Template Specificity of Isolated Chromatin*

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ABSTRACT: The ribonucleic acid synthesized *in vitro* using a chromatin template is indistinguishable by nucleic acid hybridization analysis from ribonucleic acid produced *in vivo* in the homologous tissue. Purified deoxyribonucleic acid supports a higher rate of ribonucleic acid synthesis than chromatin preparations. This is due to the fact that there is a restricted number of sites transcribed from chromatin. Within the limits of sensitivity of the hybridization assay this restriction is tissue specific. This specificity appears to be independent of the source of the ribonucleic acid polymerase as comparable results are obtained with *Escherichia coli* and mouse enzyme. Indirect evidence indicates that the synthesis of new molecules is initiated during *in vitro* transcription.

The fact that deoxyribonucleic acid–ribonucleic acid hybridization is unable to distinguish between ribonucleic acid molecules having a high degree of similarity in base composition and that incubation times used during the hybridization were too short to permit hybridization of ribonucleic acid molecules originating from sites of unique nucleotide sequence requires that strict limitations be placed on the interpretation of these results. In spite of these qualifications, it is evident that a high degree of tissue specificity is retained by isolated chromatin with respect to the synthesis of ribonucleic acid *in vitro*. It would appear that the elements determining the differential transcription of ribonucleic acid reside within the macromolecular configuration of the interphase chromosomes.

The biochemical basis of selective gene expression in metazoan cells has been investigated, *in vitro*, through the study of two kinds of subcellular preparations. Many studies have been made with cell nuclei particularly those of thymocytes (Allfrey *et al.*, 1963). More recent experiments have shown that the RNA synthesized *in vitro* in thymus nuclei reflects, to a large extent, the population of RNA molecules present in thymus tissue (Sullivan, 1968). A second approach involves the use of chromatin or interphase chromosomes for which methods of preparations from a variety of plant and animal tissue are available (Zubay and Doty, 1959; Dingman and Sporn, 1964; Bonner and Huang, 1963; Bonner *et al.*, 1967). The ability of chromatin to support RNA synthesis is restricted compared with that of DNA. This appears to be due to a limitation of the fraction of sites in DNA which is transcribed so that a restricted set of RNA molecules is produced (Marushige and Bonner, 1966; Paul and Gilmour, 1966, 1968, 1969). Paul and Gilmour (1966, 1968) have presented evidence that DNA sites transcribed from rabbit thymus and bone marrow chromatin *in vitro* resemble those sites active *in vivo*. The current experiments were designed to examine the question of template specificity in more detail. Convincing evidence for retention of a high degree of fidelity of transcription by isolated chromatin would present an opportunity for the elucidation of gene regulatory mechanisms in an *in vitro* system (Huang and Huang, 1969; Bekhor *et al.*, 1969).

Materials and Methods

Preparation of DNA. Nuclei were prepared from adult liver, kidney, and spleen or from 14-day embryos of Swiss Webster mice by a modification of the method previously described (McCarthy and Hoyer, 1964). DNA was prepared from the isolated nuclei by the Marmur procedure (Marmur, 1961) as modified by Church and McCarthy (1967a). DNA preparations were centrifuged at 18,000 rpm in the Servall SS34 head for 1 hr with 0.01% acid-washed Norit to remove traces of polysaccharides. The purity of the DNA was determined by its ultraviolet spectrum, negative color reactions for RNA (orcinol) and protein (Lowry *et al.*, 1951), and its retention by membrane filters for the duration of the hybridization reaction.

Preparation of RNA. Total RNA was isolated from freshly excised, rapidly cooled tissues by the method described by Church and McCarthy (1967a). Cytoplasmic and nuclear fractions were prepared by a modification of the procedure of Blabel and Potter (1966) as described by Church and McCarthy (1967b).

Isolation of Chromatin. Chromatin was prepared by a modification of the method described by Marushige and Bonner (1966) for rat liver. Tissue (12 g) was homogenized in 40 ml of 0.075 M NaCl and 0.024 M EDTA (pH 8.0). The homogenate was successively passed through 2, 4, 8, and 16 layers of washed cheesecloth and then through a single layer of Miracloth. The strained homogenate was centrifuged for 20 min at 2500*g* to give a crude nuclear preparation.

The nuclear pellet was washed three times by resuspension in 16 ml of the saline–EDTA buffer and centrifugation at 1500*g* for 15 min. This pellet was washed twice by resuspension in 0.5 M Tris-HCl (pH 8.0) and centrifuged at 1500*g* for 15 min.

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The pellet from the second wash was homogenized by hand in a Teflon homogenizer in 8 ml of 0.05 M Tris-HCl (pH 8.0) to break the nuclei and the chromatin was collected by centrifugation at 10,000*g* for 15 min. This pellet was resuspended by hand homogenization in 10 ml of 0.05 M Tris-HCl (pH 8.0). The crude chromatin samples were layered on top of 25 ml of 1.7 M sucrose, the interface was broken by stirring the top one-third of the tube with a glass rod, and the chromatin was sedimented through the sucrose by centrifugation in the SW25 head of the Spinco Model L ultracentrifuge for 3 hr at 23,000 rpm. The clear chromatin pellet was resuspended by hand homogenization in 5 ml of 0.01 M Tris-HCl (pH 8.0). This suspension was dialyzed overnight against 0.01 M Tris-HCl (pH 8.0) to remove the remaining sucrose and sheared by sonication. Samples (5 ml) at a concentration of approximately 0.5 mg/ml in a 50-ml beaker, cooled in an ice bath, were sonicated with a Branson S-75 sonifer equipped with a standard 0.5-in. tip stephorn mechanical transformer. Samples were sheared with five 1-min pulses at 5-min intervals with maximum output at a setting of 5. The sonicated samples were stirred for 30 min at 4° and the insoluble chromatin was removed by centrifugation at 10,000*g* for 30 min. Aliquots were quick frozen in a Dry Ice and acetone bath and stored at −65°.

