

RIBOSOME SYNTHESIS IN *TETRAHYMENA PYRIFORMIS*

AJIT KUMAR

From the Whitman Laboratory, Department of Biology, University of Chicago, Chicago, Illinois. Dr. Kumar's present address is Department of Biochemistry, Albert Einstein College of Medicine, Bronx, New York 10461

ABSTRACT

The cellular site of synthesis of ribosomal RNA in *Tetrahymena pyriformis* was studied by analyzing the purified nuclear and cytoplasmic RNA from cells pulse labeled with uridine-³H. The results of studies using zonal centrifugation in sucrose density gradients show that the ribosomal RNA is synthesized in the nucleus as a large precursor molecule sedimenting at 35S. The 35S molecule undergoes rapid transformation through two main nuclear intermediates, sedimenting at about 30S and 26S. The smaller ribosomal RNA (17S) appears first in the cytoplasm and it seems to be absent from the nucleus. The apparent delay in the appearance of the larger ribosomal RNA (26S) in the cytoplasm is due to the presence of a larger pool of its precursors in the nucleus as indicated by pulse-chase experiments. The newly synthesized ribosomal RNA's appear in the cytoplasm as discrete 60S and 45S ribonucleoprotein particles, before their incorporation into the polysomes.

INTRODUCTION

Clearly the formation and maturation of ribosomal RNA (rRNA) is one of the major synthetic processes in the nuclear metabolism of rapidly growing cells. Early radioautographic investigations showed the movement of nuclear RNA in molecular form to the cytoplasm in a variety of cell types (Goldstein and Plaut, 1955; Zalokar, 1959; Prescott, 1960). Subsequent studies have identified various molecular species of RNA's based on their sedimentation rates, metabolic instability, homology to the DNA, cellular location, and base ratio analysis (Penman, 1966; Attardi, Parnas, and Attardi, 1966; Warner, Soeiro, Birnboim, Girard, and Darnell, 1966; Penman, Vesco, and Penman, 1968; Scherrer, Latham, and Darnell, 1963; Perry, 1962; Houssais and Attardi, 1966). Some recent studies on rapidly proliferating mammalian cells in culture have shown that the newly synthesized ribosomal RNA becomes associated with proteins soon after its synthesis in the nucleus and

enters the cytoplasm in form of ribonucleoprotein particles (RNP) (Warner, 1966; Warner and Soeiro, 1967; Girard, Latham, Penman, and Darnell, 1965). The newly synthesized ribosomal precursor particles become rapidly incorporated into the functional ribosomal aggregates in the cytoplasm (Girard, Latham, Penman, and Darnell, 1965; Joklik and Becker, 1965). In addition to the ribosomal ribonucleoprotein particles, recent studies by Perry and Kelly (1968) have shown that an array of polydisperse RNP particles containing nonribosomal RNA (perhaps messenger RNA) appear in the cytoplasm.

Most of our knowledge on the subject, however, derives from studies on cultures of mammalian cells, and little is known about the synthesis and processing of ribosomal RNA in unicellular organisms. In the present study, the early events in the nuclear RNA metabolism in a holotrich ciliate, *Tetrahymena pyriformis*, is described. In particular, a

large rapidly synthesized nuclear RNA molecule identified to be the precursor to cytoplasmic ribosomal RNA was analyzed by zonal sedimentation in sucrose density gradients. Analysis of the pulse-labeled RNA following chase conditions showed the absence of stable ribosomal RNA from the nuclear fractions. The newly formed ribosomal RNA's appear first as discrete ribonucleoprotein particles in the cytoplasm, followed by their association with functional ribosomes.

MATERIAL AND METHODS

Culture Conditions

Cultures of *Tetrahymena pyriformis* (mating type 1, variety 1) were grown axenically in 2% proteose peptone (Difco Laboratories, Inc., Detroit, Mich.) supplemented with 0.2% glucose, 0.1% yeast extract, and 0.003% Sequestrene (13% Fe-ethylenediamine tetraacetate (EDTA), Geigy Chemical Corp., Ardsley, N. Y.), at 25°C with constant aeration. For incorporation studies, early log-phase cells grown in proteose peptone medium were collected and resuspended in chemically defined medium (medium B of Holz, Erwin, and Davis, 1959) for 2.5–3 hr prior to incubation with radioactive precursors. A cell density of $5-6 \times 10^5$ /ml was used in all experiments. Incorporation was terminated by pouring the aliquots over frozen isolation medium.

Pulse-labeled cells were chased with 50-fold excess unlabeled uridine in the medium, plus 0.01% cytidine and thymidine.

Cell Fractionation

The nuclear isolation procedure was based on a method reported earlier (Gorovsky, 1965). The nuclear isolation medium (NIM) contained 4% gum arabic, 0.0 M sucrose, 1.5 mM MgCl₂, and 0.1 mg/ml spermidine-HCL, pH 6.8. Cells were deposited by centrifugation at 5000 g min. The cell pellet was washed once in NIM and then resuspended in 20 volumes of NIM and homogenized with 0.6% n-octanol in a Waring Blendor at top speed for 5 sec. Light microscopic examination of the homogenate showed no intact cells, whereas most nuclei remained intact. Nuclei were collected in three centrifugations at 8000 g min in an International PR-2 centrifuge (International Equipment Co., Needham Heights, Mass.). Nuclear pellets from the three collections were pooled, washed once in NIM, and a second time in TKM buffer (15 mM Tris-HCL, pH 7.5; 1.5 mM MgCl, and 5 mM KCL) containing 0.1 mg/ml spermidine-HCL and 0.25 M sucrose. The washed nuclear pellet was immediately frozen in a Dry Ice acetone bath (–65°C) until used for RNA extraction.

Recovery of nuclei under similar conditions was about 50% (Gorovsky, 1965). Examination of the nuclear pellet under the electron microscope showed that it was largely free of cytoplasmic contaminants.

For the purification of cytoplasmic RNP particles, the cell pellet was resuspended in TKM buffer supplemented with 0.1 mg/ml spermidine-HCL and 0.25 M sucrose and was homogenized by passing through a Logeman hand mill (Cenco Instruments Corp., Chicago, Ill.). The homogenate was cleared by centrifugation at 16,000 g for 10 min. Various classes of cytoplasmic RNP particles were purified by centrifugation through a linear 15–30% sucrose gradient made in TKM buffer. In some cases cytoplasmic ribosomes were pelleted by centrifugation at 40,000 rpm for 2 hr in a Spinco 40 rotor (Beckman Instruments, Inc., Palo Alto, Calif.) at 4°C.

