RNA Synthesis in Isolated Hen Oviduct Nuclei†

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ABSTRACT: Nuclei have been prepared from the oviduct of the adult laying hen which are capable of synthesizing large amounts of RNA for long periods of time. The time course of RNA synthesis is linear through 3 h of incubation after an initial burst of activity and is inhibited 60–70% by α-amanitin. Maximum synthetic activity requires the presence of serum albumin to stabilize the nuclei, high concentrations of the four ribonucleoside triphosphates, and an incubation temperature of 25 °C for continued linear synthesis beyond 30 min. The RNA synthesized in vitro is predominantly 10–20 S with a small proportion of higher molecular weight product. Much of the 10–20S RNA is probably transcribed by RNA polymerase II and is of a size comparable to ovalbumin mRNA. A fraction of this RNA appears to contain poly(A) sequences suggesting that there is some processing of the newly synthesized RNA. These nuclei may provide a useful system for studying the control of the transcription and maturation of ovalbumin mRNA in vitro.

One of the most extensively studied systems of hormonal control of gene expression is the synthesis of ovalbumin in the tubular gland cell of chick oviduct (Palmiter, 1975). Studies on the control of the transcription and maturation of ovalbumin messenger RNA would be greatly aided by the development of an in vitro system capable of faithfully initiating, synthesizing, and processing ovalbumin mRNA.[1] Isolated cell nuclei have the potential to provide such an in vitro system. Several investigators have already reported the synthesis of RNA by isolated nuclei from a variety of cells. Marzluff et al. (1973, 1974) observed that mouse myeloma cell nuclei were capable of synthesizing RNA of various molecular weights for extended periods of time. Transcription of rRNA by RNA polymerase I has been described by Zylber and Penman (1971) in isolated HeLa cell nuclei and by Reeder and Roeder (1972) in nuclei from cultured *Xenopus laevis* cells. Nuclei from adenovirus-infected HeLa cells have been shown to synthesize adenovirus-specific RNA with most of the messenger RNA molecules transcribed by RNA polymerase II and at least one low molecular weight RNA species synthesized by RNA polymerase III (Price and Penman, 1972a,b). Recently, Jacobsen et al. (1974) demonstrated that isolated nuclei from the slime mold *Dictyostelium discoideum* could synthesize RNA molecules with properties indistinguishable from cellular precursors to both messenger and ribosomal RNA.

Besides the in vitro synthesis of RNA by all three classes of RNA polymerase, several post-transcriptional events have also been described in various isolated nuclei systems. Marzluff et al. (1973) reported that all size classes of RNA synthesized by mouse myeloma cell nuclei contained a variable proportion of poly(A) sequences. Similar observations have been made with mouse brain nuclei (Banks and Johnson, 1973) and nuclei from adenovirus-infected human (KB) cells (Raskas and Bhaduri, 1973). Jelinek (1974) demonstrated that HeLa cell nuclei were capable of synthesizing poly(A) sequences similar in size to those found in vivo and contained in heterogeneous nuclear RNA molecules. At least two nuclei preparations also show in vitro transport of in vivo synthesized RNA out of the nucleus. Raskas (1971) described the release of labeled viral RNA as ribonucleoprotein from nuclei of adenovirus-infected cells in the presence of an ATP generating system. The energy-dependent release of both ribosomal and messenger RNA as their corresponding nucleoproteins from isolated liver nuclei was reported by Webb and his collaborators (Schumm and Webb, 1972; Yu et al., 1972), who also demonstrated a requirement for cytoplasmic proteins.

Thus, isolated nuclei provide a system not only for studying transcription of defined RNA species but also the maturation and eventually the transport of RNA from the nucleus. We have prepared nuclei from the oviduct of the adult laying hen which are capable of synthesizing RNA for long periods of time (Ernest and Schutz, 1975) to ultimately study the regulation of ovalbumin mRNA synthesis. In this paper, we will describe some of the properties of our oviduct nuclei system and the types of RNA synthesized by it.

