Figure 5A shows the IPTG-mediated equilibrium release from operator of a mixture of separately renatured Q and B11 repressors. This profile serves as the control for the results obtained when Q and B11 monomers are mixed and renatured together, as shown in Figure 5B. Clearly the joint renaturation of mixtures of two kinds of monomers results in active repressors with new operator binding properties, intermediate to those of the "parental" repressor types. It also provides an independent demonstration that we are, in fact, obtaining repressor subunits by the procedure employed here; beyond that, the data presented in Figure 2 suggest the presence of three repressor types, with respect to operator binding, in the mixed tetramer population. Similar results were obtained with Q: QX86 mixed tetramer populations.

Discussion

In the present work we have found that the operator binding activity of lactose repressor can be recovered in excellent yields from repressor monomers purified in sodium dodecyl sulfate–glycerol gradients. Renatured–reassociated repressor tetramer populations generally show the same operator binding characteristics as the untreated stock repressors from which they were prepared. This technique offers a useful tool in the analysis of the repressor–operator interaction, particularly with regard to mixed tetramers, composed of normal and mutationally or chemically altered subunits.

As shown in Figure 5, the joint renaturation of two types of repressor subunit (Q and B11) leads to active repressor with operator binding properties intermediate to those of the parental repressor species. This outcome suggests that more than one repressor subunit is involved in the operator interaction.

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Fidelity of Chromatin Transcription in Vitro†

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ABSTRACT: Chromatin and DNA from Schneider's *Drosophila melanogaster* cell line 2 were transcribed in vitro with *Escherichia coli* RNA polymerase. Using mercurated UTP as precursor, the newly synthesized RNA could be separated from DNA and endogenous RNA by affinity chromatography on sulfhydryl-Sepharose 6B. Characterization of the transcription products with complementary DNA (cDNA) made from polyadenylated nuclear RNA and with fractionated cDNA probe demonstrated a fair quantitative fidelity in the in vitro transcript from chromatin which was not evident when DNA was transcribed. However, as shown by hybridization to total nuclear RNA, *E. coli* RNA polymerase transcribed both DNA strands from chromatin in vitro. We conclude that *E. coli* polymerase is able to distinguish sections of chromatin at which rapid synthesis of RNA occurs in the cell.

Early attempts to measure the fidelity with which chromatin is transcribed in vitro used relatively crude hybridization competition methods. It was concluded that the RNA made in vitro differed according to the source of the chromatin transcribed and resembled that in the cell or tissue from which it was prepared (Paul and Gilmour, 1968; Smith et al., 1969; Huang and Huang, 1969; Bekhor et al., 1969; Tan and Miyagi, 1970). However, since hybridization conditions employed allowed only the reaction of repetitive sequences, no precise conclusions could be drawn concerning the overall fidelity of in vitro transcription.

A more sensitive approach measures the concentration of a specific sequence in the in vitro transcript by hybridization to a radioactive probe (cDNA[1]) complementary to the se-

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[1] Abbreviations used: cDNA, complementary DNA; R_0t, product of the total RNA concentration (R_0) in molarity of nucleotides and time (t) in seconds; EDTA, ethylenediaminetetraacetate disodium salt; SSC, standard saline–citrate; Tris, tris(hydroxymethyl)aminomethane; poly(A), poly(adenylate); poly(U), poly(uridylic acid); PBS, 0.14 M NaCl–3 mM KCl–9.5 mM Na_2HPO_4–1.5 mM KH_2PO_4; TNE, 10 mM Tris-HCl (pH 7.4)–100 mM NaCl–1 mM EDTA.

quence of interest. Several systems have been analyzed in this way, including those for globin (Paul et al., 1973; Axel et al., 1973), histones (Stein et al., 1975), 5S ribosomal and tRNA (Marzluff and Huang, 1975), ribosomal RNA (Reeder, 1973), integrated SV40 DNA (Shih et al., 1973; Astrin, 1973), and Murine leukemia virus (Janowski et al., 1974). With the notable exception of the ribosomal genes, these reports did suggest some degree of transcriptional fidelity when either *Escherichia coli* RNA polymerase or homologous RNA polymerase was employed.

Although the use of probes for specific sequences has added another degree of sensitivity to the characterization of the in vitro transcript, this approach leaves the greater portion of the transcript uncharacterized. The experiments presented below attempt to broaden previous studies by using a probe which represents a substantive fraction of the RNA sequences transcribed in vivo, i.e., a complementary DNA synthesized with nuclear poly(A)-containing RNA as template. The experiments were conducted under conditions where contamination of the in vitro product with DNA and endogenous RNA was completely eliminated.