In order to dissociate ribosomes into large and small subunits, the ribosomal pellet was suspended in 10 mM PO₄ buffer (pH 7.5) containing 0.1 mg/ml spermidine-HCL. The ribosomal suspension was then treated with 0.3 μmole EDTA per mg ribosomes and cleared by centrifugation at 16,000 g for 10 min. The 50S and 30S ribosomal subunits were purified from the supernatant by centrifugation through a 15–30% sucrose density gradient made in TKM buffer.

Details of the centrifugation conditions are described in the explanation of the figures. Gradients were eluted through a Gilford continuously recording spectrophotometer (Gilford Instrument Labs., Inc., Oberlin, Ohio). Radioactive fractions were precipitated with 10% trichloroacetic acid (TCA) and collected on Whatman (GF/A) glass filters, washed several times with 5% TCA followed by 70% ethanol. Dried filters were counted in Packard liquid scintillation spectrometer (Packard Instrument Co., Downers Grove, Ill.).

The RNA isolation procedure, which was based on Kirby's Method II (Kirby, 1965), has been described in detail in a separate communication (Kumar, 1969).

RESULTS

Pulse-Labeled RNA

Recovery of nuclei after three centrifugations of the cell homogenate was about 50% (see Methods). However, prolonged centrifugation frequently resulted in degradation of high molecular weight nuclear RNA. Consequently, only the nuclear pellet from first centrifugation of the cell homogenate was used for analyzing pulse-labeled nuclear RNA.

Sedimentation properties of nuclear RNA from cells pulsed labeled with uridine-³H for 1 min are shown in Fig. 1 A. The two main classes of nuclear

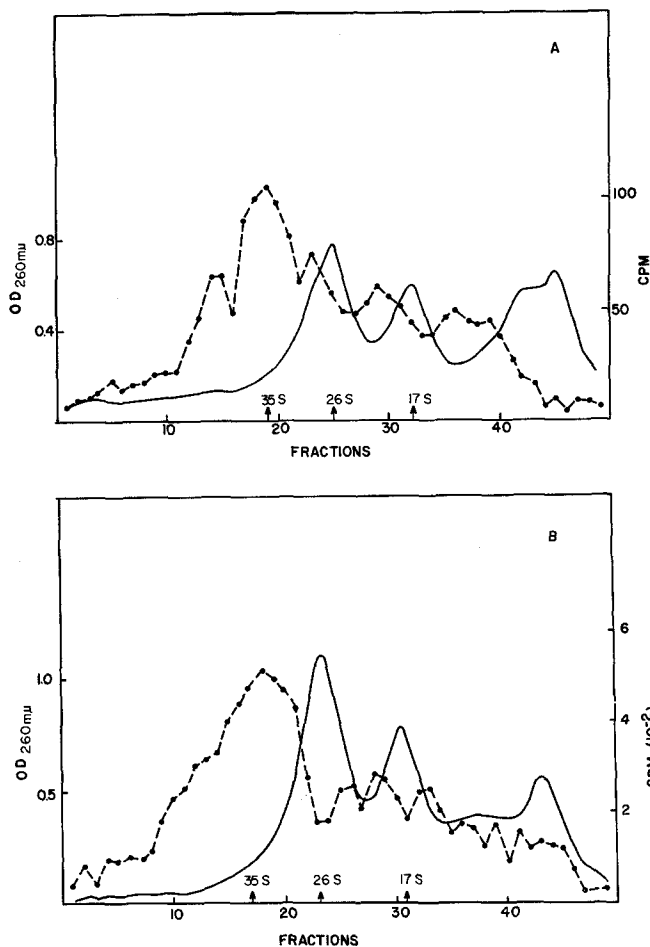


FIGURE 1 Short pulse-labeled nuclear and total cell RNA. A culture of cells labeled with uridine-³H for 1 min (1.5 μ Ci/ml; 8 Ci/mmol) was divided into two. From one fraction, nuclei were isolated, and the other half was used for total cell RNA extraction. RNA from pulse-labeled nuclei was extracted along with added unlabeled whole cell RNA and analyzed in a 15–30% (w/v) sucrose gradient dissolved in TKM buffer (A). There was no carrier RNA added to the total cell fraction (B). The gradients were centrifuged at 25,000 rpm for 12 hr at 6°C in an SW 25.1 rotor on a Spinco Model L ultracentrifuge.

RNA synthesized during this period sediment at 35S and 30S. From another fraction of the 1-min uridine-³H-labeled culture, the total cell RNA was prepared and similarly analyzed on sucrose gradients (Fig. 1 B). The majority of the RNA in whole cell fraction represents an essentially similar pattern of 35S and 30S as in the nuclear fraction. The relative excess of radioactivity in the whole cell fraction (Fig. 1 B, compare with Fig. 1 A) is apparently due to a low recovery of the nuclei. However, it is clear that there is no qualitative loss of a major class of RNA from the nuclear fraction.

Further stages in the transformation of the newly synthesized nuclear RNA was studied by analyzing nuclear RNA preparations from cells incubated with uridine-³H for 2.5–4.5 min (Figs. 2 A–2 C). The results in Fig. 2 do not represent fractions from the same experiment, and the total

acid-insoluble radioactivity in the nuclear RNA fraction varied according to the amount and specific activity of the radioactive precursor used. As such, the results are analyzed on the basis of the relative heights of the peaks of various classes of nuclear RNA. The main species of nuclear RNA synthesized within 2.5-min sediments at 35S (Fig. 2 A). Between 3 and 4 min of incorporation a new peak of nuclear RNA sedimenting at about 30S appeared (Fig. 2 B). The newly formed 30S material (Fig. 2 B) usually sedimented as a broad peak. If portions of the material had a sedimentation rate as low as 26S, it could not be resolved with these techniques. However, as is discussed later, there is no evidence for the appearance of 26S rRNA in the cytoplasm at this short period of incorporation. The radioactivity profile of 4.5 min labeled nuclear RNA showed a new distinct peak sedimenting at 26S and perhaps another one at 19S