Materials and Methods

Chemicals. Ribonucleoside triphosphates were purchased from Sigma Chemical Co. Ribonuclease inhibitor was a product of Searle Laboratories, Buckinghamshire, England. α-Amanitin was obtained from the Henley Co. Bovine serum albumin (Pentex Fraction V) was purchased from Miles Laboratories and was free of RNase activity. Uridine [5-^{3}H]-5-triphosphate (36 Ci/mmol) was a product of New England Nuclear. Oligo(dT)-cellulose was purchased from Collaborative Research, Inc. RNase-free sucrose was a product of Schwarz/Mann, Inc. All solutions were prepared in sterile, glass-distilled water.

Preparation of Nuclei. White leghorn laying hens (Shamrock Farms, New Brunswick, N.J.) were sacrificed by decapitation and the magnum portion of the oviduct was opened longitudinally and scraped with a microscope slide

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[1] Abbreviations used are: Hepes, *N*-2 hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; poly(A), poly(adenylic acid); poly(U), poly(uridylic acid); mRNA, messenger RNA.

leaving behind the serous membrane. The tissue (in 7-g portions) was homogenized at one-third full speed for 2 min in a Polytron PT20, ST homogenizer (Brinkman Instruments) in 10 volumes of 0.3 M sucrose containing 2 mM $Mg(OAc)_2$, 3 mM $CaCl_2$, and 10 mM Tris-HCl (pH 8.0). The homogenate was filtered through four layers of coarse cheesecloth and centrifuged at 1000*g* for 10 min in the Sorvall HB-4 rotor. One volume of homogenization buffer containing 0.1% Triton X-100 was added to the crude nuclear pellet which was then homogenized with five strokes of a tight pestle in a Teflon-glass homogenizer. The crude nuclear preparation was quickly diluted with 2.3 M sucrose to a final concentration of 1.90 M sucrose containing 2 mM $Mg(OAc)_2$, 3 mM $CaCl_2$, 10 mM Tris-HCl (pH 8.0), and 0.015% Triton X-100. This was then layered over a 2.0 M sucrose cushion in the same buffer and centrifuged at 70 000*g* for 60 min in the Beckman SW 27 rotor. The nuclear pellet was carefully drained and rinsed with and then resuspended in a minimum volume of 25% glycerol containing 5 mM $Mg(OAc)_2$ 50 mM NaHepes[1] (pH 8.0), and 1% bovine serum albumin. The nuclei could be used immediately or quick-frozen in liquid nitrogen and stored at −80 °C without any difference or loss in RNA polymerase activity. Nuclei prepared this way were free of cytoplasmic debris and tags as judged by light and polarizing microscopy and were obtained in yields of 25–30% as measured by nuclei counting and DNA determination.

RNA Synthesis. RNA synthesis was measured in a modified RNA polymerase I and II assay mixture as described by Reeder and Roeder (1972). The reaction mixture contained in a total volume of 50 μl: 50 mM NaHepes (pH 8.0), 5 mM $Mg(OAc)_2$, 1 mM $MnCl_2$, 2.5 mM dithiothreitol, 150 mM KCl, 10% glycerol, 1% bovine serum albumin, 1.0 mM each of the unlabeled ribonucleoside triphosphates (CTP, ATP, GTP), 0.4 mM [^{3}H]UTP (0.25–0.50 Ci/mmol), and 2.5–5.0 μg of nuclear DNA. The mixture was incubated at 25 °C and the reaction was monitored by pipetting 50-μl samples onto Whatman 3 MM filter paper discs and immediately immersing them in cold 5% trichloroacetic acid and 50 mM sodium pyrophosphate. The discs were washed three times with this solution and two times with acetone, dried, and counted in a toluene-based fluor. All cpm were corrected for background incorporation at zero time. There was a linear relationship between the observed cpm and the microliters of reaction mixture applied to the discs between 2.5 and 50 μl.

Isolation of in Vitro Synthesized RNA. The reaction mixture described above was scaled up 20- to 50-fold and the newly synthesized RNA isolated by the hot phenol–dodecyl sulfate method of Scherrer et al. (1966). A sample of the radioactive RNA (plus 50 μg/ml of rRNA carrier) was precipitated with cold 5% trichloroacetic acid and 50 mM sodium pyrophosphate, filtered, and washed on an 0.65-μm Millipore filter, and dried and counted to determine incorporation of [^{3}H]UMP into RNA.

