DIVERGENCE OF RIBOSOMAL RNA SEQUENCES
WITHIN ANGIOSPERMAE

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Manuscript received April 15, 1971
Revised copy received August 30, 1971

BY annealing ribosomal RNA (rRNA) to known amounts of DNA, one can
determine the proportion of the genome complementary to rRNA. Hybridization
experiments can also reveal the similarity among rRNA's in terms of their
nucleotide base sequences. Annealing of rRNA's isolated from various species to
the DNA of a single species provides an experimental approach for the comparis-
on of the base sequences of those rRNA's. One may conclude that rRNA's which
hybridize to the same extent to a DNA are more similar to each other than
rRNA's which differ in this regard.

Another means of comparing the nucleotide sequences of rRNA's of different
species involves thermal stability measurements of rRNA-DNA hybrids. The
rRNA that has hybridized to DNA can be released from the hybrid as a function
of temperature. The temperature at which 50% of the rRNA has dissociated is
defined as the $T_m$. Because rRNA from one species may not be the exact com-
plement to ribosomal DNA (rDNA) of another, some base pairs in the rRNA-DNA
hybrid of these two species may be mismatched. This mismatching lowers the $T_m$
of heterologous rRNA-DNA hybrids relative to that of the homologous hybrid.
The percentage of base-pair mismatching in the heterologous relative to that in
the homologous rRNA-DNA hybrid can be estimated from the difference in $T_m$'s
between the latter and the former (Laird, McConaughy and McCarthy 1969).

Several investigations employing the techniques mentioned above have been
performed to determine the similarity in base sequence of rRNA's among differ-
ent species of higher plants (Matsuda and Siegel 1967; Matsuda, Siegel and
In this study reciprocal rRNA-DNA hybridizations were performed in order
to compare the base sequences of rRNA's among Angiospermae (monocots and
dicots). The proportion of DNA complementary to a given rRNA was deter-
mined, and the thermal stability of rRNA-DNA hybrids was measured. Evidence
from these experiments permits the following conclusions: (1) The nucleotide
sequences of rRNA's of dicots have been highly conserved. (2) The nucleotide
sequences of rRNA's of monocots have diverged from each other considerably.

1 Contribution No. 1755 from the Arizona Agricultural Experiment Station. This paper is part of a dissertation to be
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There has been considerable divergence of nucleotide sequences of rRNA's between monocots and dicots.

**MATERIALS AND METHODS**

Seeds were donated by Departments of Agronomy, Horticulture, Plant Breeding and Animal Pathology at the University of Arizona as follows: Barley (*Hordeum vulgare* L., cv. Betzes), blue panicum grass (*Panicum antidotale* Retz.), wheat (*Triticum vulgare* L., cv. Siete Cerros), cotton (*Gossypium barbadense* L., cv. Pima), and pumpkin (*Cucurbita pepo* L., cv. Small Sugar).

**Extraction of DNA:** A nuclear pellet was obtained from seeds by a procedure similar to the one utilized by Matsuda and Siegel (1967). Monocots and dicots were treated in the same manner except that the nuclear pellet from dicot seeds was defatted by the method of Ergle and Katterman (1961). DNA was extracted from the pellets and purified with modifications of Marmur's procedure (1961). Purified DNA was then subjected to column chromatography with Sepharose 2B (Pharmacia Fine Chemicals).

**Labeling and extraction of rRNA:** Ten grams of monocot seedlings, about three weeks old, were excised at the base of the shoot, and the leaves were placed upright in conical containers (Nielsen and Rohringer 1963). One mCi of uridine-5H3 (Schwarz Bio Research, specific activity greater than 20 C/mM) was distributed evenly among the containers. The leaves were maintained in a small volume of distilled water for the next 48 hr. Dicot leaves were labeled according to the method of Zaitlin, Spencer and Whitfield (1968).

After the 48-hr period, leaves were rinsed several times with distilled water and blotted dry. They were ground to a fine powder with a mortar and pestle at −20°C. Thawing took place in 4V/W of 0.1% bentonite-1% sodium pyrophosphate-1% sodium dodecyl sulfate (SDS) pH 5.0. Four V/W of phenol, redistilled over zinc and equilibrated with 1×SSC (0.15 M NaCl-0.015 M trisodium citrate), were added, and the mixture was shaken vigorously in the cold for 15 min. Centrifugation for 10 min at 5,000×g separated the phases. The aqueous phase was submitted to two more phenol extractions.

