# Transcription of Ribonucleic Acid in Isolated Mouse Myeloma Nuclei†

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ABSTRACT: Nuclei have been isolated from mouse myeloma cells which are capable of synthesizing RNA at a high rate. At 37° synthesis ceases after 10–15 min but at 25° synthesis continues at the same high rate for up to 1 hr. The RNA synthesized has been characterized by sucrose gradient centrifugation and polyacrylamide gel electrophoresis. Both high molecular weight RNA (>30 S) and low molecular weight RNA species are synthesized. A significant percentage of the *in vitro* synthesized RNA contains poly(A) sequences, indicating that the nuclei may be capable of carrying out at least one specific step in RNA maturation.

The study of transcription and maturation of specific RNA sequences *in vitro* requires a system which faithfully synthesizes RNA for an extended period of time. Functionally active isolated cell nuclei provide a system intermediate between intact cells and *in vitro* RNA synthesis from a chromatin template with isolated RNA polymerase. Isolated nuclei provide an opportunity not only to study transcription of defined RNA species but also the intranuclear metabolism and eventually the release of the RNA.

Zylber and Penamn (1971) have shown that HeLa cell nuclei complete the synthesis of 45S ribosomal precursor RNA *in vitro*. In addition Reeder and Roeder (1972) have shown that *Xenopus laevis* tissue culture cell nuclei transcribe the ribosomal genes faithfully, transcribing not only the correct strand of the DNA but also only the portion of that strand transcribed *in vivo*. Both these groups concluded that RNA polymerase I transcribed the ribosomal genes *in vitro*, in accordance with earlier indirect evidence (Roeder and Rutter, 1970).

Nuclei isolated from adenovirus-infected HeLa cells have been extensively studied. These nuclei synthesize adenovirus-specific RNA (Price and Penman, 1972a,b; Wallace and Kates, 1972) similar to those species synthesized *in vivo*. A proportion of these species are transcribed by RNA polymerase II (Price and Penman, 1972a). Much of HeLa cell heterogenous nuclear RNA is also transcribed by polymerase II (Zylber and Penman, 1971). At least one adenovirus-specific RNA and some low molecular weight cellular RNA species are transcribed by a polymerase activity apparently distinct from polymerase I and polymerase II, possibly RNA polymerase III (Price and Penman, 1972b).

Raskas (1971) has studied *in vitro* the intranuclear metabolism of adenovirus-specific RNA synthesized *in vivo*. He has shown that the large molecular weight adenovirus-specific RNA is broken down to lower molecular weight species *in vitro* and that much of this RNA is released from the nuclei in an ATP-dependent reaction. In addition, a limited amount of poly(adenylic acid) (poly(A)) is attached *in vitro* to the *in vivo* synthesized RNA (Raskas and Bhaduri, 1973).

We wish to report that nuclei isolated from cultured mouse myeloma cells actively synthesize RNA *in vitro* for an extended period of time. All molecular weight classes of RNA are made and poly(A) sequences are present in some of the *in vitro* synthesized RNA. Recently, we have found that a large percentage (20–25%) of the protein synthesized by these cells is a specific immunoglobulin $\kappa$ chain. Thus, much of the messenger RNA synthesized by these cells should code for this protein. These nuclei possess potential for studying the mechanisms and control of expression of specific genes in eucaryotes.

## Methods

*Maintenance of Tumors.* The cells used in these experiments were the myeloma line 66-2, a light chain producing clone derived from MPC-11 by Coffino and Scharff (1972). The cells were maintained in stationary suspension culture in Delbecco's modified Eagles medium (GIBCO) plus 10% horse serum. The tumors were also carried subcutaneously in BALB/C mice (Jackson Labs, Bar Harbor, Maine).

*Preparation of Nuclei.* The cells (5–10 $\times$ $10^5$/ml) were centrifuged and then broken by homogenization with 20 strokes of the tight pestle in a Dounce tissue homogenizer in 0.3 M sucrose containing 2 mM $Mg(Ac)_2$, 3 mM $CaCl_2$, 10 mM Tris (pH 8), 0.1% Triton X-100, and 0.5 mM dithiothreitol. One milliliter of solution was used for $10^7$ cells. The homogenate was mixed with 1 vol of 2 M sucrose containing 5 mM $Mg(Ac)_2$, 10 mM Tris (pH 8), and 0.5 mM dithiothreitol. The mixture was layered over a 2-ml pad of the 2 M sucrose buffer and centrifuged at 20,000 rpm in the SW-50.1 rotor for 45 min. The nuclear pellet was drained and gently resuspended (0.4–1.2 mg of DNA/ml) with a pasteur pipet in 25% glycerol containing 5 mM $Mg(Ac)_2$, 50 mM Tris (pH 8), 5 mM dithiothreitol, and 0.1 mM EDTA (Reeder and Roeder, 1972). The yield of nuclei was 50–90%. Nuclei prepared from the solid tumor by a similar technique gave similar results to nuclei prepared from the cultured cells.

