Brandhorst, B. P., and McConkey, E. H. (1975), *Proc. Natl. Acad. Sci. U.S.A. 72,* 3580-3584.
Derman, E., and Darnell, J. E. (1974), *Cell 3,* 255-264.
Herman, R. C., Williams, J. G., and Penman, S. (1976), *Cell 7,* 429-437.
Levis, R., McReynolds, L., and Penman, S. (1977), *J. Cell. Physiol. 90,* 485-502.
Levis, R., and Penman, S. (1977), *Cell 11,* 105-113.
Lewin, B. (1975a), *Cell 4,* 11-20.
Lewin, B. (1975b), *Cell 4,* 77-93.
Molloy, G. R., Jelinek, W., Salditt, M., and Darnell, J. E., (1974), *Cell 1,* 43-49.
Nakazato, H., and Edmonds, M. (1974), in Control of Transcription, Biswas, B. B., Mandal, R. K., Stevens, A., and Cohen, W. E., Ed., New York, N.Y., Plenum Press.
Penman, S., Vesco, C., and Penman, M. (1968), *J. Mol. Biol. 34,* 49-69.
Perry, R. P., Kelley, D. E., and LaTorre, J. (1974), *J. Mol. Biol. 82,* 315-331.
Price, R. P., Ransom, L., and Penman, S. (1974), *Cell 2,* 253-258.
Puckett, L., Chambers, S., and Darnell, J. E. (1975), *Proc. Natl. Acad. Sci. U.S.A. 72,* 389-393.
Ross, J. (1976), *J. Mol. Biol. 106,* 406-420.
Scherrer, K., Spohr, G., Granboulan, N., Morel, C., Grosclaude, J., and Clezzi, C. (1970), *Cold Spring Harbor Symp. Quant. Biol. 35,* 539-554.
Scholtissek, C. (1971), *Eur. J. Biochem. 24,* 358-334.
Singer, R., and Penman, S. (1973), *J. Mol. Biol. 78,* 321.
Soeiro, R., Vaughn, M. H., Warner, J. R., and Darnell, J. E. (1968), *J. Mol. Biol. 39,* 112-118.
Wertz, G. W. (1975), *J. Virol. 16,* 1340-1344.

Effect of Proteolysis on Transcriptional Fidelity of Reconstituted Chromatin†

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ABSTRACT: The effect of proteolysis on the transcriptional properties of reconstituted rat liver chromatin was studied. Within the sensitivity of currently available methods, proteolysis of chromosomal proteins by chromatin-bound protease during chromatin reconstitution has no apparent effect on: (1) number of initiation sites, (2) proportion of reiterating and unique sequences of DNA transcribed, (3) size of the RNA transcribed, and (4) transcription of DNA sequences complementary to poly(A) containing messenger RNA.

Recently there have been several reports on the transcriptional fidelity of reconstituted chromatin (Gilmour and Paul, 1970; Paul et al., 1973; Spelsberg and Hnilica, 1970; Barrett et al., 1974; Stein et al., 1975). For example, both the transcriptions of globin and histone gene sequences from reconstituted reticulocyte chromatin (Paul et al., 1973; Barrett et al., 1974) and HeLa cell chromatin at S phase (Stein et al., 1975) have been demonstrated. Studies on the mechanism of chromatin reconstitution would therefore provide important information toward elucidating the role of chromosomal proteins in gene regulation.

We have found, however, that chromosomal proteins are considerably degraded during chromatin reconstitution by a chromatin-bound protease (Chae, 1975; Chae and Carter, 1974; Chae et al., 1975). Therefore, the effect that proteolysis has on the transcriptional properties of reconstituted chromatin, and the possibility that this effect could be altered by an inhibitor of protease, was of great interest. Rat liver chromatin was chosen for these studies since the patterns of protein degradation (Carter and Chae, 1976) and the protease responsible for the degradation (Carter et al., 1976) have both been studied in some detail. Also, only the transcription of redundant sequences of DNA from native and reconstituted liver chromatin was studied in earlier work (Gilmour and Paul, 1970; Spelsberg and Hnilica, 1970).

