COMPARISON OF TRANSCRIPTION OF CHROMATIN BY CALF THYMUS AND E. COLI RNA POLYMERASES

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Kinetic parameters of transcription of DNA and chromatin by calf thymus and E. coli RNA polymerases were studied. Using calf thymus RNA polymerase, the theoretical maximum velocity (Vmax) for chromatin is similar to that for DNA. The concentration of chromatin required to reach one-half the maximum velocity (Km) is greater than the Km for DNA. Using E. coli RNA polymerase, the Km for chromatin is similar to the Km for DNA, whereas the Vmax for chromatin is much less than the Vmax for DNA. These differences suggest that calf thymus RNA polymerase binds to specific, selected sites on chromatin and transcribes at all of these sites. E. coli RNA polymerase binds to many non-selected sites and transcribes at some or all of them.

The interaction of RNA polymerase with chromatin in the cell is considered to be an important control mechanism in differentiation. Therefore it is of interest to study this interaction under in vitro conditions. Previous investigators, using bacterial RNA polymerase, have reported that RNA isolated in vivo from a particular type of cell is similar to RNA synthesized in vitro when chromatin but not DNA is used as a template (Paul and Gilmour, 1968; Smith, Church and McCarthy, 1969; Bekhor, Kung, and Bonner, 1969; Huang and Huang, 1969; Tan and Miyagi, 1970.). Since transcription of chromatin appears to be restricted it is somewhat surprising that kinetic analysis of the bacterial polymerase reaction suggests that the enzyme binds to the same number of sites on chromatin as on DNA (Marushige and Bonner, 1966). The explanation offered for this apparent inconsistency is that while the enzyme binds to the same number of sites on chromatin as on DNA, RNA synthesis occurs at only some of these sites. The present investigation suggests that mammalian enzyme, in contrast, binds to specific, selected sites on chromatin and transcribes at all of these sites.

Calf thymus RNA polymerase was purified as described previously (Furth, Nicholson and Austin, 1970). The bulk of the enzyme elutes from a DEAE Sephadex column at approximately 0.23M $(NH_4)_2SO_4$ (corresponding to Form II of Roeder and Rutter, 1969) but is not inhibited by α-amanitin. E. coli RNA polymerase was obtained as described by Furth and Pizer (1966) followed by chromatography on DEAE Sephadex. The preparation contained sigma factor. Units for both enzymes were defined as theoretical maximum velocity [obtained by a Lineweaver-Burk plot (1934)] using native calf thymus DNA as template. Units for calf thymus and E. coli polymerase are expressed as nmoles UMP incorporated into RNA per 20 min and 10 min, respectively. Specific activities of the enzymes (units per mg protein) were 4.4 for calf thymus enzyme and 46.5 for E. coli enzyme. Chromatin was prepared by the method of Paul and Gilmour (1968), slightly modified. In some experiments the chromatin was sheared in a VirTis homogenizer at 60% of maximum for 1 minute at 4°. DNA was prepared from nuclei by the procedure of Marmur (1961), slightly modified.

Transcription of calf thymus chromatin and DNA by calf thymus polymerase is shown in Fig. 1, together with a parallel experiment using E. coli RNA polymerase. Chromatin, sheared and unsheared, is a much better template for mammalian enzyme than for bacterial enzyme (cf. Butterworth et al., 1971). Double reciprocal plots give similar maximum velocities for reactions with DNA and chromatin. This contrasts with results obtained with E. coli polymerase, where the Vmax is considerably lower for chromatin than for DNA. Using calf thymus RNA polymerase, the concentration of DNA required to reach half the theoretical maximal velocity, the apparent Km, is 64 μmoles deoxynucleotides per liter. For chromatin the apparent Km is 150 μmoles deoxynucleotides per liter. Therefore, $2\frac{1}{2}$ times as much chromatin as DNA is required to achieve half maximal velocity. Using E. coli RNA polymerase, the apparent Km for chromatin is approximately

