The pathway of ribosomal RNA biogenesis in *Dictyostelium discoideum* has been defined through identification, isolation, and characterization of the rapidly labeled nuclear RNAs which are intermediates in the process. Comparison of the methylation patterns, base compositions, two-dimensional oligonucleotide maps, and hybridization properties of these intermediate RNAs with those of mature rRNAs has established clearly the precursor-product sequence relationships supporting the following scheme for rRNA production and processing:

\[
37\text{S} \rightarrow 25\text{S} (28\text{S}) \rightarrow 25\text{S} \\
25\text{S} (21\text{S}) \rightarrow 17\text{S}
\]

The relationship of the 37 S RNA of *Dictyostelium* to primary rRNA transcripts of prokaryotes and other eukaryotes is discussed.

In addition to further cleavage steps, post-transcriptional modification of rRNA includes a number of site-specific methylations. In both prokaryotes and eukaryotes, at least some of the methylations occur early in the processing scheme; thus, methylation is a characteristic feature of all rRNAs and most of their precursors (9). However, almost all the eukaryotic rRNA methyl groups are ribose substitutions, and are introduced early, at the primary transcript stage; while bacterial methylations are mostly base substitutions, and many do not appear until the very last stages of ribosome maturation (1, 6).

Eukaryotes have thus diverged from prokaryotes in these and other aspects of the rRNA synthetic program. Study of this program in a variety of lower eukaryotes should provide evidence about the origins and mechanisms of the divergence.

*Dictyostelium discoideum* is a lower eukaryote of particular interest because of its simple, well defined developmental program. In the vegetative phase of its life cycle, a homogeneous population of single celled amoebae grows and multiplies. When cells are deprived of nutrients and placed on a solid surface, they synchronously aggregate into multicellular fruiting bodies, and differentiate into two new cell types: stalk cells and spore cells. Distinctive alterations in the pattern of rRNA synthesis and processing accompany the other biochemical and morphogenetic changes in the developmental sequence (10-12). One example is a marked decrease in the rate of transport of total RNA out of the nucleus in developing compared to vegetative cells, and an associated accumulation of those RNAs thought to be rRNA precursors (12). We have undertaken a thorough characterization of all the intermediates in the maturation of *Dictyostelium* rRNA. Such analysis should provide the framework for future investigations into the nature and causes of altered rRNA production during development.

Previous research has suggested that the mature rRNAs of *Dictyostelium* are ultimately derived from a common, large molecular weight precursor, via two intermediate species slightly larger than the final rRNA molecules (13). Evidence for this pathway has depended solely on examination of the kinetics of incorporation of radioactive precursors into total cellular or nuclear RNA. Attempts to chase radioactive label from short lived intermediates into mature rRNAs have failed to demonstrate clearly a precursor-product relationship, presumably because of problems in diluting the large pools of RNA precursors. We describe in this paper the isolation of...
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each of the putative precursor rRNAs as a discrete species, and demonstrate definitively the precursor-product sequence relationship between them and mature rRNAs.

EXPERIMENTAL PROCEDURES

Materials—Radioactive compounds were purchased from New England Nuclear Corp. 32P, was carrier-free, and [methyl-3H]methionine had a specific activity of 11.5 Ci/mmol.

Diethylpyrocarbonate, packed under nitrogen, was obtained from Sigma; after a bottle had been opened, it was used for only 2 weeks before being discarded. Sodium dodecyl sulfate was recrystallized twice from 95% ethanol. Phenol was redistilled. Escherichia coli K-12 strain was cultured in L broth (Difco) with or without 50 μg/ml of ampicillin, and then harvested by centrifugation and washed with ice cold Buffer MES-PDF. Twenty-milliliter cultures were incubated, with shaking, at 23°C in 50 ml of Buffer MES-PDF (β = 0.1 M NaCl, 5 mM MgSO4, 0.75 mM CaCl2, 0.3 g/liter of streptomycin sulfate, 22 mM NaH2PO4, and 11 mM Na2HPO4.

Cells—Vegetative strain AX3 were grown axenically to a density of 2 × 109/ml in Medium MES-CHL. They were then harvested and subjected to starvation conditions, either (a) on buffer filter paper or (b) in liquid suspension.