Materials and Methods

Preparation of Chromatin and DNA. Drosophila tissue culture cells (Schneider's cells, line 2) were grown as described elsewhere (McCarthy et al., 1973). Exponentially growing cells were collected by centrifugation at 4000 rpm for 10 min at 4 °C and washed once with PBS (0.14 M NaCl–3 mM KCl–9.5 mM Na_2HPO_4–1.5 mM KH_2PO_4). All further manipulations were done at 4 °C. For a typical preparation, 1 l. of cells cultured in roller bottles was broken by homogenization with a tight-fitting pestle in a Dounce homogenizer (20 strokes) in 75 ml of 0.3 M sucrose–2 mM magnesium acetate–3 mM $CaCl_2$–10 mM Tris-HCl (pH 8.0)–0.5 mM dithiothreitol–0.2% Nonidet P 40 (Shell Oil Co.). An equal volume of 2 M sucrose–10 mM Tris-HCl (pH 8.0)–0.5 mM dithiothreitol was added and the homogenate layered over a cushion of 10 ml of the above 2 M sucrose solution. After carefully stirring the interphase, nuclei were pelleted by centrifugation in a Beckman SW27 rotor at 15 000 rpm for 45 min at 4 °C. The nuclear pellet was washed once in 10 mM Tris-HCl (pH 8.0) and collected at 1000*g* for 10 min. The nuclei appeared to be free of cytoplasmic contamination as judged by phase contrast microscopy. Nuclei were taken up in 3–5 ml of 10 mM Tris-HCl (pH 8.0) and disrupted by passing through the French pressure cell at 1000 psi. The supernatant after another centrifugation at 1000*g* for 10 min in a Sorvall HB4 rotor was used within 24 h as "chromatin" in the in vitro transcription experiments. DNA was prepared from this chromatin by making the supernatant 0.5% in sodium dodecyl sulfate, 0.1 M in NaCl, and 0.01 M in EDTA and extracting once with an equal volume of phenol–chloroform (1:1) and twice with chloroform containing 4% isoamyl alcohol. DNA was precipitated overnight with 2 volumes of ethanol at −20 °C and collected at 16 000*g* for 40 min. The size distribution of the DNA in our chromatin preparation was determined on a 0.9% agarose gel using 0.09 M Tris–2.5 mM EDTA–0.09 M boric acid buffer. After electrophoresis the gel was stained with ethidium bromide and photographed and the negative was scanned in a Beckman spectrophotometer.

Preparation of Mercurated UTP. Mercurated UTP was synthesized following the procedure of Dale et al. (1975). A solution of 20 mM UTP in 0.1 M sodium acetate, pH 6.0, was incubated at 50 °C with the same volume of freshly prepared 0.1 M mercuric acetate in the same buffer. Most of the unreacted mercury ions were removed by passing the reaction mixture over a Sephadex G-10 column and the mercurated UTP was further purified by affinity chromatography on DEAE-cellulose (Whatman DE52). The compound was batch-eluted with 0.5 M triethylammonium hydron carbonate, pH 8.0, evaporated to dryness and washed three times with methanol. It was suspended at a concentration of 1–4 mg/ml in 10 mM Tris-HCl (pH 7.4) and stored at −20 °C. The mercurated UTP was characterized by its spectral properties (Dale et al., 1975) and by thin-layer chromatography on poly(ethylenimine)cellulose (Brinkman Instruments) with increasing LiCl solutions as running buffer (Randerath and Randerath, 1964). No unreacted UTP or breakdown products could be detected.

In Vitro Transcription. Chromatin or DNA was transcribed in vitro using essentially the incubation procedure described by Astrin (1975). The 5-ml reaction mixture contained 150 mM KCl, 40 mM Tris-HCl (pH 8.0), 0.1 mM dithiothreitol, 5 mM $MgCl_2$, 1 mM $MnCl_2$, 4 mM of each ATP, GTP, CTP (Sigma), 1 mM HgUTP, 20 mM 2-mercaptoethanol, and 0.5 mg/ml chromatin or DNA as template and 0.8 mg of *E. coli* RNA polymerase per mg of template purified according to Burgess (1969) through the first glycerol gradient. In experiments in which the transcript was to be labeled the CTP concentration was lowered to 1 mM and 30–100 μCi of [^{3}H]CTP (23 Ci/mmol, Schwarz/Mann) were added per ml of reaction mixture. After 4 h incubation at room temperature the solution was made 0.5% in sodium dodecyl sulfate and 10 mM in EDTA. One volume of water-saturated phenol was added and the mixture was vortexed. After adding another volume of chloroform, the mixture was vortexed again. Phases were separated by brief centrifugation (1 min, 15 000*g*) and the lower phase was removed. The aqueous phase and interphase were reextracted two or three times with chloroform + 4% isoamyl alcohol and precipitated with 2 volumes of ethanol overnight at −20 °C.

Purification of the in Vitro Synthesized RNA. The precipitated nucleic acids were dissolved in 1–2 ml of water and made 1 × TNE (10 mM Tris-HCl (pH 7.4)–100 mM NaCl–1 mM EDTA). Triphosphates were separated from the higher molecular weight material by chromatography on Sephadex G-50 medium. The material in the excluded volume was collected and precipitated again with 2 volumes of ethanol. After dissolving in 1 ml of TNE, the in vitro synthesized RNA, containing HgUTP, could be separated from DNA and endogenous RNA by affinity chromatography on sulfhydryl-Sepharose 6B. The resin was prepared according to Cuatrecasas (1970) and equilibrated in TNE. After incubating for 30 min on a 5-ml column, the unbound material was passed over the column again with the resin washed extensively with TNE until no more nucleic acid could be removed. The bound material was eluted with TNE containing 0.1 M 2-mercaptoethanol and precipitated with 2 volumes of ethanol at −20 °C. Nonspecific binding of unmercurated ^{3}H-labeled RNA to the column was negligible (see Table I).

Preparation of Nuclear RNA and Synthesis of cDNA. Nuclear RNA from *Drosophila* cells was prepared as described elsewhere (Levy W. et al., 1976 submitted for publication). The polyadenylated fraction of nuclear RNA was isolated by chromatography on poly(U)-Sepharose and the cDNA probe was synthesized from that poly(A$^+$) nuclear RNA as described previously (Levy W. and McCarthy, 1975). To obtain labeled nuclear RNA, *Drosophila* cells were grown in a spinner flask to 4 × 10^6 cells/ml (midlog) collected by centrifugation and concentrated tenfold using fresh medium.