Sucrose–Dodecyl Sulfate and Sucrose–Formamide Gradient Centrifugation. The RNA sample in 100 mM NaCl, 1 mM EDTA, 10 mM NaOAc (pH 6.0), and 0.2% dodecyl sulfate was layered over an 11-ml 5–25% linear sucrose gradient in the same buffer and centrifuged at 180 000*g* for 3.5 h at 20 °C in the Beckman SW 41 rotor. For sucrose–formamide gradient centrifugation, the RNA sample in 75% formamide, 3 mM Tris-HCl (pH 7.5), and 3 mM EDTA was heated for 5 min at 65 °C then layered over an 11-ml linear 5–15% sucrose gradient in the 75% formamide buffer and centrifuged at 180 000*g* for 24 h at 22 °C in the SW 41 rotor. Gradients were fractionated from bottom to top in 33 fractions and radioactive RNA was measured as described above. Marker RNAs (28 S, 18 S, 4 S) were prepared from [^{14}C]orotic acid-labeled Krebs ascites II cells.

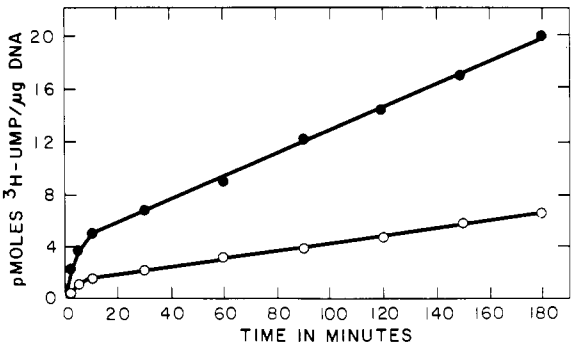

FIGURE 1: Time course of RNA synthesis in oviduct nuclei. Nuclei in the complete standard assay mixture described in Materials and Methods were incubated at 25 °C in the presence (○) and absence (●) of 2.5 μg/ml of α-amanitin. Aliquots were removed at the indicated times and acid-precipitable radioactivity was determined and converted to picomoles of [^{3}H]UMP incorporated/microgram of DNA.

Oligo(dT)-Cellulose Chromatography. Chromatography was performed by the method of Aviv and Leder (1972). The RNA sample was applied to an oligo(dT)-cellulose column (25–50 A_{260} units/ml bed volume) equilibrated with 500 mM NaCl, 10 mM Tris-HCl (pH 7.5), and 0.2% dodecyl sulfate and the column was washed with 5 to 6 volumes of the equilibration buffer. The poly(A)-containing RNA was eluted with 10 mM Tris-HCl (pH 7.5) and 0.2% dodecyl sulfate. Acid-precipitable counts in each fraction were determined and the total radioactivity (cpm) in the high ionic strength flow-through peak and low ionic strength eluate peak was calculated. Between 80 and 90% of the RNA (as cpm) was recovered from the column. Percent RNA bound was the quotient of the cpm in the eluate peak and the total recovered cpm from the column.

Results

Conditions for RNA Synthesis. The ionic composition of the assay mixture was chosen to assure that RNA polymerase I and II would both be active during the reaction. Figure 1 shows a typical time course of the incorporation of [^{3}H]UTP into an acid-precipitable product in the presence and absence of α-amanitin. Incorporation was biphasic with a burst of synthesis during the first 5 min of incubation followed by linear incorporation for at least 3 h. This burst of activity accounted for about one-quarter of the 20 pmol of [^{3}H]UTP incorporated per μg of nuclear DNA in 3 h. The rate of incorporation during the linear phase was 0.9 pmol/μg of DNA per 10 min which was one-fifth of the rate observed during the burst of activity. In the presence of the fungal toxin, α-amanitin, the same biphasic character of incorporation was evident but synthesis was reduced 60–70% throughout the incubation period. We assume that the α-amanitin sensitive activity in oviduct nuclei is RNA polymerase II since it has been shown that this concentration of α-amanitin selectively inhibits purified RNA polymerase II from rat liver, calf thymus, or sea urchin (Blatti et al., 1970). Thus, under these assay conditions, two-thirds of the incorporation is probably the result of RNA polymerase II activity.