(a) Cells designated as "developing" were plated on filters 42.5 cm in diameter, as described previously (14), except that the filters were saturated with non-nutrient Buffer MES-PDF, rather than Buffer PDF. 3 Cells so plated underwent the normal developmental sequence of biochemical and morphogenetic changes, culminating in the formation of fruiting bodies. Until the 11th h of development, each filter of vegetative cells was supported on two filter pads (Millipore, AP 1004780) also saturated with Buffer MES-PDF, at 11 h the cell filter alone was transferred to a Petri dish containing radioactive label in 0.27 ml of Temperature-controlled labeling was for 3 h; parallel filters were labeled with either 6 mCi of 32P or 5 mCi of [methyl-3H]methionine. The gel was made 0.2% (w/v) in sodium dodecyl sulfate and then 2% (v/v) in diethylpyrocarbonate. Within 10 s after addition of the diethylpyrocarbonate, 1 volume of water-saturated phenol was mixed with the sample. Then 0.1 volume of 2 M sodium acetate, pH 7.3, was added, followed by 1 volume of chloroform:isoamyl alcohol (96:4). The mixture was centrifuged for 10 min at 4,000 x g for 7 min. The two phases separated by the centrifugation were each re-extracted separately: the upper aqueous phase plus interphase was precipitated with 2 volumes of chloroform:isoamyl alcohol mixture (96:4) alone. The RNA in the first (re-extracted) organic phase was discarded. The second organic phase was removed from under the sample aqueous phase, and was re-extracted with the same 2-m1 HMK buffer phase used for the first organic phase. After this second organic phase re-extraction, the two aqueous phases, plus interphase material, were pooled and re-extracted twice with 2.5 volumes of chloroform:isoamyl alcohol mixture (96:4) alone. The RNA in the final aqueous phase was precipitated with 2 volumes of ethanol.

Cytoplasmic rRNAs were harvested by centrifugation at 10,000 x g for 10 min; nuclear RNA at 250,000 x g overnight.

Ribosomal RNAs were separated from heterogeneous, poly(A)-containing RNAs by oligo(dT)-cellulose (Collaborative Research) chromatography. RNA pellets were dissolved in 0.5 to 1.0 ml of oligo(dT)-cellulose binding buffer and applied to a 0.1 to 0.25-g column of oligo(dT)-cellulose equilibrated in the flow-through buffer; the column was washed with 10 volumes of binding buffer; the flow-through solution contained most of the RNA. RNA bound to the column was recovered when the column was washed with oligo(dT)-cellulose elution buffer; the eluate fraction was made 0.4 M in sodium acetate, pH 7.4. Both samples were stored at −20°C. All RNAs were harvested by precipitation with ethanol as described above.

Agarose Gel Electrophoresis of RNA—The gel system used was a modification of that of Sharp et al. (16). The 1.4 to 1.5% (w/v) agarose (Calbiochem or Sigma electrophoresis grade) was melted in E buffer (0.6 cm inner diameter × 12 cm long). Gels were cast in Pyrex glass tubes (0.6 cm inner diameter × 12 cm long). Slab gels were cast from the same buffered agarose solution, but without glycerol. Gels were pre-run for 15 min under the conditions used for sample electrophoresis. Samples were suspended in a small volume (10 to 15 μl) of cytoplasmic RNA sample solution, and the entire mixture was carried out at 8 mAl/gel for 3.5 to 4.5 h on cylindrical gels, and at 150 V for 1.5 h on slab gels; slab gels were cooled by a fan. After electrophoresis, cylindrical gels were cut transversely with a series of razor blades into 1-mm slices. For preparative gels, the Cerenkov radiation of each slice was measured directly (17). Analytical gel profiles were obtained by melting each slice in 1 ml of water, then determining radioactivity of the solubilized slice in 10 ml of Aquasol. Wet slab gels were covered with plastic wrap and exposed to film for autoradiography.

Fractionation of Cytoplasmic rRNAs—Cytoplasmic polysomal RNA which did not bind to oligo(dT) was collected from cells starved in suspension for 10 h and labeled with 60 mCi of 32P, during the entire starvation period. Mature 25 S, 17 S, and 4 to 5 S RNAs were separated by centrifugation through 34 ml 15 to 30% (w/v) linear sucrose gradients in Buffer A. Centrifugation was for 17 h at

1 Buffer MES-PDF is a phosphate-free variant of pad diluting fluid (Buffer PDF). Buffer PDF consists of 13 mM KCl, 5 mM MgSO4, 0.75 mM CaCl2, 0.3 g/liter of streptomycin sulfate, 22 mM NaH2PO4, and 11 mM Na2HPO4.