*RNA Synthesis.* The conditions used to assay RNA synthesis were essentially those of Reeder and Roeder (1972). The reaction mixture contained 12.5% glycerol, 5 mM $Mg(Ac)_2$, 1 mM $MnCl_2$, 25 mM Tris (pH 8), 0.05 mM EDTA, 2.5 mM dithiothreitol, 0.4 mM each of three ribonucleoside triphosphates, and 0.05 mM of the fourth ribonucleoside triphosphate (labeled either with $^3H$ or $^{32}PO_4$ in the $\alpha$ position,

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2–10 Ci/mmol). Either KCl or $(NH_4)_2SO_4$ was also present, routinely 0.15 M KCl. Aliquots of 20 μl were applied to strips of Whatman No. 3 filter paper or dissolved in 0.1 ml of 1% sodium dodecyl sulfate and 10 mM EDTA and then applied to filter paper. The papers were plunged into cold 5% $Cl_3CCOOH$ containing 0.01 M pyrophosphate for 10 min and then washed three times (5 min each) with the same solution, washed in acetone, dried, and counted by liquid scintillation in a toluene fluor in a Packard liquid scintillation counter (Model 2425). Efficiency for $^3H$ was 22%.

*Isolation of in Vitro Synthesized RNA.* The hot phenol–sodium dodecyl sulfate method was used (Wagner *et al.*, 1967). The reaction (0.2–1.0 ml) was terminated with 10 vol of 1% sodium dodecyl sulfate and 10 mM EDTA and NaAc (pH 5.1) was added to a final concentration of 0.05 M. The solution was extracted at room temperature for 10 min with 1 vol of water-saturated phenol, the aqueous phase collected after centrifugation, and the phenol and interface reextracted with 0.5 vol of pH 5 buffer for 3 min at 55°. The two aqueous phases were combined and extracted again with phenol at room temperature. The RNA was precipitated with 2 vol of 95% ethanol after addition of NaCl (final concentration 0.3 M). 28S RNA was occasionally added to facilitate the precipitation of the RNA. The yield of RNA was 60–85% of the $Cl_3CCOOH$-precipitable counts. The precipitated RNA was collected by centrifugation, washed once with 80% ethanol and once with 95% ethanol, dried, dissolved in 0.5 ml of sterile water, and treated with DNase I (10 μg/ml) for 20 min at 25°. The solution was adjusted to 1% sodium dodecyl sulfate–10 mM EDTA and layered over a linear 10–70% sucrose gradient in 0.1 M NaCl, 0.01 M EDTA, 0.01 M Tris (pH 7.5), and 0.1% sodium dodecyl sulfate and centrifuged for 19 hr at 25,000 rpm in the SW-27 rotor at 21°. The gradients were fractionated by pumping through a flow cell and the absorbance at 260 mμ recorded with a Gilford spectrophotometer. Fractions (1.0 ml) were collected. Aliquots of the fractions were applied to filter paper and $Cl_3CCOOH$-precipitable counts determined as described above.

*Formamide–Sucrose Gradient Centrifugation.* A modification of the technique of Suzuki *et al.* (1972) was used. The RNA was dissolved in 0.2 ml of 70% formamide, 3 mM Tris (pH 7.5), and 3 mM EDTA, and incubated at 37° for 5 min. The sample was layered over 5-ml linear sucrose gradients (10–40% sucrose (w/w) in 70% formamide, 3 mM Tris, and 3 mM EDTA) and centrifuged at 45,000 rpm for 4.5 hr in the SW-50.1 rotor, at 25°. The gradients were fractionated as described above and 0.25-ml fractions collected. Aliquots were dissolved in Triton–toluene (Patterson and Greene, 1965) and counted directly. The optimal density peaks were located accurately by reading each fraction.

*Poly(dT)-Cellulose Chromatography.* Poly(dT)-cellulose was prepared as described by Gilham (1964). Chromatography was performed by the method of Aviv and Leder (1972). The RNA sample was applied to the column in 0.5 M KCl–0.01 M Tris (pH 7.5) at room temperature and the column washed with 4 vol of this buffer. The bound RNA was eluted with 0.01 M Tris (pH 7.5). Over 90% of the RNA was recovered from the column.

*Polyacrylamide Gel Electrophoresis.* Electrophoresis was performed as described by Loening (1968) except that ethylene diacrylate (Choules and Zimm, 1965) was used as a cross-linking reagent to allow the gels to be solubilized. The gels (0.7 × 10 cm containing 10% acrylamide, 0.3% ethylene diacrylate, and 0.1% sodium dodecyl sulfate) were preelectrophoresced for at least 1 hr at 5 mA/gel. The samples were applied with a small amount of marker dye (Bromophenol Blue) and run at 50 V until the sample entered the gel. The voltage was then increased to 100 V and electrophoresis continued until dye reached the bottom of the gel (about 4 hr). In these gels 5S ribosomal RNA had a mobility of 0.5 relative to the dye and 4S transfer RNA had a mobility of 0.75. The gels were scanned at 260 mμ with a Gilford linear transport device, frozen on Dry Ice, and sliced into 1.7-mm fractions with stacked razor blades. Each slice was dissolved in 1 ml of 0.2 N NaOH at 37° for 24 hr, neutralized with 0.3 ml of 1 N acetic acid, and counted in Triton–toluene.