Experimental Section

Preparation of Chromatin. Nuclei were prepared by homogenizing rat liver tissue in 0.25 M sucrose, 3 mM $MgCl_2$, and 10 mM potassium phosphate (pH 6.5). All steps were done at 4 °C unless otherwise indicated. The homogenate was filtered through 4 layers of cheesecloth and centrifuged at 700–1000*g* for 5 min, and the nuclei were suspended in 2.3 M sucrose, 3 mM $MgCl_2$, and 10 mM potassium phosphate (pH 6.5) and recentrifuged at 22 000 rpm in a Beckman 30 rotor for 45 min. The nuclear pellet was then suspended in 1% Triton X-100 containing 0.25 M sucrose, 3 mM $MgCl_2$, and 10 mM potassium 1000*g* for 5 min. Further purification of the chromatin was accomplished by the procedure of Chae (1975).

Dissociation and Reconstitution of Chromatin. Chromatin was dissociated by bringing the chromatin solution to 2 M NaCl–5 M urea and 10 mM Tris-HCl (pH 7.5). The chromatin was stirred for 4 h at 4 °C. Chromatin reconstitution was carried out by a modification of the gradient dialysis procedure of Bekhor et al. (1969). The salt concentration was reduced

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[1] Abbreviations used are: Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid; cDNA, complementary deoxyribonucleic acid; SSC, standard saline-citrate (0.15 M NaCl–0.015 M sodium citrate); HnRNA, heterogeneous nuclear ribonucleic acid.

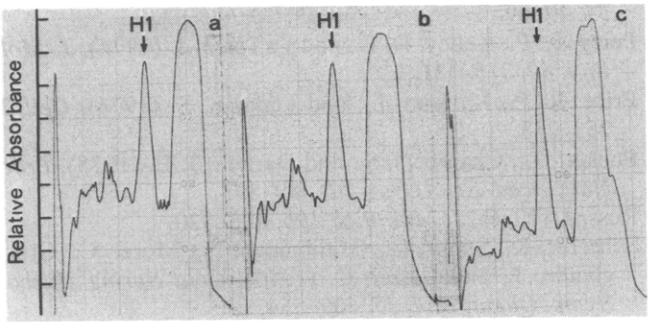

FIGURE 1: Densitometric scans of chromosomal proteins electrophoresed on sodium dodecyl sulfate–polyacrylamide gels: (a) native chromosomal proteins; (b) chromatin treated with and dissociated with phenylmethylsulfonyl fluoride before chromatin reconstitution; and (c) chromosomal proteins from reconstituted chromatin. To each gel was applied 48 μg of chromatin in DNA and electrophoretic migration was from left to right. Migration of histone H1 is indicated.

to 0.1 M over a 10-h period. Following further dialysis against 5 M urea–10 mM Tris-HCl[1] (pH 7.5) for 4 h, the chromatin was dialyzed against 10 mM Tris-HCl (pH 7.5) for 12 h.

RNA Transcription and Isolation. RNA was transcribed from chromatin in a reaction mixture containing 50 mM Tris-HCl (pH 7.9), 0.15 M KCl, 5 mM $MgCl_2$, 1 mM $MnCl_2$, 0.1 mM EDTA, 1 mM dithiothreitol, 0.4 mM ATP, GTP, CTP, and UTP, and 200 units of *Escherichia coli* RNA polymerase per mg of chromatin. Following incubation at 37 °C for 1 h, the reaction mixture was centrifuged at 12 000*g* for 15 min, and to the supernatant was added 20 μg/mL of DNase I (RNase free) purchased from Worthington Biochemical Corporation, Freehold, N.J. The fraction was incubated at 37 °C for 30 min. The solution was then brought to 20 mM EDTA and 0.5% sodium dodecyl sulfate, vortexed, allowed to stand for 10 min at room temperature, and then extracted twice with phenol and twice with phenol–chloroform (1:1). The fractions were then brought to 0.15 M LiCl and precipitated overnight with 2 vol of 95% ethanol at −20 °C. The precipitate was centrifuged at 12 000*g* for 10 min and dried and then taken up in 1.0 mL of water and applied to a Sephadex G-75 column (1 × 30 cm). The excluded peak was pooled and lyophilized to dryness.