The RNA was precipitated with 0.1 volumes of 20% potassium acetate pH 5.0 and two volumes of ethanol, and the precipitate was collected by centrifugation at 12,000×g for ten min. The pellet was suspended in a small volume of 0.5% SDS-0.15 M sodium acetate pH 8.0 and subjected to phenol extractions until protein was no longer visible at the interface. Phenol was removed from the aqueous layer by shaking three times with two volumes of cold diethyl ether. Traces of ether were evaporated by bubbling nitrogen gently through the aqueous layer. The RNA was precipitated and collected as above.

The RNA was suspended in a small volume of 0.067 M phosphate buffer pH 6.8, to which was added an equal volume of 4 M LiCl. After 12 hr, the rRNA precipitate (Avital and Elson 1969) was collected by centrifugation at 12,000×g for five min and washed sequentially with 2 M LiCl and 75% ethanol. The pellet was suspended in 0.067 M phosphate buffer and stored at −20°C. Cotton rRNA, prepared by the above methods, still had a red pigment and was further purified by the method of Bellamy and Ralph (1968).

**Gel electrophoresis of rRNA:** Ribosomal RNA was analyzed electrophoretically according to the method of Bishop, Claybrook and Spiegelman (1967). The gels were soaked in distilled water for several hours in a Petri dish, transferred to a quartz cuvette (5 mm × 100 mm × 8.5 mm) and scanned at 260 nm on a Gilford model 240 spectrophotometer equipped with a model 2410 linear transport device and a Heathkit Servo-Recorder model EUW-20A.

Gels that were to be monitored for radioactivity were frozen solid quickly by means of dry ice in a curved spatula and cut into 1.05 mm slices with a bank of evenly spaced stainless steel razor blades. The slices were digested in 0.5 ml of 30% hydrogen peroxide for 24 hr at 50°C and then counted for radioactivity (Siegel and Huber 1970).

**Annealing of rRNA to DNA:** The pH of a DNA solution (10–50 µg/ml) was raised to 12.0–12.5 by the addition of 0.1 volumes of 1 N NaOH. After ten minutes the solution was neutralized with 0.15 volumes of 1 M KH₂PO₄ and immediately diluted to 3 µg/ml with 2×SSC. The
alkaline-denatured DNA was adsorbed to Bac-T-Flex B6 nitrocellulose filters (Schleicher and Schuell Co.) essentially according to the method of Gillespie and Spiegelman (1965). Each filter was then inserted into a vial and incubated for 18 hr at 68°C in 1 ml of 0.1% SDS in 2 x SSC containing appropriate concentrations of labeled rRNA. The reaction was stopped by chilling the vials, and the filters were washed batchwise in each of the following solutions for 30 min: 6 x SSC at 50°C, 2 x SSC, 2 x SSC; 20 µg/ml of pancreatic RNAase (Calbiochem, 5 x crystallized) in 2 x SSC, and 2 x SSC. Filters were then vacuum oven-dried and monitored for radioactivity.

Thermal stability profiles of rRNA-DNA hybrids: Washed filters containing rRNA-DNA hybrids were incubated separately at 60°C for five minutes in 2 ml of 2 x SSC. The filters were transferred to other vials, and the incubation was continued at 65°C. The process was repeated in 5°C increments up to the maximum temperature (98°C), with ten minutes allotted for equilibration at each temperature. The radioactivity in the vials, representing rRNA solubilized at each temperature, was counted; also, the filters were monitored after the last incubation to determine if any rRNA remained on them. The percent of cumulative counts released was plotted against temperature, and the T_m was read directly from the graph.

Radioactivity measurements: Samples were counted at optimal gain in a Packard Tri-Carb scintillation spectrometer. Filters were counted in vials containing 0.5% PPO (2,5-diphenyloxazole) in toluene; aqueous solutions in vials containing 0.01% POPOP (1,4-bis [2-(5-phenyloxazolyl) benzene]) - 0.55% PPO in a solution 33% Triton X-100 (Rohm and Haas) and 67% toluene.

RESULTS

Characterization of DNA: The DNA's used in hybridization experiments were characterized by CsCl isopycnic centrifugation in a model E analytical ultracentrifuge in order to determine their buoyant densities and also to detect the presence of a DNA satellite. Ultraviolet photographs of the DNA’s were taken at equilibrium, and densitometer tracings of these photographs were made. The buoyant densities calculated from these figures are summarized in Table 1. With the exception of pumpkin, no other DNA in this study displays a satellite component. The buoyant densities of the native DNA’s from barley, blue panicum grass, wheat, cotton and pumpkin as well as pumpkin satellite agree closely with previously reported values (Sinclair and Brown 1967; Matsuda and Siegel 1967 and Bendich and McCarthy 1970b). The values for blue panicum grass and cotton DNA’s have not been reported before.

