNA species is random or not. We show that the post-maturation of the *Graminaceae* and of several primitive plants is peculiar and differs from that of the Dicotyledons.

Materials and Methods

Plants

A large series of plants has been examined. It includes lower non vascular plants (Liverworts, Mosses), lower vascular plants (Ferns), *Gymnospermeae (Ginkgo, Taxodium)* and higher plants chosen for their systematic position: i.e. a large number of Mono- and Dicotyledons belonging to different families (see Tables 1, 2, 3). Algae were not studied here because of the difficulty in obtaining axenic plants. Axenic *Pylaiella littoralis*, a brown alga, has been studied elsewhere (Loiseaux et al., 1979). The plants are generally grown outdoors (botanical garden, fields...), sometimes in conditioned laboratory rooms. Many species of wild plants must be harvested during spring (*Carex, Luzula, Magnolia, Gymnospermeae...*) when they have young tender leaves and a rapid growth.

Extraction of rRNA

The extractions are generally made from whole plant leaves. Sometimes, we use isolated chloroplasts obtained by the method of Joyard and Douce (1976). It is often not possible to obtain pure, intact chloroplasts because of the hardness of the cell walls or the

Table 1. Chloroplastic rRNA from lower plants

Plants	23 S (Groups of molecular weights)						Stoechiometric Ratio 0.45/0.35	16 S
	0.9	0.7	0.45	0.35	0.25	0.15	(*ca* Values)	*Mr*
Ginkgo biloba	ϵ	(+)	+	+	-	+	0.5	0.53
Taxodium distichum	*	(+)	ϵ	ϵ	-	+	-	0.54
Marchantia polymorpha	*	(+)	ϵ	ϵ	ϵ	ϵ	-	0.53
Selaginella	ϵ	(+)	+	+	-	-	0.5	0.53
Hypnum cupressiforme	ϵ	(+)	+	ϵ	-	-	0.8	0.54

Symbols: -: absence; ϵ: low amount; +: notable amount; (+): undetermined quantity because of superposition of fragments (0.7×10^6 from the 23 S together with the 18 S); *: characteristic fragment of the 23 S

Table 2. Chloroplastic rRNA from Monocotyledons

Plants	23 S (Groups of molecular weights)						Stoechiometric Ratio 0.45/0.35 (*ca* Values)	16 S
	0.9	0.7	0.45	0.35	0.25	0.15		*Mr*
Zea Mays	ϵ	+(3)	ϵ	*	-	ϵ	0.01	0.54
Triticum sativum	ϵ	(+)	-	*	-	-	0.0	0.54
Avena sativa	ϵ	(+)	-	*	-	-	0.0	0.53
Poa	ϵ	(+)	-	*	-	-	0.0	0.54
Allium porrum	ϵ	+(4)	+	+	+	+	0.3	0.53
Crocus	ϵ	(+)	+	+	+	+	0.3	0.54
Allium sativum	ϵ	(+)	+	+	-	+	0.5	0.54
Tulipa	ϵ	(+)	+	+	-	+	0.2	0.54
Luzula	*(1)	(+)	-	-	-	-	-	0.53
Carex	*(2)	(+)	-	-	-	-	-	0.54
Orchis	ϵ	(+)	+	+	-	-	0.6	0.54
Arum maculatum	ϵ	(+)	+	+	-	-	0.25	0.54
Potamogeton	ϵ	(+)	+	ϵ	-	-	2	0.53

Symbols: See Table 1
(1) $Mr = 0.80 \times 10^6$ (2) $Mr = 0.98 \times 10^6$ (3) $Mr = 0.68 \times 10^6$ (4) $Mr = 0.70 \times 10^6$

cytoplasm's viscosity. Nucleic acids are extracted by the ethanol-SDS-diethylpyrocarbonate method of Laulhere and Rozier (1976). For verification purposes, we used different pH (pH = 5, 6, 9) and the phenol extraction according to Loening and Ingle (1967). The buffer used at pH 9 is a carbonate buffer. The nucleic acids are precipitated by ethanol. The precipitate is washed with 3 M sodium acetate to eliminate DNA and soluble RNA.