Gel Electrophoresis of RNA. Polyacrylamide gel electrophoresis of RNA in the presence of 98% formamide was carried out as described by Staynov et al. (1972).

Titration of RNA Polymerase. Titration of *E. coli* RNA polymerase (1.3 units) was carried out according to the procedure of Cedar and Felsenfeld (1973). Increasing amounts of DNA or chromatin, in a 50-μL reaction mixture containing 10 mM Tris-HCl (pH 7.9), 1 mM $MnCl_2$, 0.08 mM each of ATP and GTP, and 0.02 mM [^{3}H]UTP (1.25 μCi), were incubated for 15 min at 37 °C. Initiation was stopped by adding 16 μL of 1.6 M $(NH_4)_2SO_4$, followed by the addition of 2 μL of 2.78 mM CTP and 2 μL of 0.2 M $MgCl_2$, and then further incubated at 37 °C for 20 min. Incorporation of [^{3}H]UTP was determined by precipitation with 5% trichloroacetic acid and collection on glass fiber filters.

RNA Polymerase Preparation. Isolation of RNA polymerase from *E. coli* was carried out using the procedure of Burgess and Jendrisak (1976).

Preparation of cDNA and cDNA Hybridization. Rat liver polysomal RNA was prepared by the procedure of Lee and Brawerman (1971). Poly(A) containing messenger RNA was obtained by chromatography of polysomal RNA on an oligo(dT)-cellulose column (Aviv and Leder, 1972). Synthesis of [^{3}H]cDNA from messenger RNA was carried out using RNA-dependent DNA polymerase from avian myeloblastosis virus according to the procedure of Axel et al. (1975). Hybridization of [^{3}H]cDNA to transcribed RNA was carried out by a modification of the procedure of Barrett et al. (1974). Our hybridization buffer contained 0.1% sodium dodecyl sulfate, 10 mM Tris-HCl (pH 7.9), and 0.4 M NaCl. Hybridization was carried out at 60 °C, and [^{3}H]cDNA–RNA hybrids, which were resistant to S_1 nuclease, were measured.

Preparation of Labeled RNA from Chromatin. The reaction mixture in 1.0 mL contained 50 mM Tris-HCl (pH 7.9), 0.15 M KCl, 5 mM $MgCl_2$, 0.1 mM EDTA, 1 mM dithiothreitol, 0.4 mM each of ATP, CTP, and GTP, 0.04 mM [^{14}C]UTP (10 μCi) or 0.04 mM [^{3}H]UTP (50 μCi), chromatin containing 300 μg of DNA, and 200 units of *E. coli* RNA polymerase. The reaction time was 30 min at 37 °C.

Native and reconstituted chromatin was incubated in the reaction mixture containing different isotopes and, after the reaction, the two mixtures were combined and RNA was extracted as described above. The specific radioactivity of RNA was in the order of 1×10^5 cpm/μg of RNA.

Shearing of Rat Thymus DNA. Rat thymus DNA (100 mg) in 40 mL of 0.1 × SSC (SSC = 0.15 M NaCl and 0.015 M sodium citrate) was sheared 3 times in a French pressure cell at 16 000 psi, and this sheared DNA was then made 1 × SSC and precipitated with ethanol. The DNA was then dissolved in 0.1 × SSC and dialyzed against the same solution, and the concentration of DNA was adjusted to 100 A_{260} units/mL.

Hybridization in Excess of DNA. The hybridization of labeled RNA in vast excess of DNA was done essentially by the method of Melli et al. (1971). DNA and the double-labeled RNA were mixed at a ratio of 50 000 DNA and 1 RNA by weight.

Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis. Chromatin was dialyzed against 1% sodium dodecyl sulfate–10 mM sodium phosphate (pH 7)–0.1% β-mercaptoethanol and electrophoresed through 7.5% polyacrylamide gel in 0.1% sodium dodecyl sulfate and stained with Coomassie Blue as previously described (Chae, 1975). The stained gels were scanned in an E-C densitometer (E-C Apparatus, St. Petersburg, Fla.).