Great care must be taken in the preparation of chromatin to be used as a template for RNA synthesis. Variations in the isolation procedure, especially at the sonication step, cause qualitative changes in the template capacity of the chromatin.

Preparation of RNA Polymerase. E. coli RNA polymerase was isolated from frozen *E. coli* B (General Biochemicals) by the method of Chamberlin and Berg (1962). Chamberlin and Berg's fraction 3 was used when it showed a high dependence upon added primer. If the dependence of fraction 3 upon added primer was insufficient, fraction 4 was prepared.

Mouse RNA polymerase was prepared by a modification of the Chamberlin and Berg (1962) procedure. Nuclei were isolated from embryonic mouse liver by the method of McCarthy and Hoyer (1964). A crude chromatin preparation was prepared from the nuclei by homogenization in 0.01 M Tris-HCl (pH 7.8), 0.01 M $MgCl_2$, and 0.0001 M EDTA followed by centrifugation at 10,000*g* for 20 min. The pellet was resuspended in the homogenization buffer and β-mercaptoethanol was added to a final concentration of 0.01 M. Freshly prepared 10% streptomycin sulfate (5 ml for each 100 ml of solution) was added and the mixture was centrifuged at 30,000*g* for 10 min; 1% protamine sulfate (4 ml for each 100 ml of solution) was added to the supernatant and the mixture was centrifuged at 30,000*g* for 10 min. The precipitate was washed by resuspension in the homogenization buffer containing 0.01 M β-mercaptoethanol, followed by centrifugation at 30,000*g* for 10 min. The enzyme was eluted by resuspension of the pellet in the homogenization buffer containing 0.01 M β-mercaptoethanol and 0.15 M NaCl. After centrifugation at 30,000*g* for 10 min, the supernatant was brought to 50% with ammonium sulfate (pH 7.0) to precipitate the enzyme. The precipitate was collected by centrifugation at 30,000*g* for 10 min and dissolved in 0.001 M Tris-HCl (pH 8.5), 0.01 M $MgCl_2$, 0.01 M β-mercaptoethanol, and 0.001 M EDTA, quick frozen, and stored under liquid nitrogen. This preparation corresponds to fraction 3 of Chamberlin and Berg (1962) and gives a reproducible yield of enzyme with a high dependence upon added primer. The enzyme isolated by this procedure, though free of nuclease activity, is a crude preparation and very unstable.

In Vitro Synthesis of RNA. The *in vitro* synthesis of RNA was carried out in a buffer consisting of 0.04 M Tris (pH 8.0), 0.001 M $MnCl_2$, 0.004 M $MgCl_2$, and 0.012 M β-mercaptoethanol. The reaction mixture (0.25 ml) contained 100 mμmoles each of unlabeled ATP, GTP, and UTP, 100 mμmoles of ^3H- or ^{14}C-labeled CTP (2 μCi), 250 mμmoles of primer, either as DNA or as chromatin, and 10–200 units of RNA polymerase (one unit of enzyme converts 1 mμmole of CTP into acid-insoluble material per hr). When larger reaction volumes were used, the relative concentrations of the reactants were kept constant. The reactions were carried out at 37° for the time periods indicated in the figure legends. The reactions were routinely stopped by placing the reaction tubes on ice and precipitating the RNA with cold 10% trichloroacetic acid after the addition of carrier RNA. RNA was recovered from the reaction mixtures at the end of the incubation period by extraction with hot phenol as described above.

RNA–DNA Hybridization. RNA was hybridized to DNA by the membrane filter technique as developed by Gillespie and Spiegelman (1965) and modified by Church and McCarthy (1968). The characteristics of the hybridization reaction with *in vitro* synthesized RNA are essentially identical with those with *in vivo* synthesized RNA. Maximum hybridization of about 15–20% of the input counts was attained after 16-hr incubation and no decrease was apparent by 24 hr. The thermal stability of the RNA–DNA hybrid complex formed with *in vitro* synthesized RNA was (Church and McCarthy, 1968) examined by subjecting the filter-bound complexes to increasing temperatures. The values obtained for the mean thermal dissociation temperature (about 78° in SSC)[1] were similar to those obtained in earlier studies of hybrids formed by *in vivo* synthesized RNA (Church and McCarthy, 1967). Treatment with RNase, while reducing the total counts bound to the DNA in the filter, does not increase the mean stability of the remaining material. RNase digestion was not used in the experiments to be presented.

Nonspecific binding was monitored by including a filter containing *E. coli* DNA, equal in amount to the experimental filter, for each individual point. These *E. coli* filters were carried through the same washes as the experimental filter and the counts bound were subtracted from the experimental filters to determine the level of specific hybridization. The total counts bound to the *E. coli* filters normally represented less than 0.4% of the input counts.