Two of the more striking features of this time course are the extent and duration of RNA synthesis which can be at-

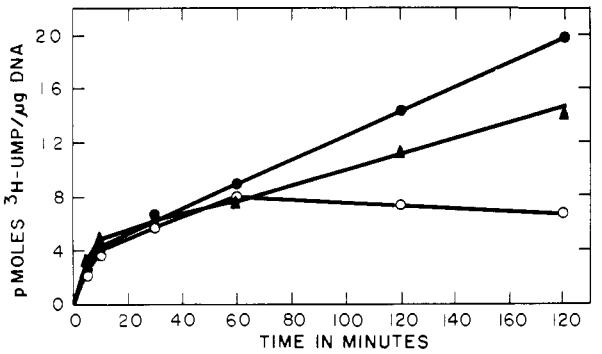

FIGURE 2: Effect of bovine serum albumin and ovalbumin on the time course of RNA synthesis. Nuclei were incubated at 25 °C in the standard assay mixture containing no supplemental protein (○), 1% bovine serum albumin (●) or 1% ovalbumin (▲). At the indicated times, acid-precipitable radioactivity was determined and converted to picomoles of [^{3}H]UMP incorporated/microgram of DNA.

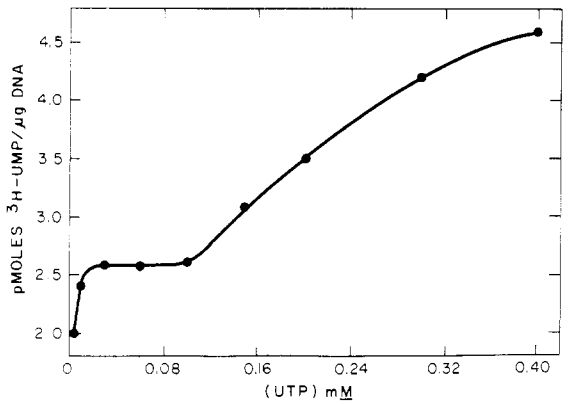

FIGURE 3: UTP concentration dependence for RNA synthesis. Nuclei in the complete assay mixture containing various concentrations of unlabeled UTP with a constant amount of [^{3}H]UTP were incubated for 15 min at 25 °C. Acid-precipitable radioactivity was determined and converted to picomoles of [^{3}H]UMP incorporated/microgram of DNA.

tributed to three factors. First is the presence of 1% bovine serum albumin in the assay mixture. The effect of bovine serum albumin on the time course of incorporation is shown in Figure 2. In the absence of bovine serum albumin, synthesis was linear from 10 to 60 min at which point the nuclei began to spontaneously lyse and incorporation stopped. Bovine serum albumin, without significantly affecting the rate of synthesis, permitted incorporation to proceed beyond 60 min. Nuclei counting in a Neubauer counting chamber revealed that nuclear counts from the 3-h incubated samples were identical with the values obtained at zero time within the 10% counting error confirming that bovine serum albumin protected the nuclei against lysis. Ovalbumin could partially substitute for bovine serum albumin (Figure 2) while polymers such as Ficoll 40 or 70 or Dextran T500 had no protective effect.

The second factor which increases incorporation is the presence of high concentrations of the ribonucleoside triphosphates and particularly the labeled nucleoside triphosphate, UTP, in the assay mixture. Figure 3 is a plot of the incorporation of [^{3}H]UTP into an acid-precipitable product after a 15-min incubation as a function of the concentration of UTP in the reaction mixture with the three unlabeled nucleoside triphosphates at a concentration of 1.0 mM. At low concentrations, UTP was rate-limiting reaching a plateau between 0.02 and 0.10 mM. Most RNA polymerase reac-

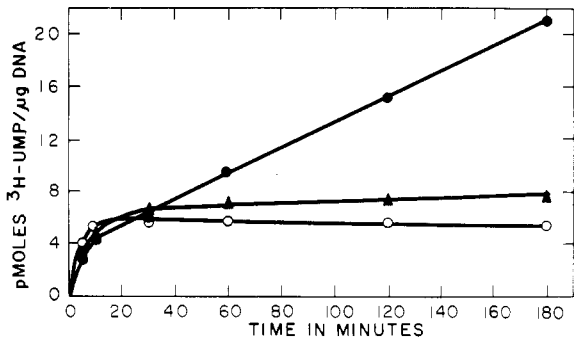

FIGURE 4: Effect of temperature on the time course of RNA synthesis. The conditions were the same as in Figure 1 (−α-amanitin) except the incubation temperature was 25 °C (●), 30 °C (▲) or 37 °C (○).