Results

Protein degradation during chromatin reconstitution has been previously documented (Chae and Carter, 1974; Chae et al., 1975). Figure 1 shows the extent of proteolytic degradation that occurs to rat liver chromosomal proteins during chromatin reconstitution. Although the proteins of reconstituted chromatin show the same general electrophoretic profile as those of native chromatin, the nonhistone proteins that migrate at a slower rate than histone H1 show extensive degradation. The degradation varies from 30 to 50% and the degraded products migrate at a more rapid rate than the smallest histone. Chromatin treated with 1 mM phenylmethylsulfonyl fluoride, a known inhibitor of rat liver chromosomal protease activity (Carter and Chae, 1976), does not show any proteolytic degradation as a result of chromatin reconstitution.

One possible result of protein degradation during reconstitution is that, in the reconstituted chromatin, regions of DNA, which were not formerly available, could become accessible to RNA polymerase. The new sites for RNA transcription would probably be located randomly along the DNA, resulting in: (1) an increase in the number of RNA polymerase initiation sites, (2) transcription of RNA products of varied molecular weights relative to RNA transcribed from native chromatin, and (3) transcription of new regions of DNA.

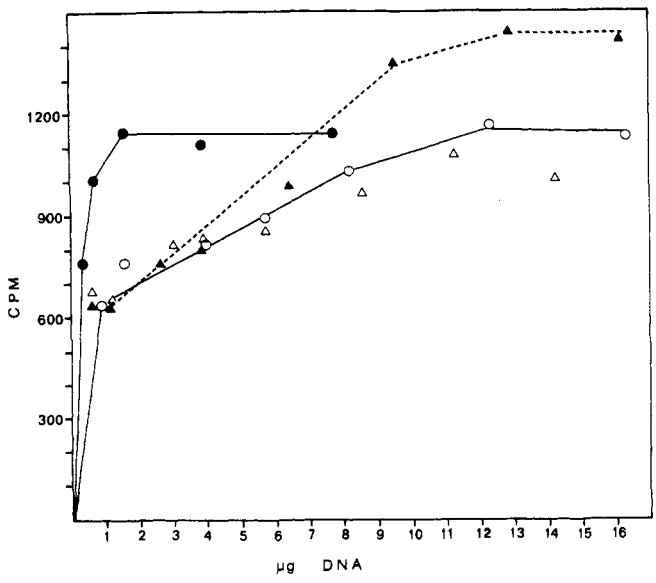

FIGURE 2: Titration of *E. coli* RNA polymerase (1.3 units) was carried out with increasing amounts of rat thymus DNA (●), native rat liver chromatin (○), reconstituted liver chromatin (▲), and reconstituted chromatin treated with 1 mM phenylmethylsulfonyl fluoride in 1% dimethyl sulfoxide for 30 min before and during dissociation (△). Experimental details are described in the Experimental Section.