<p>| TABLE 1 |</p>
<table>
<thead>
<tr>
<th>Source of DNA</th>
<th>Buoyant density g/cc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barley</td>
<td>1.701</td>
</tr>
<tr>
<td>Blue panicum grass</td>
<td>1.702</td>
</tr>
<tr>
<td>Wheat</td>
<td>1.703</td>
</tr>
<tr>
<td>Cotton</td>
<td>1.692</td>
</tr>
<tr>
<td>Pumpkin</td>
<td>1.694</td>
</tr>
<tr>
<td>Pumpkin satellite</td>
<td>1.705</td>
</tr>
</tbody>
</table>

* Plant DNA’s (3 µg) and marker DNA (1 µg) were centrifuged to equilibrium in analytical CsCl gradients. Photographs were taken and then traced with a densitometer. Buoyant densities were calculated according to the method of Sueoka (1961) from these densitometer tracings.
It is of interest to note that the DNA's of the monocot species examined have similar buoyant densities ranging from 1.701 to 1.703 g/cc and that the DNA's from both dicot species examined are characterized by somewhat lower buoyant densities. This finding is in agreement with other investigations of monocot and dicot DNA's. Green and Gordon (1967) list the buoyant densities of DNA's from several dicots. They vary from 1.690 to 1.697 g/cc. Bendich and McCarthy (1970b) assign buoyant densities of 1.700 and 1.702 g/cc respectively to the DNA's of oats and rye (monocots). However, Green and Gordon (1967) and Wells and Ingle (1970) report the buoyant density of onion DNA, a monocot, as 1.688 and 1.691 g/cc, respectively. The difference in buoyant densities between monocot and dicot DNA's may have some taxonomic significance despite the one exception noted above.

Characterization of rRNA: Ribosomal RNA preparations were routinely sub-
jected to polyacrylamide gel electrophoresis in order to determine the presence and integrity of the individual rRNA components. After electrophoresis, gels were scanned at 260 nm, and the optical density was recorded. Electrophoretic patterns of rRNA from leaves display four optical density peaks, as represented in Figure 1a. The S values have been assigned in accordance with Löening and Ingles (1967). The area under the 25S peak appears to be at least twice that under the 18S peak. Since the molecular weight (hence the nucleotide content) of the 25S rRNA is about twice that of the 18S rRNA, such a relationship would be expected if both components are present in equimolar amounts. However, there is less 23S than 16S rRNA, possibly on account of degradation of the former. Figure 1b depicts the profile for rRNA from barley roots. Root tissue contains no 70S ribosomes (Hsiao 1964) and therefore should have only the 18S and 25S rRNA components.

In order to compare the optical density profile with the radioactivity profile, a gel with labeled barley rRNA was fractionated. Labeled rRNA was subjected to gel electrophoresis as described above. After scanning for optical density, the gel was sliced, and the radioactivity of each fraction was determined. Labeled rRNA from leaf material was expected to have four peaks of radioactivity as well as four peaks of optical density. Figure 2 is a comparison of the optical density and radioactivity profiles of barley rRNA components separated by polyacrylamide gel electrophoresis. The four labeled peaks correspond closely to the four optical density peaks. The two additional peaks of radioactivity, that are lighter than 16S, probably represent degraded rRNA. The above data indicate that labeled rRNA is synthesized by plants and not by contaminating bacteria.

**Homologous and heterologous hybridization values at saturation:** In order to determine the base sequence homology of rRNA among monocots, among dicots, and between monocots and dicots, monocot and dicot rRNA’s were hybridized to monocot and dicot DNA’s in saturation-hybridization experiments. Figure 3 shows the saturation-hybridization values for a number of combinations of rRNA’s and DNA’s. The saturation–hybridization values listed in Table 2 were obtained by plotting the above data according to Bishop et al. (1969). Selected points and, when feasible, entire saturation curves were repeated with both the same and different nucleic acids. A 5 to 12% deviation from the values in Table 2 was observed when five of the saturation–hybridization experiments were repeated with a different rRNA as well as a DNA preparation.