Preparation of isolated species

The isolation method has been described (Mache et al., 1978). When the ratio cytoplasmic rRNA/chloroplastic rRNA is high, it is difficult to obtain quantities of pure 23 S and 16 S. A simplification of the method consists in the isolation of pure 25 S or 18 S species and comparison with isolated 25 S + 23 S and 18 S + 16 S. The comparison between the electrophoregrams gives satisfactory indications for each species.

rRNA analysis

Electrophoresis of rRNA is performed in aqueous SDS gels (2.5% acrylamide) according to Loening and Ingle (1967) in order to control the rRNA quality. Afterwards,

Table 3. Chloroplastic rRNA from Dicotyledons

Plants	23 S (Groups of molecular weights)						Stoechiometric Ratio 0.45/0.35 (*ca* Values)	16 S
	0.9	0.7	0.45	0.35	0.25	0.15		*Mr*
Spinacia oleracea	ϵ	+(1)	+	+	-	+	0.6	0.54
Beta vulgaris	ϵ	+(2)	*	ϵ	+	+	5.0	0.53
Magnolia	ϵ	(+)	ϵ/+	+	-	-	0.2	0.53
Ranunculus	ϵ	(+)	+	ϵ	-		0.8	0.54
Raphanus sativus	ϵ	(+)	+	+	-	+	0.7	0.54
Pisum sativum	ϵ	+	+	+	-	-	0.4	0.53
Phaseolus vulgaris	ϵ	(+)	+	+	-	+	0.5	0.54
Solanum tuberosum	ϵ	+(2)	+	+	-	-	0.5	0.53
Lactuca sativa	ϵ	+(2)	+	+	-	+	0.5	0.53
Bryophyllum	ϵ	(+)	+	+	-	-	0.8	0.54

Symbols: see Table 1
(1) *Mr* = 0.71 x 10^6 (2) *Mr* = 0.68 x 10^6

denaturing electrophoresis in 98% formamide is done according to Pinder et al. (1974a,b) with 12 cm long gels containing 4% polyacrylamide. A voltage of 10 V/cm is applied during 7 to 8 h on the gels. The rRNA dissolved in formamide is heated 2 mn at 50°C before electrophoresis. The gels are scanned at 260 nm with a Scan 400 (Joyce Loebl).

Determination of molecular weights

Apparent molecular weights (*Ma*) in aqueous SDS gels and real molecular weights (*Mr*) in formamide gels are determinated by their electrophoretic migration (Loening and Ingle, 1967); Pinder et al. (1974a,b). To determine the *Mr* values we used *Escherichia coli* rRNA as standard. It is admitted that the *Mr* of the 23 S and the 16 S of *E. coli* are respectively 1.07 x 10^6 and 0.56 x 10^6. We used two different proportions of *E. coli* rRNA for coelectrophoresis with each plant rRNA.

Results

In this work we have not tried to distinguish the rRNA precursors or the unbroken newly synthesised 23 S rRNA which are ordinarly present in very small amounts. For each plant, we have given the true molecular weight (*Mr*) of different rRNA species established in fully denaturing conditions. Values reported in Tables 1, 2, 3 have been