Results

The chemical composition of adult mouse liver and kidney chromatin is given in Table I. The relative proportions of chromatin components is similar to that of other mammalian chromatin preparations (Bonner *et al.*, 1968). Of particular importance for this study is the low quantity of RNA found in this chromatin preparation.

The rate of RNA synthesis supported by rat liver or pea chromatin in the presence of *E. coli* RNA polymerase is considerably lower than that obtained using purified DNA

[1] Abbreviation used is: SSC, standard saline citrate.

TABLE 1: Chemical Composition of Mouse Chromatin.[a]

	Content Relative to DNA			
	DNA	Histone	Non-histone Protein	RNA
Liver	1	0.96	0.73	0.045
Kidney	1	1.05	0.96	0.052

[a] Quantitative determinations of DNA and RNA are after Burton (1956) and Dische and Schwartz (1937), respectively. Histone was isolated by extracting isolated chromatin three times with 0.4 M H_2SO_4. The nonhistone protein was extracted from the histone-free chromatin in 1.0 M NaOH. Protein determinations were made by the method of Lowry *et al.* (1951). Values represent an average of three determinations on separate preparations.

as template (Bonner and Huang, 1963; Marushige and Bonner, 1966). Similar results were obtained in the present experiments with chromatin prepared from various mouse tissues whether *E. coli* or mouse RNA polymerase was used. The duration of synthesis is quite short and the incorporation of labeled triphosphates into RNA is linear for only about 10–20 min. Cessation of synthesis is probably due to the presence of nucleases in the reaction mixtures or to conditions less than optimal for the RNA polymerase. It is possible to obtain linear synthesis of RNA for up to 3 hr in systems containing a DNA template, providing higher salt concentrations are used (So *et al.*, 1967). Such conditions are not, however, compatible with accurate transcription from chromatin since chromatin is minimally soluble in the salt range of 0.2–0.4 M and higher salt concentrations disrupt the protein–nucleic acid association (Georgiev *et al.*, 1966).

Since purified DNA supports a higher rate of RNA synthesis than do chromatin preparations, it is reasonable to suppose that a much larger variety of molecules is made. This follows from the concept that the protein components of chromatin serve to block transcription from some sites. Comparisons were made between the RNA synthesized *in vitro* using a DNA template and the collection of RNA molecules present in given tissues. Figure 1 demonstrates that the RNA synthesized *in vitro* from a DNA template includes some molecules similar to those produced in the liver and the kidney but that the majority are distinguishable. Thus, both unlabeled kidney and liver RNA compete with the labeled RNA but only to a limited extent. A mixture of the two unlabeled RNA preparations gives only partial additivity.

A similar conclusion may be drawn from a different kind of experiment, the results of which are presented in Figure 2. Increasing amounts of labeled RNA synthesized *in vitro* using either liver chromatin or purified liver DNA were hybridized with mouse DNA. A much higher fraction of the DNA sites may be occupied by RNA synthesized from purified DNA than that transcribed from liver chromatin, indicating the presence of a greater diversity of base sequences.

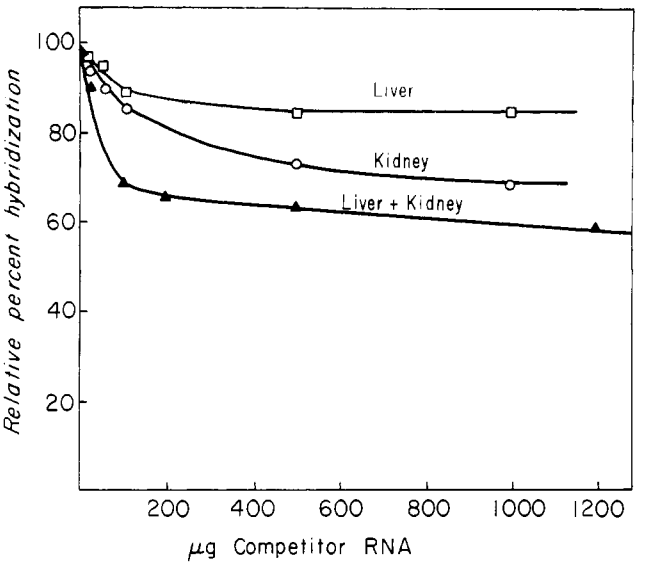

FIGURE 1: Competition of liver and kidney RNA in the hybridization reaction of RNA synthesized *in vitro* using liver DNA as a template. The RNA was hybridized with DNA in the presence of increasing amounts of unlabeled competitor RNA from liver or kidney or a mixture of equal quantities of liver and kidney RNA. Hybridization was carried out with 20 μg of DNA in 0.4 ml of 4× SSC at 65° for 18 hr; 3224 cpm was hybridized in the absence of competitor.