The data in Table 2 reflect several patterns. With one exception, the two dicot rRNA’s hybridize equally to a given monocot or dicot DNA. The exception is that cotton and pumpkin rRNA’s hybridize unequally to cotton DNA. Despite this exception, a general trend toward the conservation of rRNA sequences between dicots can be noted. This conclusion is in agreement with the findings of Matsuda and Siegel (1967).

The two monocot rRNA’s, on the other hand, do not hybridize equally to a given DNA. For instance, the saturation–hybridization value of wheat rRNA hybridized to barley DNA is only 58% of that observed for barley rRNA hybridized to barley DNA. Likewise, barley rRNA hybridizes to wheat DNA 68%
FIGURE 2.—Fractionation of labeled barley leaf rRNA on gel. Electrophoresis of 10 μg samples of radioactive rRNA as well as the tracing of the optical density profiles were carried out as described in Figure 1. The gels were then frozen solid by means of dry ice and cut into 1.05 mm slices with a bank of evenly spaced stainless steel razor blades. The slices were digested in 30% hydrogen peroxide (SIEGEL and HUBER 1970) before radioactive assay.

TABLE 2

Percent of DNA hybridized with rRNA at saturation*

<table>
<thead>
<tr>
<th>Source of rRNA</th>
<th>Source of DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Monocot</td>
</tr>
<tr>
<td></td>
<td>Barley</td>
</tr>
<tr>
<td>Monocot</td>
<td></td>
</tr>
<tr>
<td>Barley</td>
<td>0.73 (100)</td>
</tr>
<tr>
<td>Wheat</td>
<td>0.42 (58)</td>
</tr>
<tr>
<td>Dicot</td>
<td></td>
</tr>
<tr>
<td>Cotton</td>
<td>0.34 (47)</td>
</tr>
<tr>
<td>Pumpkin</td>
<td>0.29 (40)</td>
</tr>
</tbody>
</table>

* The saturation-hybridization values were calculated from the data of Figure 3 according to the method of BISHOP et al. (1969). The numbers in parentheses refer to relative percent of DNA hybridized with rRNA at saturation, with the homologous reaction normalized at 100%.
of the extent that wheat rRNA hybridizes to wheat DNA. These data suggest that about 60% of the rRNA sequences between the two monocots has been conserved.

When the dicot rRNA's hybridize to monocot DNA's, 40–60% of the homologous saturation–hybridization values are observed. In the reciprocal experiments of hybridizing monocot rRNA's to dicot DNA's, 65–95% of the homologous saturation–hybridization values are observed. This disparity between reciprocal hybridizations will be treated in the Discussion section.

The hybridization values in Table 2 involve leaf rRNA's, which include the 25S and 18S rRNA components of the cytoplasm as well as the 23S and 16S rRNA components of the chloroplast. In order to assess what proportion of rDNA codes for 25S and 18S rRNA, competition experiments were performed with unlabeled root rRNA's. In one experiment, a series of filters with barley DNA was hybridized with a fixed, saturating concentration of labeled barley leaf rRNA and increasing concentrations of unlabeled barley root rRNA. The same type of experiment was performed with cotton DNA, labeled cotton leaf rRNA, and unlabeled cotton root rRNA. The data were plotted according to the method of Bishop et al. (1969), and it is concluded that the relative contribution by cytoplasmic rRNA (25S and 18S rRNA) to the saturation–hybridization values of barley and cotton are estimated at 58 and 63%, respectively. In control experiments, hybridization of labeled leaf rRNA to DNA was reduced by more than 95% in the presence of excess unlabeled leaf rRNA.

In order to test the purity of DNA, hybridization values were obtained with DNA's that had undergone a purification procedure in addition to those routinely employed. Purified barley and cotton DNA's were submitted to preparative CsCl centrifugation. Those DNA's were then hybridized with a saturating concentration of barley and cotton rRNA's, and the results are presented in Table 3. Because there is relatively little difference between the values in Table 2 and 3, it was concluded that further purification of DNA did not alter its hybridization properties.

