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Similarities among Ribosomal RNA's of Angiospermae and Gymnospermae

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Summary. Competitive hybridization of ribosomal RNAs was used to estimate similarities of nucleotide sequences between species of *Angiospermae* and *Gymnospermae*. Similarities have been measured with respect to *Cucumis sativus*. In *Angiospermae* the nucleotide sequences are highly conserved except in the species of the family of *Compositae*, where the percentages of similarity are clearly lower. In the *Gymnospermae* the lowest similarity has been observed with *Torreya californica*. A relationship is hypothesized between conservation of rRNA nucleotide sequences and evolutionary position of the species.

Key words: rRNA Similarities/Angiospermae/Gymnospermae

INTRODUCTION

In a previous paper (Maggini, 1975) we have reported data on the homology of ribosomal RNA nucleotide sequences in monocots, showing that the examined species of the *Liliaceae* family possess a high similarity in the rRNA nucleotide sequences, while there are significant divergences among some species of the *Graminaceae* family. We correlated these results with the fact that among monocots, *Liliaceae* are primitive plants and *Graminaceae* highly evolved ones and hypothesized that the rRNA genes are not yet stabilized in the latter family. In the present work we extend our observation to some dicots and *Gymnospermae* species. Vodkin & Katterman (1971) found approximately 60% of homology between rRNA of barley and wheat, and concluded that in monocots rRNA

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Abbreviations used: MAK = Methylated albumin kieselguhr; SDS = sodium dodecyl sulphate; SSC = 0.15 M NaCl + 0.015 M Na citrate.

nucleotide sequences are less conserved than in dicots, where they observed 95% of homology between pumpkin and cotton.

In the present work too, we have utilized the competitive hybridization technique, measuring the extent of competition between rRNAs of two different species in a single rRNA/DNA hybridization experiment. We report the similarities of ribosomal RNA of fifteen species of dicots, one species of monocots and four species of *Gymnospermae*, calculated utilizing for the competition experiments *Cucumis sativus* rRNA and DNA.

MATERIALS AND METHODS

We examined sixteen *Angiospermae*, comprising the following fifteen dicots: *Cucumis sativus* (cucumber) and *Cucurbita maxima* (gourd) of the family of *Cucurbitaceae; Alyssum sp. (Alysseae); Populus nigra* (black poplar) *(Fagaceae); Anemone hortensis* (anemone) *(Ranunculaceae); Phaseolus coccineus* (bean) and *Vicia faba* (broad bean) *(Leguminosae); Albizzia julibrissin (Mimosaceae); Dianthus laciniatus* (carnation pink) *(Caryophyllaceae); Lobelia sp. (Lobeliaceae); Anthirrinum maius* (snap dragon) *(Schrophulariaceae); Lactuca virosa* (lettuce), *Bellis perennis* (daisy), *Helianthus annuus* (sunflower) and *Chrysanthemum sp.* (Chrysanthemum) of the family of *Compositae*. We examined also a monocot species, *Triticum durum* (hard wheat) of the *Graminaceae* family, and four *Gymnospermae: Pinus pinea* (pine) *(Pinaceae); Ginkgo biloba (Ginkgoaceae); Cycas revoluta* (cycad) *(Cycadaceae)* and *Torreya californica (Taxaceae).*

rRNA Extraction and Purification. Angiospermae rRNAs were obtained from roots and shoots of germinated seeds kept in the dark in sterile conditions. *Pinus pinea* and *Torreya californica* rRNAs were from roots of germinated seeds; *Ginkgo biloba* and *Cycas revoluta* rRNAs from female gametophyte. rRNA extraction was effected by the method described by Ritossa & Spiegelman (1965); rRNA was purified by MAK column chromatography (Mandell & Herschy, 1960).

