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Variability in Complementarity for Chloroplastic and Cytoplasmic Ribosomal Ribonucleic Acids among Plant Nuclear Deoxyribonucleic Acids[1, 2]

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ABSTRACT

The nuclear DNAs from a number of angiosperm species were tested for hybridization to the RNAs contained in 70 S (chloroplastic) and 80 S (cytoplasmic) ribosomes. All of the DNAs contained regions complementary to RNAs from chloroplastic as well as cytoplasmic ribosomes. DNAs from closely related plants varied widely in their proportion of coding for these RNAs. About 0.15% of the DNAs from a number of different species of *Nicotiana* were found to be complementary to the RNAs of each kind of ribosome; however, DNAs from some other members of this genus had more than three times this proportion of coding for ribosomal RNAs. These and other data suggest that hybridization percentage for ribosomal RNA is not a familial or generic characteristic.

DNAs with high amounts of coding for ribosomal RNAs have thus far been found to contain satellite DNAs of base composition more like ribosomal RNA than the main DNA component. The satellite DNA from pumpkin has been isolated and shown to contain cistrons for both chloroplastic and cytoplasmic ribosomal RNAs.

The present understanding of plant ribosomal RNAs and their relationship to nuclear DNAs has come from several types of studies. Sedimentation velocity experiments have demonstrated that roots contain 80 S ribosomes (14), whereas leaves contain 70 S chloroplastic as well as 80 S cytoplasmic ribosomes (20). Gel electrophoresis examinations have shown that each type of ribosome contains two major RNA classes that differ in molecular weight; furthermore, the molecular weights of RNAs found in 80 S ribosomes are distinct from those of 70 S ribosomes (18). Hybridization experiments have provided further insight into the nature of rRNAs and their relationships to plant nuclear DNAs. RNAs from leaves of different plants were found to hybridize to the same extent with the DNA from any given species (23). These results suggest that during the evolution of plants the base sequences of ribosomal RNAs have largely been conserved despite the evidence (1) that in other respects considerable differences can exist between DNAs of closely related species. Hybridization studies (32) have also clearly shown that the nuclear DNA from tobacco contains coding sequences for RNAs of both chloroplastic and cytoplasmic ribosomes.

In this report, we present experiments which demonstrate that the extent of DNA coding for RNA of chloroplastic and cytoplasmic ribosomes can be estimated by hybridizing the DNAs with rRNAs from roots and leaves. With this method, DNAs from a number of closely and distantly related species were examined for their content of coding sequences for these RNAs. All plant nuclear DNAs thus far examined have been found to contain coding sequences for RNA of both chloroplastic and cytoplasmic ribosomes.

METHODS

Ribosomes and labeled and unlabeled RNAs for all hybridizations were obtained primarily from roots and leaves of tobacco (*Nicotiana tabacum* L. var. Samsun). Tobacco seeds were germinated in vermiculite. When seedlings had a shoot length of about 6 inches, their roots were washed and the plants were transferred to 1-liter glazed pots containing continuously aerated complete Hoagland's medium (13), modified for rapid growth of cotton (D. R. Ergle, Department of Plant Sciences, Texas A and M University, College Station, Texas, personal communication). They were held for 3 to 4 weeks or until vigorous growth had begun. The complete medium was then replaced with −P medium for 2 days before adding 1 to 4 mc of carrier-free $H_3{}^{32}PO_4$ per pot for 3 to 4 days. Control plants were cultured in an identical manner except that label was not added. Plants of other species were also grown under greenhouse conditions and were treated like the tobacco plants except for adjustments in age so that incorporation of label occurred during vigorous plant growth. In some cases tritiated uridine was used to prepare labeled rRNAs. These preparations were made according to methods outlined by Zaitlin *et al.* (36).

For ribosome and RNA extractions, 20 to 40 g of leaves or roots were washed, then chilled in ice before grinding in sucrose-tris buffer (0.5 M sucrose, 0.01 M $MgCl_2$, 0.5 M sucrose, 0.05 M tris, 0.025 M KCl, 0.005 M mercaptoethanol, pH 8.2 [21]), with sand and an ice-cold mortar and pestle. Deribbed leaves were ground with a half-weight of buffer, roots were ground with an equal weight of buffer, and all grinding and fractionations were performed at 0 to 4 C unless otherwise noted. Centrifugations were generally conducted with a Sorvall SS-34 head in an RC-2 centrifuge. Homogenates were filtered through two layers of fine

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cheesecloth and glass wool and centrifuged at 13,000 rpm for 30 min. Supernatants were then centrifuged at 50,000 rpm in a Spinco 50 Ti head for 1.5 hr to pellet the ribosomes, which were subsequently extracted for RNA with phenol followed by alcohol precipitation overnight (10). The RNA pelleted on centrifugation at 6,000 rpm for 5 min was purified further by dissolving in 2 to 5 ml of 0.015 M Sorenson's phosphate buffer, pH 7.0, and centrifuging at 6,000 rpm for 5 min. The ribosomal RNA present in the cleared supernatant solution was then separated from the soluble RNAs by adding NaCl to 1.5 M and allowing precipitation to occur overnight. The precipitate was sedimented by centrifugation at 6,000 rpm for 5 min, and the pellet was resuspended in 0.015 M Sorenson's phosphate buffer. Concentrations were determined by spectrophotometry assuming that a 10-mm light path of a 1 mg/ml RNA solution has an A_{260} of 25. The samples were frozen and stored at −20 C.