**Thermal stability of rRNA–DNA hybrids:** Saturation–hybridization experiments measure the proportion of DNA that can be recognized by the species of RNA under study. Because both homologous and heterologous hybridization experiments have been performed, it is of interest to compare the thermal stabilities of both types of hybrids to determine if there has been a greater extent

<table>
<thead>
<tr>
<th>Source of rRNA</th>
<th>Source of DNA Barley</th>
<th>Source of DNA Cotton</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barley</td>
<td>(0.73) 0.62</td>
<td>(0.55) 0.54</td>
</tr>
<tr>
<td>Cotton</td>
<td>(0.34) 0.42</td>
<td>(0.71) 0.80</td>
</tr>
</tbody>
</table>

* The values in parentheses were taken from Table 2 for comparison, and those hybridizations were not performed with DNA's that had been subjected to preparative CsCl centrifugation.
Figure 3.—Saturation-hybridization curves of homologous and heterologous rRNA–DNA hybrids. Each hybridization experiment was done in duplicate with a set of filters containing a constant amount of DNA. One to fifty pg samples of alkaline denatured DNA were adsorbed to Bac-T-Flex B6 nitrocellulose filters (Gillespie and Spiegelman 1965). (The actual amount of DNA/filter in each hybridization experiment depended on the number of counts expected at saturation and was determined in preliminary investigations.) Each filter was inserted into a
TABLE 4

T_m of rRNA–DNA hybrids*

<table>
<thead>
<tr>
<th>Source of rRNA</th>
<th>Monocot</th>
<th></th>
<th></th>
<th>Dicot</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Barley</td>
<td>Wheat</td>
<td>Cotton</td>
<td>Pumpkin</td>
</tr>
<tr>
<td>Monocot</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Barley</td>
<td>87.0°C</td>
<td>83.0°C</td>
<td>80.5°C</td>
<td>83.5°C</td>
</tr>
<tr>
<td>Wheat</td>
<td>87.0°C</td>
<td>84.5°C</td>
<td>82.0°C</td>
<td>86.0°C</td>
</tr>
<tr>
<td>Dicot</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cotton</td>
<td>88.0°C</td>
<td>83.5°C</td>
<td>90.5°C</td>
<td>88.0°C</td>
</tr>
<tr>
<td>Pumpkin</td>
<td>88.5°C</td>
<td>81.0°C</td>
<td>86.5°C</td>
<td>92.0°C</td>
</tr>
</tbody>
</table>

* Filter-bound seed DNA was hybridized with a saturating concentration of labeled leaf rRNA, (0.5 as much rRNA as DNA) as described in Figure 3. Duplicate filters of rRNA–DNA hybrids were incubated at 60°C for five minutes in vials containing 1 ml of 2 × SSC. The filters were transferred to another pair of vials maintained at 65°C and subsequently to a series of vials ranging in temperatures from 70 to 98°C. The radioactivity released from the filters was monitored, and the cumulative percent rRNA released was plotted against temperature.

of base-pair mismatching in the heterologous than in the homologous hybrids. For this purpose, rRNA–DNA hybrids were exposed sequentially to temperatures from 60 to 98°C in 5°C increments. As the temperature increases, rRNA is released from the hybrid and is no longer filter-bound. The cumulative percent of rRNA solubilized was plotted against the temperature. Figure 4 shows the thermal stability profiles for rRNA–DNA hybrids, and Table 4 lists the T_m for each hybrid. At the end of the experiments, filters retained less than 9% of the rRNA originally bound. Five of the 16 T_m's listed on Table 4 were repeated, and the maximal difference observed between duplicate experiments was 1.5°C.

It can be seen from Table 4 that dicot rRNA–dicot DNA hybrids, on the whole, display the highest T_m's. Within this class of hybrids, the two homologous hybrids have higher T_m's than the two heterologous hybrids (see Tables 4 and 5). The difference in T_m's between the pumpkin rRNA–pumpkin DNA hybrid and the cotton rRNA–pumpkin DNA hybrid is 4.0°C. The difference in T_m's between the cotton rRNA–cotton DNA hybrid and the pumpkin rRNA–cotton DNA hybrid is also 4.0°C. Assuming that 1.5% mismatching lowers the T_m by 1.0°C (Laird, McConaughy and McCarthy 1969), which is the best estimate avail-

vial and incubated for 18 hr at 68°C in 1 ml of 0.1% SDS in 2 × SSC which contained amounts of tritium-labeled rRNA ranging from 1.25% to 50% of the amount of input DNA. The specific activity of barley, wheat, cotton, and pumpkin rRNA's were 2000, 3900, 7250, and 20500 counts/minute/μg respectively. To estimate background, a pair of filters without DNA was also incubated with the highest concentration of rRNA in that experiment.