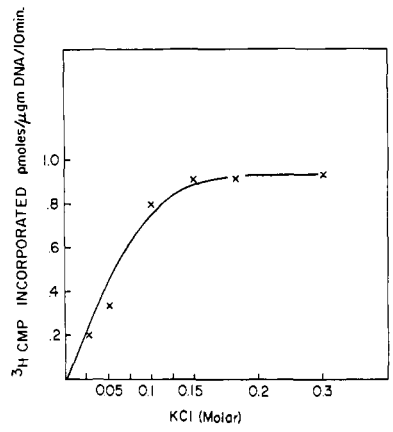


FIGURE 1: Effect of KCl concentration on RNA synthesis. Nuclei ($2.5 \times 10^6$) were incubated for 10 min at 37° as described under Methods. $Cl_3CCOOH$-precipitable counts were determined after spotting the nuclei on strips of Whatman No. 1 filter paper; 1 pmol = 5000 cpm.

*Preparation of Marker RNAs.* The cultures were labeled with $^{32}PO_4$ (100 μCi/ml) for at least two generations. The 5S and 4S ribosomal RNAs were prepared from purified ribosomes by extraction with phenol–sodium dodecyl sulfate at room temperature and sucrose gradient centrifugation as described above. The fractions containing 4S and 5S RNA were pooled, dialyzed against 0.1% sodium dodecyl sulfate, and lyophilized. The RNA was washed with 80% ethanol to remove the sodium dodecyl sulfate and dissolved in water.

*Chemicals.* Triton X-100 and DNase I (electrophorectically pure) were obtained from Sigma. α-Amanitin was purchased from the Henley Co. (New York, N. Y.). Formamide (catalog F-95) was obtained from Fisher. Ethylene diacrylate was purchased from Borden Monomer-Polymer Co. [$^3H$]CTP, [$^3H$]-UTP, and [α-$^{32}PO_4$]GTP were obtained from New England Nuclear Co.

## Results

*Isolation of Nuclei.* Different procedures for isolation of nuclei were tested and while many gave clean nuclei as assayed by microscopy, several procedures resulted in nuclei which were less active in RNA synthesis. The exposure of nuclei to concentrations of Triton greater than 0.5% during the preparation resulted in less active nuclei. Metal ion concentrations were also critical. Isolated nuclei prepared by centrifugation through 2.0 M sucrose containing calcium were less active than those prepared in the presence of magnesium. Nuclei breakage was observed in the presence of ≤2 mM magnesium in the 2.0 M sucrose.

Nuclei were stable on ice in incubation media for at least 2 hr. The nuclei could be frozen in incubation media and assayed immediately after thawing without significant loss of

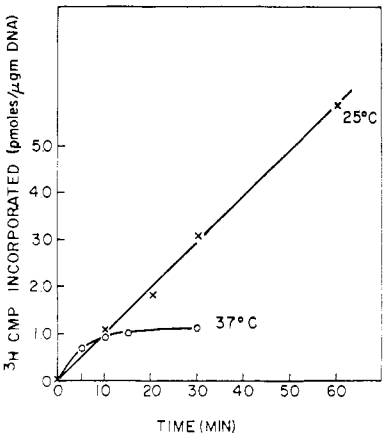


FIGURE 2: Effect of temperature on RNA synthesis. Nuclei were incubated at either 37 or 25°. At various times aliquots (2.5 × $10^5$ nuclei) were removed and $Cl_3CCOOH$-precipitable counts determined as described in Figure 1; 1 pmol = 5000 cpm: (○) 37°; (×) 25°.

activity, although they lost activity within 1 hr after thawing at 0°.

*Conditions of RNA Synthesis.* The conditions of RNA synthesis were chosen to assure that polymerase I and polymerase II would both be active (Reeder and Roeder, 1972). RNA synthesis was dependent on the monovalent cation concentration (either $K^+$ or $NH_4^+$) plateauing at 0.1 M and remaining at a constant level through 0.3 M (Figure 1). Raising the salt concentration to 0.4 M or higher resulted in spontaneous lysis of the nuclei. At the salt concentrations routinely used (0.15 M KCl) ammonium substituted equally well for potassium.

A most important variable was the temperature of the incubation. At 37° incorporation of labeled triphosphates into RNA was linear for only 10 min and essentially complete after 15 min. The rate of incorporation was 0.6–1.0 pmol/μg of DNA per 10 min (Figure 2). In contrast, at 25° synthesis continued at the initial rate for up to 60 min. Hence three to five times as much RNA was made under the same conditions at the lower temperature. Rates as high as 1.5 pmol/μg of DNA per 10 min were observed, the average being 1 pmol.

As shown in Figure 3 the rate of RNA synthesis was linearly dependent on the concentration of nuclei. This was true at either temperature. In addition, incorporation was dependent on the presence of all four ribonucleoside triphosphates.

