MOLECULAR HYBRIDIZATION OF CONIFEROUS RIBOSOMAL RNA TO DNA

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Artificial hybridization between ribosomal RNA and homologous and heterologous DNA's has been used as a research tool for nearly 10 years. Investigations with bacterial, plant, and animal sources have demonstrated:

1. Ribosomal RNA cistron sites are multiple.
2. Sites are associated at the nucleolar organizer region of higher organisms (eukaryotes).
3. The nucleotide base sequence of the rRNA cistrons are conservative (Yankofsky and Spiegelman, 1962; Ritossa and Spiegelman, 1965; Attardi, Huang, and Kabat, 1965; Oishi and Sueoka, 1965; Dubnau, Smith, More11, and Marmur, 1965; Matsuda and Siegel, 1967; Laird and McCarthy, 1968; Wimber and Steffensen, 1970; Barsacchi and Gall, 1972).

Matsuda and Siegel (1967) reported that nuclear DNA's varied by a factor of 10 in quantity in plant ribosomal RNA cistrons, and that the ribosomal RNA's from different angiospermous plant species displayed similarity to each other as determined by homologous and heterologous hybridization studies. Ingle and Sinclair (1972) demonstrated in several plant species a 40-fold variation in the percentage of total DNA which hybridized to ribosomal RNA.

The present study makes the following four conclusions:

1. DNA's from the four conifers studied vary slightly in their respective contents of ribosomal cistrons, i.e., the variation of the percentage of total DNA which hybridized to ribosomal RNA is small.

2. A general similarity of ribosomal RNA bases among species is apparent but some heterogeneity exists.

3. Cistrons 18S and 28S are tandemly arranged adjacent to each other on a short chromosome segment.

4. The prospects of the application of ribosomal RNA-DNA hybridization studies to forest genetics are favorable.

MATERIAL AND METHODS

Plant Material

Dormant seeds and germinated seedlings of *Pinus banksiana* Lamb., *Pinus resinosa* Ait., *Picea glauca* (Moench) Voss, and *Thuja occidentalis* L. were used. Germination was accomplished in a growth room in darkness in moist "perlite" at 25° C. All seeds were treated for 10 min. in 10-percent "chlorox" to reduce bacterial and fungal growth. To insure complete contact of the "chlorox" with the seed-coat surface, the seed and "chlorox" mixture was agitated with a magnetic stirrer. The seeds were then rinsed 45 min. in running tap water.

DNA Extraction and Purification

The technique reported by Stern (1968) was slightly modified for DNA extraction. Ten to 20 gms. of seed were mixed with sand in a mortar in 100 to 200 mls. of 5-percent sodium-lauryl-sulfate at 60°C; 0.05M Tris, and 0.05M sodium citrate (pH 7.4) were added to the mixture, and the suspension was then ground with a mortar and pestle. The suspension was kept for about 10 mins. at 60°C. after grinding and then filtered through cheese cloth. The filtrate was centrifuged to remove cell-wall fragments and .other insoluble materials, and 2 volumes of 95-percent ethanol were added in order to precipitate the DNA. The precipitate was suspended in 1.0M NaC1:0.01M Tris (pH 8.0): 0.05M Na-citrate and 0.1 mg. /ml. pronase (self-digested to remove endogenous nuclease activity) for 17 hrs. at 65°C. The suspension was centrifuged and solid NaC1 added to the supernate to a final concentration of 2.5 m NaCI. This solution was shaken gently for 20 mins. with an equal volume of chloroform:n-amyl alcohol (24:1). The phases were separated by centrifugation, and the aqueous layer was removed and mixed with 2 volumes of ethyl alcohol. The precipitate was collected and washed with 70-percent ethyl alcohol and resuspended in 0.15M NaCI :0.01M Tris (pH 7.0):0.015M sodium citrate. One-twentieth volume of a solution of ribonuclease (2 mg./mi.) was added to the suspension, which was incubated at 37°C. After 30 mins. the incubation was interrupted by adding solid NaC1 to a concentration of 2.5M and the DNA again deproteinized with chloroform: m-amyl alcohol. DNA was spooled-out by the addition of two volumes of 95-percent ethyl alcohol. The precipitated DNA was resuspended in saline citrate with an equal volume of 88 percent phenol, and this step was repeated three times. The DNA was precipitated with two volumes of 95-percent ethyl alcohol and suspended in 1 x SSC and loaded on a sephadex column. The

eluted DNA was precipitated and resuspended in saline citrate, dialyzed three times against 1 liter of saline citrate, 24 hrs. each. The DNA was precipitated with two volumes of 95-percent ethyl alcohol and stored in 70-percent ethyl alcohol.

DNA Buoyant Density Determination

Densities were determined with a Spinco Model E Analytical Centrifuge according to the method of Mandel, Schildkraut, and Marmur (1968). DNA (5 ugm .) was dissolved in a solution of CsCI, buffered with Tris-HCI (pH 8.5). The refractive index of the solution was adjusted to 1.4000. The ,ugm. of *E. coli* DNA were added as the reference DNA. The buoyant densities of the four species was 1.697 and no satellite peaks were observed.