2 T. Alton, personal communication.
23" and 25,000 rpm in a Beckman SW 28 rotor. The 5 S RNA was purified from material near the top of the gradient by procedures described previously. The 23 S and 25 S RNA fractions were eluted separately on 10 to 30% gradients, and the peak fractions were recovered. The purified RNA pools were concentrated by precipitation with 2 volumes of ethanol, followed by centrifugation at 10,000 \times g for 15 min. Each RNA pellet was dissolved in 0.5 ml of water and layered onto a 5-ml Sephadex G-25 M column in water. RNA was eluted from the column with water, and the salt-free void volume fractions were collected. The specific activity of the purified mature rRNAs was 1,500,000 cpm/μg.

Purification of Nuclear rRNAs—Nuclear RNA was prepared from cells starved in suspension for 1 h and labeled at the same time with 60 μCi of \( ^{32}P \). Usually further purification steps were carried out on RNA that flowed through oligo(dT)-cellulose, but 37 S RNA could also be purified from material that bound to the column under high salt conditions. In either case, nuclear RNAs were fractionated by sedimentation through 11 ml 15 to 30% (v/v) linear sucrose gradients in Buffer A; centrifugation was for 7.25 h at 23" and 37,000 rpm in a Beckman SW41 rotor. Fractions from the radioactive peaks sedimenting at roughly 21 S and 28 S were pooled separately, all material sedimenting faster than 31 S was collected in a third pool. Each RNA pool was mixed with 5 to 25 μg of carrier RNA, and was precipitated with ethanol. The RNA pellets were dried, resuspended in gel sample solution, and fractionated further by electrophoresis on agarose slab gel of the same type as used for RNA fractionations (above). Electrophoresis was at 10 V for 18 h, until the xylene cyanole dye had moved 25 S and 17 S cm. Gels were stained for 0.5 h in 0.5 μg/ml of ethidium bromide, and then illuminated with a short wave ultraviolet lamp (Ultra-Violet Products) for photography through a red filter on Polaroid 107 positive film (16). Transfer of the fractionated DNA pattern to nitrocellulose filters (Schleicher and Schuell) was by the procedure of Southern (22). DNA filters were wet uniformly with the minimum possible volume of a mixture containing 50% (v/v) formamide (MC/B), 5 x SSC, and 3,000 to 80,000 cpm of \( ^{32}P \) labeled RNA; each filter was sealed in Saran Wrap and incubated for 12 to 15 h at 42°C. In preparation for autoradiography, the filters were washed several times in 5 x SSC, incubated for several hours at 42" in a large volume of 50% formamide/5 x SSC, and then washed extensively in 5 x SSC.

RESULTS

In order to isolate and identify short lived intermediates in the program of ribosomal RNA maturation, we have taken advantage of the decreased rate of nuclear RNA processing which is a special property of developing cells of Dicystostelium. As a result, it has been possible for us to obtain accumulated supplies of rRNA precursors without disturbing normal physiological processes by the introduction of drugs or mutations. For these experiments, we have used cells starved in suspension, as well as developing cells, which are starved on filters (see "Experimental Procedures"). We can detect no differences in the overall pattern of nuclear RNAs from cells starved in suspension compared to cells starved on filters, but it is much easier to label RNA in suspension cells.

Initially, two criteria were used to identify possible rRNA precursors in the nucleus: rRNA-related species should be multiply methylated, and should not bind to oligo(dT)-cellulose columns. [methyl-\( ^{3}H \)] and \( ^{32}P \)-labeled RNAs, extracted from the nuclei of aggregation stage developing cells after a labeling period of 3 h, were passed over oligo(dT)-cellulose, and the flow-through fraction was analyzed by agarose gel electrophoresis (Fig. 1). The large molecular weight RNA's were resolved clearly on the gel into five discrete species. Two of these species co-migrate with mature, cytoplasmic 25 S or 17 S rRNA. Of the remaining RNA peaks, two are labeled "p25 S" and "p17 S," in accordance with their presumed roles as immature and which do not bind to oligo(dT)-cellulose. Two parallel developing filters were labeled from 11 to 14 h in development, one with \( ^{3}P \), and the other with [methyl-\( ^{3}H \)]methionine (see "Experimental Procedures"). At 14 h, nuclear RNA was prepared separately from each cell batch, and then appropriate quantities of the two RNAs were mixed to obtain a sample containing approximately equal \( ^{3}H \) and \( ^{32}P \) counts. The mixture was passed over oligo(dT)-cellulose column, and the material that did not bind was fractionated on a cylindrical agarose gel as described under "Experimental Procedures.