tion mixtures contain the fourth labeled nucleoside triphosphate at a concentration of 0.002–0.16 mM (Marzluff et al., 1973; Reeder and Roeder, 1972; Zylber and Penman, 1971; Jacobsen, et al., 1974; Wu and Zubay, 1974) which lie on or near the plateau of this curve. Increasing the concentration of UTP above 0.10 mM led to increasing incorporation with a doubling over the plateau value observed at 0.40 mM UTP. The inhibition by α-amanitin remained constant at 60–70% throughout the UTP concentration range indicating that high UTP concentrations did not preferentially activate one RNA polymerase enzyme form over the other (data not shown). A similar pattern was observed for UTP when [^{3}H]GTP was used as the labeled nucleoside triphosphate excluding the possibility that changes in the specific activity of UTP were responsible for this effect (data not shown).

The third factor which increases the duration of incorporation is the incubation temperature of 25 °C. The effect of temperature on the time course of incorporation is shown in Figure 4. At 37 °C, the reaction ceased within 10 min while at 30 °C, incorporation was essentially complete after 30 min. Lowering the temperature to 25 °C permitted continued linear synthesis beyond 30 min resulting in the incorporation of three to four times as much [^{3}H]UTP into acid-precipitable product at 25 °C than at 30 or 37 °C.

Thus, maximum synthetic activity requires the presence of bovine serum albumin to stabilize the nuclei, high concentrations of the four ribonucleoside triphosphates, and an incubation temperature of 25 °C for continued linear incorporation beyond 30 min. All subsequent experiments reported here were performed under these optimal conditions as described in Materials and Methods.

To demonstrate that the incorporation of [^{3}H]UTP into an acid-precipitable product by oviduct nuclei represented RNA synthesis, the effect of various RNA polymerase inhibitors was tested. Table I shows the effect of these inhibitors and various deletions from the reaction mixture on RNA synthesis at several time points during the reaction. It has already been demonstrated that 2.5 μg/ml of α-amanitin inhibited incorporation 60–70% throughout the incubation period (Figure 1) indicating that about two-thirds of the incorporation probably occurs through the activity of RNA polymerase II. Increasing the α-amanitin concentration to 500 μg/ml did not increase this inhibition any further suggesting that RNA polymerase III does not detectably participate in the reaction. Actinomycin D almost completely abolished incorporation demonstrating that synthesis was dependent upon an unmodified DNA template. Eliminating any of the three nucleoside triphosphates also

Table I: Effect of Various RNA Polymerase Inhibitors on RNA Synthesis in Oviduct Nuclei.[a]

Addition	pmol of UMP incorp./ μg of DNA at 15 min	90 min	180 min
None	5.5	12.2	21.8
α-Amanitin (2.5 μg/ml)	1.8	4.0	6.6
α-Amanitin (500 μg/ml)	1.7	4.1	6.5
Actinomycin D (5 μg/ml)	0.4	0.3	0.2
−GTP	0.2	0.1	0.1
−CTP	0.5	0.4	0.4
−ATP	0.2	0.1	0.2
RNase (5 μg)	0.1	0.1	0.1

[a] Nuclei were incubated at 25 °C in the standard assay mixture with the appropriate additions (or deletions). Acid-precipitable radioactivity was determined at 15, 90, and 180 min and converted to picomoles of [^{3}H]UMP incorporated/microgram of DNA. In experiments with RNase, samples were treated as usual but before counting, 100 μl of RNase A (50 μg/ml) in 50 mM NaHepes (pH 7.0) was applied directly to the filter paper disc. After incubation for 1 h at 25 °C, the discs were again washed with trichloroacetic acid–sodium pyrophosphate, dried, and counted. Controls not treated with RNase did not lose radioactivity during the second wash.

blocked synthesis establishing the requirement for all four ribonucleoside triphosphates and excluding the possibility that incorporation was the result of a poly(U) polymerase activity. Finally, treatment of the acid-precipitable product with RNase eliminated all incorporation proving that the labeled product was RNA.

