Ribonucleic Acid Synthesis in Isolated *Drosophila* Nuclei†

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ABSTRACT: We have developed a system for studying transcription in nuclei isolated from *Drosophila melanogaster* tissue culture cells. These nuclei synthesize RNA at the rate of 75–100 pmol/μg of DNA in 30 min. About 20% of the RNA synthesized is released from the nuclei, and both released and nonreleased RNAs have poly(A) on them. By use of mercury–agarose affinity chromatography and 5′-[γ-S]GTP or 5′-[γ-S]ATP precursors as an assay for initiation, it has been determined that approximately 16% of the radioacivity incorporated into RNA is in in vitro purine initiated RNAs. Alkaline hydrolysis of the initiated RNAs has confirmed that the sulfur is present at the 5′ end of newly synthesized RNAs and is serving as an accurate probe for initiation. Some of the in vitro initiated RNAs also have poly(A) added to them. Sucrose gradient sedimentation of in vitro synthesized RNAs shows them to sediment in a broad peak centered at about 20 S, with RNA as large as 50 S detectable. The transcription system has been optimized for NaCl, Mg^{2+}, and Mn^{2+} concentrations (100, 1.25, and 0.75 mM, respectively), and addition of BSA has been shown to stabilize the RNA once it has been synthesized. By using the inhibitors α-amanitin and rifampicin, we have been able to demonstrate that RNA polymerases Ia, Ib, II, and III are active [in addition to the poly(A) polymerase], with enzymes Ib and II accounting for about 80% of the synthesis in these nuclei. This system should prove useful for the study of transcriptional control mechanisms.

The process of producing a mature mRNA from a chromatin template is a very complex one, involving RNA synthesis, processing, and transport. A number of important steps in mRNA maturation take place within the nucleus. An isolated nuclear transcription system would be a valuable means of examining this, since studies on transcription in whole cells are complicated by the impermeability of the cell membrane to the nucleoside triphosphates as well as a number of other transcriptional modulators.

RNA synthesis has been studied in isolated nuclei from a number of systems which include many cell types (Ernest et al., 1976; Jelinek, 1974; Marzluff et al., 1973). The nuclei offer an advantage to the investigator interested in transcription in that the system is amenable to the addition of various control factors. Further, since the cytoplasm is absent, the nuclear environment can be precisely controlled. Addition of various cytoplasmic extracts to the transcription system may be carried out in order to screen for cytoplasmic regulatory molecules.

Mouse myeloma nuclei have been shown to synthesize large RNAs (Marzluff et al., 1973), add poly(A) tails (Cooper & Marzluff, 1978), initiate new RNA chains (Smith et al., 1978), and produce discrete RNA species (Marzluff et al., 1974). Poly(A) addition has been demonstrated in hen oviduct nuclei (Ernest et al., 1976) and HeLa cell nuclei (Jelinek, 1974) as well. Processing of RNA has also been reported in isolated nuclei (Marzluff et al., 1974; Ernest et al., 1976). RNA synthesis in isolated nuclei therefore shows promise of becoming a useful system for studying transcriptional control mechanisms.

In this report, we describe and characterize a nuclear transcription system from *Drosophila melanogaster* tissue culture cells. The system synthesizes substantial amounts of RNA, initiates new RNA chains in vitro, and adds poly(A) to completed chains. The size distribution of the in vitro synthesized RNA is comparable to the in vivo RNA size distribution.

Materials and Methods

Isolation of Nuclei. Nuclei were isolated by the method of Marzluff et al. (1973). Schneider line 2 *Drosophila melanogaster* cells were harvested at a cell density of (3–5) × 10^6 cells/mL and pelleted for 5 min in the International No. 279 rotor at 1700 rpm. The cells were resuspended in buffer A [0.3 M sucrose, 2 mM $Mg(OAc)_2$, 3 mM $CaCl_2$, 10 mM Tris, pH 8 at 2 °C, 0.1% Triton X-100, and 0.5 mM dithiothreitol (DTT)] at (1–3) × 10^8 cells/mL and homogenized with the tight-fitting A pestle of a Dounce homogenizer. Cell breakage was monitored by phase-contrast microscopy until at least 95% of the cells were broken (50–100 strokes). The homogenate was mixed with an equal volume of buffer S (2 M sucrose, 5 mM $Mg(OAc)_2$, 10 mM Tris, pH 8 at 2 °C, and 0.5 mM DTT) and placed in 5-mL centrifuge tubes. This mixture was underlayered with 1 mL of buffer S, the interface was stirred slightly, and then it was centrifuged at 2 °C for 20 min at 40 000 rpm in the Sorvall AH650 rotor using the reograd stop mode (Sorvall OTD-65 ultracentrifuge) to minimize mixing on deceleration. Nuclei, which form a pellet at the bottom of the buffer S pad, appear free of cytoplasmic contamination by phase-contrast microscopy.