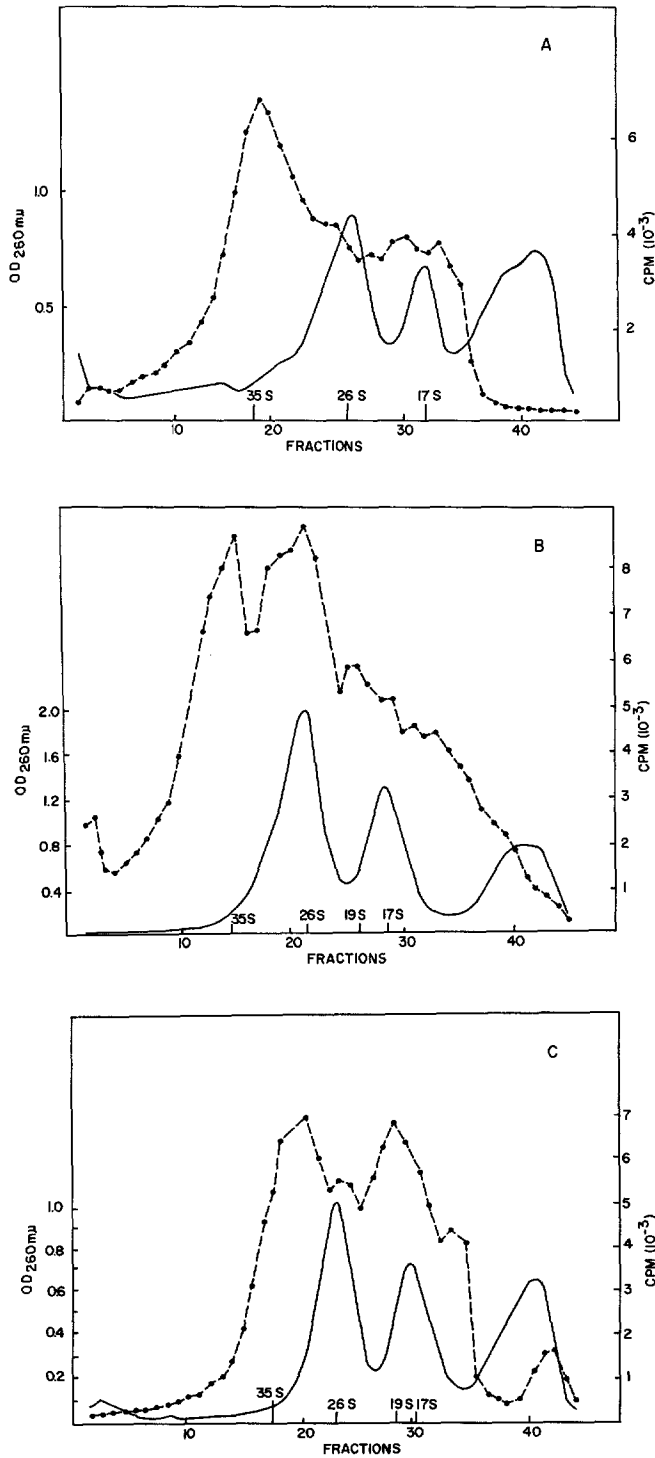


FIGURE 2 Transitions in the short pulse-labeled nuclear RNA. (A) Nuclear RNA from cells incubated with uridine-³H (0.25 μ Ci/ml) for 2.5 min was extracted and analyzed along with unlabeled whole cell RNA. Gradient conditions were the same as in Fig. 1, and centrifugation was done at 25,000 rpm for 18 hr at 6°C. (B) Nuclear RNA from cells labeled for 3.5–4 min with uridine-³H (0.25 μ Ci/ml) was purified along with added unlabeled whole cell RNA. The sample was analyzed in sucrose gradients as in the figure. Centrifugation was done for 18.25 hr at 25,000 rpm and 6°C. (C) Nuclear RNA from cells labeled for 4.5 min (uridine-³H, 0.25 μ Ci/ml) was extracted along with unlabeled whole cell carrier RNA. Gradient conditions were the same as in Fig. 1. The sample was centrifuged at 25,000 rpm for 12 hr at 6°C.