DNA Extraction. DNA was extracted from roots and shoots of germinated seeds of *Cucumis sativus*. The methods described by Stern (1971), Marmur (1961) and Flamm et al. (1969) were followed with some modifications. The material was homogenized in a mortar in cold absolute ethanol. The homogenate was squeezed through three layers of cheesecloth and centrifuged at 1000×g for 10 min. The pellet was dissolved in 1:1 absolute ethanol-ethyl ether and centrifuged at 1500×g; the precipitate was diluted in ethylether and centrifuged at 2000×g; ether was eliminated by flowing nitrogen through the sample. The nuclear preparation obtained was homogenized in saline-EDTA (0.1 M EDTA pH 8, 0.15 M NaCl, 2% SDS) and incubated at 60°C for 10 min with agitation. After chilling, $NaClO_4$ was added to a final molarity of 1 M. Subsequently, an equal volume of 24:1 chloro-

form-isoamyl alcohol was added at room temperature. The solutio was shaken for 15 min and centrifuged at 5000×g for 10 min. Thi procedure was repeated twice. An equal volume of cold 95% ethanol was added and the fibers containing the DNA spooled out. After alcohol evaporation the fibers were dissolved in 10 mM Tris pH 7.6. Solid Cs_2SO_4 was added to make the final density = 1.49g/ml. The solution was centrifuged in the Spinco 50 fixed-angle rotor at 22°C for 48 h at 44,000 r.p.m. The centrifuge tube was pierced with a gauge needle and three-drop fractions collected. The $O.D._{260}$ of each fraction was determined in a Zeiss spectrophotometer. Fractions containing DNA were pooled, dyalized against 0.1×SSC and stored at 4°C. The O.D. 260/280 and 260/230 ratios were respectively 1.83 and 2.25.

Labeling of rRNA. Forty shoots of *Cucumis sativus* obtained from germinated seeds were grown for 72 h in 20 ml of White's solution (White, 1939) containing 2 mC of uridine 5-H^3 (New England Nuclear, 28.5 C/mM), shaken by magnetic stirrer and then "chased" 6 h with cold uridine (7 mg in 40 ml of White's solution). RNA was extracted from tissues and rRNA purified by a MAK column. The rRNA specific activity was 80,000 c.p.m./µg.

Hybridization rRNA/DNA. Hybridization was performed according to the technique of Gillespie & Spiegelman (1965). For details of the method see Maggini (1975). The extent of similarity was calculated by the method of Bendich & Bolton (1967) and by another method that we have elaborated. The percentage of similarity was calculated as $\frac{w - x_1}{w - y_1} \times 100$ after 1:1 competition, as $\frac{w - x_2}{w - y_2} \times 100$ after 1:2 competition and as $\frac{w - x_3}{w - y_3} \times 100$ after a 1:3 competition. We indicate with w, the percentage of DNA hybridized in the presence only of labeled rRNA of the same species from which the DNA was obtained (3 µg of rRNA-H^3 in 3 ml of 2×SSC); x_1, x_2 and x_3 are the percentages of DNA hybridized to labeled rRNA when in the hybridization mixture are present 3 µg of rRNA-H^3 of the same species of the DNA and 3, 6 or 9 µg respectively of cold competitor rRNA of a different species; y_1, y_2 and y_3 are the percentages of DNA hybridized in the presence of 3 µg of rRNA-H^3 and of 3, 6 or 9 µg of cold rRNA of the same species from which the DNA was obtained. By our method it is possible to directly obtain the percentage of similarity from the values of percent binding after 1:1, 1:2 and 1:3 competition. Substituting w/2 for y_1 if the competition is 1:1, w/3 for y_2 if it is 1:2 and w/4 for y_3 in the Bendich and Bolton formulae, the equations become

(1) $w = \frac{2x_1}{2 - p}$ (2) $w = \frac{3x_2}{3 - 2p}$ (3) $w = \frac{4x_3}{4 - 3p}$

where p = percentage of similarity.
By equating (1) to (2) and solving for p we obtain

$$p = \frac{6(x_1 - x_2)}{4x_1 - 3x_2} \times 100 = \Delta c_1$$

By equating (2) to (3) we obtain

$$p = \frac{12(x_2 - x_3)}{9x_2 - 8x_3} \times 100 = \Delta c_2$$

By equating (1) to (3) we obtain

$$p = \frac{4(x_1 - x_3)}{3x_1 - 2x_3} \times 100 = \Delta c_3.$$

All experiments were performed twice using separately extracted DNA and rRNAs. The percent of hybridization of rRNA-H^3 to DNA is the mean of the two results.