RNA Synthesis. The nuclear pellet was gently resuspended in resuspension buffer (25% glycerol, 50 mM Tris, pH 7.3, 5 mM DTT, 0.1 mM EDTA, and 100 mM NaCl) and transferred to reaction tubes. Unless stated otherwise, the final reaction mixture contained (3–5) × 10^8 nuclei/mL, 12.5% glycerol, 25 mM Tris, pH 7.3, 2.5 mM DTT, 0.1 mM EDTA, 100 mM NaCl, 1 mM CTP, 1 mM GTP, 1 mM ATP, 0.4 mM UTP, 10 μCi/mL [^{3}H]UTP (41 Ci/mmol; Amersham), 1.25 mM $Mg(OAc)_2$, 0.75 mM $MnCl_2$, and 1% (w/v) bovine serum albumin (BSA). Reactions with ^{32}P contained 250 μCi/mL [α-^{32}P]UTP (470 Ci/mmol; New England Nuclear) instead of the [^{3}H]UTP. For investigation of RNA initiation, reactions contained either 5′-[γ-S]ATP or 5′-[γ-S]GTP (Boehringer-Manheim) instead of ATP or GTP, respectively. Incubations were for 30 min at 25 °C unless indicated otherwise. RNA synthesis was monitored by spotting 5 μL of the reaction mix on Whatman No. 1 filter paper and precipitating the RNA with 5% cold trichloroacetic acid (Cl_3-

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AcOH). The filters were washed 3 times in 5% Cl_3AcOH and once in acetone, air-dried, and counted in toluene fluor (Yorktown Research; LSC Complete).

Isolation of Synthesized RNA. After the synthesis reaction, the nuclei were treated with 50 μg/mL DNase (Worthington; RNase free) for 5 min at room temperature, diluted with 10 volumes of 1% (w/v) sodium dodecyl sulfate ($NaDodSO_4$), 0.1 M NaOAc, pH 5.1, and extracted once with 2 volumes of water-saturated phenol and then 2 times with phenol–chloroform (1:1). RNA was precipitated from the final aqueous phase by addition of NaCl to 0.3 M, followed by 2 volumes of −20 °C, 95% ethanol. In some cases, after the synthesis reaction, the nuclei were pelleted for 4 min in a Beckman microfuge. RNA extracted from the supernatant was defined as the released RNA, and RNA from the nuclear pellet was defined as the nonreleased RNA.

UMP–Sepharose Chromatography. Agarose–5′-[(*p*-aminophenyl)phosphoryl]uridine 2′(3′)-phosphate (Miles-Yeda, Ltd.) was used to remove RNase. The BSA stock at 1 g/mL in 0.02 M NH_4OAc, pH 5, was applied to the column equilibrated in the same buffer, and the column was washed until the BSA came through. The fractions containing the BSA were lyophilized and resuspended in water to give a stock solution of 1 g/mL. This stock was stored frozen at −20 °C. UMP–Sepharose can be regenerated by eluting with 0.2 M acetic acid to remove the RNase.

Mercury–Agarose Chromatography. This was done by the method of Reeve et al. (1977). The mercury–agarose resin was a generous gift from Dr. Anthony Reeve (Johns Hopkins). The RNA precipitate was resuspended in NEST buffer (0.1 M NaCl, 1 mM EDTA, 0.1% $NaDodSO_4$, and 10 mM Tris, pH 7.9) and slowly applied to the column until the nonbinding RNA had completely run through the column. The bound RNA was eluted with 10 mM DTT in NEST buffer. The column was regenerated between each use by washing in the following buffers: (1) 0.1 M ammonium formate, (2) 10 mM $HgCl_2$, 20 mM EDTA, and 50 mM NaOAc, pH 4.8, (3) 0.2 M NaCl, 1 mM EDTA, and 0.1 M sodium phosphate, pH 6.0, and (4) NEST.