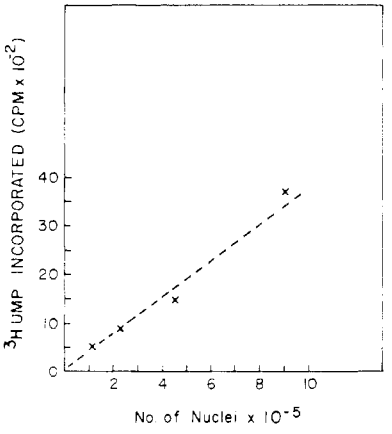


FIGURE 3: Effect of nuclei concentration on RNA synthesis. The indicated number of nuclei were incubated for 10 min at 25° and $Cl_3CCOOH$-precipitable counts determined as in Figure 1; 1 pmol = 500 cpm.

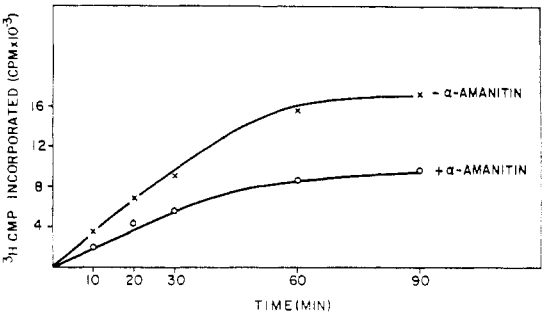


FIGURE 4: Effect of α amanitin on RNA synthesis. Nuclei were incubated at 25° in either the presence or absence of α-amanitin (5 μg/ml). Aliquots were withdrawn (2 × $10^5$ nuclei) at various times and $Cl_3COOH$-precipitable counts determined as in Figure 1; 1 pmol = 1000 cpm: (×) no α-amanitin; (○) plus α-amanitin.

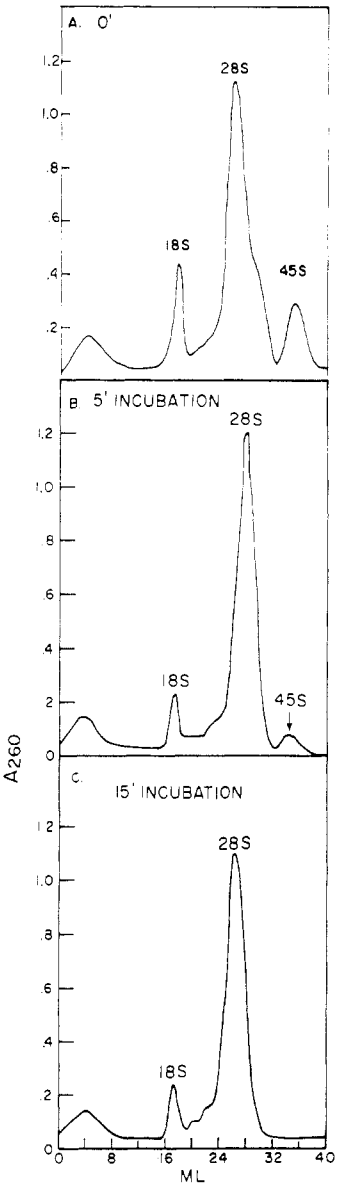


FIGURE 5: Stability of RNA in isolated nuclei. Nuclear RNA was prepared as described under Methods, either immediately after preparation (A) or after incubation at 37° for either 5 (B) or 15 min (C). Incubation was performed under the exact conditions of RNA synthesis. The RNA was analyzed on 10–70% sucrose gradients for 19 hr at 21° in the SW-27 rotor as described under Methods

*Effect of α-Amanitin on RNA Synthesis.* α-Amanitin specifically inhibits RNA polymerase II (Roeder *et al.*, 1970). Figure 4 shows that RNA synthesis was inhibited 40% by α-amanitin at 0.15 KCl. Synthesis was still linear in the presence of the drug for as long as it was in the absence of the drug. Thus, under the routine assay conditions 40% of the activity was due to polymerase II. The effect of α-amanitin was not seen at low salt concentrations (<0.05 M KCl), indicating that the increase in RNA synthesis with increasing salt concentration was due primarily to the activation of the polymerase II activity, in agreement with several other studies (Zylber and Penman, 1971; Reeder and Roeder, 1972).

*Analysis of RNA Synthesized in Vitro.* Mouse myeloma nuclei possessed negligible ribonuclease or deoxyribonuclease activity. Figure 5 shows the sucrose gradient analysis of nuclear RNA prepared from nuclei which had been incubated under conditions of RNA synthesis for either 5 (B) or 15 (C) min. The major components found were identical with those found in nuclei which had not been incubated (A). The small amount of 18S RNA relative to 28S RNA indicates that there was little cytoplasmic contamination of the nuclei (Penman, 1966). The high molecular weight RNA (45S) disappeared during the incubation, consistent with the possibility that specific maturation of RNA species could be occurring *in vitro*. Similar results were found for nuclei incubated at 25°. The stability of the major RNA species during the incubation does not rule out small amounts of nuclease activity in the nucleoplasm. Hence it might be expected that these nuclei could synthesize discrete RNA species without extensive random degradation of the RNA by contaminating nucleases. In addition, the DNA template is not degraded during incubation and there is no detectable DNase activity present in the nuclei (Dr. Henry Berger, personal communication).