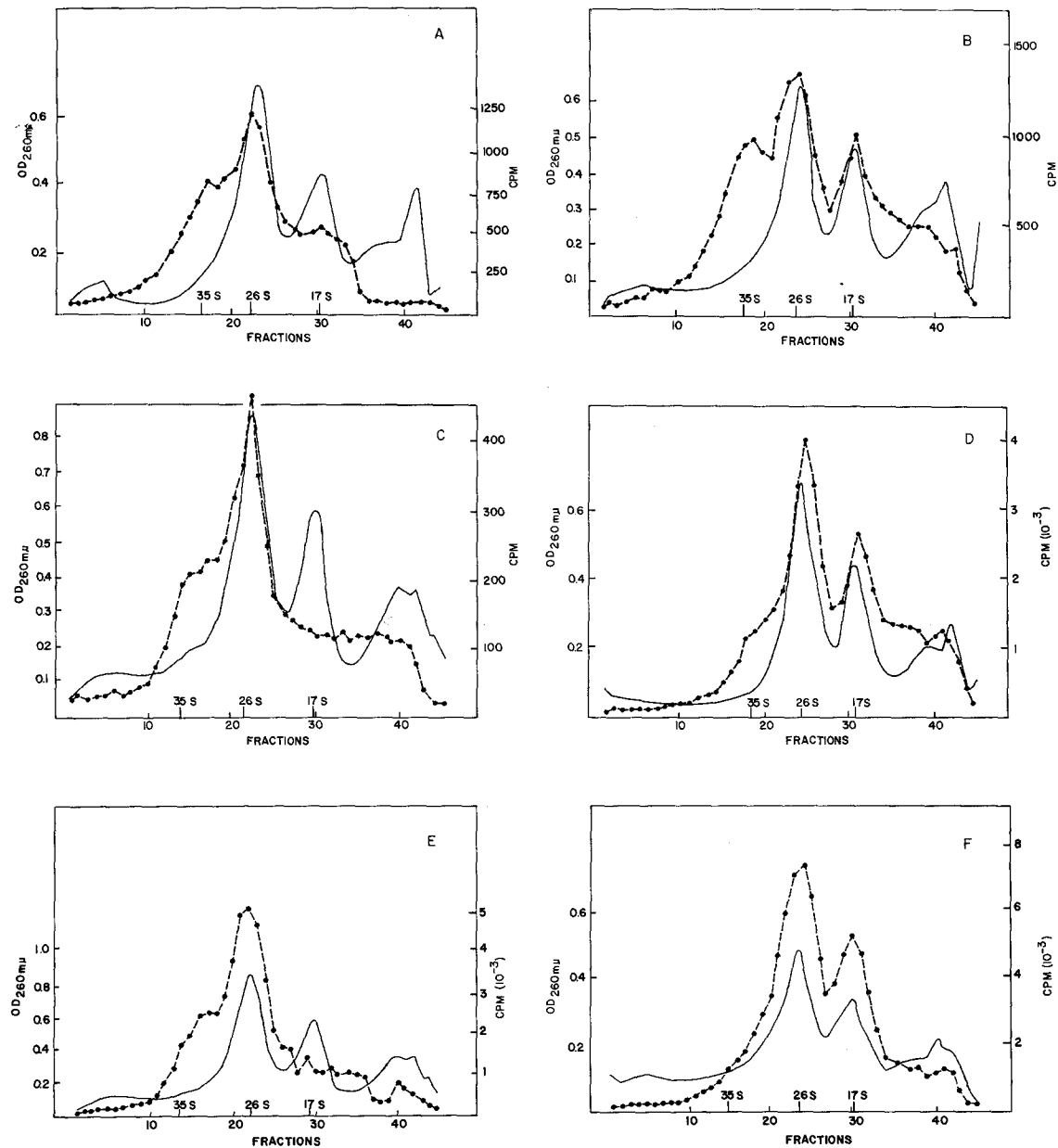


FIGURE 3 Nuclear and ribosomal RNA synthesized between 15 min and 1 hr. A culture at 5.5×10^5 cells/ml was incubated with uridine- ^3H ($0.25 \mu\text{Ci/ml}$; 20 Ci/mmole), and three equal samples were taken after 15 min, 30 min, and 1 hr. One-half of each sample was taken for nuclear isolation, and the other half was used for whole cell RNA. Nuclear RNA was extracted along with added unlabeled whole cell RNA as carrier. 15–30% sucrose gradients were prepared as in Fig. 1 and centrifuged at 25,000 rpm. The duration of the centrifugation was as follows: (A) 15 min nuclear RNA centrifuged for 13.25 hr at 6°C ; (B) 15 min total cell RNA centrifuged for 12.5 hr at 6°C ; (C) 30 min nuclear RNA centrifuged for 13.25 hr at 6°C ; (D) 30 min whole cell RNA centrifuged at 12.5 hr at 6°C ; (E) 1 hr nuclear RNA centrifuged for 13.25 hr at 6°C ; and (F) 1 hr total cell RNA centrifuged for 12.5 hr at 6°C .

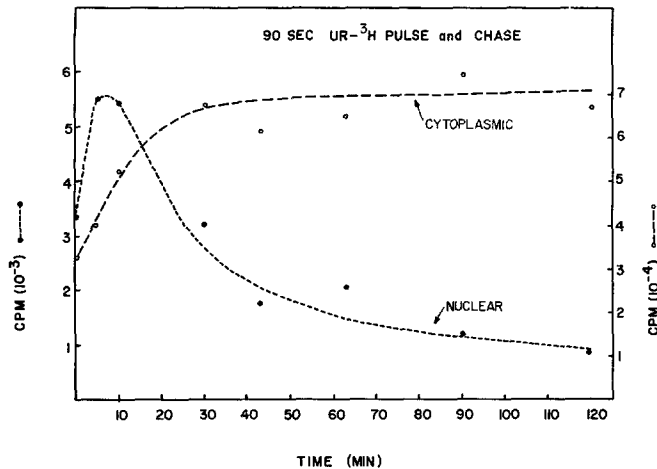


FIGURE 4 Chase kinetics. A 250 ml culture in chemically defined medium with cell density at 5×10^5 cells/ml was incubated with $0.5 \mu\text{Ci/ml}$ uridine- ^3H (specific activity 20 Ci/mmole) for 1.5 min. The cells were deposited by centrifugation and immediately suspended in fresh medium containing 50-fold excess uridine, plus 0.01% cytidine and thymidine. Samples of 12.5×10^6 cells were taken at indicated times after suspension in the chase medium. Nuclei were purified from each sample, and the total acid-precipitable radioactivity of each nuclear and postnuclear supernatant (cytoplasmic) fraction was determined.

(Fig. 2 C). The pattern of radioactive RNA's present in the nucleus after 2.5–4.5 min of incorporation suggests the following: (a) an increase in the relative amount of radioactivity in the 30S region compared to the first formed 35S material (Figs. 2 B and 2 C); and (b) by 4.5 min of incorporation (Fig. 2 C), an increase in the radioactivity in 26S and 19S regions compared to the 30S region. It is also important to note that, during these transition stages of the rapidly synthesized nuclear RNA, there is no major radioactivity peak cosedimenting with the rRNA optical density peaks. On correcting for the recovery of nuclei from cells labeled for 4.5 min with uridine- ^3H , it appears that about 85% of the total cell acid-insoluble radioactivity is retained in the nucleus.

Longer Labeling Time

In order to examine further the pattern of transformation in the newly synthesized ribosomal RNA a culture of cells was incubated with uridine- ^3H , and three equal aliquots were taken at 15-min, 30-min, and 1-hr intervals. Nuclear and whole cell RNA's from half of each fraction were purified and analyzed in sucrose gradients (Fig. 3). Since the sedimentation profiles of the radioactivity in nuclear and total cell RNA's shown in Figs. 3 A–3 F represent different periods of incorporation from the same culture of cells, analysis of the relative amounts of radioactivity in each peak would suggest their possible precursor product relationship. Between 15 and 60 min of incorporation, the nuclear RNA gradually shows a relative decrease in the radioactivity of the 35S region with the concurrent increase in the 26S region (Figs. 3 A,

3 C, and 3 E). It is also evident that the 17S rRNA must leave the nucleus soon after its formation since it is virtually absent in the 15 min nuclear RNA profile (Fig. 3 A), although it is clearly present in the total cell RNA at that time (Fig. 3 B). The specific activity in the 26S rRNA region equals that of 17S rRNA only after 30 min of incorporation. The delayed appearance of 26S rRNA in the cytoplasm is analyzed in detail later. The lines for the rate of formation of 26S and 17S rRNA, taken from the whole cell RNA data (shown in Figs. 3 B, 3 D, and 3 F), extrapolate to a common point, suggesting a common origin of the two mature ribosomal RNA's (illustration not included).

Pulse-Chase

A culture of cells was labeled with uridine- ^3H for 90 sec and then transferred to the chemically defined medium (Holz et al., 1959) containing excess of unlabeled uridine, cytidine, and thymidine. The rate of decay in total acid-insoluble radioactivity in the cytoplasmic and nuclear fractions under these chase conditions is represented in Fig. 4. After 2 hr of chase, the nuclei retained only about 15% of the acid-precipitable, pulse-labeled RNA present at the beginning of the chase period.