For sedimentation velocity studies the ribosomal pellet was washed twice without resuspending with a buffer consisting of 5 mM tris (Sigma), 7 mM magnesium acetate, and 5 mM mercaptoethanol, pH 7.5, to remove adhering sucrose grinding solution. The pellet was then resuspended in a fresh aliquot of buffer, using 2 ml of buffer for each 20 g of original tissue. The suspension was clarified by centrifuging at 8,000 rpm for 10 min, and the supernatant was examined at 34,000 rpm in the An-D head of the Spinco model E. In the sedimentation studies, the 70 S and 80 S values assigned, respectively, to chloroplastic and cytoplasmic ribosomes are nominal.

DNA was obtained from *Micrococcus lysodeikticus* according to methods outlined by Marmur (22). Plant DNAs were obtained from leaves of tobacco and from *N. tabacum* L. var. Xanthi, *N. acuminata* (Grah.) Hook, *N. rustica* L., *N. occidentalis* Wheeler, *N. paniculata* L., *N. glauca* Grah., *N. sylvestris* Speg. and Comes, *N. glutinosa* L., *N. benthamiana* Domin., *N. bigelovii* var. multivalvis Lindl.; and, also, from pumpkin, *Curcurbita pepo* L. var. Small Sugar, Chinese cabbage, *Brassica pekinensis* (Lour. Rupr.) var. Wong Bok; Alaska and Little Marvel peas, *Pisum sativum* L.; pinto and red kidney beans, *Phaseolus vulgaris* L.; blue panicum grass, *Panicum antidotale*, Retz; and wheat germ, *Triticum vulgare* L. Prior to extracting for DNA, leaves were washed, chilled in ice, and homogenized with the materials and procedure outlined for ribosome preparation, except that the grinding buffer contained 0.01 M $CaCl_2$ rather than 0.01 M $MgCl_2$. The filtered homogenate was centrifuged at 2000 rpm for 4 min at 2 C, and the pellet from each 100 g of original tissue was resuspended in 40 ml of grinding buffer that contained 3.5% Triton X-100 (Rohm and Haas) and centrifuged again as before. The washing procedure was repeated twice more, and the final pellet containing nuclei was essentially free of chlorophyll color. The pellet was then extracted and purified for DNA by a modification (23) of the procedure of Marmur (22). DNAs subjected to alkali denaturation were further purified by CsCl preparative centrifugation (7) followed by passage through a Sepharose 4B column (Pharmacia Fine Chemicals Inc.). In the case of pumpkin, the DNA was partially fractionated by CsCl centrifugation (7) into two components. The main component, representing about 94% of the total DNA, had a mean buoyant density of 1.697; the remaining, or satellite fraction, had a buoyant density of 1.705. In all cases, DNA concentrations were estimated by assuming that a solution of 1 mg of native DNA per ml had an A_{260} of 20 (6). Concentrations estimated in this way for tobacco, red kidney beans, and Alaska peas were in close agreement with values obtained with diphenylamine (17).

Hybridizations were conducted according to Gillespie and Spiegelman (11). In early experiments, DNA samples (50 μg of total nuclear, 10 μg of pumpkin satellite DNA) in 2.5 ml of 0.1 × SSC (0.015 M NaCl, 0.0015 M trisodium citrate, pH 7.0 [22]) were heat denatured by maintaining at 97 C for 10 min then quick cooling in ice water. The buffer solution was then made to 2 × SSC and the DNAs were embedded on 25-mm nitrocellulose membranes (Schleicher and Schuell, B-6). In later studies, DNAs were alkali-denatured. Solutions containing 50 μg of DNA or less per ml of SSC were adjusted to pH 12.5 with 1 N NaOH and held for 10 min before rapidly neutralizing to pH 6.5 with 1 M KH_2PO_4. Unless otherwise stated, hybridizations were conducted for 12 hr at 68 C in 5 ml of 2 × SSC that contained 10 μg of labeled RNA. DNAs were embedded just prior to use since aging of membranes resulted in reduction of hybridization. All comparisons of root and leaf rRNA hybridization were made with identically prepared and treated DNA-embedded membranes. Radioactivities were determined with either the Nuclear-Chicago thin window counter or Packard Tricarb liquid scintillation counter. Net hybridized radioactivity values were determined by subtracting radioactivities obtained from identical treatments with blank membranes. Values obtained with tritium- or phosphorus-labeled RNAs were identical.