Analysis of RNA Synthesized in Vitro. Examination of the RNA synthesized in vitro was initially complicated by the high endogenous RNase activity of oviduct nuclei (Cox, 1973) and in preliminary experiments only 4S to 5S RNA was observed. However, when 30 units/ml of RNase inhibitor from rat liver cytosol was added, there was a significant improvement in the size of the newly synthesized RNA. The addition of RNase inhibitor preserved the size of the RNA without affecting the total amount or time course of RNA synthesis as seen in the previous experiments (data not shown).

Figure 5 shows sucrose-dodecyl sulfate gradient profiles of the RNA synthesized in vitro under several conditions. In the absence of α-amanitin, the RNA synthesized during the 0–15 min burst of activity was predominantly 10–20 S with a small but significant amount of product in the higher molecular weight region (Figure 5A). Under these same conditions, mature ovalbumin mRNA sediments at 16–18 S (Shapiro and Schimke, 1975). When RNA from the 20 to 180 min linear phase of synthesis was analyzed (Figure 5B), it showed an almost identical size profile to the RNA made during the initial burst of activity suggesting that there was a continuous synthesis of similar RNA species during the entire 3-h incubation period. α-Amanitin, which inhibits RNA synthesis 60–70% in oviduct nuclei, led to a preferential reduction of newly synthesized RNA in the 10–20S region during both the early (Figure 5C) and late (Figure 5D) phases of synthesis suggesting that RNA polymerase II was responsible for transcribing much of the RNA in the 10–20S size range.

To assure that this was the true size of the in vitro synthesized RNA and not the result of aggregation during isolation, samples of RNA used for the gradients in Figure 5 were subject to sedimentation through denaturing sucrose–

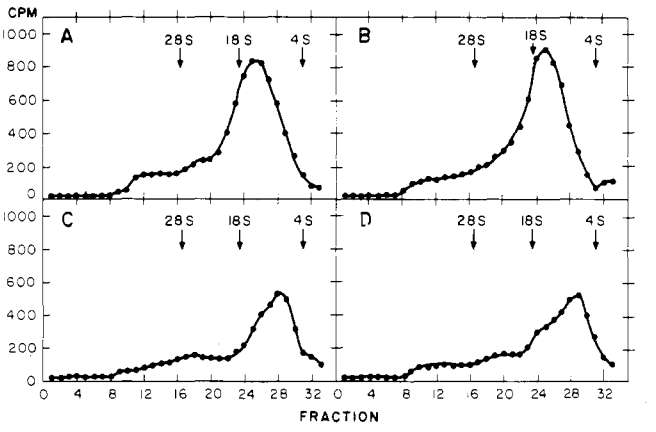

FIGURE 5: Sucrose-dodecyl sulfate gradient profiles of in vitro synthesized RNA. RNA was prepared from nuclei incubated under several conditions and analyzed by sucrose-dodecyl sulfate gradient centrifugation as described in Materials and Methods. The incubation conditions were: (A) 0–15 min at 25 °C in the complete assay mixture, (B) 20–180 min at 25 °C (preceded by 0–20 min in the absence of [^{3}H]UTP), (C) same as (A) + 2.5 μg/ml of α-amanitin, (D) same as (B) + 2.5 μg/ml of α-amanitin.

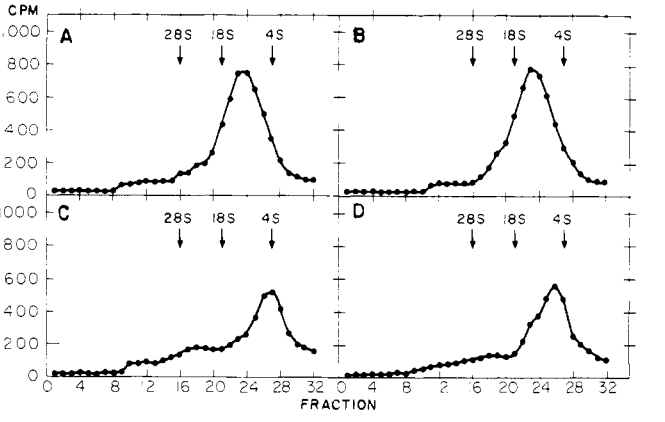

FIGURE 6: Sucrose-formamide gradient profiles of in vitro synthesized RNA. Conditions were the same as Figure 5 except sucrose-formamide gradients were used.

formamide gradients and those results are shown in Figure 6. These profiles were almost identical with those observed under the nondenaturing conditions except for a slight reduction in the *s* value of the peak from 15 S to 12 S.