Within the limits of sensitivity of the assay, it can be shown that the restriction of synthesis of RNA *in vitro* from chromatin template leads to a collection of RNA molecules indistinguishable from that produced *in vivo* in the corresponding tissue. The first series of experiments compared labeled RNA synthesized *in vitro* from liver, kidney, or brain chromatin with unlabeled RNA prepared from various tissues. For

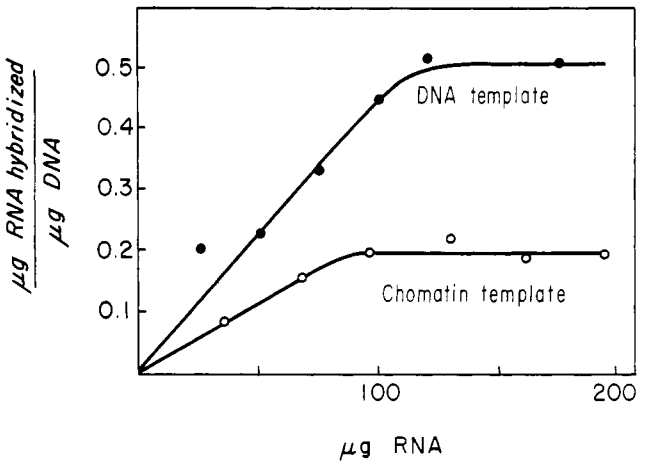

FIGURE 2: Saturation of DNA and RNA synthesized *in vitro* using liver chromatin or DNA isolated from liver chromatin as primer. RNA was synthesized in 50 ml of the standard reaction mixture using 50 μmoles of either liver chromatin or DNA isolated from liver chromatin. The reaction was run for 10 min and then the RNA was extracted with hot phenol. Hybridization was carried out using increasing amounts of RNA with 20 μg of DNA in 0.2 ml of 4× SSC at 65° for 18 hr. Specific activity of RNA synthesized on a DNA template was 810 and RNA synthesized from chromatin was 720 cpm/μg.

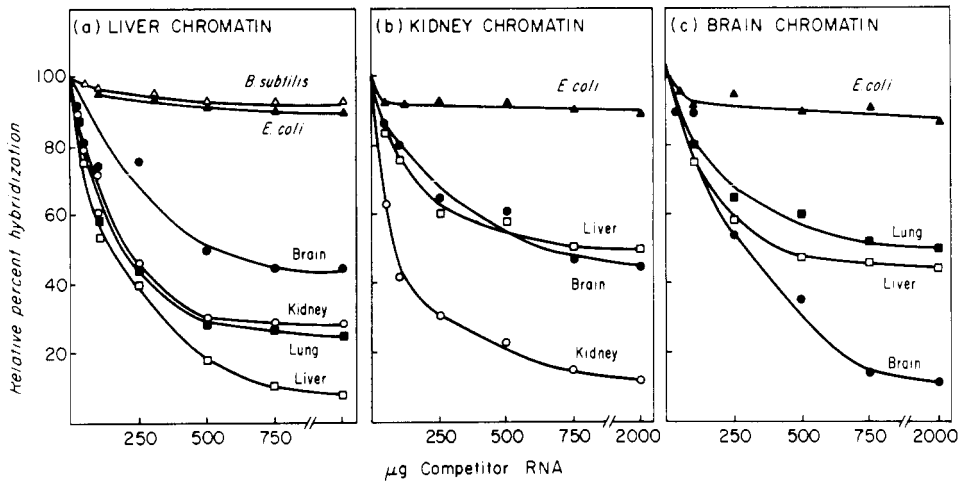

FIGURE 3: Tissue specificity of RNA synthesized from chromatin originating from various mouse organs using *E. coli* RNA polymerase. (a RNA was synthesized using a mouse liver chromatin template and *E. coli* RNA polymerase. The reaction was run at 37° for 10 min and the RNA was then extracted with hot phenol. RNA was extracted from various tissues and used as competitor for the hybridization of the *in vitro* synthesized RNA to DNA. Hybridization was with 12 μg of DNA in 0.4 ml of 2× SSC at 67° for 18 hr; 1211 cpm was hybridized in the absence of competitor. (b) RNA was synthesized using a mouse kidney chromatin template and *E. coli* RNA polymerase. The reaction was run at 37° for 10 min and the RNA was then extracted with hot phenol. RNA extracted from various tissues was used as competitor for the hybridization of the *in vitro* synthesized RNA to DNA. Hybridization was with 12 μg of DNA in 0.4 ml of 2× SSC at 67° for 18 hr; 406 cpm was hybridized in the absence of competitor. (c) RNA was synthesized using a mouse brain chromatin template and *E. coli* RNA polymerase. The reaction was run at 37° for 10 min and the RNA was then extracted with hot phenol. RNA extracted from various tissues was used as competitor for the hybridization of the *in vitro* synthesized RNA to DNA. Hybridization was with 12 μg of DNA in 0.4 ml of 2× SSC at 67° for 38 hr; 504 cpm was hybridized in the absence of competitor.