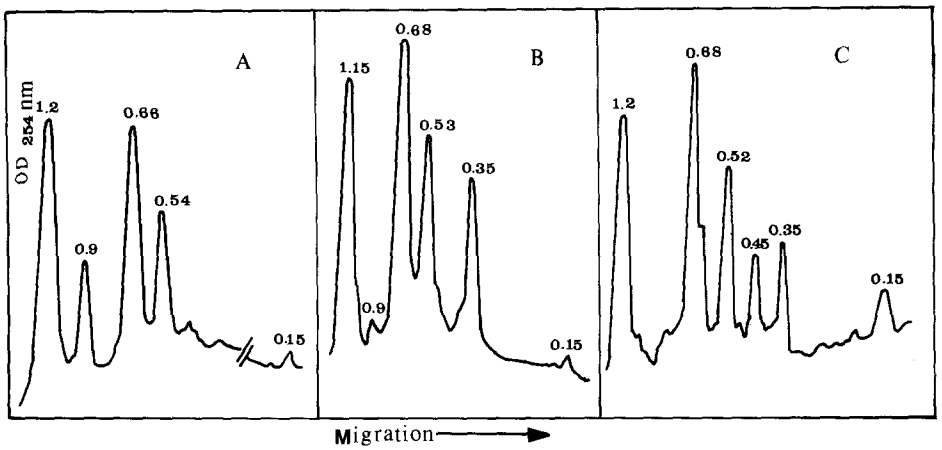

Fig. 1. Main types of 23 S fragmentation. (a) *Taxodium distichum (Gymnosperma)* with a primitive pattern of 23 S fragmentation, the 23 S gives an important 0.9 x 10^6 dalton fragment. This is also found for *Marchantia* (Liverwort), *Carex* (Monocot)... (b) *Zea Mays. Graminacea* (Monocot). The 23 S fragmentation shows several small pieces. The main characteristics is the presence of a great amount of the 0.35 x 10^6 dalton fragment and practically no 0.45 x 10^6 dalton fragment. All the *Graminaceae* studied here show this fragmentation pattern. (c) *Lactuca sativa* (Dicot). This evoluted type shows a fragmentation into several small pieces. It is characterized by nearly the same amount of the two smaller fragments 0.45 and 0.35 x 10^6 dalton. This fragmentation pattern is that of all the Dicots studied here (*Beta vulgaris* excepted), as well as *Hypnum* (Bryophyt) *Selaginella* (Pteridophyta), *Ginkgo* (Gymnosperma) and *Orchis* (Monocot)...

obtained either from isolated rRNA species (*Spinacia oleracea, Zea Mays, Allium porrum*) or from isolated plastids (*Spinacia oleracea, Lactuca sativa, Beta vulgaris*, ssp cicla) or from whole rRNA extracts. Electrophoregrams using formamide gels show a great number of rRNA fragments. We found 3 types of electrophoregrams which are reported on Fig. 1.

The *ca* 1.2 x 10^6 fragment, present in large relative amounts, belongs to the cytoplasmic 25 S rRNA as verified from isolated 25 S species in the case of spinach (Mache et al., 1978) and maize (not shown). Payne and Dyer (1972) have shown that the 25 S is composed of one large polynucleotide chain of ca 1.2 x 10^6 dalton and of one smaller chain of ca. 50,000 dalton (5.8 S). We found that the *Mr* of the large polynucleotide chain falls between 1.15 and 1.25 x 10^6 for all the plants studied. The small species is not present in our electrophoregrams because of its low molecular weight and because of our electrophoretic conditions.

The cytoplasmic 18 S species when isolated and electrophoresed under denaturing conditions gives one unique fragment in the range of 0.65-0.73 x 10^6 dalton (not shown) as in spinach (Mache et al., 1978). In many cases, when using total rRNA, the cytoplasmic 18 S species cannot be distinguished from a fragment of the plastid 23 S species with a close molecular weight. In some cases, the difference between the *Mr* of the 18 S species and of the 23 S fragment is large enough to allow a separation after electrophoresis [*Lactuca sativa* (Fig. 1c), most *Liliaceae, Solanum tuberosum...*].

The plastid 16 S rRNA is an unbroken polynucleotide chain (Loening and Ingle, 1967; Ingle, 1968; Mache et al., 1978); all our analyses of the isolated species in denaturing conditions are in agreement with this conclusion. In all plants analyzed here, the *Mr* of the 16 S is smaller than the *Mr* of *Escherichia coli:* $0.54 \pm 0.01 \times 10^6$ instead of 0.56×10^6.

As already known, the plastid 23 S rRNA produces several fragments. Electrophoresis of isolated 23 S species from some plants (spinach, maize, leek) establishes their origin. No fragment has been detected with a molecular weight larger than 1.0×10^6. For some primitive plants recorded in Table 1, as well as for *Carex,* (Table 2), a fragment of ca 0.9×10^6 dalton is detected in notable amounts. For other plants, this fragment is only present in very low quantities.