The RNA synthesized in vitro was also chromatographed on an oligo(dT)-cellulose column which is expected to bind only RNA containing poly(A) sequences (Aviv and Leder, 1972) which are present in most eukaryotic mRNA and heterogeneous nuclear RNA (Darnell et al., 1971a,b). Samples of RNA from the 10–20S and the >20S regions of the gradients in Figure 5 were applied to the column at high ionic strength and, after washing, RNA retained by the absorbent was eluted at low ionic strength. Table II shows the percent RNA bound to oligo(dT)-cellulose for the RNA samples. 18% of the radioactive 10–20S RNA synthesized during the 0–15 burst of activity and 21% of the labeled 10–20S product made during the 20–180 min linear phase of incorporation were bound to the column. Very little binding of the >20S RNA was observed under the same conditions. The presence of α-amanitin during synthesis substantially reduced the binding of both early and late synthesized 10–20S RNA. These results strongly suggest that about 20% of the newly synthesized, 10–20S RNA contains poly(A) sequences and most of this RNA was probably

Table II: Poly(A) Content of RNA Synthesized in Vitro.[a]

Conditions (min)	RNA Size	% Bound
	− α-Amanitin	
0–15	10–20 S	18
0–15	>20 S	5
20–180	10–20 S	21
20–180	>20 S	4
	+ α-Amanitin	
0–15	10–20 S	3
0–15	>20 S	2
20–180	10–20 S	3
20–180	>20 S	3

[a] RNA was isolated from nuclei incubated under the conditions described in Figure 5. The RNA was fractionated on sucrose–dodecyl sulfate gradients as in Figure 5 and various size fractions were pooled and assayed for their ability to bind to oligo(dT)-cellulose as described in Materials and Methods.

transcribed by RNA polymerase II which is the enzyme form expected to synthesize cellular mRNA.

Discussion

Most of the previously described systems used for examining RNA synthesis by isolated nuclei are short-lived (10–15 min) and show very little net synthesis of RNA (0.1–2.0 pmol of nucleoside monophosphate incorporated/μg of nuclear DNA). Two noteworthy exceptions are the nuclei preparations of Huang (Marzluff et al., 1973) and Zubay (Wu and Zubay, 1974). Huang and her collaborators have devised conditions under which the synthesis of RNA by mouse myeloma nuclei was linear for 1 h at 25 °C resulting in the incorporation of 6 pmol of [^{3}H]CMP/μg of nuclear DNA. Zubay and his co-workers have chosen incubation conditions that are optimal for protein synthesis to study RNA transcription by Krebs II ascites cell nuclei. Using nuclei in the presence of cell-sap, RNA synthesis proceeded rapidly for 5–10 min followed by a slower rate of linear synthesis over the next 2 h. After 2 h of incubation, from 5 to 15 pmol of [^{3}H]GMP was incorporated per μg of DNA. In this paper, we have described the preparation of hen oviduct nuclei which are capable of incorporating 20 pmol of [^{3}H]UMP/μg of DNA in 3 h. After a 5–10 min burst of activity, RNA synthesis is linear for at least 3 h resulting in the synthesis of approximately 24 ng of RNA per μg of nuclear DNA.