TABLE I: Functional Test of Sulfhydryl-Sepharose 6B Column.[a]

Preparation Applied to the Column	^{3}H-Ribosomal RNA	^{3}H-Nonpolyadenylated RNA + Unlabeled in Vitro Synthesized Mercurated RNA 1:1 Ratio	^{203}Hg
% not retained	94	91	2
% eluted with TNE + 2-mercaptoethanol	0.05	0.2	97.3
% remaining in column	UD[b]	UD[b]	0.7

[a] ^{3}H-Labeled ribosomal RNA (8×10^5 cpm), [^{3}H]poly(A$^-$) nuclear RNA (43.8×10^3 cpm) mixed in 1:1 ratio with unlabeled in vitro synthesized mercurated RNA, or ^{203}Hg (New England Nuclear, 7.6×10^5 cpm) in TNE was loaded on a 1-ml sulfhydryl-Sepharose 6B column and incubated for 30 min. The unbound material was washed off with 20 volumes of TNE and the bound material was eluted with TNE + 0.1 M 2-mercaptoethanol. Total (in the case of ^{203}Hg) or Cl_3CCOOH-precipitable radioactivity was determined in the combined fractions. [b] UD, undetectable.

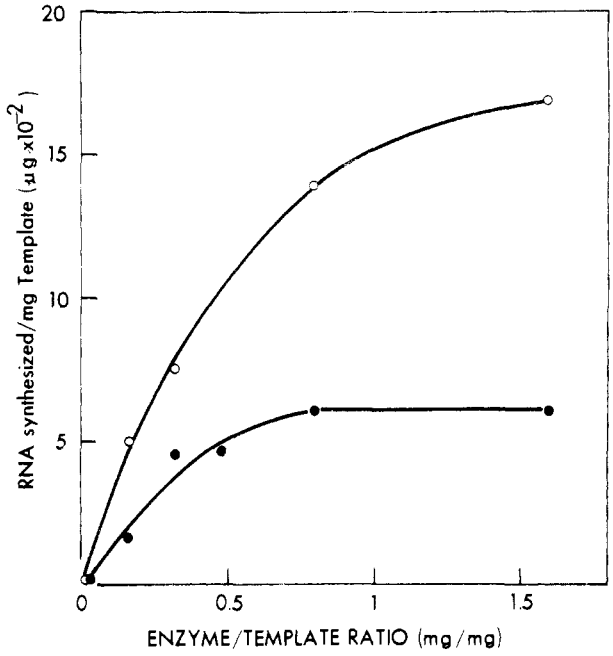

FIGURE 1: In vitro transcription of chromatin (●) and DNA (○) with increasing amounts of *E. coli* RNA polymerase. After 3 h of incubation of the reaction mixture with [^{3}H]CTP (5 μCi/ml), the Cl_3CCOOH-precipitable radioactivity was determined.

RNA was labeled by adding 10 μCi/ml of [^{3}H]uridine (28 Ci/mmol; Schwarz/Mann) and incubating for 20 min.

cDNA Fractionation. Aliquots of cDNA were annealed with nuclear RNA to a R_0t of 12 M s and the double-stranded cDNA fraction was separated from unreacted material by adsorption on hydroxylapatite as described earlier (Levy W. and McCarthy, 1976).

Hybridization Reactions. Hybridizations of the in vitro transcripts with the total or fractionated cDNA probe made from poly(A$^+$) nuclear RNA were carried out in 0.24 M equimolar phosphate buffer containing 1 mM EDTA and 0.01% sodium dodecyl sulfate. cDNA (500–1000 cpm) and the RNA or DNA were sealed in 5-μl capillaries, boiled for 10 min, and incubated at 70 °C for the desired periods of time. At the end of the incubation, the samples were challenged with S1 nuclease as described by Leong et al. (1972). A zero time hybridization value (less than 10%) was subtracted as background from the experimental points. Hybridizations of unlabeled in vitro transcribed RNA with labeled total nuclear RNA were performed in 6 × SSC, 45% formamide at 50 °C as described recently (Obinata et al., 1975) except that 0.01% sodium dodecyl sulfate was added. After the desired incubation time the samples were diluted into 1 ml of 2 × SSC and incubated with 5 μg/ml pancreatic RNase (Sigma) for 30 min at 37 °C. Cl_3CCOOH-precipitable material was collected on glass-fiber filters (Whatman, GF/C) and washed with cold 5% Cl_3CCOOH + 10 mM sodium pyrophosphate and ethanol. Filters were counted in Omnifluor solution in a Beckman liquid scintillation spectrometer.

Results

Characterization of in Vitro Transcription. In most in vitro transcription systems studied, it has proved difficult to attain a precise characterization of the transcription product due to two inherent difficulties. In the first place, a high yield of the in vitro synthesized transcript must be obtained to permit completion of RNA excess hybridization reactions. Second, this transcript must be isolated from the reaction mixture free of DNA and the endogenous RNA associated with chromatin since both contaminants complicate interpretation of the results of hybridization assays. The first problem has been solved through use of the transcription system described by Astrin (1975) in which transcription continues for longer periods of time; the second, by applying the mercuration technique of Dale et al. (1975) and Dale and Ward (1975).

Data in Figure 1 illustrate the amount of RNA synthesized from DNA or chromatin at increasing polymerase/template ratios. Transcription was conducted as described in Materials and Methods except that all four triphosphates were present at 4 mM and 5 μCi/ml [^{3}H]CTP (specific activity 23 Ci/mmol) was added. No HgUTP was used in this experiment. Cl_3CCOOH-precipitable radioactivity was determined after 3 h of incubation. DNA was transcribed with a higher efficiency than was chromatin. The rate of transcription of chromatin was maximal at a polymerase/template ratio of about 0.8. This same ratio was used in all further experiments in which chromatin and DNA were transcribed.