The RNA product synthesized at 37° was analyzed on 10–70% sucrose gradients. The RNA was extremely heterogenous ranging from 4 to greater than 45 S (Figure 6). After synthesis for 15 min at 37°, at which time RNA synthesis had ceased, the high molecular weight RNA was no longer present (Figure 6B). The high molecular weight RNA was converted to lower molecular weight RNA as there was no loss in total counts incorporated. The same result was obtained after a chase period started after 5 min by adding an excess of unlabeled triphosphate and continuing to 15 min (not shown). Whether the formation of lower molecular weight RNA species with time was due to normal maturation of the RNA or to slight nucleolytic activity is not clear.

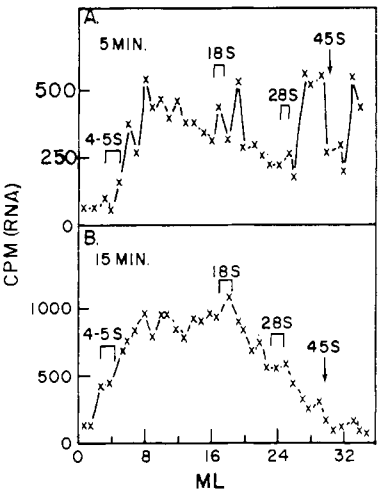


FIGURE 6: Sucrose gradient analysis of RNA synthesized at 37°. RNA was prepared from nuclei after incubation for either 5 or 15 min at 37° in the presence of [$^3$H]CTP. The RNA was analyzed as described in Figure 5 and $Cl_3CCOOH$-precipitable counts determined on 0.05-ml aliquots of each 1-ml fraction. The position of various marker ribosomal RNAs is indicated: (A) 5-min incubation; (B) 15-min incubation.

In contrast, when nuclei were incubated at 25° there was a continuous accumulation of both high and low molecular weight RNA species (Figure 7). Even after synthesis *in vitro* for 30 min a large portion of the RNA was of high molecular weight. Apparently, in addition to continued synthesis of RNA there is also a slower conversion of the high molecular weight species to lower molecular weight RNA (unpublished observations). Significantly there was continued synthesis of all molecular weight classes of RNA during the entire incubation.

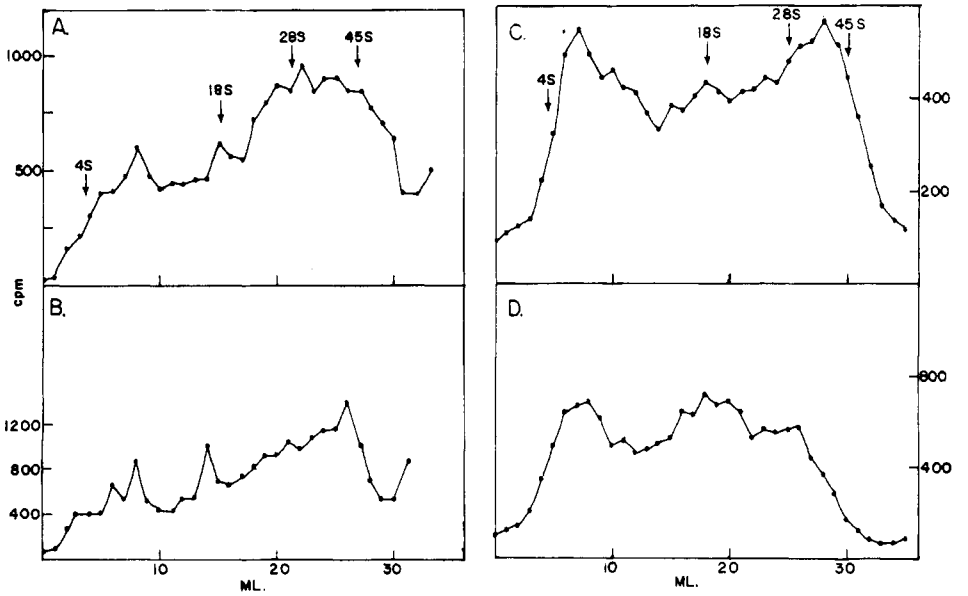


FIGURE 7: Sucrose gradient analysis of RNA synthesized at 25°. RNA was prepared from nuclei incubated for either 15 or 30 min in the presence or absence of α-amanitin. The RNA was analyzed as described in Figure 6: (A) 15-min incubation; (B) 30-min incubation; (C) 15-min incubation + α-amanitin; (D) 30-min incubation + α-amanitin.