Oligo(dT)–Cellulose Chromatography. Oligo(dT)–cellulose was obtained from Collaborative Research. RNA samples were applied to the column in 0.5 M NaCl, 10 mM Tris, pH 7.3, and 0.1% $NaDodSO_4$, and the column was washed with this buffer until no more RNA washed through. The bound RNA was eluted in 0.1% $NaDodSO_4$ and 10 mM Tris, pH 7.3.

Sucrose Gradients. RNA size was analyzed by sedimentation in 10–70% (w/v) sucrose gradients in 0.1 M NaCl, 0.01 M EDTA, 0.01 M Tris, pH 7.5, and 0.1% $NaDodSO_4$. The sample was heated for 3 min at 65 °C, rapidly cooled, and sedimented in the Sorvall TV865B vertical rotor for 135 min at 60 000 rpm at 22 °C. A Sorvall OTD-65 ultracentrifuge was used with slow B acceleration and reograd stop.

Tetraphosphate Analysis. This was done by the method described by Reeve et al. (1977). In vitro transcribed RNA that bound to the mercury column was hydrolyzed for 16 h at 37 °C in 0.3 M NaOH in a reaction volume of 100 μL. Samples were neutralized with HCl, diluted to 2 mL with 0.1 M NH_4OAc, 10 mM EDTA, and applied to the mercury–agarose column (equilibrated in 0.1 M NH_4OAc). The column was washed with 0.1 M NH_4OAc, pH 7, until no more counts eluted, and then the bound nucleotides were released with 10 mM DTT in 0.1 M NH_4OAc. The fractions containing the thiol-substituted analogues were pooled and lyophilized. The lyophilized samples were resuspended in 10 mM $Hg(OAc)_2$, 10 mM EDTA and analyzed by thin-layer chromatography on PEI plates (with fluorescent indicator). Standards were run in parallel tracks. 5′-[γ-S]GTP or 5′-[γ-S]ATP ran as GTP or ATP when complexed with the mercury (Reeve et al., 1977). The chromatogram was developed with 0.75 M KH_2PO_4 adjusted to pH 3.4 with HCl (Goody & Eckstein, 1971), and autoradiography was carried out with Kodak XR-5 X-ray film with a Du Pont Cronex Lightning Plus screen.

Results

Before examining the RNA transcripts, we set about determining the optimum conditions for RNA synthesis. This was done by varying the magnesium and manganese concentrations, ionic strength, incubation temperature, and nuclear concentration and by adding BSA. With the system optimized, greater than 95% of the Cl_3AcOH-precipitable counts were sensitive to digestion with pancreatic RNase, and 5 μg/mL actinomycin D eliminated greater than 98% of the incorporation of [^{3}H]UTP into Cl_3AcOH-precipitable material. Thus, the material being synthesized is RNA.

To show that the incorporation is due to the nuclei and to determine optimal nuclear concentrations, we monitored RNA synthesis as a function of nuclear concentration. The synthesis of RNA is directly proportional to the nuclear concentration up to at least 5×10^8 nuclei/mL. All reactions are therefore carried out between 1×10^8 and 5×10^8 nuclei/mL.

Varying the NaCl concentration from 0 to 200 mM demonstrates a maximum incorporation between 100 and 150 mM NaCl. We routinely use 100 mM NaCl in the assays, since this concentration is optimal for a mixture of the major *Drosophila* RNA polymerases (Gross & Beer, 1975). This concentration is also in the physiological range (Ephrussi & Beadle, 1936).

Magnesium and manganese concentrations were determined through a reiterative process. Initial assays were done by using 2.1 mM Mg^{2+} and 1.5 mM Mn^{2+}, concentrations which are optimal for isolated RNA polymerases (Gross & Beer, 1975). With the Mn^{2+} concentration fixed at 1.5 mM, the Mg^{2+} concentration was varied from 0 to 8 mM to determine a new optimum. Then, with the Mg^{2+} concentration at its new optimum, the Mn^{2+} concentration was varied. The procedure was repeated until the optima no longer changed. The results are shown in Figure 1. Our standard assay contains 1.25 mM Mg^{2+} and 0.75 mM Mn^{2+}. The double peak shown for Mn^{2+} is very reproducible and probably represents optima for two different RNA polymerases.