The size of DNA in the slightly sheared chromatin preparation used in the experiments was determined by electrophoresis on a 0.9% agarose gel (Figure 2). DNA fragments of known size were obtained by digestion of *E. coli* phage λ DNA with the Eco RI restriction endonuclease (Thomas and Davis, 1975). The DNA fragments isolated from chromatin showed a broad size distribution with most in the higher molecular weight range up to about 14×10^6.

The in vitro transcription kinetics from DNA and chromatin as template using a polymerase/template ratio of 0.8 and 4 mM of each ATP, GTP, and CTP, 1 mM UTP, and 5 μCi/ml [^{3}H]CTP are shown in Figure 3. Under these conditions the reaction progressed for 4 h and about 0.6 mg of RNA was synthesized for each mg of chromatin and more than twice as much from DNA. We assume that reinitiation of new RNA chains occurred in vitro. Although chromatin acted as a restricted template in vitro compared with DNA, the amount of RNA synthesized was sufficient to carry out the desired hybridization assays. Endogenous polymerase attached to chromatin remained active for about 20 min and synthesized about 5 μg of RNA per mg of chromatin. Attempts have been

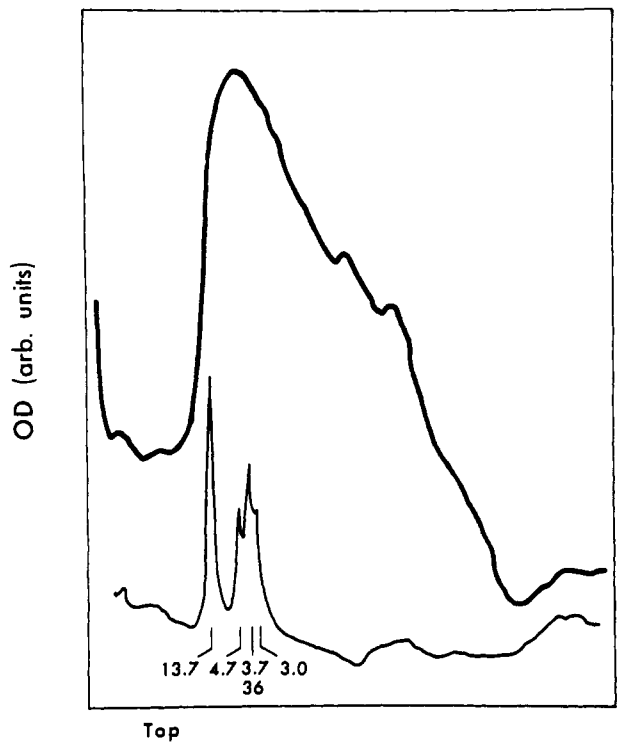

FIGURE 2: Size distribution of DNA in chromatin (upper curve). Scan of a photograph taken from a 0.9% agarose gel stained with ethidium bromide. Eco RI endonuclease fragments of λ phage DNA were used as size markers (lower curve).

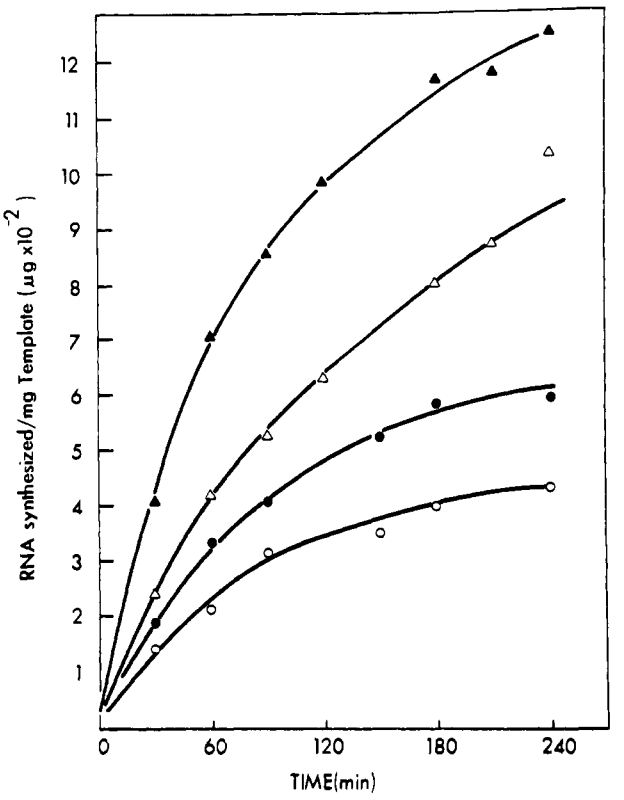

FIGURE 3: Kinetics of in vitro transcription reaction from chromatin or DNA as template. ATP, GTP, and CTP were 4 mM; 5 μCi/ml [^{3}H]CTP. DNA template, 1 mM UTP (▲); DNA template, 1 mM HgUTP (△); chromatin template, 1 mM UTP (●); chromatin template, 1 mM HgUTP (○).

made to isolate this RNA synthesized by the endogenous enzyme but yields were not sufficient for analysis by hybridization.

Substitution of HgUTP for UTP in the reaction mixture reduced the rate of RNA synthesis with either DNA and chromatin as template. This is in agreement with the results of Dale and Ward (1975) who reported similar kinetics using poly[d(A-T)] as template. The amount of RNA synthesized after 4 h with HgUTP as precursor was about 0.4–0.5 mg/mg of chromatin and about 1 mg/mg of DNA.