RNA Extraction

The ribosomal fraction was isolated by differential centrifugation in a buffered sucrose solution containing 0.2 mg./ml. potassium polyvinylsulfate (Eastman Organic Chemicals). Fifty grams of dry seed were partially ground in a mortar and pestle, then further ground in a Virtis homogenizer with 250 ml. of 0.35 M sucrose, 0.01 M Tris-HCI (pH 8.1), 25 mM KCI, 1 mM $MgCl_2$ per 0.2 mg./ml. of polyvinylsulfate.

Twenty-five grams of germinated seedlings were chopped with a scissors and blended in a Virtis homogenizer in the same above buffer. Homogenates from seeds and seedlings were centrifuged at 40,000 x g for 20 mins. after filtration through flannellette, and the supernatant was centrifuged at 80,000 x g for 150 mins. to pellet ribosomes. The pellet was washed once in the same buffer and centrifuged at 100,000 x g for 90 mins. The ribosomal fraction was suspended in the buffer without sucrose and treated with 88-percent phenol three times followed by an ethyl alcohol precipitation. The precipitate was washed with 3 M Na-acetate and centrifuged 5000 x g for 15 mins. and dissolved in 0.01 M Tris (pH 7.8) in 1 mM EDTA followed by an ethanol precipitation.

Labeling of RNA

$C^{14}O_2$ gas generated from $NaHC^{14}O_3$ in an enclosed chamber was taken up by seedlings under light. The resulting specific activity of the rRNA after extraction and purification was 300 to 600 cpm/,ugm. Purified rRNA was also labeled by an exchange reaction with H^3 dimethylsulfate (New England nuclear, 5 mC/m1) in Tris-HC1 buffer. The H^3 rRNA specific activity was 200 x 10^3 cpm/ugm .; Nuclear-Chicago and Beckman scintillation counters were used.

Nucleotide Separation

Ribosomal RNA's from three species were fractionated by 5- to 25-percent sucrose gradient centrifugation and pelleted by further centrifugation after dilution of the sucrose with buffer (rRNA extraction methods). After washing in 70-percent ethyl alcohol, the rRNA's were hydrolyzed with 0.5N KOH at 37°C. for 18 hrs. Total rRNA (100,000 x g, 90 min. pellet, see RNA extraction methods) was also subjected to a 0.5N KOH, 37°C., 18 hr. hydrolysis. The alkaline hydrolysates were acidified with 6N perchloric acid in the cold and centrifuged. The supernatant was adjusted to pH 8.0 with 6N KOH and the resulting potassium Perchlorate was removed. Nucleotide analysis was performed according to the technique of Osawa, Takata, and Hotta (1958) on an AR1 formate column.

Hybridization and Thermal Dissociation

The DNA was denatured with 0.3 M NaOH and immobilized on nitrocellulose membrane filters (Gillespie and Spiegelman, 1965). Each filter (1.5 cm. in diameter) containing up to 100 ,ugm. DNA was incubated with rRNA in 0.5 ml of 6 X SSC at 67°C for 15 hrs. unless specified otherwise. Thermal dissociation profiles were obtained in 1 X SSC by measuring the remaining radioactivity on the filter at each temperature increase.

RESULTS

Sedimentation Patterns and Nucleotide Composition

Ribosomal RNA from seedlings (C^{14} labeled) and from seeds (H^3) were co-sedimented in a sucrose 5- to 25-percent gradient and yielded identical patterns (Figure 1). A separate gradient run with the marker rRNA of *E. colt** labeled with H^3 uridine, 28S and 18S components of the conifers was estimated. No size change of the rRNA's before and after germination was observed.

The results of column chromatography show differences between seed and seedling nucleotides in *Pinus banksiana.* Ribosomal RNA of dry seed has a lower cytosine content and a higher purine/pyrimidine ratio than seedling rRNA. Also, the dry seeds possess a rather large amount of unknown nucleotide between the adenine and guanine, and it was identified as an adenine compound. Seedling rRNA displayed little or no unknown component (Table 1, Figure 2).

Saturation of rRNA Cistrons

The factors that affect stabilization of hybrid formation between rRNA and DNA are incubation temperature, concentration of RNA, and length of incubation (Kennel, 1971). Therefore, the rRNA-DNA hybrids were incubated at 67°C. (approximately 20° below the expected average melting temperature of rRNA and DNA) in the presence of a high concentration of RNA to generate a time curve of hybrid formation. Figure 3 demonstrates that a saturation condition of 0.6-percent rRNA per genome is obtained within 10 hrs. and remains stable through 20 hrs. Hybrid formation between rRNA and DNA was then studied against various concentrations of rRNA. About 5 ugm . of rRNA were required to saturate rRNA cistrons under the experimental conditions used in this study (Figure 4).

Determination of the rRNA Cistron Size per Genome and the Number of rRNA Genes

After establishing the physical parameters necessary for artificial hybridizations, cross hybridizations between

rRNA and whole genome DNA were made among several coniferous species (Table 2). Three features of the table are of interest:

1. All of the DNA's tested proved to have regions complementary to rRNA from homologous intraspecific and intergeneric heterologous hybridizations.