The following experiments were designed to determine the relative instability of various species of pulse-labeled nuclear RNA under a similar chase condition. Nuclear and ribosomal RNA's were prepared from cells labeled for 1.5 min with uridine- ^3H , then transferred to a similar chase medium. Aliquots were taken at 15 and 30 min

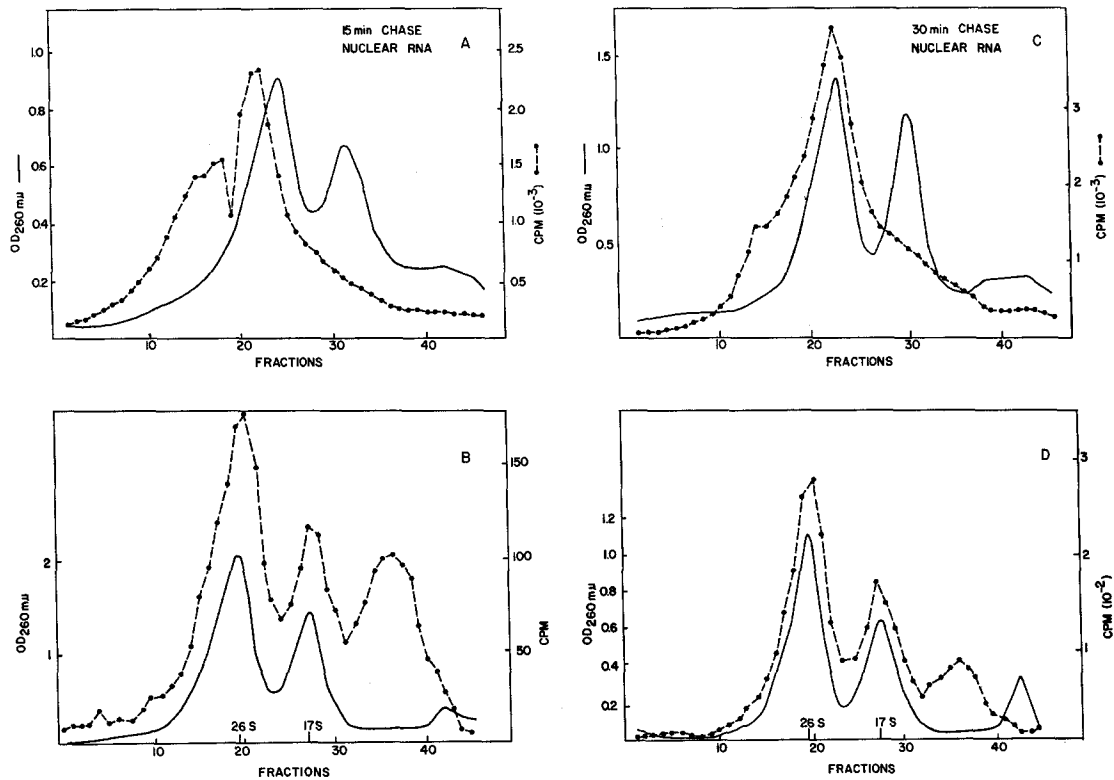


FIGURE 5 Pulse-chase nuclear and ribosomal RNA. Cells were labeled for 1.5 min with uridine- ^3H and chased as in Fig. 4. Samples were taken out after 15-min (A) and 30-min (C) chases. The nuclear RNA fractions were layered on a 4.5 ml 15–30% (w/v) sucrose gradient made in TKM and centrifuged in an SW39 rotor on a Spinco Model L ultracentrifuge at 39,000 rpm for 4.5 hr at 6°C. From the rest of the samples the corresponding ribosomal RNA (B and D) was prepared and centrifuged in a 30 ml gradient as in Fig. 1. The centrifugation time was 14 hr at 25,000 rpm.

after chase, from which nuclear and ribosomal RNA's were purified and analyzed in sucrose density gradients. After a 15 min chase, the main nuclear radioactivity was in the 35S and 30S regions (Fig. 5 A), whereas after 30 min the dominant peak of nuclear radioactivity was in the 26S RNA (Fig. 5 C). The appearance of radioactivity in the cytoplasmic ribosomal RNA in the corresponding 15- and 30-min chase samples is shown in Figs. 5 B and 5 D, respectively. The absence of radioactivity in the 17S region of the nuclear fraction even after a 15 min chase demonstrates that the 17S rRNA has a relatively short life-span in the nucleus. After a 1 hr chase (results not illustrated), the small amount of remaining radioactivity in nuclear RNA was heterogeneously sedimenting material with no recognizable ribosomal RNA species.

Appearance of Newly Formed Ribosomes in the Cytoplasm

The next experiments were designed to determine the forms and the steps through which the newly synthesized rRNA's appear in the cytoplasm. A cell culture was labeled with uridine- ^3H for 4.5 min and the cytoplasmic ribonucleoprotein particles were analyzed in sucrose gradients (Fig. 6 A). It is likely that nuclease has caused the degradation of many of the polysomes and thus enriched the single ribosome region. In addition, there are two main radioactive peaks of RNP particles sedimenting at 60S and 45S. The specific activity in 60S and 45S peaks is higher than that of either polysomes or single ribosomes after 4.5 min labeling with uridine- ^3H .