Nucleic acids were extracted from the transcription mixture after 4 h of incubation as described in Materials and Methods and separated from excess triphosphates by chromatography on Sephadex G-50. After a second ethanol precipitation they were applied to a sulfhydryl-Sepharose 6B column and eluted with TNE buffer. The bound material was recovered with TNE + 0.1 M 2-mercaptoethanol. This separation is shown in Figure 4, using [^{3}H]CTP labeled in vitro synthesized RNA which had incorporated HgUTP. The nucleic acids which did not contain mercury were washed off the column and appeared in the first few fractions (not shown). Some mercury-containing material did not bind to the resin as could be shown by rechromatography. This could be due to a relatively slow replacement of the mercury-bound 2-mercaptoethanol by the sulfhydryl groups of the resin or simply by an overloading effect. The portion of the mercurated RNA that did not bind to the column varied between 10 and 40%. The RNA bound and eluted with TNE–2-mercaptoethanol was ethanol percipitated and applied to a 15–30% sucrose gradient for size determination. As shown in Figure 5, the RNA sedimented with a peak at about 5 S although a major fraction was greater than 10 S.

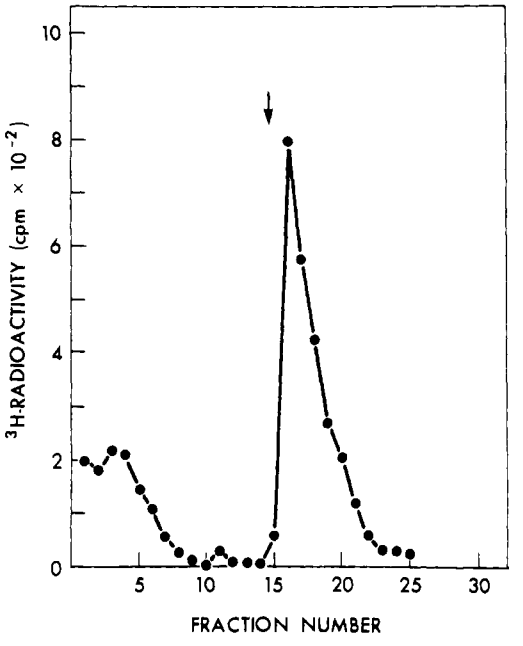

FIGURE 4: Chromatography of [^{3}H]CTP labeled in vitro synthesized RNA containing HgUTP on a sulfhydryl-Sepharose 6B column. The arrow indicates the beginning of the elution with TNE + 0.1 M 2-mercaptoethanol. Fractions of 1 ml were collected and the Cl_3CCOOH-precipitable radioactivity was determined.

Characterization of the in Vitro Transcription Product. Several hybridization experiments were performed with the RNA transcribed in vitro from chromatin or DNA. Figure 6 shows the kinetics of the reaction with a cDNA probe complementary to polyadenylated nuclear RNA. The hybridization kinetics of this cDNA probe with its own template is included for comparison. The experimental data show that the in vitro

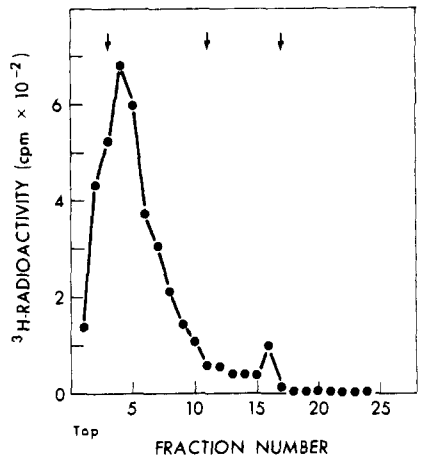

FIGURE 5: Size distribution of in vitro synthesized RNA from chromatin after purification on sulfhydryl-Sepharose 6B column. [^{3}H]CTP labeled RNA was loaded on a 15–30% sucrose gradient in 10 mM Tris-HCl, pH 7.4, 100 mM NaCl, 1 mM EDTA, 0.5% sodium dodecyl sulfate in a SW40 rotor and centrifuged at 25 000 rpm for 15 h at 24 °C. Reading from left to right the arrows indicate the positions of 5, 19, and 26S ribosomal RNA markers run in a parallel gradient.

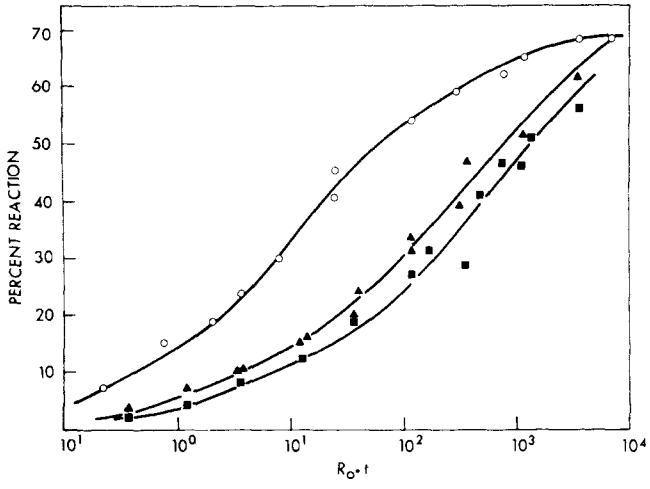

FIGURE 6: Kinetics of the hybridization of cDNA made from polyadenylated nuclear RNA to its own template (○), to RNA synthesized in vitro from chromatin (▲) and from DNA (■).

synthesized RNA reacts with the cDNA probe to the same extent as does polyadenylated nuclear RNA, implying that *E. coli* RNA polymerase had transcribed most of the sequences represented by that probe. However, the reaction between the in vitro transcript and the probe was slower by a factor of about ten than the reaction with the in vivo poly(A$^+$) nuclear RNA, implying dilution of those sequences by others present in the in vitro transcript. The relatively small size of the transcript would also tend to produce slower kinetics of hybridization.