2. The proportion of different DNA's complementary to rRNA varied by a factor of nearly two over all of the experiments.

3. The highest complementarity was displayed among the homologous hybridizations, and the lowest degree of complementarity resulted from heterologous determinations.

The amount of DNA complementary to rRNA is calculated from the diploid genome size and is the percent of the DNA that will hybridize to this RNA. The value (in Dalton's) is divided by the combined number of rRNA genes per diploid genome of the four conifers (Table 3). The most striking feature of the table is the large number of genes or high multiplicity of the rRNA gene system.

Use of Competitor rRNA to Estimate rRNA-DNA Hybrid Specificity

The existence of heterogeneity in rRNA is illustrated in Figure 5. *P. glauca* rRNA did not successfully compete for *P. resinosa* DNA sites until a large amount had been added (Figure 5B). This suggests that *Pinus resinosa* rRNA cistrons contain more heterogeneity than the *Picea glauca* rRNA cistrons. The heterorgeneity infers mismatching of the bases between rRNA of *Picea glauca* and the DNA of *Pinus resinosa.*

Thermal Stability of rRNA-DNA Hybrids

The thermal stability of the artificial hybrids formed after RNase treatment was tested. The results shown on Figure 6 indicate the following points: (1) Homologous rRNA-DNA hybrids display more stability than the heterogeneous hybrids. (2) The thermal denaturation curves were very similar except for the hybrid between *Pinus resinosa* DNA and *Picea glauca* rRNA. (3) The difference in melting points for all hybrids at the 50-percent melting point was 3°C., indicating 2 percent of mismatch bases or 98-percent complementarity. (4) Conifer rRNA-DNA hybrids show greater heterogeneity than *E. coli* despite the average percent G + C content difference of only 5 percent (conifers, guanine + cytosine 55 percent and *E. cola'* 60 percent).

Structural Characterization of rRNA Cistron

DNA from *Pinus resinosa* and *Picea glauca* was centrifuged in cesium chloride, and each main band DNA fraction was denatured by alkaline 0.2 NaOH and absorbed on membrane filters. The filters were incubated with the respective *Pinus resinosa* and *Picea glauca* H^3 rRNA. The DNA which hybridized with H3 rRNA was of higher density *(1.7*16) than the main band DNA (1.697). The separation was made clearer by treating the loaded filters at 80°C. for 20 mins. in 1XSSC, and the 80°C. resistant fraction was counted. Despite 18S and 28S RNA possessing different percent guanine + cytosine contents, 52 percent and 56 percent, respectively, only one density peak was found in the 54-percent guanine + cytosine region. The results indicate that both rRNA species combine with the same DNA segment, such that the 28S and 18S cistrons lie next to each other on a relatively short DNA strand.

DISCUSSION

High molecular weight rRNA's, 28S and 18S, and the lower molecular weight, 4-5S, were present in the conifers studied. These results agree with the Barnett, Shih, and Adams (1970) sedimentation studies of *Pinus lambertiana* and also confirm that rRNA of the conifers studied fits in the higher plant group. However, our base composition results were not similar to their finds as we detected a high guanine level similar to the base composition results of Kupila-Ahvenniemi and Pihakaski (1966).

The procurement of the above qualitative information was necessary also to establish nucleic acid separation and preparation procedures for the hybridization studies.

The formation of stable complexes of rRNA with denatured strands of complementary DNA is predicated upon incubation time, temperature, and concentration of rRNA. The parameters were established and illustrated in Figures 3 and 4. Further discussion of the physical and chemical aspects of RNA-DNA hybrid formation is not applicable to this presentation. Readers desiring such information are requested to examine a recent coverage of the topic by Kennel (1971).

The matter at hand is to compare the present rRNA-DNA hybridity results with other published works and ascertain the feasibility of the application of molecular-level research to tree-improvement programs.

The percentage of total DNA which hybridized to rRNA was small in over-all variation (a factor of 1.8) and relatively high (mean of 0.60 percent) over-all hybrid in the four conifers studied. The variation in hybridity demonstrated by artificial membrane hybridization selfing, i.e., a given species DNA crossed with its own rRNA (homologous) was near unity at 1.3, or smaller than the over-all variation (Table 2). Ingle and Sinclair (1972) found the percentage of total DNA which hybridized to rRNA varied by a factor of 40 in homologous hybridizations using seven angiosperm species of different phylogenetic origins. Tobacco rRNA was hybridized homologous to DNA and heterologous DNA of pinto bean, pumpkin, and Chinese cabbage by Matsuda and Siegel (1967) and a 10-fold variation in percent hybridization was reported.

The above findings superficially indicate a greater variation in percent hybridization between DNA and rRNA in angiosperms than is found in confiers. In reality, the large angiosperm hybrid variation may be misleading, since some rRNA-DNA hybrids produced from the aniosperms were formed with DNA containing satellite DNA. A satellite DNA component offers a large number of regions in the genome that are complemen-

tary to rRNA; and, therefore, greater hybridization is registered in certain cases. For example, a homologous tobacco cross yielded 0.07 percent hybridization, and a heterologous cross between wheat DNA and tobacco rRNA produced 0.06 percent hybridization, whereas tobacco rRNA hybridized with Chinese cabbage DNA registered 0.76 percent as a result of satellite DNA (Matsuda and Siegel, 1967).