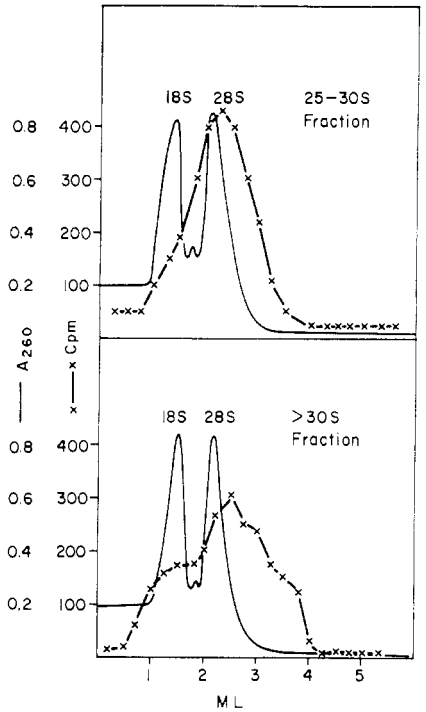


FIGURE 8: Analysis of RNA on formamide–sucrose gradients. RNA was isolated from nuclei incubated in the presence of [$\alpha$-$^{32}PO_4$]GTP for 30 min at 25°. The RNA was fractionated as shown in Figure 7B and the 26–30S and >30S regions pooled, dialyzed against 0.1% sodium dodecyl sulfate, and lyophilized. The RNA was washed in 80% EtOH to remove the sodium dodecyl sulfate and dissolved in 70% formamide–3 mM Tris–3 mM EDTA (pH 7.5). One $A_{260}$ unit of 18 S was added to the 26–30S sample and 1 $A_{260}$ unit of both 18 and 28 S was added to the >30S sample. The samples were incubated at 37° for 15 min and then layered onto 10–40% sucrose (w/w) gradient prepared in 70% formamide–3 mM Tris–3 mM EDTA (pH 7.5). The gradients were centrifuged for 4.5 hr at 45,000 rpm in the SW-50.1 rotor, at 23°. The gradients were pumped through a flow cell and 0.25-ml fractions collected. An aliquot of each fraction was counted directly and the $A_{260}$ peaks located accurately by reading each fraction: (—) $A_{260}$; (×) cpm of $^{32}P$; (A) 26–30 S; (B) >30 S.

The inhibitor, $\alpha$-amanitin, significantly reduced the amount of RNA synthesized but all size classes of RNA were still synthesized (Figure 7C,D). There was a relatively greater reduction in synthesis of RNA in the 7–20S region than in the other regions of the gradient, although the RNA product was very heterogenous.

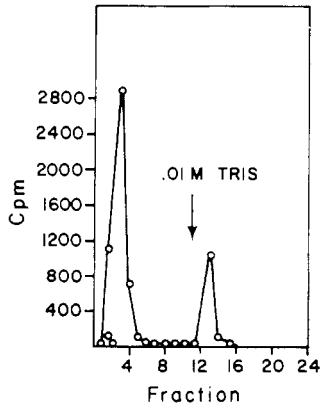


FIGURE 9: Chromatography of RNA on poly(dT)-cellulose. The RNA >30 S (Figure 8) was applied to a 4-ml poly(dT)-cellulose column. Fractions (4 ml) were collected and 0.5-ml aliquots of each fraction were counted. The sample was applied on 0.5 M KCl–0.01 M Tris (pH 7.5) and the column washed with this buffer. The bound RNA was eluted with 0.01 M Tris (pH 7.5), starting at the point indicated by the arrow. The RNA was prepared from nuclei incubated in the presence of [$^{32}PO_4$]GTP for 30 min at 25°. The RNA was first fractionated on a sucrose gradient similar to Figure 7.

TABLE 1: Chromatography of RNA on Poly(dT)-Cellulose.[a]

| RNA Size (S) | Synthesis | % Retained[b] I | II |
|---|---|---|---|
| 7–15 | *In vitro* | 33 | 5 |
| 16–21 | *In vitro* | 22 | 5 |
| 22–29 | *In vitro* | 17 | 6 |
| >30 | *In vitro* | 14 | 4 |
| Ribosomal RNA, 28 + 18 | *In vivo* | 1 | |
| 7–15 | *In vivo* | 25 | |

[a] RNA was isolated from nuclei incubated for 30 min at 25° in the presence of [$\alpha$-$^{32}PO_4$]GTP. The RNA was fractionated on a sucrose gradient as shown in Figure 7. Various size fractions of the RNA were pooled and assayed for their ability to bind to poly(dT)-cellulose as described in the legend to Figure 9. The polysomal RNAs (*in vivo*) were isolated from microsomes prepared from 66-2 tumors and analyzed in an identical manner. [b] Either in the absence (I) or presence (II) of $\alpha$-amanitin.

The RNA synthesized *in vitro* which sedimented in the high molecular region of the gradient was actually of high molecular weight and not simply aggregated during the preparation. Samples of RNA collected from the 26–30S and >30S regions of the gradient were analyzed by centrifugation in the presence of 70% formamide. Under these conditions the secondary structure of the RNA is destroyed and any aggregates dissociated (Suzuki *et al.*, 1972). The RNA from both these size classes remained high molecular weight when centrifuged in the presence of formamide (Figure 8). Thus, *in vitro* there was significant incorporation into truly high molecular weight RNA throughout the entire 30-min incubation period.