There are a few reports in the literature that BSA can enhance incorporation in isolated nuclear transcription systems [e.g., Ernest et al. (1976)]. Before determining the effects of BSA on our system, we first removed any contaminating RNase activity by passing BSA through cUMP–Sepharose (J. Stanchfield and K. Mullinex, personal communication). Two transcription reactions were run (one with 1% BSA, one without), and aliquots were withdrawn over a 2-h period. As seen in Figure 2, BSA has no significant effect for the first 60 min but seems to enhance RNA survival between 60 and 120 min.

Drosophila is unique among eucaryotes in that it has an RNA polymerase which is sensitive to the procaryotic RNA polymerase inhibitor rifampicin (*Drosophila* RNA polymerase Ib is >95% inhibited by 5 μg/mL; Gross & Beer, 1975). Thus, with low α-amanitin (1 μg/mL), high α-amanitin (100 μg/mL), and rifampicin, we can determine individual enzyme activities in isolated nuclei. This is shown for enzymes Ia, Ib, and II in Figure 3. Incorporation rates and periods may vary

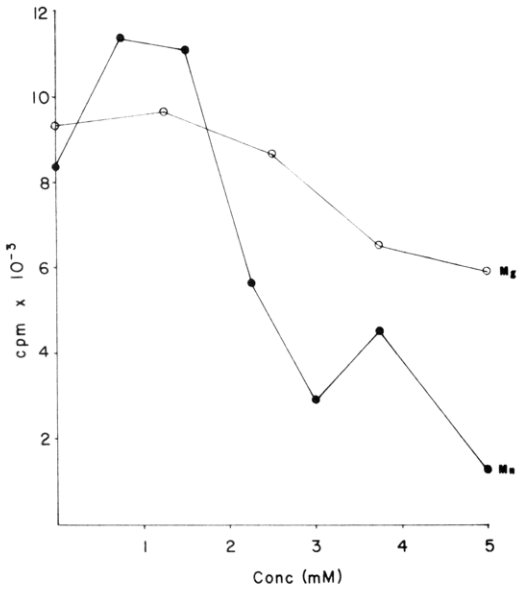

FIGURE 1: RNA synthesis and divalent cation concentration. Nuclei were prepared as described and incubated in the appropriate Mg^{2+} or Mn^{2+} concentrations. All reactions also contained 100 mM NaCl. Aliquots were withdrawn after 20 min at 25 °C and assayed by Cl_3AcOH precipitation. Mg^{2+} dependence (O) was determined at 0.75 mM Mn^{2+}, and Mn^{2+} dependence (●) was determined at 1.25 mM Mg^{2+}, as described in the text.

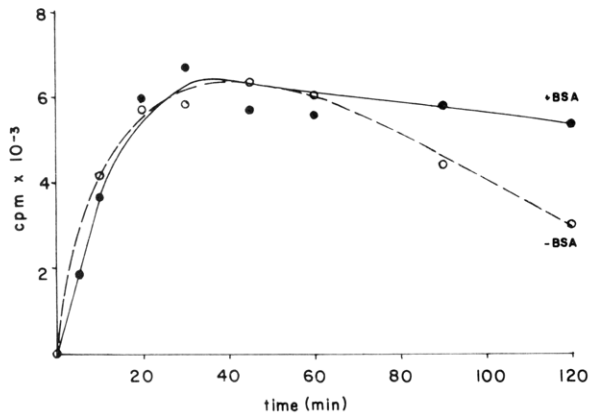

FIGURE 2: Effect of bovine serum albumin on RNA synthesis. Nuclei were incubated under standard conditions in two parallel tubes, one containing 1% BSA (RNase free as described under Materials and Methods). Aliquots were withdrawn at various times and assayed by Cl_3AcOH precipitation. (●) 1% BSA present; (O) no BSA present.

somewhat among experiments. By using high α-amanitin concentrations (100 μg/mL) we estimate that there is some enzyme III activity (Phillips & Sumner-Smith, 1977) in the isolated nuclei, making up about 5–15% of the total activity. This activity is too low, however, to enable us to determine the time course of enzyme III activity. In typical reactions (30 min) RNA polymerase Ia synthesizes 15 ± 5% of the RNA, Ib synthesizes 40 ± 8%, II synthesizes 40 ● 5%, and enzyme III synthesizes 10 ± 5% of the RNA.