The use of rRNA-DNA hybridity to estimate genetic relatedness between organisms is, therefore, not a valid analysis unless the DNA under study is fully characterized. Crosses should be made with main band and satellite DNA (if present) to fully ascertain relatedness.

Another interesting feature of the conifer rRNA-DNA crosses is the high total percentage hybridization (Table 2) without the apparent presence of satellite DNA (Miksche and Hotta, 1972; Hall, Stairs, Miksche, 1972). The high cross values indicate redundancy of the genes that code for ribosomal RNA. In fact, the hybridization values are generally higher than values reported for angiosperms. The number of copies of the rRNA gene per interphase nucleus varied from 7.5×10^4 to 3.3×10^5 genes (Table 3) which may correspond to the number of genes per nucleolar organizer region, assuming all nuclear organizers are equivalent in function. The number of rRNA gene copies per diploid genome in 8 angiosperms varied from 1.6×10^3 to 1.3×10^4 and excluding polyploidy 2.6×10^2 to 6.7×10^3 copies (Ingle and Sinclair, 1972).

The high rRNA multiplicity reported here and elsewhere (Matsuda and Siegel, 1967; Ingle and Sinclair, 1972) supports the contention of Birnstiel, Chipchase, and Spiers (1971) that the amount of genome consisting of ribosomal cistrons is higher in plants than in animals. The particularly high rRNA multiplicity in the conifers is a reflection of their large DNA quantity per cell which is generally larger than the DNA per cell in angiosperms (Miksche, 1967).

The size of the rRNA cistron per genome may have an important application to tree breeding programs. Ribosomes are protein-synthesizing particles and the number of ribosomes determines the general rate of protein synthesis in the cell (Spirin and Gavrilova , 1969). If, for example, in comparing fast and slow growing forms of the same species, the fast performer has a larger rRNA locus per genome than the slow grower, it may tentatively be assumed that the fast grower performs as a result of increased protein synthesis due to the large rRNA locus per genome size.

The competition experiments (Figure 5) estimated the specificity of rRNA for common DNA sites in three conifers and demonstrated the existence of heterogeniety in the rRNA cistrons. A further evaluation of the rRNA cistron heterogeniety was pursued by thermal stability studies of the rRNA-DNA complexes (Figure 6).

Bendich and McCarthy (1970) utilized thermal stability studies to compare rRNA homologies among distantly related organisms and demonstrated the conservative nature of plant rRNA in evolution. Conservativeness implies a small change in rRNA molecules in time and, therefore, rRNA is useful in genetic and evolutionary studies. They used the *Pisum sativium* rRNA-DNA homologous thermal stability hybridization value as a reference against the results of heterologous artificial hybridizations between pea rRNA and DNA from six angiosperms and one gymnosperm, and reported the extent of base sequence divergence of the various rRNA cistrons of the seven species. That is, a decrease in the thermal stability of the nucleic acid heteroduplexes apparently relates linearly to the extent of base sequence divergence (Bautz and Bautz, 1964; Laird, McConaughty, and McCarthy, 1969) such that the lower the thermal stability, the greater the divergence.

The thermal stability results reported here (Figure 6) were generated from more closely related or less divergent species than the species studied by Bendich and McCarthy (1970); and, as expected from the linear relation between relatedness and thermal stability, only a 3°C. difference in thermal stability was observed.

The 3°C. difference in thermal denaturation of the rRNA-DNA coniferous hybrids studied may indicate that the respective extant conifer DNA evolved between 30 and 70 million years ago. The geologic time span corresponds roughly to the Upper Cretaceous and Tertiary periods, the time when the extant coniferous genera became differentiated (Scagel, Bandoni, Rouse, Schofield, Stein, and Taylor, 1966). Britten and Kohne (1968) presented the method of estimating the origin of DNA species on the basis of thermal stability of reassociated pairs of DNA strands formed between members of each family of DNA repeated sequences. Since our data display close complementarity between conservative rRNA and DNA, the evolutionary interpretation can be presented with a certain degree of confidence.

Proof of gene linkage or gene mapping of rRNA by biochemical techniques rather than genetical analysis was first established in a eukaryote by Birnstiel, Spiers, Pardom, Jones, and Loening (1968). In the present study, an adjacent gene linkage between 28S and 18S rRNA of *Picea glauca* and *Pinus resinosa* was estimated by biochemical methods (Figure 7).

Summary of molecular hybridization results between coniferous rRNA and DNA with emphasis on possible applications to forest genetics:

1. The membrane filter technique of molecular hybridization offers a means of demonstrating general genetic relatedness, which may augment the study and understanding of phylogenetic origins of tree species.

2. Thermal stability of the rRNA-DNA molecular hybrids is predicated on nucleic acid base pair homology, i.e., the closer the base pair complementarity the greater the degree of relationship between and among species, and conversely, the lesser the base complementarity the greater the genetic divergence.