![Fig. 1](http://www.jbc.org/) Profile of developing cell nuclear RNAs which are methylated and which do not bind to oligo(dT)-cellulose.

* B. Batts-Young and H. F. Lodish, manuscript in preparation.
diate precursors to 25 S RNA and 17 S RNA, respectively. The last and largest RNA is most likely candidate for the primary rRNA transcription product. We designate this species "37 S" RNA, in accordance with the terminology used to describe other primary transcript rRNAs; however, by this title we mean to indicate only an approximate value for the sedimentation coefficient of the RNA (see below). Although all of the discrete nuclear RNAs can incorporate substantial quantities of label from [methyl-3H]methionine, as would be expected of ribosomal RNAs and their precursors, there is a marked difference in the ratio of $^3$H to $^{32}$P in "precursor" compared to 25 S and 17 S RNAs, demonstrating that the precursors are not aggregates or conformational variants of the mature rRNAs. Thus, the $^3$H/$^{32}$P ratio for both 25 S and 17 S RNA is approximately 0.8 (1x), while the comparable ratio is 1.55 (2x) for p25 S and p17 S RNA, and 3.6 (4x) for p37 S RNA.

We have examined nuclear RNA profiles from developing and suspension starved cells labeled with $^{32}$P, for periods ranging from 10 min to 10 h (data not shown). In no case do we detect any discrete large molecular weight RNAs other than the five described above. The radioactive discrete nuclear RNAs isolated from cells labeled for short periods consist almost exclusively of the putative precursor species; only after an hour does label begin to appear in mature rRNAs, and these then are probably cytoplasmic contaminants. The purity of labeled "precursor" RNAs in the nuclei of cells labeled for only 1 h is illustrated by the gel profile of Fig. 2A.

In order to obtain profiles as clean as that illustrated in Fig. 2A, it is essential first to pass the RNA preparation over an oligo(dT)-cellulose column in high salt binding buffer, and material which flowed through the column under these conditions was collected. The RNA which had bound to the column was then recovered by elution with low salt elution buffer (see "Experimental Procedures"). All base composition figures represent averages of at least two determinations.

![Fig. 2. Profiles of nuclear RNAs from suspension starved cells labeled with $^{32}$P, for only 1 h: fractionation by oligo(dT)-cellulose column chromatography. Cells were starved in suspension (see "Experimental Procedures"), and were labeled for 1 h with 60 uCi of $^{32}$P, immediately after the onset of starvation. At the end of the hour, the cells were harvested and nuclear RNA was extracted from them. Individual RNA species were purified from total nuclear RNA as described in the text. In the last step, RNA eluted from an agarose gel was suspended in 0.25 ml of Buffer A (A to C), or in 30 ml of a solution consisting of 27 ml of deionized formamide (MC/B) plus 3 ml of Buffer A (D). The RNA in formamide was incubated at 65° for 5 min, quick-cooled, and diluted to 0.25 ml with Buffer A. All samples were immediately layered onto 11 ml 15 to 30% (w/v) linear sucrose gradients for 10.25 h (A) or 7.25 h (B to D). The Cerenkov radiation of each gradient fraction was measured directly (17). The positions of markers of 35 S poliovirus RNA, Chinese hamster ovary cell rRNA, and Dictyostelium cytoplasmic (25 S + 17 S) RNA were obtained from parallel gradients. Peak fractions from sample gradients were pooled as indicated by the bars. A, p17 S RNA; B, p25 S RNA; C, 37 S RNA; D, another preparation of 37 S RNA, after formamide denaturation.](http://www.jbc.org/)

**Table I**

Base compositions of discrete nuclear RNA species compared to mature RNAs

| RNA sample | CMP | AMP | GMP | UMP | G + C |
|------------|-----|-----|-----|-----|-------|
| 17 S RNA   | 18.3| 28.5| 24.2| 29.0| 42.5  |
| p17 S RNA  | 19.0| 30.3| 22.0| 28.6| 41.1  |
| 25 S RNA   | 17.5| 28.5| 25.6| 28.1| 43.4  |
| p25 S RNA  | 18.0| 28.8| 24.1| 29.2| 42.1  |
| 37 S RNA   | 17.9| 29.8| 22.1| 29.3| 40.9  |
Fig. 3 demonstrates that pure, discrete precursor rRNAs can in fact be isolated from nuclear RNA preparations comparable to that shown in Fig. 2A. The purification procedure is detailed under "Experimental Procedures" and involves fractionation of the individual RNA species on two sucrose gradients and an agarose gel. A to C of Fig. 3 represent characteristic profiles of the final purification gradients for p17 S RNA, p25 S RNA, and 37 S RNA. D provides additional evidence that 37 S RNA is not simply an aggregate of smaller RNAs. A preparation of 37 S RNA was heated at 65°C in 90% formamide before being layered onto the final sucrose gradient. Under these denaturing conditions, the RNA did not dissociate into smaller rRNA species (Fig. 3D). Peak fractions from the gradients were pooled as indicated in the figure, and were concentrated by precipitation with ethanol. From the mobility of sample peaks relative to marker RNAs on such gradients, we can estimate the size of each discrete nuclear RNA; thus, 37 S RNA actually has a sedimentation coefficient somewhere be-