A low reaction temperature, the presence of bovine serum albumin and the use of high concentrations of the ribonucleoside triphosphates all appear to contribute to the high synthetic capacity of these nuclei. One component of RNA synthesis is clearly sensitive to temperature being slowly inactivated (over 30 min) at 30 °C and rapidly inactivated (within 10 min) at 37 °C (Figure 4). Lowering the incubation temperature to 25 °C preserves this component for at least 3 h resulting in linear synthesis of RNA. It is not known whether RNA polymerase or some other factor involved in RNA synthesis is the temperature-sensitive component. The presence of bovine serum albumin in the reaction mixture is very important for preserving the integrity of the nuclei during the incubation period. Aside from providing a milieu similar to cytosol, bovine serum albumin probably binds to the nuclear membrane rendering the nuclei more resistant to the mechanical stresses resulting from 3 h of shaking during incubation (Williams, 1973). High concentrations of the ribonucleoside triphosphates enhance RNA synthesis without preferential activation of one RNA polymerase activity over the other. The concentrations used in our assays were 10- to 50-fold greater than the K_m values for the ribonucleoside triphosphates for purified calf thymus RNA polymerase I and II (Chambon, 1974). It is possible that high concentrations of the ribonucleoside triphosphates stimulate RNA synthesis by increasing the intranuclear concentration of these nucleotides in oviduct nuclei which otherwise may not be freely permeable to them. Alternatively, the free concentration of the ribonucleotides may be lowered by binding to bovine serum albumin which would be overcome at high concentrations. However, neither of these suggestions provides a satisfactory explanation for the incorporation plateau between 0.02 and 0.10 mM UTP (Figure 3). It is unlikely that a change in the concentration of UTP from 0.10 to 0.40 mM would cause structural changes in the DNA template leading to the increased transcription of RNA observed above 0.1 mM UTP.

The question of whether these oviduct nuclei initiate synthesis of new RNA molecules in vitro is still unresolved. The extended time of RNA synthesis and the continuing accumulation of newly synthesized RNA after 3 h in the range of 10% of the nuclear RNA content suggested that there might have been some in vitro initiation. In this regard, it is attractive to suggest that the burst of activity seen during the first 10 min of incubation may represent elongation and termination of in vivo initiated RNA molecules while the linear phase of synthesis from 20 to 180 min represents in vitro initiation (and elongation) at a much lower efficiency. However, at present, there is no evidence to support this hypothesis. If in vitro initiation of RNA transcription was occurring in these oviduct nuclei, then it should be possible to label the 5′-terminal end of the RNA synthesized in vitro. Incubation of oviduct nuclei in the standard reaction mixture with [γ-^{32}P]GTP or [γ-^{32}P]ATP did not result in the detectable incorporation of ^{32}P label into RNA as would be required for a 5′ terminus containing G (pppGp . . .) or A (pppAp . . .). Nuclei were also incubated with [α-^{32}P]GTP or [α-^{32}P]ATP followed by hydrolysis of the labeled RNA in 0.3 M KOH and high voltage paper electrophoresis. In either case, no ^{32}P-labeled material was detected in the position of authentic pGp or pAp which would be the product if the 5′-terminal base was G or A. Based on the specific activities of the ^{32}P-labeled nucleoside triphosphates and the size and amount of the newly synthesized RNA, we conclude that if there is any initiation of RNA transcription in these oviduct nuclei, it occurs in less than 0.05% of the 10–20S RNA molecules synthesized in vitro. Therefore, we infer that our 10–20-fold increase in RNA synthesis probably reflects a more efficient completion and release of RNA chains than in the previously described nuclei systems.

Much of the RNA synthesized in vitro by the oviduct nuclei was probably transcribed by RNA polymerase II and was of a size comparable to mature ovalbumin mRNA (Shapiro and Schimke, 1975), which apparently is not synthesized as a high molecular weight precursor (McKnight and Schimke, 1974). Approximately 20% of the newly synthesized 10–20S RNA appears to contain poly(A) sequences. This is the first evidence that these nuclei may be capable of some processing of the newly synthesized RNA since poly(A) sequences are thought to be added post-transcriptionally to the 3′ terminus of messenger and heterogeneous nuclear RNA (Darnell et al., 1971b; Adesnik et al., 1972). The critical question that remains to be answered is:

are ovalbumin mRNA molecules synthesized in vitro by oviduct nuclei? Detection by hybridization to complementary DNA made to ovalbumin mRNA is complicated by the presence of as much as 12 ng of unlabeled ovalbumin mRNA per μg of nuclear DNA most of which is the result of cytoplasmic contamination (M. J. Ernest and G. Schutz, unpublished observations). Once this problem is overcome, isolated oviduct nuclei should provide an attractive system for studying the regulation of ovalbumin mRNA synthesis at a level intermediate between the whole oviduct and isolated chromatin.

Acknowledgment

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