The rate of hybridization of the in vitro DNA transcript with the cDNA probe was displayed by a further factor of two to three. While all sequences represented by the probe were also transcribed from naked DNA, they make up a smaller proportion of the total mass than in the case of chromatin.

These experiments show only that all of the polyadenylated nuclear RNA sequences produced in vivo are represented in the in vitro product. A question of more critical interest is whether any quantitative distinction is made in the in vitro system such that the frequency distribution of the population of in vitro RNA reflects that in vivo.

In the case of cytoplasmic polyadenylated RNA of *Drosophila* cells, it is already clear that the frequency distribution in vivo is very broad (Levy W. and McCarthy, 1975). Parallel experiments have permitted the same conclusion for nuclear polyadenylated RNA (unpublished results). We therefore sought to ask the same question for the in vitro product. The cDNA probe representing nuclear polyadenylated RNA was fractionated by incomplete annealing with nuclear RNA. This resulted in two cDNA probes enriched for representation either of frequent or rare sequences in the population of nuclear RNA. Successful fractionation was validated by annealing with nuclear RNA, the kinetics of which are illustrated in Figure 7. Previously hybridized cDNA reacted much more rapidly than did the unreacted material. In comparing the kinetics in Figures 6 and 7, it should be stressed that polyadenylated nuclear RNA was used to construct reference curves in the former case and total nuclear RNA in the latter. Since polyadenylated nuclear RNA comprises some 10% of the total nuclear RNA (Levy W. and McCarthy, 1976), $R_0t_{1/2}$ values are correspondingly tenfold higher when total nuclear RNA is the driver.

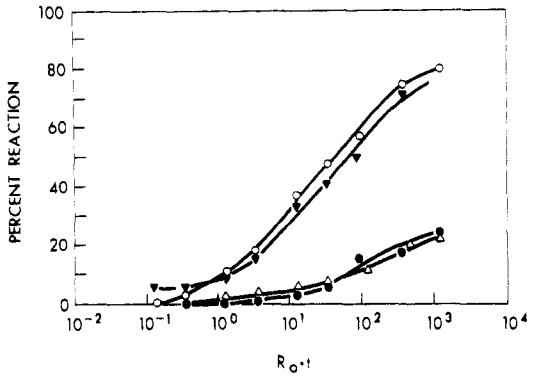

FIGURE 7: Hybridization kinetics of the fractionated cDNA probe to different RNA populations. Probe enriched in frequent sequences hybridized to total nuclear RNA (○), to in vitro chromatin transcript (▲); probe enriched in infrequent sequences hybridized to total nuclear RNA (△) and to in vitro chromatin transcript (●).

A similar displacement between the two curves was obtained when the transcript from chromatin was used to drive the fractionated cDNA. Therefore it appears that the *E. coli* RNA polymerase preferentially transcribes sequences in chromatin which are also represented by the most abundant RNA in the cell nucleus. Experiments using RNA synthesized with DNA as template resulted in only a threefold lateral displacement between the curves for abundant or total cDNA (Figure 8A), whereas the displacement using in vitro chromatin transcript was tenfold (Figure 8B). Therefore it is clear that the chromatin transcript is much more representative of the in vivo frequency distribution than is the case for the transcript from DNA. However, the difference obtained in Figure 8B remains to be explained. Perhaps some vestiges of the preferential transcription remain when deproteinized DNA is the template. Alternatively, the result could be attributed to gene dosage if some of the nuclear cDNA represents repetitive DNA. That the latter explanation is correct is demonstrated by the kinetics of hybridization of fractionated nuclear cDNA with DNA (Figure 9), showing that random transcription together with some gene dosage effect can account for the hybridization kinetics of the DNA transcript with fractionated cDNA. The much greater differences observed in the case of the chromatin transcribed cannot be explained in this manner.

Evidence for Incorrect Strand Selection. To examine the symmetry of in vitro transcription, an excess of transcribed

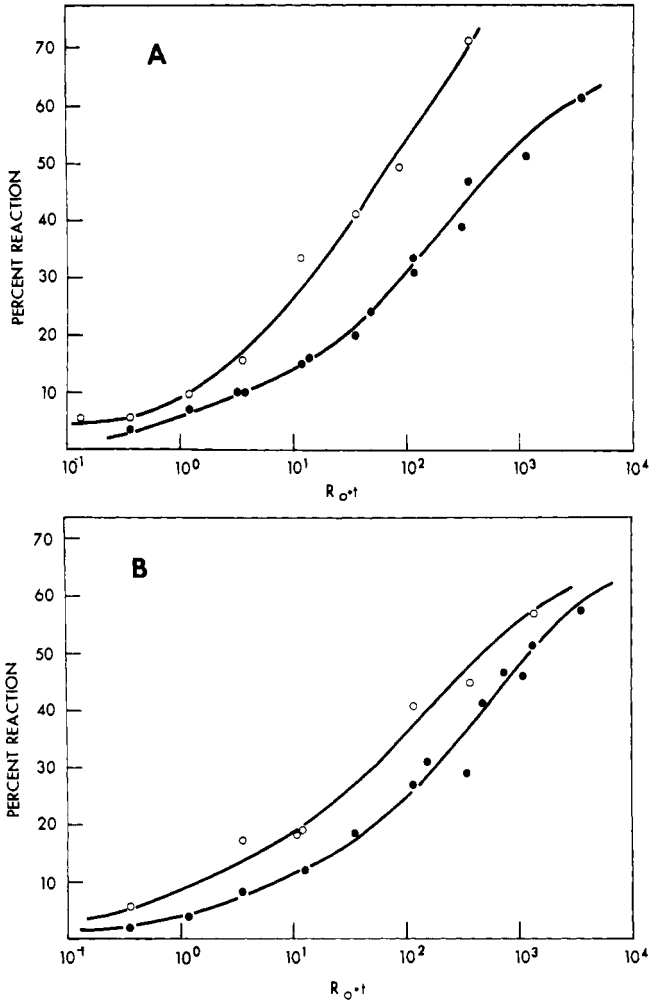

FIGURE 8: Kinetics of the hybridization of in vitro chromatin transcript (A) and in vitro DNA transcript (B) to cDNA complementary to polyadenylated nuclear RNA (●) and cDNA probe enriched in frequent nuclear sequences (○).