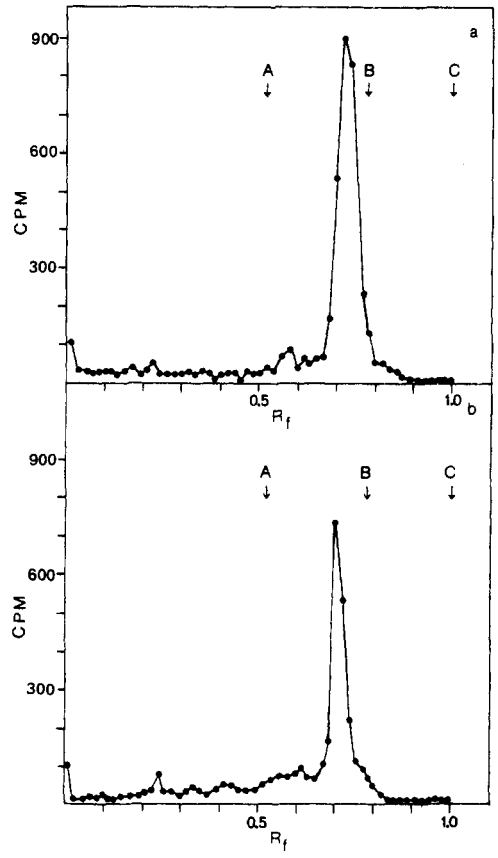

FIGURE 3: Electrophoresis of [^{3}H]RNA, transcribed from 26 μg of reconstituted rat liver chromatin by 2 units of *E. coli* RNA polymerase in 100 μL of initiation reaction mixture under the conditions used in Figure 1, was carried out on 10% polyacrylamide gels containing 98% formamide. RNA was transcribed from reconstituted chromatin treated with 1 mM phenylmethylsulfonyl fluoride in 1% dimethyl sulfoxide (a) and reconstituted chromatin not treated with phenylmethylsulfonyl fluoride (b). Following chain elongation, the fractions were brought to 1.0 mL with water, and 20 μg of RNase-free DNase I was added to the reaction mixture. The fractions were incubated at 37 °C for 10 min and were then made 0.1% sodium dodecyl sulfate and extracted with phenol-chloroform (1:1). To the aqueous phase was added 50 μg of yeast tRNA, and the RNA was precipitated overnight by the addition of 3 vol of 95% ethanol. The precipitate was washed three times with 70% ethanol, air dried, and taken up in 98% formamide. Xylene cyanol (A), yeast tRNA (B), and bromophenol blue (C) were used as markers.

Reconstituted chromatin was examined for the purpose of determining whether the known proteolytic degradation that occurs during reconstitution altered the transcriptional properties of the chromatin. The number of initiation sites for RNA transcription was determined by titrating *E. coli* RNA polymerase with rat thymus DNA, as well as native and reconstituted chromatin of rat liver, using the procedure of Cedar and Felsenfeld (1973). The end-point titration for native chromatin and chromatin reconstituted in the presence and absence of 1 mM phenylmethylsulfonyl fluoride appeared to be very similar and approximately ten times greater than protein-free DNA (Figure 2). This is consistent with the findings of Cedar et al. (1976) and Felsenfeld et al. (1975) that reconstituted chromatin retains the same number of initiation sites as native chromatin. The reconstituted chromatin not treated with phenylmethylsulfonyl fluoride consistently reached a higher plateau of [^{3}H]UTP incorporation over DNA, native chromatin, and chromatin reconstituted in the presence of phenylmethylsulfonyl fluoride. It was first thought that this greater incorporation of [^{3}H]UTP was a result of longer RNA products, but polyacrylamide gel electrophoresis in the presence of 98% formamide (Figure 3) showed that RNAs transcribed from chromatin reconstituted in the presence and absence of phenylmethylsulfonyl fluoride are the same when transcribed under conditions that prevented a second round of initiation.

The proportions of the reiterated and unique sequences of DNA in chromatin transcribed by *E. coli* RNA polymerase were determined. *E. coli* RNA polymerase was used to transcribe RNA from both native and reconstituted chromatin. The native chromatin reaction mixture contained [^{3}H]UTP, and the reconstituted chromatin reaction mixture (not treated with a protease inhibitor) contained [^{14}C]UTP. Following incubation at 37 °C for 1 h, the two reaction mixtures were combined, and RNA was extracted as described in the Experimental Section. The specific radioactivity of the pooled RNA was in the order of 1×10^5 cpm/μg of RNA. The pooled, labeled RNA fraction was then hybridized to excess sheared rat thymus DNA. The ratio of DNA to RNA was 5×10^4. The hybridization patterns of the RNA, transcribed from native and reconstituted chromatin to DNA, appear to be very similar (Figure 4). In the lower C_0t range which represents repeating DNA sequences, the hybridization patterns of the two RNAs from native and reconstituted chromatin are identical; however, there were small differences in the two patterns at C_0t values over 1000 which represent unique DNA sequences. The similar hybridization patterns obtained across a wide C_0t range suggest that *E. coli* RNA polymerase transcribes the same proportion of reiterated and unique sequences from the native and reconstituted chromatin. The hybridization pattern of the RNA transcribed from chromatin is very similar to the hybridization pattern of liver HnRNA to vast excess DNA (Melli et al., 1971).