3. The use of molecular hybridization to predict success of field crosses between and among species deserves exploration.

4. The relationship between ribosomal RNA locus size and protein synthesis may offer a means of predicting growth. Since ribosomes are directly related to protein production, it may follow that the larger the ribosomal locus size the greater the amount of protein synthesized the greater the potential for growth.

5. In view of the long generation time in most tree species and the related delay in procurement of genetic information, the use of biochemical methods to investigate gene structure has significant meaning to forest genetics.

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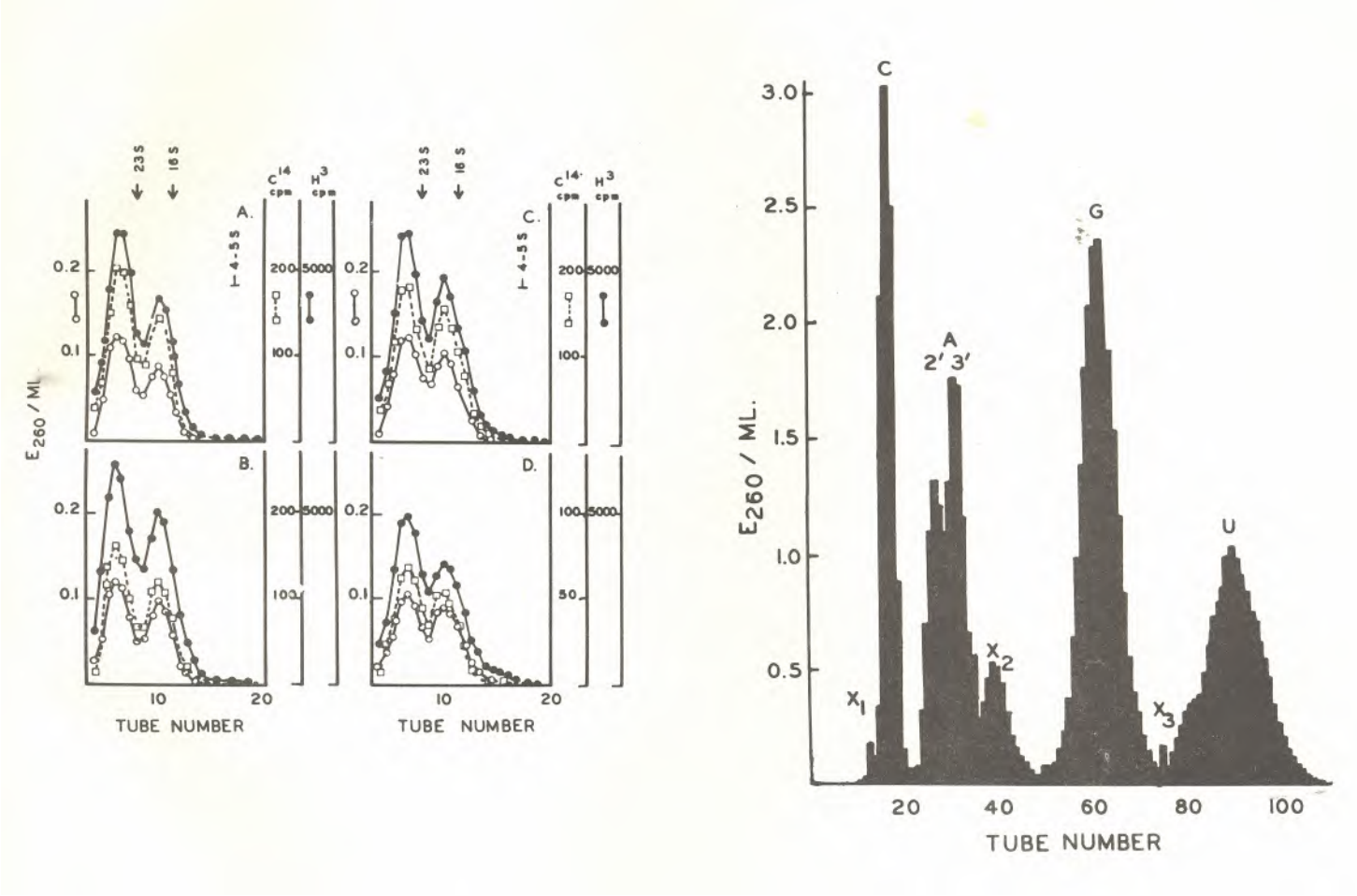

Figure 1. Sedimentation pattern of rRNA's in a 5-25 percent sucrose gradient. The following components, 50-60 ,ug rRNA consisting of 8-12ug C ^{14} seedling rRNA (200 cpm/mg) and 40-50 pg of H ^{3} seed rRNA (1050 cpm/,ug) were placed on a sucrose gradient with 0.1 M Tris pH 7.3 - 0.1 mNaCl - 1mMEDTA. A Spinco SW401 rotor was used and centrifuged at 20,000 rpm for 16 hrs. at 2°C. Samples were collected from the bottom and radioactivity of TCA precipitable material was counted after reading optical density of E260. The relative sedimentation values were compared against a separate buoyant density run with H ^{3} uridine labeled *E. coli* RNA's that demonstrated the 23S, 16S, and 45S components. A. *Pinus banksiana;* B. *Pinus resinosa;* C. *Picea glauca;* D. *Thuja occidentalis.*

Figure 2. Chromatographic separation of mononucleotides from *Pinus banksiana* rRNA. C, A, G, and U are cytidylic acid, adenyllic acid, quanylic acid, and uridylic acid, respectively. X $_{1}$, X2, and X3 are unknown nucleotides significantly present in rRNA isolated from seeds.