RESULTS

Classes of Ribosomes in Preparations from Roots and Leaves. One of the objects of this study was to determine the extent of complementarity of plant nuclear DNA to the RNA contained in 70 S and 80 S ribosomes. For this purpose it was necessary to have sources of the two types of RNA. Although we found it possible to prepare purified 80 S ribosomes from both roots and leaves of different plant species, several attempts to prepare substantial amounts of purified 70 S ribosomes by a variety of methods proved unsuccessful. An alternate strategy was, therefore, adopted for estimating the amount of DNA complementary to RNA of 70 S ribosomes. This involved the comparison of the behavior of leaf rRNA with that of root rRNA in artificial hybridization experiments. This strategy is dependent on the reported (20) presence of both 70 S and 80 S ribosomes in leaves and the presence of only 80 S ribosomes in roots (14). In order to confirm that this situation existed, leaf and root ribosome preparations from tobacco, peas (Little Marvel and Alaska), Chinese cabbage, pumpkin, and red kidney beans were examined in the analytical centrifuge. In every instance, leaf preparations contained both 70 S and 80 S ribosomes in addition to 17 S protein, whereas the root preparations contained only 80 S ribosomes. A typical set of sedimentation patterns illustrating this point is shown in Figure 1.

Characterization of Leaf and Root rRNAs. RNAs extracted from ribosomal preparations of leaves and roots and fractionated to eliminate the 5 S components were examined by sucrose density centrifugation. As seen in Figure 2, the preparations contain the two typical rRNA components normally distinguishable on sucrose gradients, the heavier 23 to 25 S component and the lighter 16 to 18 S component. In root preparations, the ratio of heavy to light component was consistently close to 2:1, whereas this ratio for the leaf rRNA samples varied from 1:0.9 to 1:2. The variability found in leaf preparations may result from differences in the relative amounts of 70 S and 80 S ribosomes in different leaf extracts and the known tendency of the heavy RNA component of the 70 S ribosome to separate and appear as a lighter component of about 13 to 16 S (18, 30).

In the experiment reported in Figure 2 and in all other studies on rRNAs obtained from plants given a 3-day label, leaf rRNAs as well as root rRNAs contained low and high molecular weight fractions of equal specific radioactivity. In leaf rRNA samples this suggested that the RNAs of 70 S ribosomes had specific radioactivities equal to those of 80 S ribosomes. This was later verified by gel electrophoresis examinations of total leaf RNAs by the procedures outlined by Ingle (15).

Hybridization of Tobacco Root and Leaf rRNAs with Homolo-

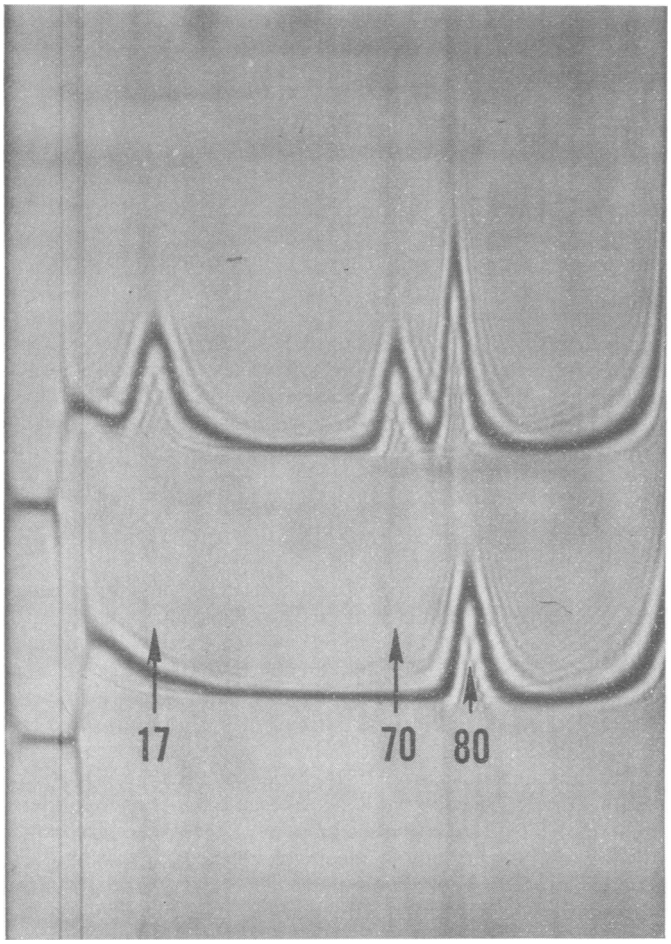

FIG. 1. Sedimentation patterns from ribosomal preparations of tobacco leaves (top) and roots (bottom). Sedimentations were conducted in the Spinco AnD rotor at 34,000 rpm and at a temperature of approximately 22 C. Direction of sedimentation is from left to right, and the photograph was taken at a Schlieren bar angle of 60°, 21 min after speed was reached.