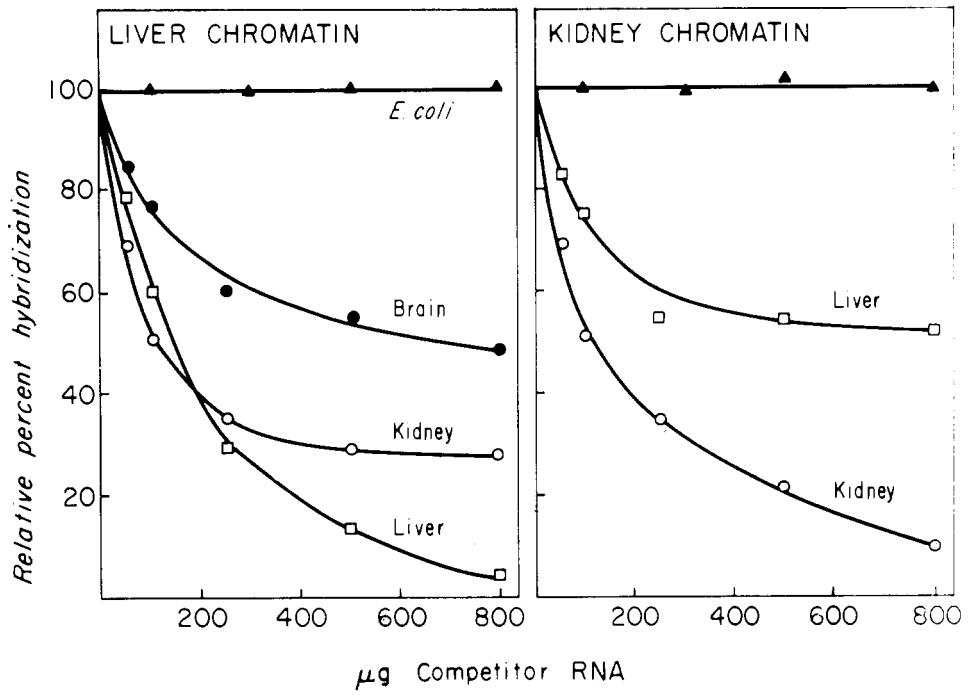

FIGURE 4: Tissue specificity of RNA synthesized from chromatin originating from liver or kidney using mouse RNA polymerase. (a) RNA was synthesized *in vitro* from a mouse liver chromatin template using mouse RNA polymerase. The reaction was run in 5 ml buffer at 37° for 10 min and the RNA was extracted with hot phenol. RNA isolated from various tissues was used as competitor for the hybridization of the *in vitro* synthesized RNA to DNA. Hybridization with 12 μg of DNA in 0.2 ml of 2× SSC at 67° for 18 hr; 384 cpm was hybridized in the absence of competitor. (b) RNA was synthesized *in vitro* from a mouse liver chromatin template using mouse RNA polymerase. The reaction was run in 5 ml buffer at 37° for 10 min and the RNA was extracted with hot phenol. RNA isolated from various tissues was used as competitor for the hybridization of the *in vitro* synthesized RNA to DNA. Hybridization was with 12 μg of DNA in 0.2 ml of 2× SSC at 67° for 18 hr; 336 cpm was hybridized in the absence of competitor.

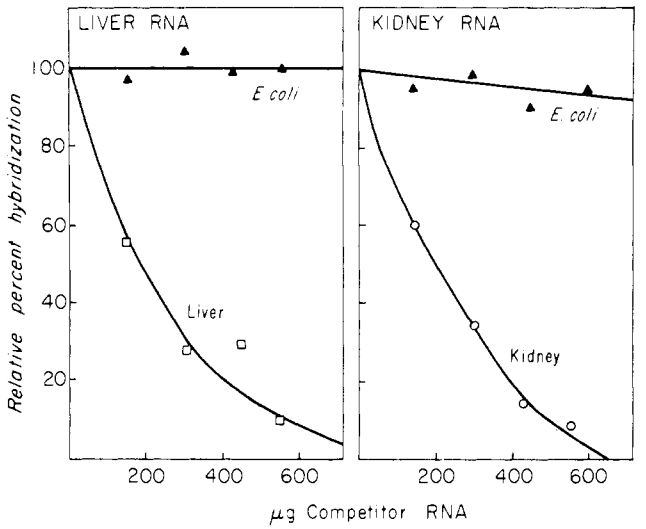

FIGURE 5: Competition of RNA synthesized *in vitro* from chromatin in the hybridization reaction of RNA labeled *in vivo*. (a) ^{32}P liver RNA (5 μg) labeled *in vivo* was hybridized with 12 μg of DNA in the presence of increasing amounts of RNA synthesized *in vitro* with liver chromatin. Hybridization was in 0.2 ml of 4× SSC for 18 hr at 67°; 520 cpm was hybridized in the absence of competitor. (b) ^{32}P kidney RNA (5 μg) labeled *in vivo* was hybridized with 12 μg of DNA in the presence of increasing amounts of RNA synthesized *in vitro* with kidney chromatin. Hybridization was in 0.2 ml of 4× SSC for 18 hr at 67°; 620 cpm was hybridized in the absence of competitor.

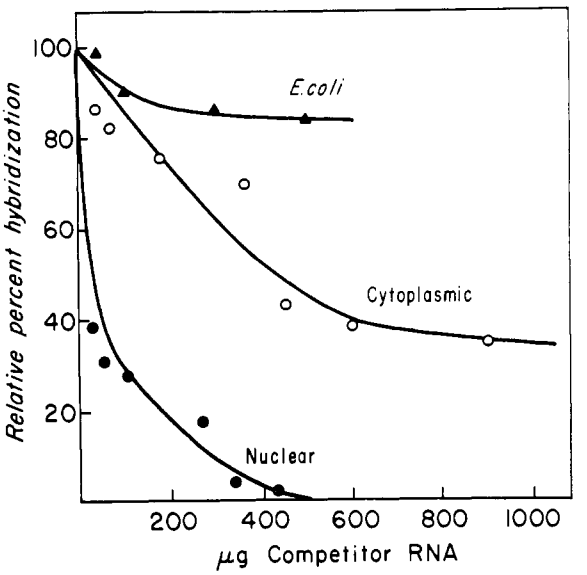

FIGURE 6: Competition of nuclear and cytoplasmic RNA in the hybridization reaction of RNA synthesized *in vitro* using liver chromatin as a template. The RNA was hybridized with DNA in the presence of increasing amounts of unlabeled competitor RNA from liver nuclei or liver cytoplasm. Hybridization was performed with 12 μg of DNA in 0.2 ml of 2× SSC at 67° for 20 hr; 266 cpm was hybridized in the absence of competitor.