In order to characterize the newly formed 60S

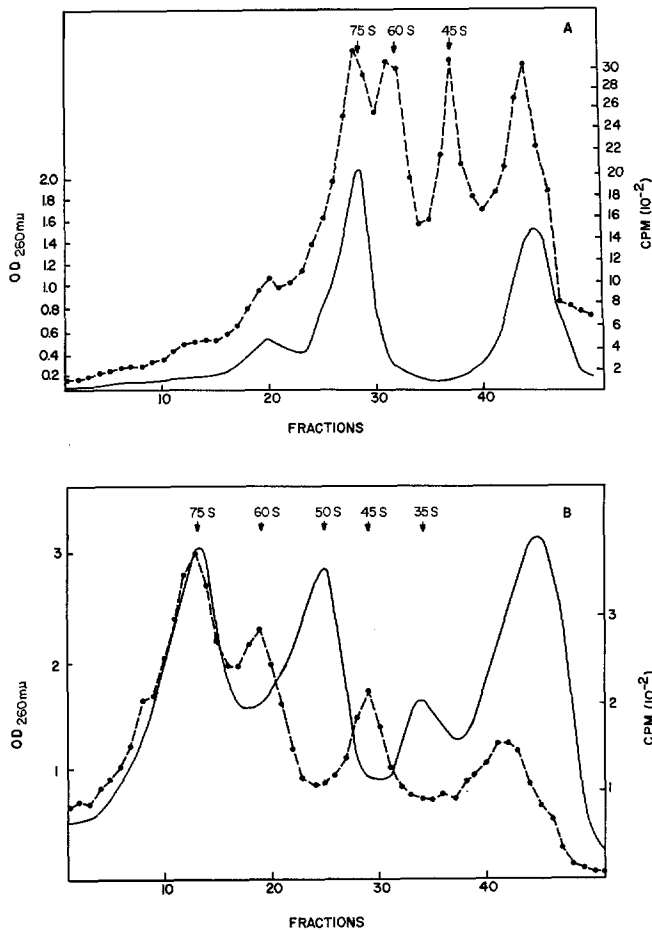


FIGURE 6 Pulse-labeled cytoplasmic ribonucleoprotein particles. (A) Cells were labeled with 2.5 $\mu\text{Ci/ml}$ uridine- ^3H (8 Ci/mmol) for 4.5 min. Cleared cell lysate was layered on a 30 ml 15–30% sucrose gradient made in TKM buffer and centrifuged in the SW 25.1 rotor at 25,000 rpm for 4 hr at 6°C. (B) In order to further resolve the radioactive 45S and 60S RNP regions, another sample of the 4.5 min uridine- ^3H pulse-labeled cytoplasm was centrifuged in a similar sucrose gradient as in Fig. 6 A, along with unlabeled EDTA-dissociated 50S and 30S ribosomal subunits as optical density markers. Centrifugation was done in an SW 25.1 rotor at 25,000 rpm for 10 hr at 6°C.

and 45S RNP particles on the basis of their RNA content, the cytoplasm from 4.5 min uridine- ^3H -labeled cells was centrifuged longer through sucrose gradients along with unlabeled EDTA dissociated 50S and 30S ribosomal subunits as optical density markers (Fig. 6 B). Radioactive RNA's from polysome (pellet), monosome, 60S and 45S RNP regions from the gradient (Fig. 6 B) were released by treatment with 0.2% dodecyl sodium sulfate (SDS), and their sedimentation properties were analyzed in sucrose density gradients. As is shown in Fig. 7 A, the newly formed 60S RNP particles contain largely 26S rRNA, and the 45S RNP contains 17S rRNA. The pulse-labeled RNA from the monosomes and polysomal region contains little or no newly synthesized ribosomal RNA's. The main class of pulse-labeled RNA associated with the monosomes and polysomal fraction was the heterogeneously sedimenting 9–12S material (Figs. 7 A and 7 B).

In order to determine the relative turnover rate of the newly synthesized ribosomal 60S and 45S RNP particles, a culture of cells was labeled with uridine- ^3H for 4.5 min and then subjected to a chase condition similar to the one discussed earlier. Aliquots were taken at various times after chase, and cytoplasmic RNP particles were prepared and resolved by sedimentation through sucrose density gradients. The sedimentation profile of the newly synthesized cytoplasmic RNP particles at various times after being in chase medium is shown in Figs. 8 A and 8 B. The entire chase data are summarized in Fig. 9 as percentage of the total radioactivity in each class of RNP particles with respect to the duration of the chase. It is clear that the newly synthesized 60S RNP particles have a longer existence in the cytoplasm as free RNP particles than the 45S RNP particles. After a 20 min chase, both newly synthesized 26S and 17S rRNA's appeared in the polysomal fraction,

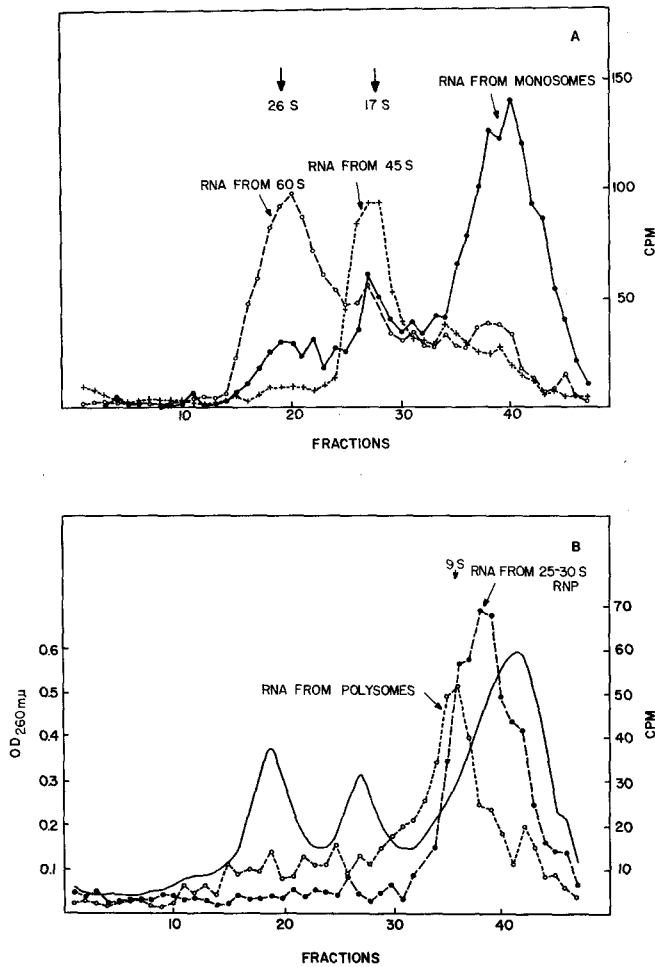


FIGURE 7 RNA from pulse-labeled cytoplasmic RNP particles. (A) RNA from the monosome as well as the 60S and 45S RNP regions from the gradient shown in Fig. 6 was extracted with 1% SDS and analyzed in a 15–30% sucrose gradient as described for Fig. 1. (B) RNA from the polysomal region as well as the heterogeneously sedimenting 25–30S region of the gradient shown in Fig. 6 was similarly extracted with SDS and analyzed in sucrose gradients. All radioactive RNA samples were centrifuged along with added unlabeled whole cell RNA as optical density marker. Centrifugation was done in SW 25.1 rotor at 25,000 rpm for 16 hr at 10°C. The continuous line in Fig. 7 B represents absorbance at 260 m μ , and the broken line represents the radioactivity in various samples as indicated.

although the specific activity in the 26S rRNA region at this time was less than that of 17S rRNA (results not illustrated). The delayed appearance of the newly formed 60S ribosomal RNP particles in the cytoplasm, as compared to that of the 45S RNP particles, may be attributed to a larger pool of the nuclear precursors to 26S rRNA and/or a longer time of processing.