To investigate whether the synthesis we are getting is due solely to elongation of in vivo initiated RNA or whether new RNA chains are being initiated in vitro, we used the technique of Reeve et al. (1977). Synthesis was carried out in the presence of either 5′-[γ-S]ATP or 5′-[γ-S]GTP to replace ATP or GTP, respectively. A newly initiated RNA in these reactions will contain a sulfhydryl group at its 5′ end, whereas elongated (but not in vitro initiated) RNAs will have no sulfurs. The newly initiated RNAs are then detected by affinity chromatography on mercury–agarose columns. Using

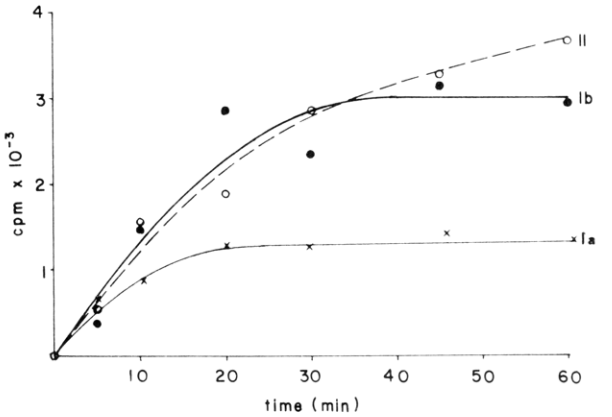

FIGURE 3: RNA synthesis due to individual RNA polymerases. Nuclei were incubated in tubes containing either 5 μg/mL α-amanitin, 10 μg/mL rifampicin, 100 μg/mL α-amanitin plus 10 μg/mL rifampicin, or no inhibitors (control). RNA polymerase Ia (×) activity was the activity present in high α-amanitin concentration and rifampicin. RNA polymerase Ib activity (●) was calculated as the difference between the control and rifampicin reactions. RNA polymerase II activity (O) was calculated as the difference between the control and low α-amanitin concentration reactions. Aliquots were withdrawn at various times and assayed by Cl_3AcOH precipitation.

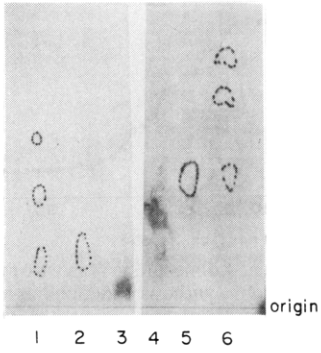

FIGURE 4: Thin-layer chromatography of alkaline hydrolysis products. In vitro initiated RNAs were hydrolyzed and the sulfur-containing products were purified on mercury–agarose as described under Materials and Methods. The PEI thin-layer chromatogram was developed in 0.75 M KH_2PO_4, pH 3.4. Lane 1, mixture of GTP, GDP, and GMP; lane 2, 5′-[γ-S]GTP; lane 3, mercury-retained radioactivity from 5′-[γ-S]GTP synthesis reaction; lane 4, mercry-retained radioactivity from 5′-[γ-S]ATP synthesis reaction; lane 5, 5′-[γ-S]ATP; lane 6, mixture of ATP, ADP, and AMP. Samples 2–5 were applied in 10 mM $HgCl_2$, 10 mM EDTA. Samples 1 and 6 were applied in water. The positions shown in lanes 3 and 4 are autoradiographic spots; the positions in 1, 2, 5, and 6 were detected by UV absorbance and outlined in ink. Lanes 1–3 were run at a different time from lanes 4–6.

5′-[γ-S]ATP, 8.7 ± 3.1% of the synthesized RNA bound to the mercury column while 7.9 ± 4.3% of the 5′-[γ-S]GTP synthesized RNA bound. These results are the average of four separate determinations. Control reactions were run without thiol-substituted analogues, and no detectable counts bound to the column. When the RNA that bound from the 5′-[γ-S]ATP or 5′-[γ-S]GTP reactions was reapplied to the mercury–agarose, greater than 90% bound again. Therefore, about 16% of the RNA synthesized in the isolated nuclei was initiated in vitro with a purine.