Fig. 4. Autoradiograms and tracings of two-dimensional separations of oligonucleotides produced by complete RNase A digestion of p25 S, 25 S, p17 S, and 17 S RNAs. First dimension separation, on cellulose acetate, pH 3.5, is based mainly on oligonucleotide charge; while second dimension homochromatography fractionates mainly on the basis of size, with smaller oligonucleotides moving toward the top of the plate (19). The tracings are composites of the fingerprints for a precursor rRNA and the corresponding mature rRNA. Open circles represent those oligonucleotides common to both; filled circles, those unique to the precursor species; and cross-hatched circles, those found exclusively, or in far greater quantity, in the mature rRNA. Oligonucleotides outlined in dots are faint, but reproducible in many fingerprints. Most of the faint oligonucleotides near the top of the fingerprints are not reproducible, and appear more frequently when the batch of digesting enzyme is old; they have therefore been omitted from the fingerprint tracings. The regions outlined with a dashed line represent characteristic oligonucleotide groupings that distinguish the p25 S-25 S RNA pair from the p17 S-17 S RNA pair.

oligo(dT)-cellulose column under such conditions that heterogeneous, poly(A)-containing RNAs are retained on the column. Fig. 2B indicates the composition of the material that binds to the column when salt concentration is high. There is considerable nonspecific sticking of the putative rRNA precursors to the oligo(dT)-cellulose; however, there does not appear to be preferential binding of any one of these species relative to another. Most of the bound radioactive material is heterogeneous, and presumably represents poly(A)-containing message-like RNAs (22). A large portion of the heterogeneous RNA migrates only slightly faster than p17 S RNA. Roughly 30 to 40% of total nuclear RNA binds to oligo(dT)-cellulose under the conditions used here. Thus in unfractionated nuclear RNA, heterogeneous material would obscure the profile of distinct species, particularly in the region of 17 S and p17 S RNA. This problem has made it impossible to determine from previous studies whether or not the proposed rRNA species were intact, discrete species.
between 36 S and 38 S, and the sedimentation coefficients for p25 S and p17 S RNAs are approximately 28 S and 21 S, respectively.

**Base Compositions**—The nuclear genome of *Dictyostelium discoideum* has a low, 22 to 23% G + C content (23-25), as does the total mRNA (26). In marked contrast is the 42 to 44% G + C content of mature ribosomal RNA (26) (see also Table I). As would be predicted for ribosomal RNA precursors, 37 S RNA, p25 S RNA, and p17 S RNA do not differ significantly in base composition from mature ribosomal RNAs (Table I).

**Oligonucleotide Fingerprint Analysis**—More compelling evidence for rRNA nucleotide sequence relationships has been obtained from examination of oligonucleotide map “fingerprints” of the RNase A digestion products of p25 S and p17 S RNAs, compared to equivalent fingerprints of the mature rRNAs (Fig. 4). Because RNase A recognizes both cytidine and uridine residues as cleavage sites, a large number of small oligonucleotides are generated by its action; such small fragments are expected to be distributed commonly among RNA molecules as large as rRNAs. Thus the upper portions of all the RNase A oligonucleotide maps are almost identical, even for such relatively unrelated species as 17 S RNA and 25 S RNA. However, in the lower regions, where large unique oligonucleotides are located, the fingerprints for 17 S RNA and

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**Fig. 5.** Autoradiograms and tracings of two-dimensional separations of oligonucleotides produced by complete RNase T1 digestion of p25 S, 25 S, p17 S, and 17 S RNAs. The separation system and conventions for fingerprint tracings are as described for Fig. 4. In this case we have omitted from the fingerprint tracings a few oligonucleotides from the extreme right side of the 25 S RNA fingerprint which clearly would have run off the edge of the p25 S RNA fingerprint. The p17 S RNA shown here is contaminated with a small amount of p25 S RNA degradation product; however, we know which oligonucleotides are true p17 S RNA constituents from another fingerprint with no obvious p25 S RNA contamination.
EcoRI restriction endonuclease and is fractionated by agarose gel electrophoresis, nine discrete fragment bands, representing those extra oligonucleotides which distinguish each precursor from its mature RNA counterpart.