RESULTS AND DISCUSSION

When rRNA-H^3 of *Cucumis sativus* is hybridized with homologous DNA, a saturation plateau is reached at 1.121%. This value is in agreement with the data of other authors for the same species and for other species of the family of *Cucurbitaceae* (Ingle & Sinclair, 1972; Goldberg et al., 1972). In our conditions there is saturation with 3 µg of rRNA in 3 ml of 2×SSC; therefore we used this amount of *Cucumis sativus* rRNA-H^3 for the competition experiments, and these results are reported in the Table 1. All the similarities are calculated with respect to *Cucumis sativus* rRNA. Nucleotide sequences of *Cucurbita maxima* rRNA show perfect similarity with those of *Cucumis sativus*: in fact both species belong to the *Cucurbitaceae* family. The rRNA of *Alyssum sp.* and *Populus nigra* (subclass *Dilleniidea*, comprising also the *Cucurbitaceae* family); of *Anemone hortensis* (subclass *Magnoliidae*); of *Phaseolus coccineus, Vicia faba* and *Albizzia julibrissin* (subclass *Rosidae*) are highly conserved since we calculated similarities of approximately 95%. With *Dianthus laciniatus* (subclass *Caryophyllidae*), *Lobelia sp.* and *Anthirrinum maius* (subclass *Asteridae*) we note fairly high rRNA similarities (about 90%). With regards to the species of the *Compositae* family (subclass *Asteridae*) we calculated similarities of 85.4 with *Lactuca virosa*, of 80.3 with *Bellis perennis*, of 76.4 with *Chrysanthemum sp.* and of 75.1 with *Helianthus annuus*. The 80.9 similarity obtained in the monocot *Triticum durum* is in good agreement with our previous work (Maggini, 1975). For the *Gymnospermae* species, the percen-

Table 1
Experiments of competition performed with *Cucumis sativus* DNA

Subclass	Family	Competitor rRNA	% DNA hybridized			Percentages of similarity[a]						
			1:1	1:2	1:3	1:1	1:2	1:3	Δc_1	Δc_2	Δc_3	Avg[b]
		Angiospermae										
Dilleniidae	Cucurbitaceae	*Cucumis sativus*	0.598	0.406	0.303	101.3	99.8	100.0	98.1	100.5	99.3	99.8
		Cucurbita maxima	0.602	0.408	0.301	100.7	99.5	100.2	98.3	101.6	100.0	100.0
	Alysseae	*Alyssum sp.*	0.632	0.444	0.348	95.7	95.0	95.0	94.3	95.0	94.7	94.9
	Fagaceae	*Populus nigra*	0.658	0.467	0.368	91.4	92.2	92.8	93.1	94.4	93.7	92.9
Magnoliidae	Ranunculaceae	*Anemone hortensis*	0.643	0.445	0.346	93.9	94.9	95.3	96.0	96.0	96.0	95.3
Rosidae	Leguminosae	*Phaseolus coccineus*	0.628	0.439	0.346	96.4	95.7	95.3	94.9	94.3	94.6	95.2
		Vicia faba	0.639	0.443	0.355	94.6	95.2	94.3	95.8	92.1	94.1	94.3
	Mimosaceae	*Albizzia julibrissin*	0.645	0.642	0.366	93.6	92.8	93.1	91.9	93.6	92.8	93.0
Caryophyllidae	Caryophyllaceae	*Dianthus laciniatus*	0.660	0.473	0.390	91.1	90.8	90.4	91.9	87.6	90.0	90.3
Asteridae	Lobeliaceae	*Lobelia sp.*	0.663	0.484	0.396	90.1	90.1	89.8	89.5	88.9	89.2	89.6
	Schrophulariaceae	*Anthirrinum maius*	0.651	0.468	0.380	92.6	92.1	91.5	91.5	90.1	90.9	91.4
	Compositae	*Lactuca virosa*	0.694	0.521	0.436	85.5	85.5	85.4	85.6	84.9	85.3	85.4
		Bellis perennis	0.718	0.559	0.480	81.5	80.8	80.5	79.8	79.6	79.7	80.3
		Chrysanthemum sp.	0.760	0.598	0.520	74.6	76.0	76.1	78.0	76.6	77.4	76.4
		Helianthus annuus	0.775	0.614	0.534	72.1	74.0	74.6	76.8	76.6	76.7	75.1
Commelinidae	Graminaceae	*Triticum durum*	0.731	0.566	0.478	79.4	79.9	80.7	80.7	83.1	81.8	80.9
		Gymnospermae										
	Pinaceae	*Pinus pinea*	0.707	0.542	0.446	83.9	82.9	84.2	82.3	87.9	84.9	84.3
	Ginkgoaceae	*Ginkgo biloba*	0.741	0.574	0.492	77.7	79.0	79.2	80.7	80.0	80.4	79.5
	Cycadaceae	*Cycas revoluta*	0.755	0.596	0.523	75.4	76.2	75.8	77.4	74.2	76.1	75.8
	Taxaceae	*Torreya californica*	0.782	0.643	0.564	71.0	70.4	71.3	69.6	74.4	71.6	71.4