Upon completion of the reaction time, the filters were washed batchwise in the following solutions for 30 min: 6 × SSC at 50°C, 2 × SSC, 2 × SSC with 20 μg/ml of pancreatic ribonuclease and finally with 2 × SSC. Filters were then dried under vacuum and assayed for radioactivity. The background determined from the blank filters (25–30 counts/min) was subtracted from the counts of each test filter. The plateau region of the graph represents between 150 (for the barley rRNA–wheat DNA hybrid) to 400 (for the pumpkin rRNA–pumpkin DNA hybrid) net counts/min.
TABLE 5

Difference in $T_m$'s between heterologous and homologous rRNA–DNA hybrids

<table>
<thead>
<tr>
<th>Source of rRNA</th>
<th>Barley $T_m$</th>
<th>Source of DNA</th>
<th>Cotton $T_m$</th>
<th>Pumpkin $T_m$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barley</td>
<td>—</td>
<td>1.5°C</td>
<td>10.0°C</td>
<td>8.5°C</td>
</tr>
<tr>
<td>Wheat</td>
<td>0.0°C</td>
<td>—</td>
<td>8.5°C</td>
<td>6.0°C</td>
</tr>
<tr>
<td>Cotton</td>
<td>-1.0°C</td>
<td>1.0°C</td>
<td>—</td>
<td>4.0°C</td>
</tr>
<tr>
<td>Pumpkin</td>
<td>-1.5°C</td>
<td>3.5°C</td>
<td>4.0°C</td>
<td>—</td>
</tr>
</tbody>
</table>

* The $T_m$ of each heterologous rRNA–DNA hybrid listed in Table 4 was subtracted from the corresponding homologous rRNA–DNA hybrid.

These differences translate into a 6% base-pair mismatching in both heterologous dicot rRNA–dicot DNA hybrids. In comparing the $T_m$'s between homologous and heterologous monocot rRNA–monocot DNA hybrids, it can be seen from Tables 4 and 5 that the $T_m$'s show little difference. Thus, there is no evidence of base-pair mismatching in the heterologous monocot rRNA–monocot DNA hybrids, as compared to the homologous hybrids.

The homologous monocot rRNA–monocot DNA hybrids, however, have lower $T_m$'s than the homologous dicot rRNA–dicot DNA hybrids. The thermal stability profiles of the former are broader than those of the latter (see Figure 4). Moore and McCarthy (1968) observed a broad thermal stability profile and a relatively low $T_m$ of the rabbit rRNA–DNA hybrid and concluded that the nucleotide sequences coding for rabbit rRNA were heterogeneous. Such a heterogeneity would result in base-pair mismatching in homologous monocot rRNA–monocot DNA hybrids and could explain their relatively low $T_m$'s. Alternatively the GC content of dicot rRNA's could be higher than that of monocot rRNA's. Such a difference in GC content would also cause a decreased $T_m$, though not a broader melting profile for homologous monocot rRNA–monocot DNA hybrids. The effect of GC content on the $T_m$ of DNA–DNA hybrids has, in fact, been quantitated by Marmur and Doty (1959) as a 1°C decrease in $T_m$ per 2.5% decrease in GC content. The maximum decrease in $T_m$'s among homologous monocot rRNA–monocot DNA hybrids and homologous dicot rRNA–dicot DNA hybrids is 7.5°C (see Table 4), which would be equivalent to an 18.8% difference in GC content. However, it is uncertain whether this quantitative analysis is applicable to the $T_m$'s of rRNA–DNA hybrids. In conclusion, either of the two above hypotheses could explain the lower $T_m$'s of homologous monocot rRNA–monocot DNA hybrids.

**Figure 4.**—Thermal stability profiles of homologous and heterologous rRNA–DNA hybrids. Washed filters containing 300–800 counts/minute of rRNA (tritium labeled)–DNA hybrids (hybridized under saturating conditions) were incubated separately at 60°C for 5 min in 2 ml of 2× SSC. The filters were transferred to other vials and the incubation was continued at 65°C. The process was repeated in 5°C increments up until the maximum temperature (98°C) with 10 min for equilibration at each temperature. The rRNA solubilized at each temperature was determined and the percent of cumulative counts released was plotted against temperature. The $T_m$ value was read directly from the graph.
The dicot rRNA–monocot DNA hybrids display approximately the same Tₘ's as those of the corresponding monocot rRNA–monocot DNA hybrids. Therefore, both types of hybrids have the same percentage of base-pair mismatching. By contrast, the monocot rRNA–dicot DNA hybrids have Tₘ’s considerably lower than those of the corresponding dicot rRNA–dicot DNA hybrids. For example, the barley rRNA–cotton DNA hybrid has a Tₘ 10.0°C lower than that of the cotton rRNA–cotton DNA hybrid. The monocot rRNA–dicot DNA hybrids apparently have 9 to 15% more base-pair mismatching than was observed in dicot rRNA–dicot DNA hybrids.