25 S RNA differs significantly; some of the distinguishing oligonucleotide groupings are outlined in the fingerprint tracings. The fingerprints and tracings of Fig. 4 demonstrate clearly that all the unique oligonucleotides characteristic of 25 S RNA are also found in p25 S RNA. In fact, except for four extra large oligonucleotides in p25 S RNA, the fingerprints of p25 S RNA and 25 S RNA appear identical. Essentially the same type of relationship is seen between the fingerprints of p17 S RNA and 17 S RNA, although 17 S RNA contains one distinctive oligonucleotide, probably representing one end of the intact RNA, which is not found in p17 S RNA.

T1 RNase, with a greater degree of specificity than RNase A, cleaves next to guanosine residues only. Consequently, a T1 RNase digest contains many more large, distinctive oligonucleotides than does an RNase A digest. Fig. 5 illustrates the many sequences distinguishing 17 S RNA from 25 S RNA which are revealed in T1 oligonucleotide maps. The striking correlation of T1 oligonucleotide patterns in p25 S RNA compared to 25 S RNA, and in p17 S RNA compared to 17 S RNA (fingerprints and tracings of Fig. 5), therefore provides strong substantiating evidence for the precursor-product relationships suggested by the RNase A fingerprints. As is demonstrated by the tracings of Figs. 4 and 5, the RNase T1 fingerprints also expose more clearly than RNase A fingerprints those extra oligonucleotides which distinguish each precursor from its mature rRNA counterpart.

Hybridization with EcoRI Fragments Which Code for rRNA—When Dictyostelium nuclear DNA is digested with EcoRI restriction endonuclease and is fractionated by agarose gel electrophoresis, nine discrete fragment bands, representing reiterated DNA sequences, are resolved above a background of heterogeneous fragments. One of these DNA bands (actually a doublet) hybridizes specifically with 25 S RNA; a second band hybridizes with 17 S RNA; and a third band hybridizes with both rRNAs (20, 27). We have used these rRNA-specific restriction fragment bands as probes for the detection of rRNA sequences in proposed precursor rRNAs.

For the hybridization analysis, total nuclear DNA was digested with EcoRI, and the digest was fractionated on an agarose slab gel as described under “Experimental Procedures.” The entire fractionated DNA pattern was then transferred from the gel to a nitrocellulose membrane by the procedure devised by Southern (21). 32P-labeled 5 S, 17 S, p17 S, 25 S, p25 S, and 37 S RNAs were allowed to hybridize with the DNA on parallel strips of the nitrocellulose filter, each derived from one slot of the same gel. As shown in Fig. 6, the p25 S RNA hybridizes specifically to those EcoRI DNA restriction fragments complementary to 25 S RNA; similarly, the pattern of p17 S RNA hybridization is identical with that for 17 S RNA. Purified 37 S RNA anneals with all three of the rRNA-specific restriction fragment bands. Thus, this large RNA species contains the proper sequence complement required for a common precursor to 25 S and 17 S rRNAs.

**DISCUSSION**

We have characterized three discrete, rapidly labeled RNA species, all extensively methylated, from the nuclei of starved and developing Dictyostelium discoideum. These RNAs correspond in mobility and methylation properties to very transient radioactive species observed by Iwabuchi et al. (13) in sucrose gradient profiles of whole cell RNA from vegetative NC-4 Dictyostelium (NC-4 is parent to the axenic strain we have used). We assume that the comparable vegetative and developing cell RNA intermediates are the same. The one possible exception is a 30 S RNA intermediate, observed as a shoulder in Iwabuchi's gradients of vegetative RNA, which we do not detect in high resolution agarose gel profiles of nuclear RNA from starved or developing cells. Kinetics of RNA labeling in the vegetative cells suggested that the short lived intermediates were probably rRNA precursors. We have exploited the special properties of starved and developing cells to demonstrate further that each intermediate can be isolated as a discrete species, uncontaminated by heterogeneous material, incomplete chains, or aggregates of smaller rRNAs. It has not been possible to establish for Dictyostelium an effective pulse-chase procedure for demonstrating clearly the flow of label from precursor to product RNAs (13). We have instead applied fingerprinting and hybridization techniques, in order to obtain unequivocal evidence for the derivation of mature rRNAs from the sequences of the proposed precursors.