gous and Heterologous DNAs. Various amounts of labeled root and leaf rRNAs allowed to react with a number of different DNAs in order to determine the amount of rRNA required to obtain maximum binding. These studies consistently showed that 10 μg of either root or leaf rRNAs were sufficient to saturate or virtually saturate the available sites in 50 μg of DNA. In the example shown in Figure 3, root and leaf rRNAs were hybridized with DNAs from two closely related plants, *N. tabacum* and *N. occidentalis*. At saturation, root rRNAs reacted with 0.15% of *N. occidentalis* and 0.05% of *N. tabacum* DNAs. These values were similar to values obtained in other studies using these DNAs and 10 and 20 μg of root rRNAs (see data for heat-denatured DNAs, Table II). Since roots contain only 80 S ribosomes, these values represent the amount of DNA coding for cytoplasmic rRNA. Leaves, however, contain both 70 S chloroplastic and 80 S cytoplasmic ribosomes, and rRNAs from this source hybridize to 0.19% of *N. occidentalis* and to 0.11% of *N. tabacum* DNAs. The difference in the hybridization value obtained with the two sources of rRNAs is presumed to indicate that fraction of DNA which is complementary to the RNA from 70 S ribosomes. In the example given here, 0.04% (0.19 minus 0.15) of *N. occidentalis* DNA is complementary to 70 S rRNA, and the same value for *N. tabacum* is 0.06% (0.11 minus 0.05).

The above calculations are made on the assumption that the RNAs from 70 S and 80 S ribosomes are complementary to separate, distinct DNA sites. This assumption is supported not only by the hybridization-saturation experiments reported above, but also by the results of competition-hybridization experiments. In these experiments, saturating amounts of labeled leaf rRNAs were challenged by the addition of unlabeled root or leaf rRNA to the reaction mixture. In the example given in Figure 4, a saturating amount of leaf rRNA was found to hybridize to 0.21% of *N. occidentalis* and 0.12% of *N. tabacum* DNAs. Reactions containing unlabeled leaf rRNAs from identically grown plants reduced the binding of labeled leaf rRNAs by amounts expected due to dilution by unlabeled, but otherwise identical, RNAs. In contrast

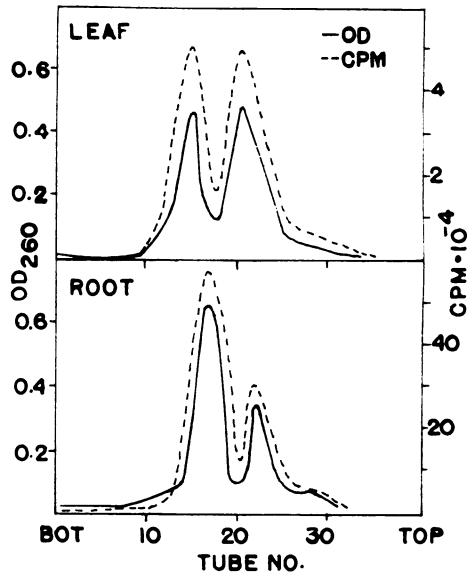

FIG. 2. Separation of labeled leaf and root rRNAs in sucrose gradients. Gradient: 0.2 M to 1.0 M sucrose in 0.02 M potassium phosphate buffer, pH 7.0. Centrifugation was for 8 hr in Spinco SW-39 head at 35,000 rpm (rotor temperature 2–4 C). Two drop fractions were diluted to 1 ml for A_{260}. Specific radioactivity of leaf and root rRNAs were 6,800 and 23,000 cpm/μg, respectively.

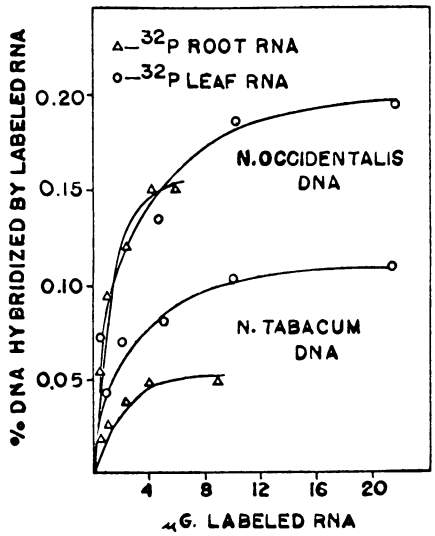

FIG. 3. The reactivity of heat-denatured DNAs from *N. occidentalis* and *N. tabcum* to rRNAs from tobacco roots and leaves. Except for use of different amounts of rRNAs, reaction conditions were identical to those of Table I.