To ensure that the RNA was binding to the mercury column through its 5′ end and truly represented newly initiated RNA [i.e., not binding through a sulfur transferred enzymatically to an in vitro elongated RNA; see Spencer et al. (1978)], reactions were carried out with 5′-[γ-S]GTP or 5′-[γ-S]ATP and [α-^{32}P]UTP as the label. RNA that bound to the mercury–agarose column was alkaline hydrolyzed, and the

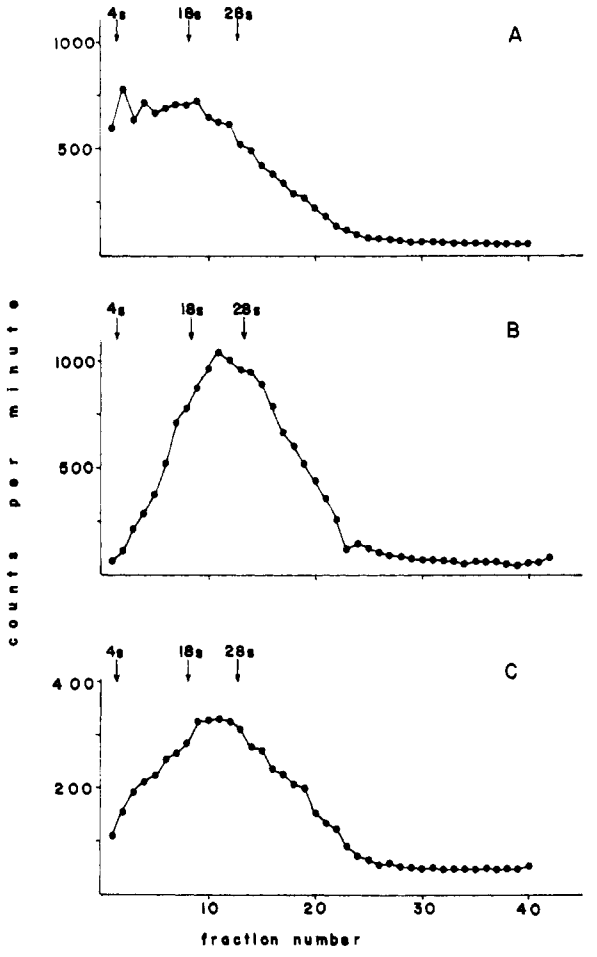

FIGURE 5: Sucrose gradient sedimentation of in vitro synthesized RNA. RNA was extracted from synthesis reactions as described under Materials and Methods. Samples were sedimented into 10–70% (w/v) sucrose gradients, fractions were collected, and aliquots were counted. Internal markers of chick brain poly(A–) RNA were detected by absorbance at 260 nm. (A) RNA synthesized in control nuclei under standard conditions; (B) RNA synthesized in 5′-[γ-S]ATP-containing reactions which did not bind to mercury–agarose; (C) RNA synthesized in 5′-[γ-S]ATP-containing reactions which bound to mercury–agarose.

hydrolysis products which bound to the mercury column (i.e., those hydrolysis products containing sulfur) were analyzed by thin-layer chromatography on PEI plates. Figure 4 shows that the majority of the radioactivity migrates as a tetraphosphate with no detectable radioactivity migrating as a monophosphate.

Next we determined the extent to which the synthesized RNAs were being polyadenylated. Oligo(dT)–cellulose bound 5 ± 1% of the released RNAs and 3 ± 1% of the nonreleased RNAs. In addition, 0.9 ± 0.4% of the 5′-[γ-S]ATP-initiated RNAs and 1.1 ± 0.2% of the 5′-[γ-S]ATP-initiated RNAs will bind to oligo(dT)–cellulose (i.e., these RNAs bind to both mercury–agarose and oligo(dT)–cellulose columns). Although there is some variation in the percent binding to oligo(dT)–cellulose for the released and nonreleased RNAs, in all experiments, a higher percent of the released RNA bound than the nonreleased RNA. In general, about 1.5 times as much released RNA bound to oligo(dT)–cellulose as did nonreleased RNA. The fact that some of the ATP- and GTP-initiated RNAs also have poly(A) added to them suggests that at least some of the in vitro initiated RNAs are completed and polyadenylated.