The evidence presented in this paper suggests that the 25 S and 17 S RNA genes in Dictyostelium are associated in a large common transcription unit, which produces a 37 S RNA primary transcription product that includes sequences of both 25 S and 17 S mature rRNAs. Cleavage of the primary transcript yields the two immediate precursors of mature rRNA:
p25 S RNA, a molecule containing a small amount of transcribed spacer linked with the precise sequence composition of mature 25 S RNA, and p17 S RNA, which is related in much the same way to mature 17 S RNA. Mapping of restriction fragments produced from the rDNA of Dictyostelium demonstrates a very close association of the 25 S and 17 S RNA genes (20) (Fig. 7), and also provides some evidence that these genes lie on the same DNA strand. Therefore, the organization of the rDNA is at least consistent with production of a single primary transcript rRNA encompassing sequences programmed by both rRNA genes.

Moreover, by combining data from the rDNA restriction fragment map with our evidence about the properties of 37 S RNA, we can eliminate the possibility that this RNA actually consists of two 36 to 38 S RNA populations, one containing 25 S RNA sequences, and the other 17 S RNA sequences, in each case linked to long stretches of transcribed spacer. We have shown that 37 S RNA hybridization is restricted to the same EcoRI fragments which anneal with the mature rRNAs. Mobility of 37 S RNA relative to markers on gradients and gels, and analogy with the 36 to 38 S primary rRNA transcripts of other lower eukaryotes (28-30), allow us to set a lower limit for the length of Dictyostelium's primary rRNA transcript at approximately 8,000 nucleotides. Then if there were two independently transcribed 37 S RNAs, they would be coded by, and would anneal with, two different DNA regions, representing a total length of at least 16,000 base pairs. In particular, an 8,000-nucleotide-long 37 S RNA containing 25 S RNA, but not 17 S RNA sequences, would have to be encoded by a stretch of DNA extending from some point within the R1 fragment to a region at least 2,000 base pairs into the R1 7 fragment (see map in Fig. 7). However, the combination of the four EcoRI restriction fragments containing sequences complementary to 37 S RNA accounts for only 11,000 base pairs, and we can detect no hybridization of 37 S RNA with the R1 7 fragment. Therefore, we conclude that the 37 S RNA which we have isolated represents a single species, the common primary transcript product of 25 S and 17 S rRNAs. We can estimate an upper limit for the size of this primary transcript, since it must be generated from a stretch of DNA no more than 11,000 base pairs long, sufficient to code for an RNA no bigger than 3.5 x 10^4. The actual molecular weight of 37 S RNA is probably closer to 2.6 to 2.8 x 10^4, as is the case for other 36 to 38 S rRNA transcripts found in a variety of lower eukaryotes (28).

The rRNA synthesis and maturation sequence defined here for Dictyostelium fits entirely within the general pattern observed for all eukaryotes (31). Especially striking is the close similarity of the processing events in Dictyostelium to those in yeast, another lower eukaryote (30). One notable feature of the rRNA synthetic apparatus of yeast is the association of 5 S RNA genes with cistrons for the other rRNAs (31). Despite their close physical linkage, these two gene classes remain functionally segregated as separate transcription units (32). This arrangement appears to represent an intermediate phase in the evolution of eukaryotic from prokaryotic rDNA. Thus, Dictyostelium, like yeast, maintains the 5 S DNA-rDNA linkage relationship characteristic of prokaryotes. As is indicated in Fig. 7, the 5 S RNA gene is located somewhere in a 7000 base pair fragment (R1 7) which is neighbor to the terminal 25 S RNA gene fragment (20). What remains to be resolved is whether the primary rRNA transcription unit in Dictyostelium extends to include 5 S RNA sequences. The absence of obvious hybridization of 37 S RNA with the R1 7 fragment band (Fig. 6) argues against this possibility. However, the gene coding for 5 S RNA might be located at the very end of the R1 7 fragment, immediately adjacent to the rRNA genes. In such a case, 37 S RNA could include 5 S RNA with the addition of no more than 120 nucleotides, representing less than 2% of the total RNA length, in sequences complementary to R1 7. Because extremely small quantities of radioactive purified 37 S RNA were available for these experiments, it is quite possible that hybridization of 37 S RNA to such a small piece of R1 7 would not be detected. Substantial evidence that 5 S RNA is not transcribed as part of a precursor common to 25 S and 17 S rRNAs has been obtained very recently. We can demonstrate that both 5 S RNA and p17 S RNA are initiated with a 5′-triphosphate residue in Dictyostelium. It should be of interest to characterize the relationship of 5 S RNA to primary rRNA transcript in a variety of lower eukaryotes, since the discovery of aspects of rRNA synthesis characteristic to intermediate level organisms can provide some insight into molecular mechanisms of evolution.