The extent of hybridization in Figure 4, approximately 30%, is lower than would be expected for hybridization of transcribed RNA to a large excess of DNA. Similar low hybridization values have been reported for hybridization of mRNA (Klein et al., 1974; Spradling et al., 1974) and heterogeneous nuclear RNA (Spradling et al., 1974) to large excess of DNA.

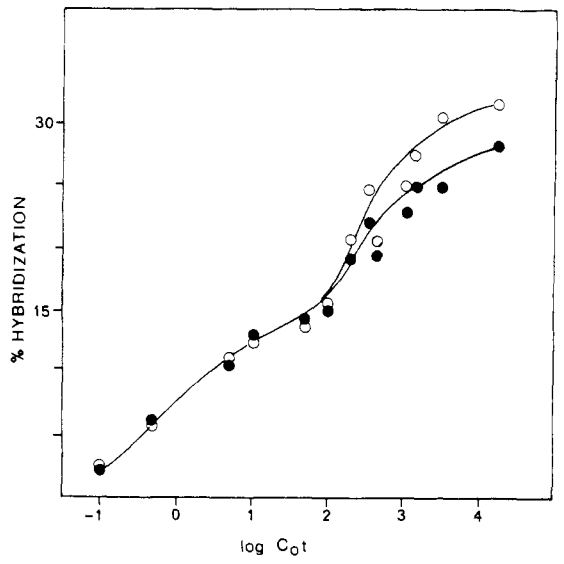

FIGURE 4: Hybridization of [^{3}H]RNA transcribed from native chromatin (●) and [^{14}C]RNA transcribed from reconstituted chromatin (○) to excess sheared DNA; native and reconstituted chromatin were incubated in separate RNA transcription reaction mixtures containing [^{3}H]UTP and [^{14}C]UTP, respectively. After incubation for 30 min at 37 °C, the two reaction mixtures were combined and RNA was extracted as described in the Experimental Section. The ratio of sheared DNA to double-labeled RNA was 5×10^4.

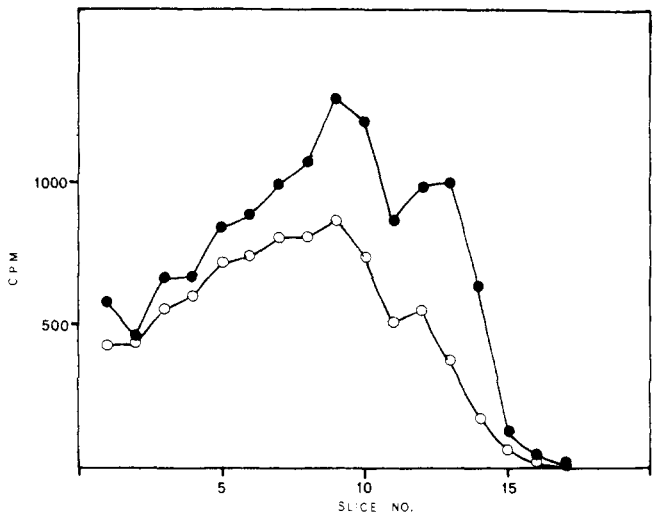

FIGURE 5: Relative mobility of RNA transcribed from native (●) and reconstituted (○) chromatin on 10% polyacrylamide gels containing 98% formamide. The double-labeled RNA (Figure 3) was dissolved in formamide and heated at 90 °C before electrophoresis. After electrophoresis, the gel was sliced into 1.5-mm sections and counted for radioactivity.

The explanation given by Klein et al. (1974) for the failure to achieve a greater degree of hybridization is that some sequences are represented in very high frequency in the RNA population. If single copy sequences are transcribed a relatively large number of times, it would be difficult to saturate the labeled RNA with DNA. We feel this same argument can be applied here. The extent of hybridization could have been made greater by increasing the ratio of DNA to RNA but this was limited in our experiment by the specific radioactivity of the labeled RNA, in particular [^{14}C]RNA. The results are still meaningful since the relative frequency of various RNA species still determines the kinetics of hybridization across the C_0t range reported, and the conclusion that the proportion of re-