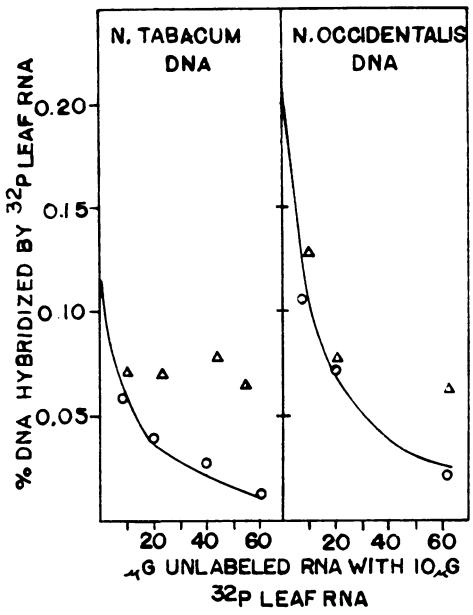

FIG. 4. The effect of unlabeled root and leaf rRNAs on hybridization by ^{32}P-tobacco leaf rRNA. Indicated amounts of unlabeled leaf or root rRNAs were mixed with 10 μg of ^{32}P-leaf rRNA prior to reacting with 50 μg of heat-denatured DNA for 12 hr at 68 C in 2 × SSC. Theoretically expected hybridizations by the labeled leaf rRNA in the presence of unlabeled but otherwise identical rRNAs are indicated by the solid line; actual values obtained in the presence of root rRNAs are indicated by triangles; and values obtained in the presence of leaf rRNAs are indicated by circles. *N. tabacum* and *occidentalis* DNAs retained 370 and 650 cpm, respectively, when allowed to react with ^{32}P-leaf rRNA (6200 cpm/μg) in absence of unlabeled rRNAs.

even large amounts of unlabeled root rRNA had only limited potential for reducing the hybridization of labeled leaf rRNA to tobacco DNA. In mixtures containing unlabeled root rRNAs, the residual hybridization in both DNAs corresponded closely to that estimated from saturation-hybridization experiments as being due to RNA of 70 S ribosomes (Fig. 3). Results of this type can occur only if the RNAs of the 80 S ribosomes of roots and leaves are identical and if these RNAs are distinct from those of 70 S ribosomes. We concluded, therefore, that hybridizations with root RNAs will provide an estimate of coding for RNAs of 80 S cytoplasmic ribosomes. Furthermore, since leaves contain 70 S and 80 S ribosomes and their RNAs have equal specific radioactivity, any additional hybridization obtained with leaf rRNAs is a measure of complementarity to RNAs of 70 S chloroplastic ribosomes.

Most of the experiments presented in this paper consist of artificial hybridization of DNAs from several plant species with rRNAs from tobacco. This single plant source was used because of convenience and because we had previously shown that rRNAs from widely different plants are alike in their hybridization specificity (23). Trewavas and Gibson (33), however, have recently presented data suggesting that the rRNAs of Alaska peas, for example, hybridized differently than rRNAs from other legumes. We have conducted numerous reciprocal hybridization experiments of the type shown in experiment 1 of Table I, using nucleic acids from a number of plant species, and have consistently found that rRNAs from tobacco roots or leaves hybridize like rRNAs from roots or leaves of other dicot species. These results indicate that the coding sequences for both chloroplastic and cytoplasmic rRNAs have been conserved during the evolution of higher plant species.

Although rRNAs from distantly related plants hybridized

Table I. *Hybridization of Various Plant rRNAs to Plant and Bacterial DNAs*

Ten-microgram amounts of each of the indicated rRNAs in 5 ml of 2 X SSC were allowed to react with 50 μg of heat-denatured DNAs for 12 hr at 68 C (see "Methods"). RNAs were from leaves or roots of tobacco or Alaska peas. Each value represents the mean from duplicate replications and net hybridized cpm were obtained by subtracting hybridization values from identical reactions with blank membranes. Specific radioactivities as cpm/μg RNA are expressed parenthetically.

DNA	Labeled rRNA	Net Hybridization	^{32}P-RNA Hybridization
		cpm	*μg/100 μg DNA*
Experiment 1			
Tobacco	Tobacco leaf (1,980)	89	0.090
Tobacco	Tobacco root (11,200)	349	0.062
Tobacco	Alaska pea leaf (600)	34	0.11
Tobacco	Alaska pea root (5,680)	203	0.072
Alaska pea	Tobacco leaf (1,980)	179	0.18
Alaska pea	Tobacco leaf (11,200)	810	0.14
Alaska pea	Alaska pea leaf (600)	57	0.19
Alaska pea	Alaska pea root (5,680)	328	0.12
Experiment 2			
Tobacco	Tobacco leaf (4,130)	188	0.092
Micrococcus	Tobacco leaf (4,130)	1	...
Tobacco	Tobacco root (15,000)	398	0.051
Micrococcus	Tobacco root (15,000)	7	...

alike and to significant amounts of plant DNAs, rRNAs from either roots or leaves were found to bind only slightly to the DNA from the bacterium, *Micrococcus lysodeikticus* (experiment 2, Table I). The results are similar to those obtained by others (5, 32) and provide further evidence that rRNA reactions to plant DNAs are not random; rather they represent binding to specific and complementary sites.