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REFERENCES

1. Perry, R. P. (1976) Annu. Rev. Biochem. 45, 695-629
2. Brown, D. D., and Weber, C. S. (1968) J. Mol. Biol. 34, 661-680
3. Pardue, M. L., Brown, D. D., and Birnstiel, M. L. (1973) Chromosoma 47, 191-201
4. Winfrey, D. E., and Steffensen, D. M. (1971) Science 170, 639-641
5. Aloni, Y., Hatlen, L. E., and Attardi, G. (1971) J. Mol. Biol. 56, 555-563
6. Pace, N. R. (1973) Bacteriol. Rev. 37, 562-563
7. Ginsburg, D., and Steitz, J. A. (1975) J. Biol. Chem. 250, 5647-5654
8. Hayes, F., Vasseur, M., Nikolajev, N., Schlessinger, D., Sri Widada, J., Krol, A., and Brandlnt, C. (1975) FEBS Lett. 56, 86-91
9. Starr, J. L., and Sells, B. H. (1969) Physiol. Rev. 49, 623-669
10. Coccossi, S. M., and Sussman, M. (1979) J. Cell Biol. 45, 399-407
11. Misukami, Y., and Iwabuchi, M. (1970) J. Biochem. (Tokyo) 67, 501-504
12. Keiser, R. H. (1973) Dev. Biol. 31, 242-251
13. Iwabuchi, M., Misukumi, Y., and Sameshima, M. (1971) Biochim. Biophys. Acta 228, 693-700
14. Tuchman, J., Altton, T., and Lodish, H. F. (1974) Dev. Biol. 40, 118-129
15. Gerisch, G. (1968) Curr. Top. Dev. Biol. 3, 157-197
16. Sharp, P. A., Sugden, B., and Sambrook, J. (1973) Biochemistry 12, 3055-3063
17. Clausen, T. (1968) Anal. Biochem. 22, 70-73
18. Rosc, J. K. (1975) J. Biol. Chem. 250, 6000-6004
19. Davie, B. G. (1971) in Procedures in Nucleic Acid Research (Cantoni, G. L., and Davies, D. R., eds.) Vol. 2, pp. 751-779, Harper & Row, New York
20. Maizels, N. (1970) Cell 9, 431-440
21. Southern, E. M. (1975) J. Mol. Biol. 98, 503-517
22. Firtel, R. A., and Lodish, H. F. (1973) J. Mol. Biol. 79, 295-314
23. Schildkraut, C. L., Mandel, M., Levisohn, S., Smith-Sonneborn, J. E., and Marmur, J. (1965) Nature 196, 795-796
24. Sussman, R., and Rayner, E. P. (1971) Arch. Biochem. Biophys. 144, 127-137

*G. Frankel, A. F. Cockburn, K. L. Kindle, and R. A. Firtel, manuscript submitted for publication.
25. Firtel, R. A., and Bonner, J. (1972) J. Mol. Biol. 66, 339-361
26. Jacobson, A., Firtel, R. A., and Lodish, H. F. (1974) J. Mol. Biol. 82, 213-230
27. Firtel, R. A., Cockburn, A., Frankel, G., and Hershfield, V. (1976) J. Mol. Biol. 102, 831-852
28. Perry, R. P., Cheng, T. Y., Freed, J. J., Greenberg, J. R., Kelley, D. E., and Tartof, K. D. (1970) Proc. Natl. Acad. Sci. U. S. A. 65, 609-616
29. Loening, U. E., Jones, K. W., and Birnsteil, M. L. (1969) J. Mol. Biol. 45, 353-366
30. Udem, S. A., and Warner, J. R. (1972) J. Mol. Biol. 65, 227-242
31. Rubin, G. M., and Sulston, J. E. (1973) J. Mol. Biol. 79, 521-530
32. Trapman, J., and Planta, R. J. (1975) Biochim. Biophys. Acta 414, 115-125
Precursors of ribosomal RNA in the cellular slime mold Dictyostelium discoideum. 
Isolation and characterization.
B Batts-Young, N Maizels and H F Lodish

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