Figure 5A shows the sucrose gradient sedimentation profile for RNA synthesized in a standard synthesis reaction. RNA as large as 50 S is detectable. The size distribution for RNAs synthesized with 5′-[γ-S]ATP instead of ATP in the reaction is shown in parts B and C of Figure 5. RNA that was elongated in vitro after initiation in vivo (i.e., radioactivity not binding to the mercury–agarose) is shown in Figure 5B, and RNA initiated in vitro is shown in Figure 5C. We have obtained similar results for 5′-[γ-S]GTP-containing reactions.

Discussion

As shown in Figure 5, most of the RNA synthesized in our system falls between 4 and 28 S, but RNA up to 50 S is synthesized. Levy W. & McCarthy (1975) have found that the mRNA population in Schneider line 2 cells sediments in a broad peak between 10 and 20 S. MacLeod (1976) shows a mRNA distribution centered around 18 S for adult *Drosophila* mRNA. Lengyel et al. (1975) find that Schneider line 2 cells have nuclear RNA that sediments with a peak of about 29 S. No sedimentation peaks are observed for rRNA or pre-rRNA in these nuclei. Thus, the size of the RNA being synthesized in the isolated nuclei is comparable to that of the in vivo synthesized RNA. The size distribution of the in vivo initiated, in vitro elongated RNA is similar to that of the in vitro initiated RNA, as shown in Figure 5. The relative decrease in RNAs smaller than 18 S in [γ-S]ATP-containing reactions (parts B and C of Figure 5) is not currently understood.

Poly(A) is added to 3–5% of the RNA synthesized in our nuclear transcription system ([^{3}H]UTP label). Cytoplasmic RNA labeled in vivo shows about 1–2% binding to oligo(dT)–cellulose by our extraction procedures, while this percentage is slightly less for in vivo synthesized nuclear RNAs. The in vitro synthesized RNA population therefore seems to be slightly enriched for poly(A)-containing RNAs. Cooper & Marzluff (1978) find that the addition of poly(A) to RNA synthesized in isolated mouse myeloma nuclei is dependent on a soluble protein extract from crude nuclei. They assay their results by binding to poly(U)–Sepharose and point out that their results differ if oligo(dT)–cellulose is used. Jelinek (1974) finds that poly(A) addition in HeLa cells is dependent on the addition of a cytosol fraction. However, Ernest et al. (1976) find that hen oviduct nuclei add poly(A) without the addition of other cellular fractions. In our system we know that enough oligo(A) is synthesized to allow binding to the oligo(dT)–cellulose, but the length of the oligo(A) region has not been determined.

Approximately 18–20% of the RNA synthesized in the isolated *Drosophila* nuclei is released from the nuclei into the buffer; the remaining (nonreleased) RNAs apparently do not leave the nucleus. This is not the result of nuclear breakage, since the nuclear concentration remains unchanged during the standard 30-min reaction (as assayed by counting in a hemocytometer). It is interesting to note that the released RNAs always have a 1.5 times greater fraction that binds to oligo(dT)–cellulose than do the nonreleased RNAs. This is consistent with the theory that the poly(A) tail may aid in the transport of the RNA out of the nucleus (Jelinek et al., 1973; Brandhorst & McConkey, 1975).

Enzyme activity has been reported that can phosphorylate 5′-mono- and -diphosphate RNA termini to 5′-triphosphates by using ATP as the phosphate donor (Spencer et al., 1978). The possibility that this kind of reaction was being carried out in the *Drosophila* nuclei and caused in vivo initiated RNA to bind to the mercury–agarose (by transferring a thiophosphate from 5′-[γ-S]ATP or 5′-[γ-S]GTP to the 5′ end of an in vivo initiated RNA) was examined by alkaline hydrolysis of [α-^{32}P]UTP-labeled RNA. Alkaline hydrolysis of RNA will yield 2′- or 3′-monophosphates from nucleosides internally

Table I: Comparison of Optimum Incubation Conditions[a]

reaction/ enzymes	Mg^{2+} (mM)	Mn^{2+} (mM)	NaCl (mM)
Ia	2.5	3	<100
Ib	1–8	1.5	<100
II	2.5	1.0	150
nuclei	1.25	0.75 and 3.5	100–150