Hybridization Values Obtained with Heat- and Alkali-denatured DNAs. Nuclear DNAs from a number of closely and distantly related higher plants were examined for the presence and amount of coding for RNAs of chloroplastic and cytoplasmic ribosomes. In early experiments, hybridizations were performed with heat-denatured DNAs. The results were reproducible, but later studies showed that higher hybridization values were nearly always obtained with alkali denatured DNAs. The values obtained with DNAs denatured in both ways are presented in Table II.

An over-all examination of the results provided a basis for making several statements regarding complementarity between plant rRNAs and nuclear DNAs. Most significantly, the data show that all DNAs examined have coding sites for RNAs of chloroplastic as well as cytoplasmic ribosomes. We believe that this situation will apply to all or nearly all nuclear DNAs from angiosperms.

The data of Table II show also that plant DNAs can differ widely in the amount of coding for RNAs of both 70 S and 80 S ribosomes. Since DNAs from members of the same family (*i.e.*, peas and beans) and even members of the same genus (*i.e.*, *N. rustica* and *N. paniculata*) differed in their proportion of coding for rRNAs, hybridization percentage to ribosomal RNA was not considered to be a useful taxonomic characteristic.

In animals, the DNA sequences coding specifically for rRNAs (rDNA) are located in the nucleoli or nucleolar organizer regions of chromosomes (27, 34). Because extensive cytological studies have been made on members of the genus *Nicotiana* (12), a

Table II. *Proportion of Heat- and Alkali-Denatured DNAs Reactive to RNAs of 70 S and 80 S Ribosomes*

The values given here are considered to be representative of a large number of trials. Calculations of percentages were made using the procedure shown in Table I.

DNA	Heat-denatured DNAs Reactive to rRNAs			Alkali Denatured DNAs Reactive to rRNAs		
	Roots (80 S)	Leaves		Roots (80 S)	Leaves	
		(70 + 80 S)	(70 S)		(70 + 80 S)	(70S)
	%	%	%	%	%	%
N. acuminata	...	0.15	...	0.14	0.30	0.16
N. benthamiana	...	...	...	0.16	0.35	0.19
N. bigelovii	...	...	...	0.16	0.35	0.19
N. glauca	...	...	...	0.16	0.35	0.19
N. glutinosa	0.08	0.14	0.06	0.25	0.67	0.42
N. occidentalis	0.14	0.21	0.07	0.14	0.40	0.26
N. paniculata	0.23	0.37	0.14	0.35	0.80	0.45
N. rustica	0.08	0.14	0.06	0.16	0.25	0.09
N. sylvestris	0.30	...	...	0.30	0.95	0.65
N. tabacum	0.06	0.12	0.06	0.14	0.30	0.16
N. tabacum var. Xanthi	0.10	0.16	0.06	...	...	...
Chinese cabbage	0.70	0.83	0.13	0.63	1.80	1.17
Pumpkin						
Whole	0.40	0.52	0.09	...	...	...
Main band	0.17	0.24	0.07	...	...	...
Satellite	2.1	2.8	0.7	6.5	12.5	6.0
Alaska pea	0.14	0.18	0.04	...	...	...
Little marvel pea	0.13	...	...	...	...	...
Red kidney bean	0.26	0.42	0.16	...	...	...
Pinto bean	0.20	0.35	0.15	...	...	...
Blue Panicum grass	0.41	0.50	0.09	...	...	...
Wheat germ	0.05	0.08	0.03	...	...	...

number of species in this group were studied in the hope that some simple correlation could be made between cytological observations and the hybridization percentages for rRNAs in the nuclear DNAs. As yet, no clear relationships of this type have been found.

Table II shows that in most cases, cytoplasmic and chloroplastic rRNAs were far more reactive to alkali than to heat-denatured DNAs. Since the reaction conditions were otherwise identical, these data suggested that the heat denaturation procedure resulted in DNAs that either were incompletely denatured or became partially renatured. Support for the latter possibility was provided on examination of heat- and alkali-treated pumpkin satellite DNAs following isopycnic centrifugation (Fig. 5). The alkali-treated DNA yielded one band (Fig. 5C) which had a bouyant density corresponding to that expected for denatured DNA (31). Conversely, the heat-denatured DNA (Fig. 5B) contained an additional band which had a bouyant density similar to that expected for renatured DNA. These data illustrate how perturbations in a small amount of the total DNA (the satellite DNA of pumpkin is about 6% of the total DNA) can have a significant effect on quantitative hybridization results. They indicate also that hybridization values obtained with alkali-denatured DNAs are quantitatively more correct than those obtained with heat-denatured DNAs but even the most accurate data available today may be subject to revision since many aspects of denaturation and hybridization are still only partially understood (24, 25).