[a] The percentages of similarity of 1:1, 1:2, 1:3 columns are calculated by the Bendich and Bolton's method and the percentages of Δc_1, Δc_2, Δc_3 are calculated by our method (see text).

[b] Average among the percentages of similarity calculated by the two different methods.

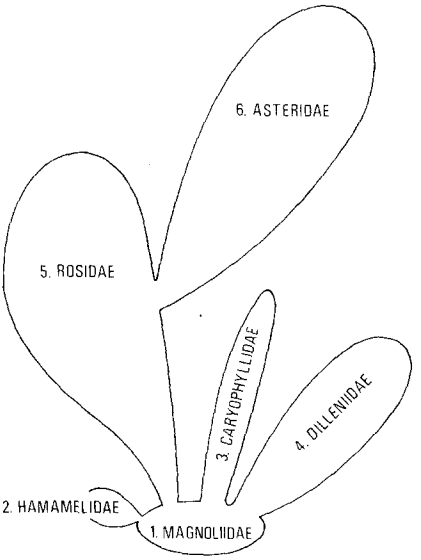

Fig.1
Probable relationships among the subclasses of Magnoliatae (dicots). The size of the balloon for each subclass is proportional to the number of species. (From Cronquist, 1968)

tages of similarity are 84.3 with *Pinus pinea*, 79.5 with *Ginkgo biloba*, 75.8% with *Gycas revoluta* and 71.4% with *Torreya californica*. The above reported values are the arithmetical mean of the values obtained by the Bendich and Bolton's method and by the method we have elaborated (Table 1).

Even if ribosomal RNA genes represent a peculiar system in evolutionary terms (Birnstiel et al., 1971; Brown et al., 1972), it is possible to draw interesting phylogenetic considerations comparing the rRNA similarities in monocots (Maggini, 1975) with the similarities of the dicots reported in the present paper. From Figure 1 results the probable relationship among the dicots (*Magnoliatae*) subclasses, deduced on the basis of morphological characteristics (Cronquist, 1968). Vodkin & Katterman (1971) reported a 95% similarity between pumpkin and cotton. Their data agree with our results since both species belong to the *Dilleniidae* subclass. With the species of the *Magnoliidae*, *Rosidae* and *Caryophyllidae* subclasses we find high percentages of similarity and this is evidence for a good conservation of the rRNA genes in dicots. The *Asteridae* subclass comprises, among others, the families *Lobeliaceae (Lobelia)*, *Schrophulariaceae (Anthirrinum)* and *Compositae*; this latter family is regarded as highly evolved among dicots. It is significant that we find the lowest similarities with species of *Compositae*. These results agree with the monocots data: indeed with the some species of the *Graminaceae* family, regarded as highly evolved among monocots, we have obtained lower similarity than with the rRNA of primitive *Liliaceae*. With regards to *Gymnospermae*, the lowest value of similarity (75.1%) has been obtained with *Torreya californica*, as could be expected since *Torreya* is considered fairly evolved among *Gymnospermae* on the basis of the

reduction of archegonial cells in the female gametophyte (Battaglia, 1951). On the other hand *Cycas revoluta* and *Ginkgo biloba*, two species regarded as primitive plants in *Gymnospermae*, have lower similarities than *Pinus pinea*, a species surely less primitive. In the latter case it is important to note that *Cycas* and *Ginkgo* are phylogenetically very distant from *Cucumis sativus*; this evolutionary consideration could be enough to explain the low similarities observed.

We are aware that our conclusions are drawn from hybridization data only; however our results still provide evidence in favour of the hypothesis that, in the highly evolved species, ribosomal RNA genes and perhaps the many genes that are functionally connected with them have not yet attained a definitive stabilization.

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