*Poly(adenylic acid) Content of in Vitro Synthesized RNA.* Much of the messenger RNA in eucaryotes is distinguished by the presence of poly(adenlyic acid) (poly(A)) at the 3′ terminal (Edmonds *et al.*, 1971; Darnell *et al.*, 1971b; Lee *et al.*, 1971). Some of the heterogenous nuclear RNA also contains poly(A) at the 3′ terminal (Darnell *et al.*, 1971b). These regions are thought to be added posttranscriptionally in the nuclei (Darnell *et al.*, 1971a; Adesnik *et al.*, 1972). These RNAs may be distinguished from other cellular RNAs by their ability to bind specifically to poly(dT)-cellulose. The various *in vitro* synthesized RNAs were tested for their ability to bind to poly(dT)-cellulose. Figure 9 shows that a fraction of the high molecular weight RNA (>30 S) synthesized in 30 min bound to the column. A certain proportion of each size class of RNA bound to the column (Table I). For comparison the proportion of various cellular RNAs which bind to the column are shown. The messenger RNA for the immunoglobulin light chain secreted by these cells binds specifically to the column (E. C. M., unpublished results; Table I, line 6), as does the messenger RNA for the light chain secreted by MOPC-41 (Swan *et al.*, 1972; Mach *et al.*, 1973). Ten–fifteen per cent of the RNA synthesized in 30 min has poly(A) attached to it.

A much lower percentage of the RNA synthesized in the presence of $\alpha$-amanitin bound to poly(dT)-cellulose (Table I,

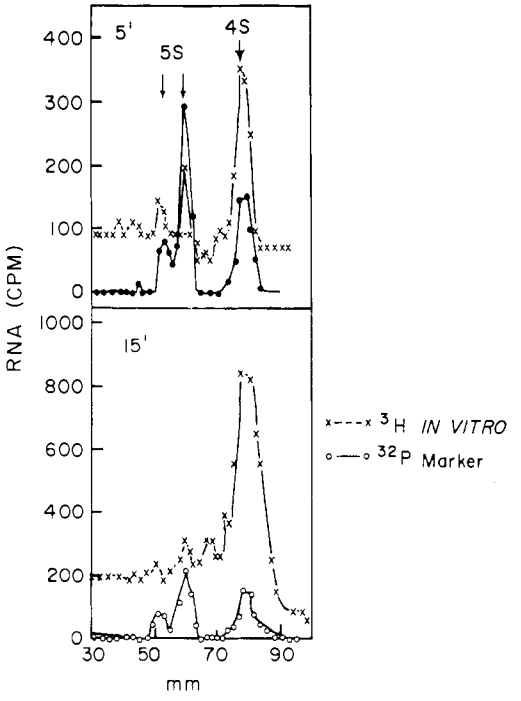


FIGURE 10: Gel electrophoresis of low molecular weight RNA. RNA from fractions 2–8 in Figure 6 was dialyzed against 0.1% sodium dodecyl sulfate and lyophilized. The sodium dodecyl sulfate was removed by washing with cold 80% EtOH and the RNA dissolved in sterile water for electrophoresis. $^{32}PO_4$-Labeled 5S and 4S RNA (1500 cpm) prepared from ribosomes was added to each sample. The gels were 0.7 × 10 cm and were run under the conditions described under Methods. Fractions (1.7 mm) were cut hydrolyzed in base and counted as described under Methods. The RNA was prepared from nuclei incubated at 37° for either 5 or 15 min in the presence of [$^3$H]CTP: (X) [$^3$H]CMP *in vitro;* (●) $^{32}PO_4$ marker RNA *in vivo;* (A) 5 min, (B) 15 min.

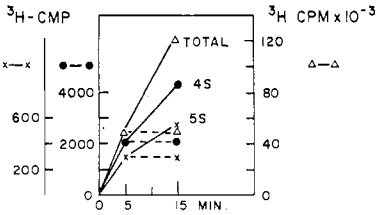


FIGURE 11: Kinetics of synthesis of low molecular weight RNA at 37°. RNA was analyzed as in Figure 10 and the total counts in the 5S and 4S regions summed. A chase experiment was performed by adding a 20-fold excess of unlabeled CTP to the incubation after 5 min and continuing incubation for 10 more minutes. This effectively stopped incorporation of [$^3$H]CTP: (Δ) total $Cl_3CCOOH$-precipitable counts; (●) 4 S; (X) 5 S; (- - -) the chase period.

column II). Hence much of the poly(A)-containing RNA was probably transcribed by RNA polymerase II. This nucleoplasmic RNA polymerase may synthesize at least some of the cellular messenger RNAs.