DISCUSSION

After pulse labeling *Tetrahymena* with uridine-³H, the main class of RNA from purified nuclei sediments at 35S. The 35S RNA attained a high specific activity in a relatively short period of incubation (0.5% of the generation time) with uridine-³H, indicating its high rate of turnover. Its synthesis appears to be localized in the nucleus. Soon after its synthesis, there is a relative decrease

in the radioactivity of the 35S region accompanied by the simultaneous appearance of the two main classes of more slowly sedimenting nuclear RNA's (Fig. 2). The acid-insoluble radioactivity in the nuclei from uridine-³H pulse-labeled cells is largely conserved and incorporated into the finished cytoplasmic rRNA's following a chase with excess unlabeled pyrimidine nucleosides. The curves of the rates of appearance of the newly synthesized rRNA's extrapolate to a common origin, suggesting a common precursor. These observations suggest that the rapidly synthesized 35S RNA serves as the nuclear precursor to the cytoplasmic rRNA's.

The details of transformation of the 35S nuclear precursor molecule and the interrelationship of nuclear intermediates are not clear at present. Cleavage of a single 35S precursor RNA into two

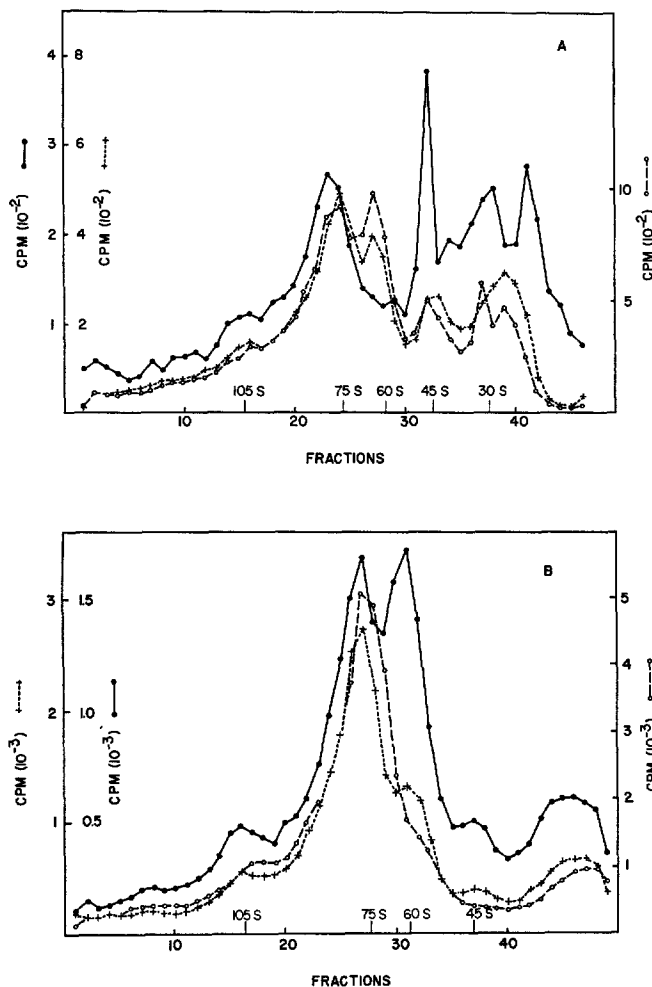


FIGURE 8 Chase kinetics of pulse-labeled RNA in cytoplasmic RNP particles. (A) Cells were labeled with $2.5 \mu\text{Ci/ml}$ uridine- ^3H (specific activity 8 Ci/mmol) for 4.5 min. Labeled cells were collected and resuspended in chase medium containing excess unlabeled pyrimidine nucleosides. Equal volume samples were taken out at 0.5, 2.5, and 5 min after the beginning of the chase, and the cell lysate was analyzed in 15–30% sucrose (in TKM buffer) gradients. Centrifugation was done for 4.5 hr at 25,000 rpm at 4°C . The counts represent only one-eighth of each fraction. The rest of the fraction was used for RNA extraction. ●—● cpm in 0.5 min chase +—+—+ cpm in 2.5 min chase ○—○ cpm in 5 min chase. (B) Continuation of the experiment in Fig. 8 A. Cytoplasmic particles were prepared from cells taken out at 10, 20, and 40 min after start of the chase. Centrifugation conditions were similar to those of Fig. 8 A. Centrifugation times: 4 hr. ●—● cpm in 10-min chased samples +—+—+ cpm in 20-min chased samples ○—○ cpm in 40-min chased samples. The counts represent only a portion of the fractions as in fractions as in Fig. 8 A.

products sedimenting at about 30S and 26S would probably involve major conformational changes. However, the possibility that two 35S molecules independently give rise to a 30S and a 26S RNA cannot be ruled out on the basis of the present results. A culture of cells was labeled for 2.5 min with uridine- ^3H , and the total cell RNA was prepared from one-half of the culture and the other half was subjected to a 20 hr chase with excess unlabeled uridine, cytidine, and thymidine. Ribosomal RNA was prepared from these cells at the end of the chase period. Estimates of the radioactivity under the 35S peak after a 2.5 min pulse and that of rRNA at the end of chase period indicated that the radioactivity under the 35S peak was largely conserved. This suggests that a major size change during interconversion of the ribosomal precursor molecule would be less likely. However,

conservation of the nuclear label could also be due to the possible degradation and reentry of the nucleotides into the pool, with subsequent re-incorporation. No data on the rates of turnover of the endogenous pool are available at present to support this argument.

The nuclear 30S RNA possibly goes through the nuclear intermediaries 26S and 19S before its appearance as the cytoplasmic 26S and 17S rRNA, respectively.