The $0.65\text{-}0.73 \times 10^6$ dalton fragment migrates with the cytoplasmic 18 S. It is not possible with whole extracts to give quantitative estimations. We know from the rRNA analyzed from isolated plastids (*Lactuca, Spinacia, Beta, Zea*) or from isolated 23 S (*Zea, Allium, Spinacia*) that this species is generally present in large amounts, but sometimes in a small amount as in *Beta vulgaris* (Fig. 2). The values of this 23 S fragment are reported in Tables 1-3 whenever it is possible (isolated plastids).

The 0.45×10^6 and 0.35×10^6 dalton fragments are both present in all primitive and dicotyledonous plants analyzed, with two exceptions: the Dicot *Beta vulgaris* which has no or a very low amount of 0.35×10^6; the young leaves of *Magnolia* which have no detectable 0.45×10^6 fragment during the bloom. However, this fragment appears later on in small amounts. In order to quantify the relative amount of these two fragments we determined their stoechiometric ratio in the different plants (see Tables 1-3). In the *Gymnospermea Taxodium* and in the Liverwort *Marchantia polymorpha,* which have a large amount of the 0.9×10^6 fragment, there are very low amounts of the 0.45 and 0.35×10^6 fragments. Among monocotyledonous plants the *Carex* has also a large 0.9×10^6 dalton peak and almost no 0.45 or 0.35×10^6. The monocot family

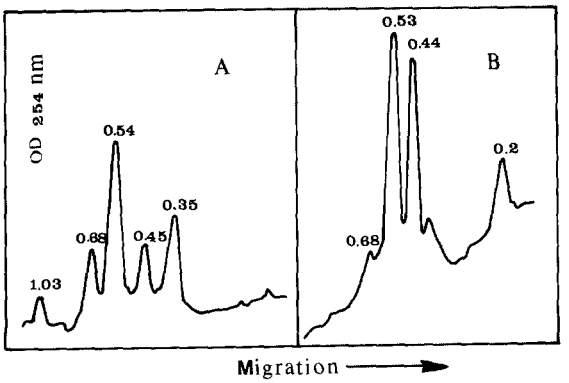

Fig. 2a and b. 23 S RNA in isolated plastids of the Dicots *Lactuca sativa* (**a**) and *Beta vulgaris* (**b**). Both figures show the presence of the 0.68×10^6 fragment as a product of the breaking of the 23 S (the 16 S being unbroken as verified by electrophoresis of the isolated species in formamide). In Fig. 2b, the unusual importance of the 0.45×10^6 dalton fragment must be noticed in *Beta vulgaris*

Graminaceae presents a remarkable feature: there is practically no 0.45 x 10^6 fragment: this latter always represents less than 1% of the number of the 0.35 x 10^6 molecules, in all *Graminaceae* studied.

The 0.15 x 10^6 fragment is present in many plants. It occurs only as traces in the *Graminaceae*. Some other occasional fragments may occur with *Mr* of 0.2 to 0.27 x 10^6. We have shown for spinach that they derive from the 23 S (Mache et al., 1978). A 0.8 x 10^6 dalton fragment is present in *Luzula* (not shown). A small amount of *ca* 0.6 x 10^6 dalton fragment appears in the isolated 23 S of maize.

Discussion

The most significant remarks concern the chloroplastic rRNA. It is already known that post-maturation occurs in 23 S plastid rRNA and results in a fragmentation of the rRNA (Leaver, 1973; Munsche and Wollgiehn, 1974; Mache et al., 1978). We have studied the molecular weights of these fragments using fully denaturing conditions, in several families of plants (with the exception of *algae*). Different methods of rRNA extraction give identical results: we obtained the same electrophoretic profiles for *Zea Mays* when extracted by the phenol method of Loening and Ingle (1967), as when extracted by our method (Laulhere and Rozier, 1976) at various pH. The physiological state of plants seems to have little influence on the post-maturation of rRNA: similar results are found in normal or etiolated maize. The stage of development of young *Magnolia* leaves influences only slightly the 0.45/0.35 ratio.