It should be noted, however, that the Tₘ analysis refers only to the rRNA and DNA that have hybridized. In the case of homologous hybridizations, all or nearly all of the rDNA sequences have hybridized with rRNA sequences. In the case of heterologous hybridizations, some sequences of rRNA have diverged to such an extent that they no longer anneal to heterologous DNA’s. Therefore, the extent of base-pair mismatching in heterologous hybrids as compared to that of homologous hybrids does not take into account widely divergent sequences of rRNA’s and can thus over-estimate the proportion of the nucleotide sequence shared by the homologous and heterologous rRNA’s.

Hybridization of rRNA to DNA fractionated from a preparative CsCl gradient:
As in other organisms investigated, plant rDNA clusters in CsCl gradients with a buoyant density characteristic of the GC content of rRNA (Sinclair and Brown 1967). In order to resolve rDNA from the bulk of DNA, plant DNA’s were centrifuged to equilibrium in CsCl, and fractions of the gradient were collected. The optical density at 260 nm of each fraction was determined, and alternate fractions were hybridized with labeled leaf rRNA. By splitting a filter containing the denatured DNA of a single fraction, it was possible to hybridize the same fraction of DNA with various rRNA’s. Figure 5 shows that barley rRNA hybridizes to DNA slightly less dense than the bulk of barley DNA, which has a buoyant density of 1.701 g/cc and a GC content calculated from it as 42% by the method of SchilDKRAUT, MARMUR and DOTY (1962). Chen and Osborne (1970) reported a similar observation for wheat rRNA and wheat DNA. In Figure 6, cotton DNA with a buoyant density of 1.692 g/cc and a calculated GC content of 33% allows for a better resolution of rDNA from the bulk of the DNA. In all cases homologous and heterologous rRNA’s hybridize to DNA of the same buoyant densities.

DISCUSSION
Accurate values for the extent of rRNA–DNA hybridization depend heavily on the purity of the nucleic acids. The DNA’s used in the present study are judged to be pure on the following basis: a purification procedure, subsequent to those routinely employed, did not alter the hybridization properties of DNA’s. Ribosomal RNA’s are judged to be pure because they underwent rigorous purification procedures and duplicate preparations from the same species yielded essentially the same results.

Labeled leaf rRNA shows no evidence of being synthesized by contaminating
RIBOSOMAL RNA IN ANGIOSPERMS

Figure 5.—Distribution of rDNA in barley DNA banded on a preparative CsCl gradient. 200 μg of purified DNA in 3.25 ml of 0.05 M Tris buffer (pH 8.5) was adjusted to a density of 1.700 g/cc with solid cesium chloride. The solution was placed in a polyallomer tube and overlaid to the top with mineral oil. Samples were centrifuged at 35,000 r.p.m. for 3 hr at 20°C in a number 50 angle rotor.

After centrifugation, 15 drop fractions were collected and read at 260 nm. Alternate fractions were hybridized with tritium-labeled rRNA as described in Figure 3.

bacteria, because peaks of radioactivity corresponded closely to the optical density profile of the four rRNA components separated by polyacrylamide gel electrophoresis. The above criterion for judging freedom from bacterial contamination may be inadequate, because the 23S and 16S rRNA components of chloroplasts coincide with the 23S and 16S rRNA components of bacteria. However, a fraction of the labeled leaf rRNA behaves as if it were homologous to chloroplastic rDNA in the following experiment: under conditions where excess unlabeled leaf rRNA depressed hybridization of labeled leaf rRNA by more than 95%, excess unlabeled root rRNA depressed hybridization only by 60%. Messenger RNA was also not detected as a contaminant because representative rRNA preparations hybridized specifically to DNA (rDNA) of a buoyant density greater than the bulk of cotton DNA. Therefore, all four components of leaf rRNA, 25S, 23S, 18S and 16S, and only those four components, were judged to be labeled.

The hybridization data reported in this study agree essentially with those from the literature. CHEN and OSBORNE (1970) cite a value of 0.32% for wheat rRNA.
hybridized to wheat DNA at saturation; the value cited in this study is 0.22%. According to Bendich and McCarthy (1970a), barley contains twice as much rDNA as wheat. Assuming that the true value for the proportion of wheat DNA homologous to wheat rRNA is the average of the value in this study and that of Chen and Osborne (1970), 0.54% of barley DNA should code for barley rRNA. The value cited in this study is 0.73%. Goldberg (1970) assigns for pumpkin DNA a value of 1.95%, which is very close to the value of 1.88% assigned in this study. The fact that various dicot rRNA's generally hybridize equally to a given DNA extended similar observations by Matsuda and Siegel (1967).