Whether the poly(A) regions were transcribed or attached posttranscriptionally cannot be determined from these data. Studies by Darnell *et al.* (1971a) and Adesnik *et al.* (1972) indicate that poly(A) sequences are added posttranscriptionally *in vivo.*

*Synthesis of Defined Low Molecular Weight RNA Species.* *In vivo* only a limited number of defined low molecular weight RNA species are present. The predominant species are the ribosomal 5S RNA and the 4S transfer RNA. In addition there are several discrete low molecular weight RNA species present in the cell in lesser amounts which are confined to the nucleus (Weinberg and Penman, 1968; Moriyama *et al.*, 1969). The low molecular weight RNA species synthesized *in vitro* were analyzed by polyacrylamide gel electrophoresis. RNA species were made *in vitro* which migrated identically with 4S and 5S cytoplasmic RNAs (Figure 10). These species were found after both 5 and 15 min of incubation at 37°. They did not accumulate further during a chase period (Figure 11), suggesting that they did not arise from breakdown of higher molecular weight RNA. The data indicate that any precursor of these species must necessarily be short lived. These low molecular weight species accumulated during the total period of synthesis in parallel with total RNA synthesis (Figure 11). A more detailed characterization of these RNA species will be the subject of a later report (Marzluff, W. F., Murphy, E. C., and Huang, R. C. C., manuscript in preparation).

## Discussion

Isolated nuclei active in the synthesis and metabolism of RNA are a potentially useful *in vitro* system for studying the regulation of gene expression in eucaryotes. Nuclei isolated from mouse myeloma cells fulfill several requirements necessary for study of RNA synthesis *in vitro.* (1) RNA synthesis continues at a linear rate for an extended period of time. (2) There is little endogenous nonspecific ribonuclease or deoxyribonuclease activity in the nuclei. (3) High molecular weight RNA is synthesized and possibly matured in the nuclei. (4) Some of the RNA synthesized *in vitro* contains poly(A) sequences, as do many messenger RNAs. (5) Distinct RNA species as assayed by polyacrylamide gel electrophoresis are synthesized.

The most striking feature of RNA synthesis in these nuclei is the effect of temperature. One component of the RNA synthetic apparatus is temperature sensitive, being rapidly inactivated at 37°. Possibly the temperature-sensitive component is a regulatory nuclear component other than RNA polymerase, as all activity ceases abruptly, not just the activity of a single polymerase. It is unlikely that initiation of RNA synthesis is the temperature-sensitive step, as in that case one would expect the accumulation of low molecular weight RNA to stop before that of high molecular RNA and this was not observed (Figure 11). Rather low molecular weight RNA species accumulated in parallel with total RNA, and RNA synthesis ceased abruptly rather than tapering off.

It is unlikely that the difference in synthetic capacity with temperature is due to a temperature-sensitive nuclease activity. No loss of synthesized RNA occurs during a chase period (Figure 11) and there is no production of random low molecular weight species (4–6 S) during a chase period. Rather it appears more likely that transcription stops for an unknown reason after a short time at 37°.

A significant percentage of RNA synthesized *in vitro* contains poly(adenylic acid) sequences. The poly(A) regions were present in all size classes of the RNA made *in vitro.* Whether the poly(A) was transcribed or added posttranscriptionally could not be determined, although Raskas and Bhaduri (1973) have shown that the poly(A) sequence may be attached *in vitro* to previously synthesized RNA in isolated HeLa cell nuclei. It has been shown that *in vivo* poly(A) sequences may be added to specific RNAs very soon after transcription (Adesnik *et al.*, 1972). Whether this indicates that specific messenger RNAs have been synthesized in the nuclei has not been established. However, messenger RNAs are a major class of RNA

which contain poly(A) (Edmonds *et al.*, 1971; Darnell *et al.*, 1971b; Lee *et al.*, 1971). Much of the RNA containing poly(A) was synthesized by polymerase II. Adenovirus messenger RNAs are also transcribed by polymerase II (Price and Penman, 1972a) and it is likely that many messenger RNAs in eucaryotes are transcribed by this nucleoplasmic polymerase. The recent development of an assay system, based on RNA–DNA hybridization, for specific messenger RNAs (Ross *et al.*, 1972) makes it feasible to answer this question in the near future.

The mouse myeloma nuclei are as active in RNA synthesis as the nuclei described by Reeder and Roeder (1972) although the myeloma nuclei synthesize RNA for a much longer time at moderate salt concentrations. In this respect they are comparable in activity to the nuclear preparation recently obtained by Gurney *et al.* (1972) using a nonaqueous technique. A central problem remaining to be answered is whether these nuclei initiate synthesis of new RNA chains. The extended time period of RNA synthesis and the continued accumulation of low molecular weight RNA species in parallel with total RNA synthesis strongly suggest that some initiation of RNA chains *in vitro* is occurring, although rigorous chemical proof of this has not been obtained as yet. Price and Penman (1972b) have shown that a synthesis of low molecular weight adenovirus-specific RNA is initiated in nuclei isolated from adenovirus-infected HeLa cells using similar arguments.

If these nuclei are to be useful in studying control of gene expression they must initiate synthesis of RNA chains. However, the capacity of these nuclei to synthesize and mature RNA *in vitro* together with the recent development of assay procedures for specific messenger RNAs should allow one to answer many pressing questions concerning intranuclear RNA metabolism using isolated nuclei.

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