Although the data obtained with heat- and alkali-denatured DNAs differed quantitatively, they both showed that nuclear DNAs from closely related species could differ widely in their amount of coding for chloroplastic and cytoplasmic rRNAs. Since values of the type shown in Table II were obtained consistently when reactions were run under comparable conditions, we think that the basic principles found in these studies will continue to be valid.

Distribution of rRNA Coding Sites in DNAs Partially Fractionated in CsCl. Evidence from a number of sources indicates that in many plant DNAs the base sequences that code for rRNA (rDNA) are located on DNA components that have a molar GC[3] content distinct from that of the main DNA fraction but similar to that of rRNA. We found earlier (23) that low hybridizing DNAs (such as those from tobacco) showed the presence of a single band on CsCl equilibrium centrifugation, but DNAs from high hybridizers (such as those from pumpkin and Chinese cabbage) contained, in addition, satellite DNAs of buoyant density greater than that of the main band. The satellite and main band fractions of pumpkin were isolated, and base composition calculations based on buoyant density values (28) showed that the GC content of satellite and main bands was 48 and 36%, respectively. The GC content of the satellite fraction was clearly closer to the reported (19, 26, 35) 53 to 60% of plant rRNAs than the bulk of the DNA. Hybridization experiments (see Table II) also showed that the satellite DNA of pumpkin had a high proportion of rDNA. In order to gain a clearer understanding of the relationships existing between the satellite and rDNA of pumpkin, hybridization experiments of the type outlined by Birnstiel *et al.* (2) were conducted. In this study, samples of purified pumpkin satellite and main band components were banded preparatively in CsCl gradients (7), and fractions (18 drops per tube) were collected and tested for A_{260} and for hybridization capacity for rRNA from leaves. The results in Figure 6 show that the absorbance and hybridization profiles superimpose for the purified satellite component (Fig. 6A). In contrast, it is evident that the rDNA found in the main band (Fig. 6C) and also in unfractionated pumpkin DNA (Fig. 6B) is of higher buoyant density

[3] Abbreviations: GC: guanine plus cytosine.

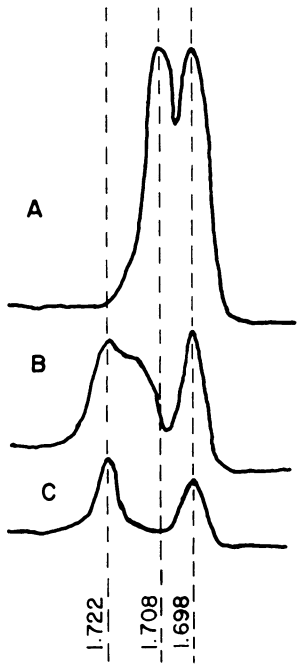

FIG. 5. Isopycnic banding in CsCl of pumpkin satellite DNA. A: Native; B: heat-denatured; C: alkali-denatured. The band at 1.698 g/cc is tobacco nuclear DNA used as a marker.