The 16 S of all plants studied here has a *Mr* of 0.54 (± 0.01) x 10^6 which is smaller than the *Mr* of *Escherichia coli* (0.56 x 10^6). We arrived at this conclusion in 1977, for *Spinacia oleracea* (Mache et al., 1977) and now we can generalize the fact.

The hidden breaks of the plastid 23 S rRNA in denaturing conditions characterize the 23 S of all plants studied here, whilst the 23 S of *bacteria* is unbroken (*Escherichia coli*) or nicked for some other species (Marrs andKaplan, 1970; Meier and Brownstein, 1976; Schuch and Loening, 1975). In plants, it must be noted that the size of fragments is not randomly distributed but belongs to the following main groups with molecular weights of ca: 0.9 x 10^6, 0.7 x 10^6, 0.45 x 10^6, 0.35 x 10^6, 0.15 x 10^6. In some plants, fragments belonging to one, two, or three (*Carex*) classes are absent or scarcely detectable. Some additional fragments may also sometimes occur: 0.2, 0.27. But, on the whole, there is a remarkable conservation of the plastid 50 S ribosomal subunit structure that allows formation of regularly distributed hidden breaks after the biogenesis of mature ribosomes. It is especially clear for the four major fragments: 0.9 x 10^6, 0.7 x 10^6, 0.45 x 10^6, 0.35 x 10^6 dalton.

Another notable fact is the following: the relative importance of the main groups of fragments of the 23 S is well correlated with the systematic position, despite some discrepancy in details.

- The presence of the 0.9 x 10^6 dalton fragment is a characteristic of primitive plants such as *Marchantia* (Liverwort), *Taxodium (Gymnospermeae)* or of a peculiar phylum, among higher plants: *Carex* (Monocot).
- The Dicotyledons form a relatively homogenous group with a 23 S fragmentation pattern similar to that of spinach: 0.9 + 0.7 + 0.45 + 0.35 + 0.15. An exception is *Beta vulgaris* (ssp cicla), a *Chenopodiacea* such as *Spinacia oleracea*, which presents no or a very low amount of 0.35 x 10^6 dalton fragment.

– The Monotyledons 23 S fragmentation shows more diversity. The *Carex (Cyperaceae)* presents an important 0.98 x 10^6 fragment, the *Luzula (Juncaceae)* shows a large peak at 0.80 x 10^6 dalton. Both plants have no, or hardly any, 0.35 x 10^6 or 0.45 x 10^6 dalton fragments. The *Orchidaceae* are very similar to the Dicotyledons and so are the *Liliaceae*, but with a lower 0.45/0.35 stoechiometric ratio. The most striking and constant pattern is that of the *Graminaceae* with a very large 0.35 x 10^6 dalton fragment and a 0.45/0.35 ratio practically equal to zero. It is already known that plants especially among *Graminaceae* have a particular metabolism of CO_2 fixation (C_4 metabolism). The disappearance of the 0.45 x 10^6 dalton fragment is not restricted to the C_4 plants. Nevertheless, the Monocot group has been the template of important modifications and is not as homogeneous as the Dicot group.

The present data relative to 23 S post-maturation may, along with many others (Swain, 1963, 1966; Mabry et al., 1968; Harborne, 1970), contribute to a chemical or genetic plant taxonomy. Correlations of biochemical characters with classical morphological taxonomy appear. It seems, schematically, that the more highly evolved the plant is, the more the 23 S molecular species is fragmentable into small pieces.

The reasons for which the fragmentation of the 23 S rRNA is not random are to be discussed. These fragmentations may be either due to the rRNA structure or to the surrounding proteins. The differences in the fragmentation may be due to different nucleotide sequences of rRNA, as a relationship has been hypothesized between the conservation of the rRNA nucleotidic sequences and the evolutionary position of the plants (Maggini et al., 1976). But no firm conclusion can be drawn since Thomas and Tewari (1974) have found no detectable difference between the rDNA coding for the 23 S species in the *Graminaceae* and the Dicotyledons. The differences in the fragmentation pattern of mature rRNA may also be due to the various interactions between the nucleic acids and the constitutive or enzymatic proteins of ribosomes. Clearly further experiments are necessary to determine which of these hypotheses is correct.

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