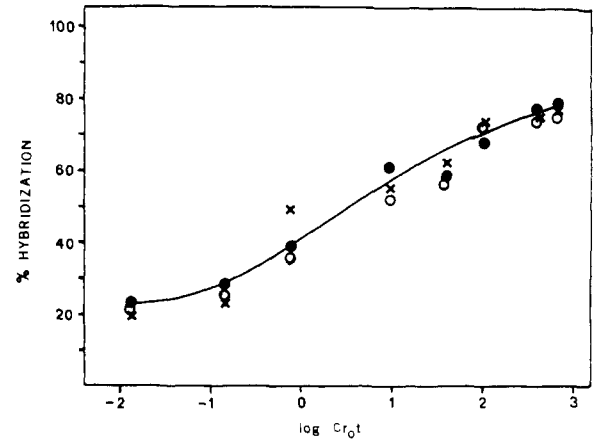

FIGURE 6: Kinetics of hybridization of [^{3}H]cDNA (complementary to liver messenger RNA) to RNA transcribed from native chromatin (●), reconstituted chromatin (○), and chromatin reconstituted in the presence of 1 mM phenylmethylsulfonyl fluoride (×).

iterated and unique DNA sequences transcribed from native and reconstituted chromatin is similar is still valid.

Formamide polyacrylamide gel electrophoresis of the double-labeled RNA fraction transcribed from native and reconstituted chromatin was carried out (Figure 5). Except for the leading peak, which probably represents degraded RNA, the ratio of ^{3}H/^{14}C of RNA remained constant across the gel. Therefore, there is no apparent difference in the length of the RNA transcribed from native chromatin or chromatin reconstituted in the absence of a protease inhibitor under the conditions used for RNA transcription by *E. coli* RNA polymerase.

Finally, we compared transcription of messenger RNA sequences from native and reconstituted chromatin. RNA transcribed from chromatin was annealed to the [^{3}H]cDNA, which is complementary to rat liver poly(A) containing messenger RNA. Annealing reactions were carried out for RNA transcribed from native chromatin, reconstituted chromatin, and chromatin reconstituted in the presence of 1 mM phenylmethylsulfonyl fluoride. The results of this experiment are shown in Figure 6. The hybridization pattern of RNA transcribed from reconstituted chromatin, and chromatin incubated with and dissociated in the presence of phenylmethylsulfonyl fluoride, is very similar to the hybridization pattern of RNA transcribed from native chromatin. It was found that no RNA could be detected at the final step for isolating transcribed RNA from chromatin (Sephadex G-75 chromatography) when inactive RNA polymerase was present in the transcription mixture. Thus, the reconstitution results do not reflect hybridization of [^{3}H]cDNA to endogenous chromatin-bound RNA. The results suggest a similarity in the transcription of cytoplasmic poly(A) containing messenger RNA sequences between native and reconstituted chromatin; also, this transcription does not appear to be affected by proteolytic degradation as far as rat liver chromatin is concerned.

Discussion

The purpose of this report was to determine the fidelity of reconstituted chromatin with respect to RNA transcription. At the present time, our laboratory is involved in the isolation and characterization of chromatin-associated proteases from rat liver chromatin. Being acutely aware of the degradation that occurs to nonhistone and histone proteins under the conditions routinely used for chromatin dissociation and recon-

stitution, we were looking for a correlation between chromatin degradation and changes in RNA transcription. Reconstituted chromatin and native chromatin appear to have a similar number of initiation sites, and the RNAs transcribed are similar with respect to hybridization to excess DNA, molecular weight, and hybridization of cDNA made from mRNA. The only difference noted was the increased amount of [^{3}H]UTP incorporated into the RNA transcribed from reconstituted chromatin under the conditions of high salt used to restrict reinitiation. This increased incorporation is not a result of an increase in the length of the RNA transcribed, but may be a result of altered transcription (due to proteolytic degradation) of DNA sequences resulting in increased transcription of certain DNA sequences, for example, DNA rich in A·T base pairs or incorrect DNA strands.