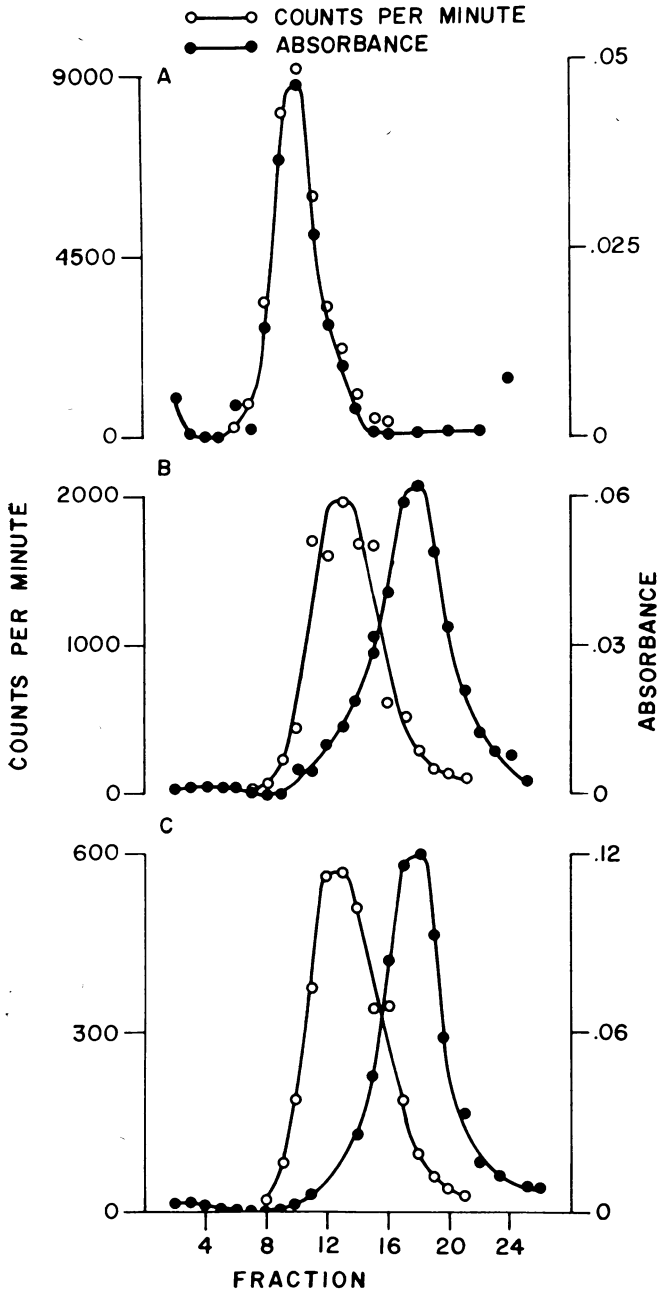

FIG. 6. Fractionation of pumpkin nuclear DNA. A: 11 μg of purified satellite component; B: 34 μg of unfractionated DNA; C: 42 μg of purified main band component. A 0.2-ml DNA solution was mixed with 3.05 ml of 0.05 M tris buffer and 4.1 g of CsCl. Centrifugation was for 64 hr at 35,000 rpm in the small No. 50 Spinco rotor. Eighteen drop fractions were collected and the A_{260} was determined after addition of 0.95 ml of 2 × SSC to each fraction. Appropriate fractions were then embedded in B-6 membranes after alkali denaturation and were hybridized with ^{3}H-labeled pumpkin leaf rRNA of specific activity 40,000 cpm/μg.

than the bulk of these DNAs. This suggests that the rDNA of the satellite and the main bands of pumpkin are identical and that the initial separation of satellite and main DNA bands was incomplete.

In experiments conducted with tobacco DNA, a low hybridizer with no detectable satellite component, results similar to those obtained with the main DNA band of pumpkin were obtained. The rDNA was found to have a buoyant density similar to that of pumpkin rDNA and distinctly greater than that of the bulk of tobacco DNA. Although the results may be fortuitous, these data indicate that rDNAs from other plants may have buoyant densities similar to rDNAs from pumpkin.

DISCUSSION AND CONCLUSIONS

The data presented here are part of a continuing study on the relationships existing among plant DNAs and their rRNAs. Earlier preliminary studies suggested that nuclear DNAs from tobacco, Chinese cabbage, and pumpkin contained sites coding for RNAs of chloroplastic as well as cytoplasmic ribosomes (23). We have since made far more extensive studies of the hybridization behavior of these and other plant DNAs to determine if this situation generally exists in plants. The results of many experiments consistently showed that coding for RNAs of both kinds of ribosomes are present in DNAs obtained from purified nuclei (Table II). These findings agree with those of Tewari and Wildman (32), who obtained nucleic acids from tobacco using methods not generally used in this study, but they stand in marked contrast to results reported for *Euglena* (29). In *Euglena*, coding for chloroplastic rRNA appears to be confined to chloroplastic but not nuclear DNA. It appears that findings with this organism should be used cautiously when generalizing about the function and formation of angiosperm DNAs.

The studies presented here have shown also that DNAs from even closely related species can differ greatly in the amount of coding for RNAs of 70 S and 80 S ribosomes. Examinations of various DNAs in CsCl have continued to show that DNAs with low amounts of binding to rRNAs (*i.e.*, tobacco) have a single band. In contrast, DNAs containing clearly higher complementarity to rRNAs (*N. occidentalis*, etc.) have been found to contain a distinguishable satellite fraction of GC content that is greater than the GC content of about 35 to 40% found in most plant DNAs. The amount of satellite DNA, however, varies considerably from species to species, and the relative proportions of the satellite to the main band are not always related to the amount of hybridization to rRNAs. We interpret this to mean that in some species the DNAs coding specifically for rRNAs may be only a part of a complex mixture of DNAs that comprise the satellite fraction. Even in the case of the pumpkin satellite DNA, which appears to be the most homogenous of the DNAs studied, it is not yet clear if this fraction is a single DNA containing coding sites for RNAs of 70 S and 80 S ribosomes, or if it is a mixture of DNAs.

The hybridization results obtained here are, in some respects, analogous to those obtained with animals. The more extensive studies done with the latter organisms have provided avenues of approach that may be used in future plant studies. In the amphibian, *Xenopus*, rDNAs were isolated early (3) and were shown to have a base composition similar to that of rRNA. In *Xenopus* (2, 9, 16, 34, 35) and in *Drosophila* (27), there is now abundant evidence that coding sites for rRNAs are located in the nucleolar organizer region. Interestingly, in *Xenopus* a very pronounced and selective multiplication of rRNA coding sites is known to occur during oocyte formation (4, 8), and this selective amplification is associated with the formation of a great many free nucleoli that contain DNA distinct from chromatin DNA.

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