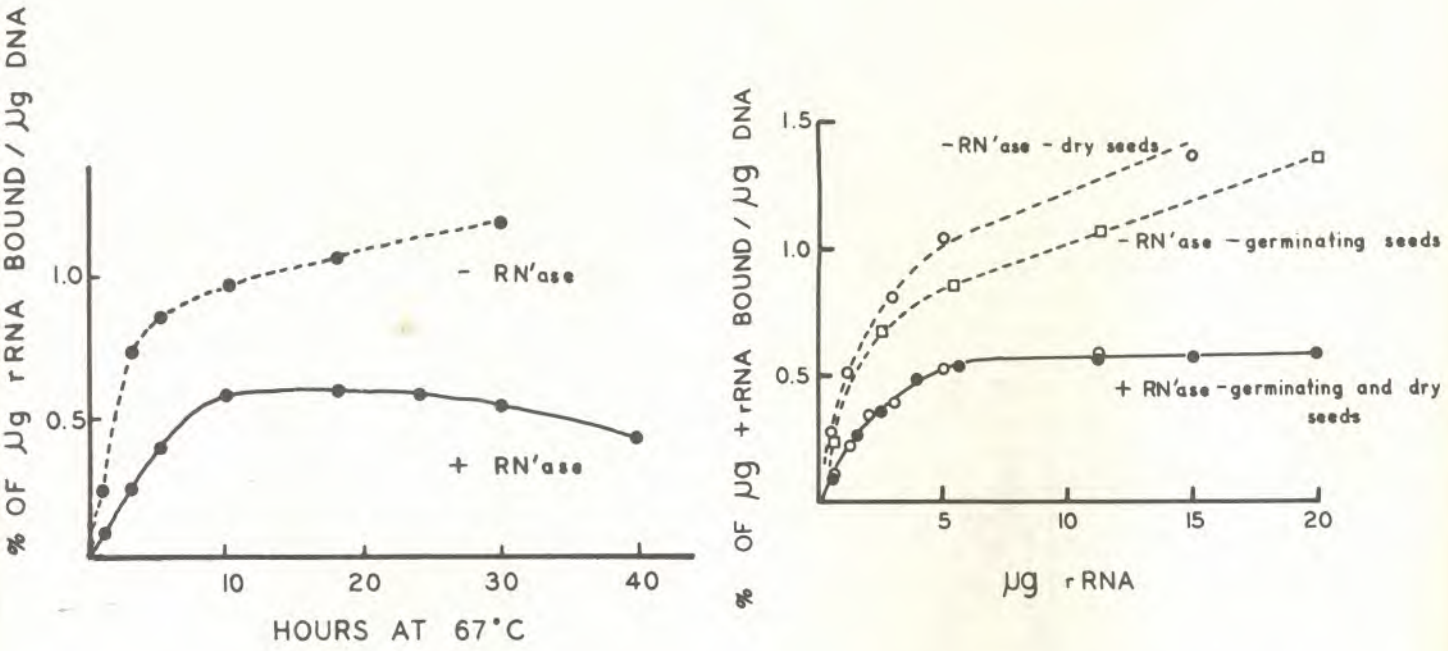

Figure 3. Time course for rRNA - DNA hybrid formation. 53.3 µg of *P. banksiana* DNA fixed on the filter was incubated with 10 µg of H^3-*P. banksiana* rRNA from germinating seedlings (10,000 cpm/µg) at 67°C. for various lengths. The remaining activity was measured with •————————• and w/out •– – – – – – – – – – – –•. RNase treatment.

Figure 4. Hybrid formation between rRNA and DNA. DNA filter containing 53.3 µg of *P. banksiana* DNA was incubated with various amounts of *P. banksiana* rRNA isolated either from dry seeds 0----0 (-RNase), or from germinating seedlings □————□ (-RNase), •————• (+ RNase) for 12 hrs. at 67°C. Specific activity of rRNA was about 10,000 cpm/µg labeled by H^3.