Other laboratories also have reported that reconstituted chromatin has many of the properties of native chromatin as well as similar transcriptional properties. The most likely possibility is that the RNA–DNA hybridization method is not sensitive enough to detect minor changes in the concentration of liver mRNA sequences in the total chromatin transcript. It is possible that the 50% degradation of nonhistone protein during chromatin reconstitution (Figure 1) might have resulted in a 50% change in the concentration of mRNA sequences in the total chromatin transcript. According to our calculation this would cause a shift of the log $Cr_0t_{1/2}$ by 0.3 in the RNA/DNA hybridization curve shown in Figure 6. Since the slope of the hybridization curve of [^{3}H]cDNA and liver chromatin transcript is very low, due to the high complexity of the mRNA sequences transcribed in the liver, it is difficult for us to detect with any accuracy this small shift in the hybridization curve. Also, there is the uncertainty about the specificity of RNA chain initiation in chromatin by *E. coli* RNA polymerase. Although our results show no significant effect of proteolysis on the transcriptional fidelity of reconstituted chromatin, it still will be desirable to carry out dissociation and fractionation of chromosomal proteins and chromatin reconstitution under the condition which minimizes proteolytic degradation of chromosomal proteins.

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References

Aviv, H., and Leder, P. (1972), *Proc. Natl. Acad. Sci. U.S.A. 69,* 1408–1412.

Axel, R., Cedar, H., and Felsenfeld, G. (1975), *Biochemistry 14,* 2489–2495.

Barrett, T., Maryanka, D., Hamlyn, P. H., and Gould, H. J. (1974), *Proc. Natl. Acad. Sci. U.S.A. 71,* 5057–5061.

Bekhor, I., Kung, G. M., and Bonner, J. (1969), *J. Mol. Biol. 39,* 351–364.

Burgess, R. R., and Jendrisak, J. J. (1975), *Biochemistry 14,* 4634–4638.

Carter, D. B., and Chae, C.-B. (1976), *Biochemistry 15,* 180–185.

Carter, D. B., Efird, P. H., and Chae C.-B. (1976), *Biochemistry 15,* 2603–2607.

Cedar, H., and Felsenfeld, G. (1973), *J. Mol. Biol. 77,* 237–254.

Cedar, H. C., Solage, A., and Zurucki, F. (1976), *Nucleic Acids Res. 3,* 1659–1670.

Chae, C.-B. (1975), *Biochemistry 14,* 900–906.

Chae, C.-B., and Carter, D. B. (1974), *Biochem. Biophys. Res. Commun. 57,* 740–746.

Chae, C.-B., Gadski, R. A., Carter, D. B., and Efird, P. H. (1975), *Biochem. Biophys. Res. Commun. 67,* 1459–1465.

Felsenfeld, G., Axel, R., Cedar, H., and Sollner-Webb, B. (1975), *Ciba Found. Symp. 28,* 29–41.

Gilmour, R. S., and Paul, J. (1970), *FEBS Lett. 9,* 242–245.

Klein, W. H., Murphy, W., Attardi, G., Britten, R. J., and Davidson, E. H. (1974), *Proc. Natl. Acad. Sci. U.S.A. 71,* 1785–1789.

Lee, S. Y., and Brawerman, G. (1971), *Biochemistry 10,* 510–516.

Melli, M., Whitfield, C., Rao, K. V., Richardson, M., and Bishop, J. O. (1971), *Nature (London), New Biol. 231,* 8–12.

Paul, J., Gilmour, R. S., Birnie, A. G., Harrison, B. P., Hell, A., Humphries, S., Windass, J., and Young, B. (1973), *Cold Spring Harbor Symp. Quant. Biol. 38,* 885–890.

Spelsberg, T. C., and Hnilica, L. S. (1970), *Biochem. J. 120,* 435–437.

Spradling, A., Penman, S., Campo, S., and Bishop, J. O. (1974), *Cell 3,* 23–30.

Staynov, D. Z., Pinder, J. C., and Gratzer, W. B. (1972), *Nature (London), New Biol. 235,* 108–110.

Stein, G., Stein, J., Thrall, C., and Park, W. (1975), Chromosomal Proteins and Their Role in the Regulation of Gene Expression, New York, N.Y., Academic Press, pp 1–17.