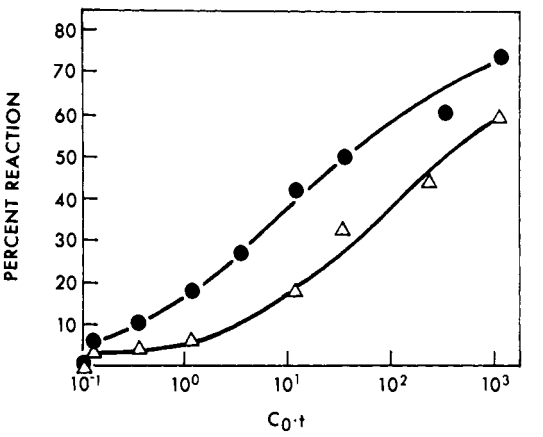

FIGURE 9: Kinetics of the hybridization of total DNA (sheared at 12 000 psi) to the fractionated cDNA probe. Hybridization between frequent nuclear cDNA and DNA (●) and between infrequent nuclear cDNA and DNA (△).

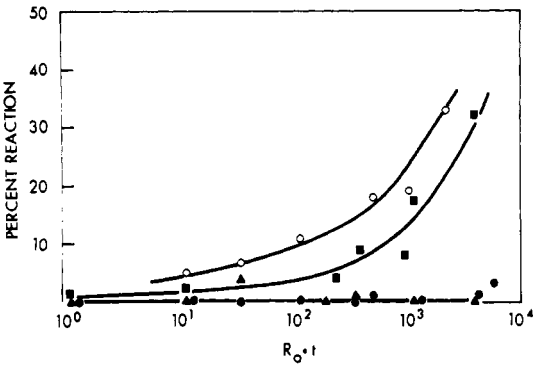

FIGURE 10: Hybridization kinetics of labeled total nuclear RNA to in vitro chromatin transcript (○), in vitro DNA transcript (■), total unlabeled nuclear (▲) and cytoplasmic RNA (●).

RNA was hybridized with labeled total nuclear RNA. As shown in Figure 10, the transcripts from chromatin and DNA both contain RNA derived from the incorrect strand of DNA. The observed hybridization of the transcripts to nuclear RNA was not ascribable to self-annealing of the nuclear RNA or DNA contamination. Due to the low frequency of these transcripts of the incorrect strand in the total RNA population the hybridization reaction with total nuclear RNA could not be driven to completion. However, when compared with the hybridization kinetics to the cDNA probe, the curves of the hybridization to nuclear RNA were shifted some 20-fold to higher R_0t values. This was true both for the transcript from chromatin or DNA, suggesting that *E. coli* polymerase preferentially transcribes the right strand from accessible regions of chromatin or from DNA. Again, the hybridization curve of the DNA transcript was displaced to higher R_0t values compared with that of the chromatin transcript. Control experiments in which the driver was nuclear or cytoplasmic RNA showed very little double-stranded RNA formation demonstrating that in vivo RNA has a high degree of strand asymmetry.

Discussion

The question as to whether isolated chromatin retains its specific restriction in vitro and whether signals which are involved in regulation of gene expression are recognized by *E. coli* RNA polymerase has been the subject of several investigations. However, the analysis of the in vitro transcribed RNA sequences from chromatin has proved difficult because of several technical problems: first, the in vitro transcript must be free of DNA which also would hybridize to the probe. Some workers have centrifuged the mixture after transcription to remove most of the DNA, leaving the RNA in the supernatant (Paul and Gilmour, 1968). Treatment of the isolated nucleic acids with DNase as recommended by Axel et al. (1973) proved unsatisfactory in our hands since the fragments of degraded DNA could not be totally separated from the newly synthesized RNA by gel filtration. This procedure may also cause degradation of RNA as a result of impurities in the DNase. A further difficulty arises from the necessity to separate the newly synthesized RNA from endogenous RNA associated with the chromatin template. In some cases, the amount of endogenous RNA can be equal to the newly synthesized RNA (Wilson et al., 1975a).

The use of mercurated UTP as precursor obviates both the difficulties and allows high yields of in vitro synthesized RNA (Crouse et al., 1976; Smith and Huang, 1976). As shown by Dale and Ward (1975), this precursor is incorporated into RNA in the presence of a 20-fold excess of 2-mercaptoethanol. The kinetics of the RNA transcription reaction were only slightly slower as compared when using UTP, reflecting the higher K_M value of *E. coli* RNA polymerase with the mercurated compound. In the experiments reported here HgUTP concentrations of 1 mM were used in the absence of UTP to

avoid any preferential incorporation of the unmodified precursor. Under those conditions the RNA synthesis continued for 4 h at room temperature resulting in a high yield of newly synthesized RNA. Because of the high specificity of the sulfhydryl-Sepharose 6B column, this RNA could be obtained free from other nucleic acids which would interfere with the hybridizations.