Although these data represent only two species within each classification (i.e., dicots and monocots), some tentative conclusions as to trends of nucleotide conservation within and between classes can be drawn. The data from Table 2 suggest that there has been some divergence of rRNA base sequences both among monocots and between monocots and dicots. On the basis of reciprocal rRNA–DNA hybridizations, approximately 60% of the nucleotide sequence of barley rDNA is homologous to the nucleotide sequence of wheat rRNA; 60% of the nucleotide

**Figure 6.** Distribution of rDNA in cotton DNA banded on a preparative CsCl gradient. Same procedure as Figure 5.
sequence of wheat rDNA is homologous to the nucleotide sequence of barley rRNA. From the data of Bendich and McCarthy (1970b), it can be seen that 72% of wheat DNA is homologous to barley DNA and that 48% of barley DNA is homologous to wheat DNA. However, the latter experiments involved the hybridization of redundant sequences at nonrestrictive conditions of hybridization, and the values obtained from them were regarded as over-estimates of the true extent of DNA homology. Therefore, the nucleotide sequence of rRNA's of barley and wheat is conserved at least as much and probably more than that of the bulk of the DNA's of barley and wheat.

Because dicot rRNA's hybridize equally to a given DNA, it is concluded that the nucleotide sequence of rRNA's among the dicots studied has been conserved. Bendich and Bolton (1967) found a 5-55% homology in the nucleotide sequence among dicot DNA's. Again these values probably over-estimate the true homologies. Therefore, the nucleotide sequence of rRNA's between dicots has been conserved more than that of the bulk of the dicot DNA's. Moreover, the conservation of the rRNA sequence among dicots is greater than that observed among monocots.

The divergence of the rRNA base sequence between monocots and dicots is difficult to determine. Reciprocal hybridizations between monocots and dicots are unequal. The two estimates of nucleotide sequence conservation between monocot and dicot rRNA's obtained from the reciprocal hybridizations are 40-60% and 65-95%. Bendich and Bolton (1967) found a maximum of 10% nucleotide sequence conservation between monocot and dicot DNA's. Thus, whichever estimate of the reciprocal hybridizations is accepted, the rRNA nucleotide sequences of monocots and dicots have been conserved more than those of the rest of their genomes.

Several models can be invoked to explain the unequal reciprocal hybridizations noted above. One model depends on the heterogeneity of rRNA that may have been observed within monocots. Each monocot may thus have a family of related genes coding for rRNA. Apparently the dicot rRNA hybridizes only to some of these related genes under restrictive conditions. Thus, not all of the monocot rDNA is available to hybridize to dicot rRNA. In the reciprocal experiment involving dicot DNA and monocot rRNA, all of the dicot rDNA is available to hybridize with some of the related monocot rRNA's. Because rRNA is in excess, the saturation-hybridization value can approach 100% of the homologous value. Thus, reciprocal hybridizations, under these circumstances, are expected to yield different estimates of conservation of rRNA sequence.

The above interpretation is subject to qualification, and other explanations are also possible. For instance, unequal specific activities of the four rRNA components can lead to inaccurate estimations of the saturation-hybridization values. Even if the specific activities of the four major rRNA components were equal, unequal labeling of heterogeneous subpopulations of the rRNA components can also cause problems in estimating the saturation-hybridization values. Therefore, all hybridization data should be judged with a certain amount of caution.
SUMMARY

DNA's have been isolated from nuclei of barley, blue panicum grass, wheat, cotton, and pumpkin. Ribosomal RNA's have been isolated from leaves of barley, wheat, cotton, and pumpkin and are characterized by four optical density peaks with polyacrylamide gel electrophoresis.—On the basis of reciprocal hybridizations and thermal stability profiles, the divergence of rRNA nucleotide sequences has been estimated among monocots, among dicots, and between monocots and dicots. The rRNA nucleotide sequence however has been conserved relative to that of the rest of the genome.—A comparison of the thermal stability profiles and T°'s between homologous monocot rRNA—monocot DNA hybrids and homologous dicot rRNA—dicot DNA hybrids suggest a possible heterogeneity for monocot rRNA's or a difference in GC content between monocot and dicot rRNA's.

LITERATURE CITED


GOLDBERG, R. B., 1970  Committee on Genetics, The University of Arizona (Unpublished observations).