example, in Figure 3 it is apparent that liver RNA is the most effective competitor for RNA synthesized from liver chromatin and that this competition is essentially complete. Lung, kidney, and brain RNA are progressively less effective indicating only partial similarity while bacterial RNA has no effect. Similar results were obtained with RNA transcribed from kidney and brain chromatin (Figure 3b,c); in each case homologous RNA gave essentially complete competition and heterologous RNA only partial effects. This series of experiments implies that each chromatin preparation serves as a template for a specific array of RNA molecules. Furthermore, all of the RNA molecules synthesized are counterparts of similar molecules present in the parental tissue and no new sites are activated as a result of the purification of chromatin.

The experiments illustrated in Figure 3 employed *E. coli* polymerase. In order to compare the effects of homologous and heterologous enzyme, some of the same experiments were repeated using mouse RNA polymerase. A very similar set of results was obtained (Figure 4) demonstrating that the fidelity of transcription is to a large extent independent of the enzyme used as a catalyst. Some slight but consistent differences in the shapes of the competition curves obtained when the two enzymes are used suggested the possibility of minor distortions in the relative proportions of RNA molecules synthesized. For example, although liver RNA competitor gives complete competition with RNA synthesized from liver chromatin whichever enzyme is used, consistently larger amounts of competitor are necessary in the case of the *E. coli* enzyme. This is apparent from comparison of Figures 3a,b and 4a,b. These differences are minor, however, and the conclusion is tentative.

While the experiments illustrated in Figures 3 and 4 suggest that no new sites of RNA synthesis are revealed through the isolation and purification of chromatin, they are not sensitive to the possibility that some RNA molecules synthesized

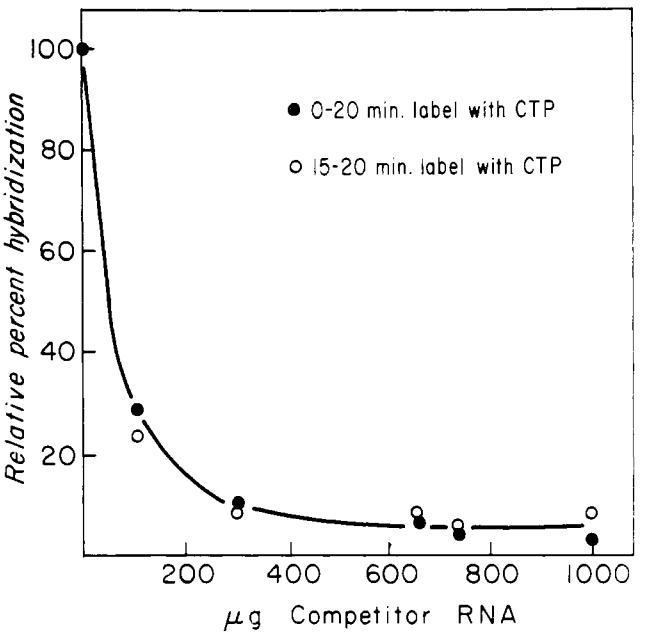

FIGURE 7: Competition by liver RNA in the hybridization reaction of RNA synthesized *in vitro* from liver chromatin. RNA was synthesized for 20 min at 37° with labeled CTP present throughout or only during the final 5 min. Hybridization was to filters containing 12 μg of DNA in 0.2 ml of 2× SSC at 67° for 18 hr; 210 cpm was hybridized in the absence of competitor.

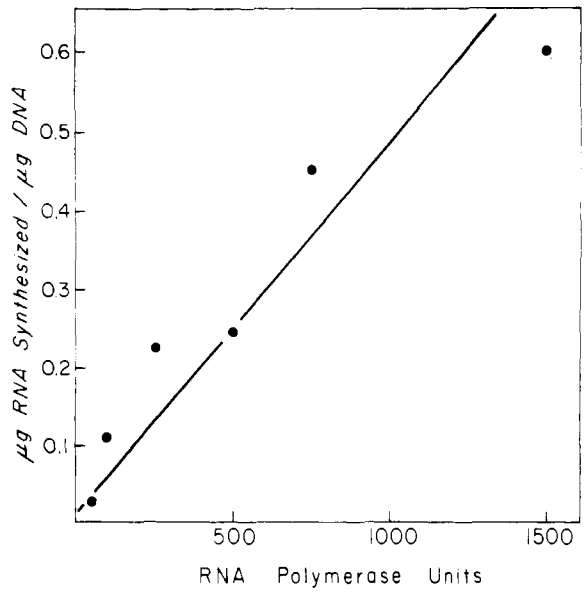

FIGURE 8: Relationship of the amount of RNA synthesized *in vitro* per unit chromatin template to the amount of RNA polymerase present. RNA synthesized *in vitro* using 11.8 μg of liver chromatin as a primer with increasing amounts of RNA polymerase. Fraction 4 RNA polymerase was used and the incubation was carried out for 20 min. The ratio of the total amount of RNA synthesized to the amount of chromatin DNA is plotted against increasing amounts of RNA polymerase.