Pulse-labeled nuclear RNA after a brief chase shows that the newly synthesized 17S rRNA leaves the nucleus soon after its synthesis. On the other hand, the predominant peak of radioactivity in pulse-labeled nuclear RNA after a 30 min chase is in the 26S region, indicating a larger pool of nuclear 26S RNA (Fig. 5). The interpretation is further substantiated by the delay in equilibration

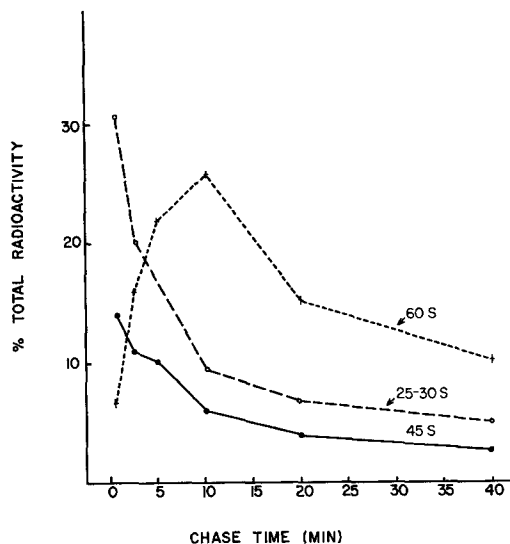


FIGURE 9 Kinetics of chase of pulse-labeled cytoplasmic RNP particles. The radioactivity in 60S, 45S, and the heterogeneously sedimenting 25-30S sub-ribosomal RNP particle regions of the sucrose gradients shown in Figs. 8 A and 8 B was estimated and plotted as per cent of the total counts (in respective gradient) as a function of chase time.

of a specific activity in 26S rRNA as compared to that of the 17S rRNA (Fig. 3). In other words, the smaller of the two ribosomal RNA's (i.e. 17S) appears in the cytoplasm soon after its synthesis in the nucleus. The 26S rRNA, on the other hand, shows a comparatively delayed appearance in the cytoplasm probably due to its larger precursor pool in the nucleus.

The newly synthesized rRNA's appear in the cytoplasm as 60S and 45S RNP particles which seem to have a finite existence as free particles before their incorporation into the functional ribosomal population of the cells. The evidence from chase kinetics of the pulse-labeled cytoplasmic RNP particles (Fig. 9) shows that the newly formed 45S RNP particles appear first in the cytoplasm followed by the 60S RNP. Moreover, the radioactivity in the 45S RNP region under these pulse-chase conditions declines at a faster rate than that of the 60S RNP particles. The evidence from the

experiments on the appearance of newly formed ribosomal precursor particles in the cytoplasm substantiates the interpretation of the kinetics of ribosomal RNA synthesis, suggesting a larger pool of nuclear precursor to the cytoplasmic 26S rRNA. The other possibility, that a longer time is taken for processing the nuclear precursors of 26S rRNA, cannot be ruled out.

It appears, therefore, that the first step in formation of ribosomes in *Tetrahymena* is the synthesis of a 35S nuclear precursor of both 26S and 17S cytoplasmic ribosomal RNA's. The apparently smaller size of the 35S precursor molecule could be related to the relatively small size of the finished cytoplasmic rRNA's (26S and 17S as compared to 28S and 18S in most higher organisms). It also appears from the pulse-chase data that, during the course of synthesis of the finished cytoplasmic rRNA, the 35S precursor molecule is largely conserved. In mammalian cells, on the other hand, only about half of the 45S precursor molecule is conserved (Weinberg, Loening, Willems, and Penman, 1967). The rapidity of synthesis of ribosomal RNA in *Tetrahymena*, along with the small difference in size of the nuclear intermediaries of the precursor molecule, interferes with the detailed analysis of the maturation of 35S molecule. However, it appears likely that the basic steps in ribosome formation in *Tetrahymena* are not unlike those reported for mammalian cells (Penman, 1966). The relatively rapid rate of ribosomal RNA synthesis in *Tetrahymena* could be partly related to the faster rate of growth in these unicellular organisms.

I am grateful to Dr. Hewson Swift for his valuable suggestions and encouragement throughout the course of this work. I am thankful to Dr. Jonathan R. Warner for critical reading of the manuscript and to Dr. Martin Gorovsky for the use of his unpublished nuclear isolation procedure and many valuable suggestions.

This work was supported by United States Public Health Service Grants HD-1612 and Training Grant HD-174.

Received for publication 11 March, 1969 and in revised form 24 December 1969.

REFERENCES

- ATTARDI, G., H. PARNAS, M. HWANG, and B. ATTARDI. 1966. *J. Mol. Biol.* 20:145.
- GIRARD, M., H. LATHAM, S. PENMAN, and J. DARNELL. 1965. *J. Mol. Biol.* 11:187.

3. GOLDSTEIN, L., and W. PLAUT. 1955. *Proc. Nat. Acad. Sci. U.S.A.* **40**:874.
4. GOROVSKY, M. 1965. *J. Cell Biol.* **27**:37A.
5. HOLZ, G. G., JR., J. ERWIN, and R. DAVIS. 1959. *J. Protozool.* **6**:149.
6. HOUSSAIS, J., and G. ATTARDI. 1966. *Proc. Nat. Acad. Sci. U.S.A.* **56**:616.
7. JOKLIK, W. K. and Y. BECKER. 1965. *J. Mol. Biol.* **13**:496.
8. KIRBY, K. S. 1965. *Biochem. J.* **96**:266.
9. KUMAR, A. 1969. *Biochim. Biophys. Acta.* **186**:326.
10. PENMAN, S. 1966. *J. Mol. Biol.* **17**:117.
11. PENMAN, S., C. VESCO, and M. PENMAN. 1968. *J. Mol. Biol.* **39**:49.
12. PERRY, R. P. 1962. *Proc. Nat. Acad. Sci. U.S.A.* **48**:2179.
13. PERRY, R. P., and D. E. KELLY. 1968. *J. Mol. Biol.* **35**:37.
14. PRESCOTT, D. M. 1960. *Exp. Cell Res.* **19**:29.
15. SCHERRER, K., H. LATHAM, and J. DARNELL. 1963. *Proc. Nat. Acad. Sci. U.S.A.* **49**:420.
16. WARNER, J. R. 1966. *J. Mol. Biol.* **19**:383.
17. WARNER, J. R., R. SOEIRO, C. BIRNBOIM, M. GIRARD, and J. E. DARNELL. 1966. *J. Mol. Biol.* **19**:349.
18. WARNER, J. R., and R. SOEIRO. 1967. *Proc. Nat. Acad. Sci. U.S.A.* **58**:1984.
19. WEINBERG, R. A., U. LOENING, M. WILLEMS, and S. PENMAN. 1967. *Proc. Nat. Acad. Sci. U.S.A.* **58**:1088.
20. ZALOKAR, M. 1959. *Nature (London)*, **183**:1330.