[a] Data for the first three rows (the isolated enzymes) are taken from Gross & Beer (1975). The NaCl concentrations were calculated to give the same cationic strength as the $(NH_4)_2SO_4$ data presented in the original paper. The concentrations in the table are those which give the maximum synthesis of RNA.

located and will generate a tetraphosphate (3′-mono,5′-triphosphate) from the 5′ terminus. If the RNA has a 5′-mono- or -diphosphate terminus, alkaline hydrolysis will yield di- or triphosphates (the new 3′-phosphate originating from the second base in the RNA). Alkaline hydrolysis of the RNA synthesized in the *Drosophila* nuclei with [α-^{32}P]UTP and 5′-[γ-S]ATP (or 5′-[γ-S]GTP) produced predominantly [^{32}P]tetraphosphates. The only way this result could occur is for initiation to have occurred with either 5′-[γ-S]ATP or 5′-[γ-S]GTP, followed by the [α-^{32}P]uridine as the second nucleotide. This means that the RNA binding to the mercury affinity column represents RNA synthesis initiated in vitro.

The temperature at which the reaction is carried out is quite important. At 37 °C, the nuclei synthesized RNA very rapidly but for only 5–10 min, while, at 15 °C, synthesis was linear for at least 2 h. In neither case, however, was the total amount of RNA synthesized as great as that at 25 °C for 30 min (our standard conditions). The reason for the decline in RNA synthesis is not understood. Attempts to "revive" transcription by adding fresh precursors to reactions at later times were not successful. Perhaps a nuclear–cytoplasmic interaction is needed for synthesis to continue.

In mouse myeloma nuclei, RNA is incorporated at the rate of 12 pmol of RNA per μg of DNA per 30 min (Marzluff et al., 1973). Stallcup et al. (1978) report 2.2 pmol of RNA per μg of DNA per 30 min in GR cell nuclei (a mouse mammary tumor cell line), Ernest et al. (1976) report 8 pmol of RNA per μg of DNA per 30 min for hen oviduct nuclei, and, in HeLa cell nuclei, this rate is 0.9 pmol of RNA per μg of DNA per 30 min (Jelinek, 1974). The *Drosophila* nuclei typically synthesize 75–100 pmol of RNA per μg of DNA per 30 min, a considerably higher synthesis rate. The reason for this is not known.

Ernest et al. (1976) found that the addition of BSA to their hen oviduct nuclear transcription system enhanced the synthesis of RNA. They determined that the BSA prevented the lysis of nuclei and therefore allowed more RNA synthesis. In our system it appears that the BSA prevents degradation of already synthesized RNA without significantly affecting the rate of synthesis (Figure 2).

It is interesting to compare the various optima for RNA synthesis in isolated nuclei to the optima for partially purified RNA polymerases (Gross & Beer, 1975). This is done in Table I. The NaCl optimum in nuclei corresponds to physiological ionic strength (Ephrussi & Beadle, 1936), a satisfying (but not overly significant) piece of information. The double Mn^{2+} optima seen in the nuclei would seem to indicate that more than one RNA polymerase is active, including RNA polymerase Ia. The major difference in the optima lies in the Mg^{2+} ion concentration, which may be a function of nuclear stability. Phillips & Forrest (1973) found that *Drosophila* embryo nuclei tended to clump at concentrations above 1.5 mM Mg^{2+}, while lower Mg^{2+} concentrations led to deterioration of nuclear structure.

Drosophila offers many advantages as an organism for the study of molecular genetics. The availability of an isolated nuclear transcription system which initiates RNA synthesis in vitro should make it possible to investigate the effects of a number of regulatory molecules (e.g., ecdysone receptors) on transcription. Through the use of specific mRNA probes, we hope to be able to determine if RNA processing is taking place in these nuclei and then to follow this processing if it is occurring.

All four *Drosophila* RNA polymerases (Ia, Ib, II, and III) are active in these nuclei, as is poly(A) polymerase, and a significant fraction of the synthesized RNA is released from the nuclei to the surrounding medium. Since some of the RNA is initiated in vitro, the system should prove useful for the study of specific gene induction in vitro.

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