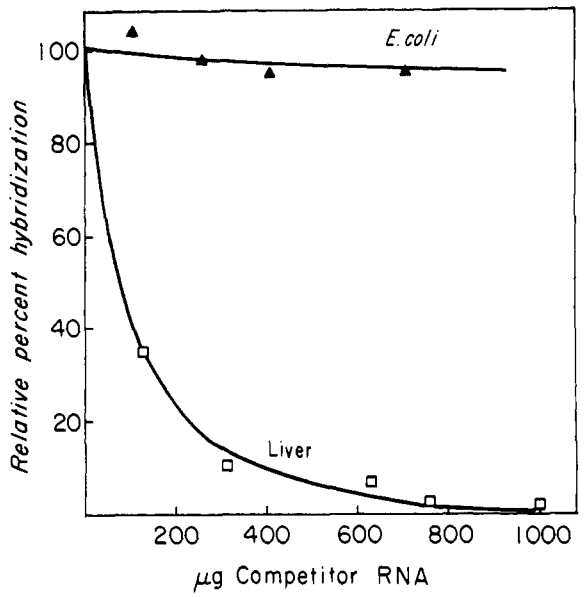

FIGURE 9: Competition by liver RNA or *E. coli* RNA in the hybridization reaction of RNA synthesized *in vitro* from liver chromatin under conditions of net synthesis. RNA synthesized *in vitro* using 10 μg of liver chromatin as a template and 1000 units of *E. coli* RNA polymerase fraction 4. The incubation was carried out for 20 min. Mouse liver RNA or *E. coli* RNA was used as competitor in increasing amounts. Hybridization was in 0.2 ml of 2× SSC at 67° for 18 hr; 1105 cpm was hybridized in the absence of competitor.

in vivo are absent in the *in vitro* synthesized RNA. This was tested by examining the competitive efficiency of RNA synthesized from chromatin with the hybridization of labeled RNA isolated from the appropriate parental tissue. In the case of both liver and kidney RNA complete competition could be obtained with unlabeled RNA transcribed from the corresponding chromatin preparation (Figure 5).

A further estimate of the fidelity of transcription from chromatin was made through examination of the relative amounts of cytoplasmic and nuclear RNA produced. Previous work has shown that some RNA molecules are restricted to the nucleus of mouse L cells or liver cells although the majority are represented in both nucleus and cytoplasm (Shearer and McCarthy, 1967; Church and McCarthy, 1967b). Thus when the hybridization of labeled nuclear RNA is studied, unlabeled nuclear RNA is the better competitor and cytoplasmic RNA leads to incomplete competition. Figure 6 shows that the same result is obtained when liver nuclear and cytoplasmic RNA are compared with labeled RNA transcribed from liver chromatin.

Most of the experiments described were carried out using RNA synthesized *in vitro* under conditions of low RNA polymerase to primer ratio where only small amounts of RNA were produced. Thus, it is possible to account for the apparent specificity by assuming that chain termination accounts for the RNA synthesized *in vitro*. That is, the added polymerase *in vitro* merely completes the synthesis of RNA molecules initiated *in vivo*. Under these circumstances one would expect to retain specific *transcription in vitro* since most of the labeled molecules were not initiated in the incubation. Thus, it is essential to establish that chain initiation occurs *in vitro* and that all potential templates within the chromatin are transcribed.

The uniformity of transcription throughout the duration of the incubation is illustrated in Figure 7. Two parallel reactions were carried out using 5 μg of liver chromatin and 100 units of *E. coli* RNA polymerase. In one reaction [^{3}H]CTP was added for the entire 20-min incubation time and the RNA was then extracted and purified. In the second reaction, unlabeled CTP was present for the first 15 min of the reaction and [^{3}H]CTP added for the final 5 min of synthesis. Both RNA preparations were then hybridized to DNA in the presence of increasing amounts of competitor RNA isolated from mouse liver. The two RNAs gave essentially identical competition curves indicating that new RNA chains initiated in the latter part of the incubation period are undistinguishable from the total population.

Much larger amounts of RNA per unit of chromatin DNA may be synthesized by using high RNA polymerase to chromatin ratios (Marushige and Bonner, 1966). This is illustrated in Figure 8 where it is apparent that at high ratios the amount of RNA synthesized is comparable with the total amount of DNA template present. The ratio of RNA synthesized per unit of DNA approaches 0.5 at the highest level of enzyme used. This is equivalent to the total amount of potential template present assuming one-strand transcription. Since only a few per cent of the chromatin sites are actually active, several RNA molecules must be transcribed from each active site.

When the template specificity of chromatin was assayed under conditions of high enzyme to chromatin ratio, results identical with those of Figure 3 were obtained. An example

is shown in Figure 9 depicting competition of liver RNA with RNA synthesized from liver chromatin.