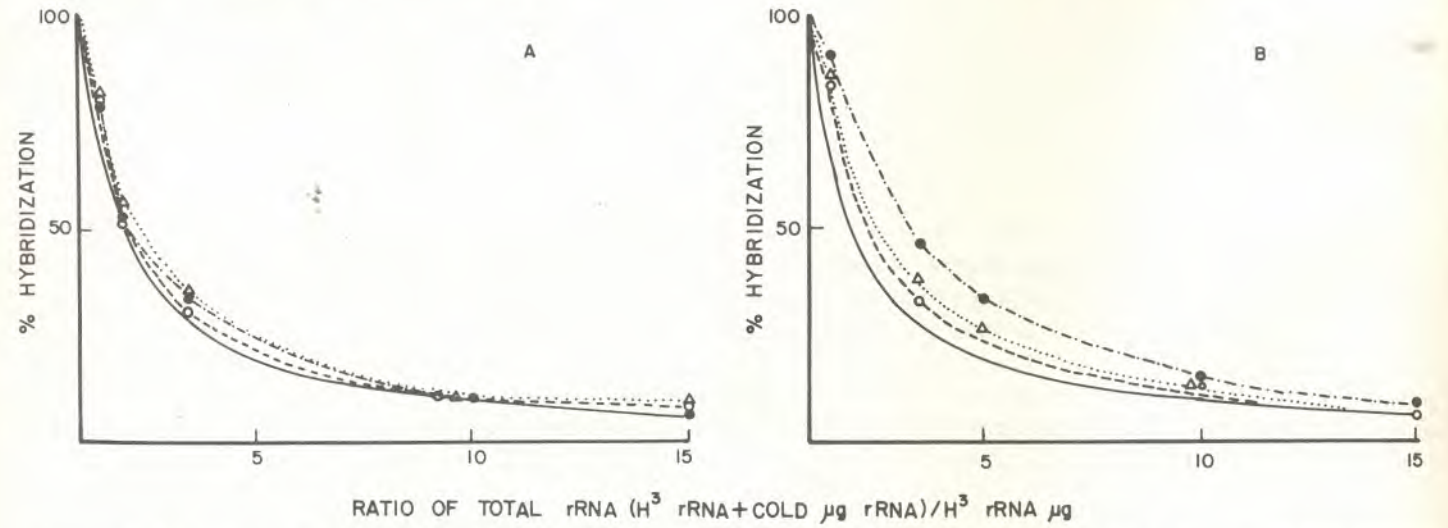

Figure 5. Competition of rRNA for sites. 2.8 µg of homologous rRNA (H^3 spec. act. 50 90 x 10^3 cpm/µg) was reacted with denatured DNA at 68°C. for 12 hrs., with or without addition of various amounts of cold homologous or heterologous rRNA. After incubation, all the samples were treated with RNase and counted. ———— theoretical curve. A. *P. glauca* DNA fixed on filter. B. *P. resinosa* DNA fixed on filter. o—.—.—o *P. glauca* rRNA, △ . . . . . △ *P. banksiana* rRNA, o----------o *P. resinosa* rRNA. Percent hybridization was calculated against no addition of cold rRNA after subtracting the background value at each point.