Although *E. coli* RNA polymerase binds to different sites in chromatin compared with eucaryotic RNA polymerases (Keshgegian and Furth, 1972; Cedar, 1975), it does appear to recognize some structural features in chromatin. This conclusion was drawn from experiments in which the presence or absence of a particular RNA sequence in the in vitro transcript was analyzed by hybridization to the appropriate cDNA probe (Axel et al., 1973; Paul et al., 1973; Stein et al., 1975; Chiu et al., 1975). In contrast, other experiments have revealed the transcription of DNA sequences representing parts of the genome known to be repressed in vivo (Reeder, 1973; Honjo and Reeder, 1974; Wilson et al., 1975b). The latter findings have raised some doubts about the degree of fidelity of chromatin transcription.

In the light of these conflicts we sought to carry out tests of fidelity with a probe representing a large fraction of the total RNA synthesized in vivo. Using a cDNA made from poly(A)-containing nuclear RNA, we observed about a tenfold shift of the $R_0t_{1/2}$ values between the hybridization curve of this probe to in vivo poly(A^+) nuclear RNA and in vitro synthesized RNA from a chromatin template. Although most of the sequences present in the poly(A^+) nuclear RNA population were also transcribed in vitro, they appear to be diluted with other sequences not represented in the probe. However, the use of the hybridization curve of the cDNA to its own template as reference presents several difficulties. In the first place it does not take account of nuclear sequences lacking poly(A) which are probably also synthesized in vitro. Further, it must be stressed that the abundance of different RNA molecules in the nucleus reflects a balance between the rates of biosynthesis and of processing. In the chromatin transcription system the relative abundance of different sequences at the end of the reaction depends only upon the relative efficiency with which they are produced by the *E. coli* polymerase. Therefore there need be no agreement between the range of R_0t values in which in vitro and in vivo RNA drives the same cDNA probe. It can only be concluded that all the sequences represented by the nuclear cDNA probe are synthesized in vitro and that the proportion of the total population which they represent is about three times higher for the chromatin transcript than for that from DNA. If we assume that the transcript from DNA is essentially random, then it seems to follow that in this case transcription from chromatin as template is only some three times better than random.

This line of reasoning may not, however, be correct, for it neglects the fact that the frequency distribution of RNA molecules in vivo is very broad. As a result of differences in the rate of biosynthesis and processing some sequences in both nuclear and cytoplasmic RNA exist in much higher numbers than others (Bishop et al., 1974; Levy W. and McCarthy, 1975; Ryffel and McCarthy, 1975; Getz et al., 1975). This phenomenon suggests that more meaningful questions can be posed regarding the total population of RNA transcribed from chromatin. Are chromosomal sites, which code for the most abundant RNA in the cell nucleus, preferentially transcribed in the chromatin system? The use of a cDNA probe fractionated to represent the most abundant sequences in nuclear RNA allowed us to approach this question. This experiment yielded a very positive result in that the rate of hybridization with abundant cDNA was some hundred fold greater than for the probe representing rarer sequences. Therefore it is clear that different sites in chromatin are transcribed with relative efficiencies which differ by two orders of magnitude. We can conclude that this difference in efficiency is related to in vivo abundance. However, we cannot eliminate the formal possibility that relative abundance is controlled predominantly by processing rather than synthesis in vivo and that this correlation between in vivo and in vitro abundance is a fortuitous result of aberrant transcription. Nevertheless, we feel that this result together with those concerned with the transcription of specific RNA sequences from chromatin (Paul et al., 1973; Axel et al., 1973; Shih et al., 1973; Astrin, 1973; Reeder, 1973; Stein et al., 1975) implies that the *E. coli* enzyme can recognize some features of chromatin structure which directly relate to preferential transcription in the cell. That this is attributable to chromatin structure rather than DNA base sequence is demonstrated by the failure of the DNA transcript to preferentially drive abundant cDNA to a degree greater than that explicable by gene dosage effects. Among other reports that the transcription of eucaryotic DNA by *E. coli* polymerase is essentially random is that of Maio and Kurnit (1974) who showed that about 10% of the transcript of mouse L-cell DNA represented satellite DNA.

Despite the preferential transcription of chromatin sites, it does appear that some aberrant transcription occurs. Certainly a considerable amount of transcription from the incorrect strand is evident. This conclusion is in agreement with several other reports of poor strand selection (Reeder, 1973; Honjo and Reeder, 1974; Astrin, 1973; Wilson et al., 1975b). In addition, it is clear that some normally nontranscribed regions, e.g., 5S or ribosomal spacer are transcribed in isolated chromatin by the *E. coli* enzyme (Reeder, 1973).

How can these sets of conflicting reports be reconciled? On the one hand, using cDNA complementary either to individual mRNAs or to the overall population of polyadenylated RNA, it does appear that selective transcription occurs at some sites which is related to activities in the cell or tissue from which the chromatin originated. On the other hand, there is no reason to believe that the *E. coli* enzyme correctly recognizes initiation and termination signals. Indeed binding of *E. coli* enzyme and homologous enzyme are noncompetitive (Cedar, 1975). In addition, there can be little doubt that incorrect strand selection and other types of aberrant transcription occur. The most straightforward explanation is that the foreign polymerase can recognize some perturbation of chromatin structure which is preserved during isolation and correlated with transcriptional activity. The ability of DNase II, another DNA binding protein, to preferentially attack transcriptionally active chromatin would seem to be an analogous finding (Gottesfeld et al., 1974). Future work with either of these proteins as probes will be directed to the nature of the perturbation of structure in active chromatin which both can apparently recognize.

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