Conclusions

Within the limits of sensitivity of the comparisons made (Church and McCarthy, 1968), RNA transcribed from chromatin *in vitro* is indistinguishable from that synthesized *in vivo* in the organ from which chromatin was purified. On the other hand, liver, kidney, and brain chromatin do support the synthesis of different RNA molecules and these represent only a restricted part of those which may be transcribed from purified DNA. Similar conclusions have been drawn by Paul and Gilmour (1966, 1968) for calf thymus chromatin and Bekhor *et al.* (1969) for pea chromatin. However, no final judgment can yet be made as to the limits of this fidelity of transcription. As a result of the occurrence of many similar nucleotide sequences in the DNA of higher organisms (Britten and Kohne, 1968), annealing reactions of these nucleic acids are not completely locus specific. Thus a given RNA molecule may recognize and form a hybrid with DNA sites other than that responsible for its synthesis. Since the number of related nucleotide sequences constituting a family may be large, cross-reactions among different DNA sites and their transcripts will dominate the DNA–RNA hybrids formed. The actual extent of cross-reaction is dependent upon the incubation conditions which allow more or less mispairing of bases in the hybrids formed (Church and McCarthy, 1968). Thus RNA molecules having a high degree of similarity in base sequence are not distinguishable. Moreover the incubation times are much too brief to permit hybridization of RNA molecules originating from genes of unique nucleotide sequence (Britten and Kohne, 1968). Thus no information is obtained relative to the specificity of transcription from this part of the genome.

In spite of these qualifications, it is apparent that a high degree of tissue specificity is retained by isolated chromatin with respect to the synthesis of RNA *in vitro*. This is equally true whether homologous or heterologous RNA polymerase is employed and whether RNA chains are merely completed *in vitro* or actually initiated during the incubation. Thus it would appear that the elements determining the differential transcription of RNA reside within the macromolecular configuration of the interphase chromosomes themselves. This conclusion suggests the possibility of elucidating the mechanisms of control of gene activity in eucaryotic cells through further study of the structure of chromatin. Recent studies of the dissociation and reassociation of chromatin *in vitro* implicate chromosomal RNA (Bekhor *et al.*, 1969; Huang and Huang, 1969) or acidic proteins (Paul and Gilmour, 1969) as determinants of the specificity of transcription.

References

Allfrey, V., Littau, V. C., and Mirsky, A. E. (1963), *Proc. Natl. Acad. Sci. U. S. 49*, 414.

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

Blabel, G., and Potter, V. R. (1966), *Science 154*, 1662.

Bonner, J., Chalkley, G. R., Dahmus, G. R., Fambrough, D., Fujimura, F., Huang, R. C., Huberman, J., Jensen, R., Marushige, K., Ohlenbusch, H., Olivera, B., and Widholm, J. (1967), *Methods Enzymol. 12*, 3.

Bonner, J., Dahmus, M. E., Fambrough, D., Huang, R. C., Marushige, K., and Tuan, D. Y. H. (1968), *Science 159*, 47.

Bonner, J., and Huang, R. C. (1963), *J. Mol. Biol. 6*, 169.

Britten, R. J., and Kohne, D. E. (1968), *Science 161*, 529.

Burton, K. (1956), *Biochem. J. 62*, 315.

Chamberlin, M., and Berg, P. (1962), *Proc. Natl. Acad. Sci. U. S. 48*, 81.

Church, R. B., and McCarthy, B. J. (1967a), *J. Mol. Biol. 23*, 459.

Church, R. B., and McCarthy, B. J. (1967b), *Proc. Natl. Acad. Sci. U. S. 58*, 1548.

Church, R. B., and McCarthy, B. J. (1968), *Biochem. Genet. 2*, 55.

Dingman, C., and Sporn, N. (1964), *J. Biol. Chem. 239*, 3482.

Dische, J., and Schwartz, K. (1937), *Mikrochim. Acta 2*, 13.

Georgiev, G. P., Ananieva, L. N., and Kozlow, J. V. (1966), *J. Mol. Biol. 22*, 365.

Gillespie, D., and Spiegelman, S. (1965), *J. Mol. Biol. 12*, 829.

Huang, R. C., and Huang, P. C. (1969), *J. Mol. Biol. 39*, 365.

Lowry, O. H., Rosebrough, N. J., Farr, A. C., and Randall, R. J. (1951), *J. Biol. Chem. 193*, 265.

Marmur, J. (1961), *J. Mol. Biol. 3*, 208.

Marushige, K., and Bonner, J. (1966), *J. Mol. Biol. 15*, 160.

McCarthy, B. J., and Hoyer, B. H. (1964), *Proc. Natl. Acad. Sci. U. S. 52*, 915.

Paul, J., and Gilmour, R. (1966), *J. Mol. Biol. 15*, 242.

Paul, J., and Gilmour, R. (1968), *J. Mol. Biol. 34*, 305.

Paul, J., and Gilmour, R. (1969), *J. Mol. Biol. 40*, 137.

Shearer, R. W., and McCarthy, B. J. (1967), *Biochemistry 6*, 283.

So, A. G., Davies, E. W., Epstein, R., and Tissières, A. (1967), *Proc. Natl. Acad. Sci. U. S. 58*, 1739.

Sullivan, D. T. (1968), *Proc. Natl. Acad. Sci. U. S. 59*, 846.

Zubay, G., and Doty, P. (1959), *J. Mol. Biol. 1*, 1.