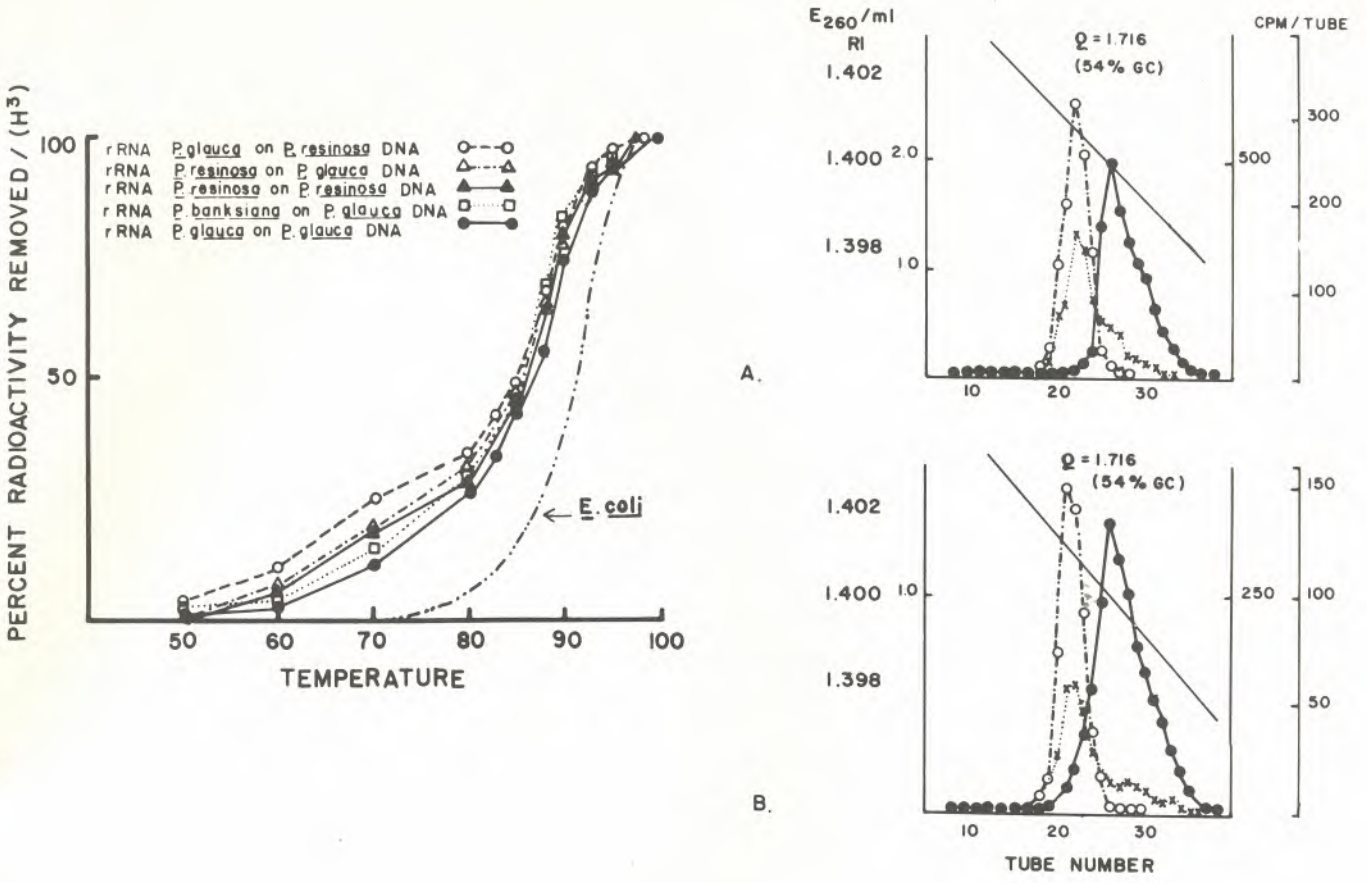

Figure 6. Thermal stability of rRNA-DNA hybrids formed at saturation conditions 10-12 hrs. with 50-80 μg DNA on retaining filters and incubated with 100-150 μg rRNA (H^3 sp.act 5-10 x 10^3 cpm/μg) at 69°C. for 12-15 hrs. in total volume of 5 ml of 6XSSC. After RNase treatment and washing, these filters were incubated at various temp. in 2XSSC for 20 mins. (1 filter/vial containing 10 ml. of 2XSSC) and the filter washed with 2XSSC was counted. The background radioactivity was subtracted in each case and the percent of release was calculated.

Figure 7. Determination of rRNA cistron structure by CsCl buoyant density centrifuge methods. Purified DNA was centrifuged in CsCl solution using a Spinco rotor #30, spun at 30,000 rpm for 48 hrs. Fractions collected from the bottom were denatured by alkaline and absorbed on the filter. Filters were incubated with 15 μg of rRNA (sp. act. μ70 cpm/μg) in 10 ml. of 6XSSC at 68°C. for 12 hrs. After hybrid formation, the filter was treated with RNase and one filter set was used to count their radioactivity directly, and the other set was incubated in 1XSSC at 80° C. for 20 mins. and the 80°C. heat resistant portion of radioactivity was counted. A. *Pinus resinosa*, B. *Picea glauca*, OD_{260} •——•, CPM rRNA hybridized after RNase treatment x·····x, CPM of 80°C. heat resistant fraction o—.—.o

Table 1. Nucleotide composition of seed and seedling coniferous rRNA

	Cytidylic	Adenylic	Guanylic	Uridylic	Unidentified Nucleotide
Pinus banksiana (seed)	15.4	22.0	33.9	22.7	6.0
Pinus banksiana (seedling)	20.4	21.6	33.4	24.6	*
Picea glauca (seedling)	20.2	20.5	36.2	22.9	*
Pinus resinosa (seedling)	21.0	21.6	34.8	26.6	*

*Present in minute quantities.

Table 2. Hybridization of coniferous ribosomal RNA to coniferous DNA

DNA source	µg DNA on filter	rRNA source	µg rRNA hybridized	rRNA hybridized as % of DNA
Pinus banksiana	106.6	*Pinus banksiana*	0.65	0.60
Pinus banksiana	106.6	*Pinus resinosa*	0.65	0.60
Pinus banksiana	106.6	*Picea glauca*	0.61	0.57
Pinus banksiana	106.6	*Thuja occidentalis*	0.46	0.43
Pinus resinosa	204.0	*Pinus resinosa*	1.65	0.80
Pinus resinosa	204.0	*Picea glauca*	1.03	0.50
Pinus resinosa	204.0	*Thuja occidentalis*	1.32	0.64
Picea glauca	56.0	*Picea glauca*	0.36	0.64
Thuja occidentalis	64.0	*Thuja occidentalis*	0.42	0.65

Table 3. Number of rRNA genes

	DNA picograms per nucleus	% DNA hybridized*	Amount of DNA complementary to rRNA (Daltons)	Genes per nucleus
Picea glauca	60	0.64	$2.3 \times 10''$	1.15×10^5
Pinus banksiana	69	0.60	$2.5 \times 10''$	1.25×10^5
Pinus resinosa	138	0.80	$6.6 \times 10''$	3.3×10^5
Thuja occidentalis	39	0.65	$1.5 \times 10''$	7.5×10^4

*Total rRNA was used for the hybridization experiments. Hybridization techniques are given in the methods section. The amount of DNA per nucleus was determined by cytophotometry and biochemical assay methods (Miksche 1967).

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