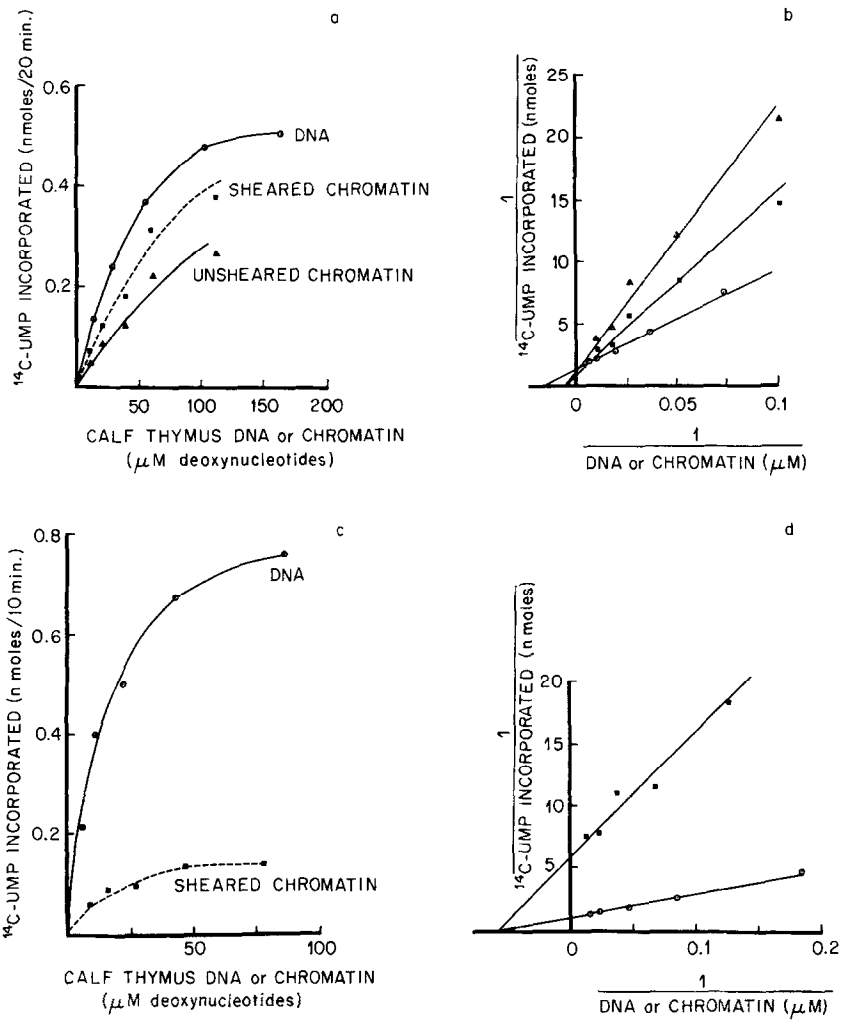

Figure 1. Transcription of calf thymus DNA and calf thymus chromatin.
(a) Effect of varying concentration of template with calf thymus RNA polymerase: ⊖——⊖ calf thymus DNA, ▣---▣ sheared calf thymus chromatin, △——△ unsheared calf thymus chromatin.
(b) Double reciprocal plot of (a). Km values: DNA, 64 μmoles deoxynucleotides per liter; sheared chromatin, 150; unsheared chromatin, 150. Vmax values: DNA, 0.75 nmoles UMP incorporated per 20 min; sheared chromatin, 1.0; unsheared chromatin, 0.75.
(c) Effect of varying concentration of template with E. coli RNA polymerase: ⊖——⊖ calf thymus DNA, ▣---▣ sheared calf thymus chromatin.
(d) Double reciprocal plot of (c). Km values: DNA, 18 μmoles deoxynucleotides per liter; chromatin, 17. Vmax values: DNA, 0.95 nmoles UMP incorporated per 10 min; chromatin, 0.17.
The reaction mixture (0.25 or 0.5 ml) contained 50 mM Tris-maleate buffer (pH 7.6), 1 mM $MnCl_2$, 12 mM $MgCl_2$, 0.2 M KCl, 0.01 mM EDTA, 8 mM dithiothreitol, 80 μM ^{14}C-UTP (6 X 10^6 cpm/μmole), 0.32 mM of each of the other three nucleoside triphosphates, DNA or chromatin, and 0.75 units of calf thymus enzyme or 0.95 units of E. coli enzyme.
The reaction was terminated (20 min, calf thymus enzyme; 10 min, E. coli enzyme) and the rate of RNA synthesis determined as described previously (Furth and Ho, 1965).

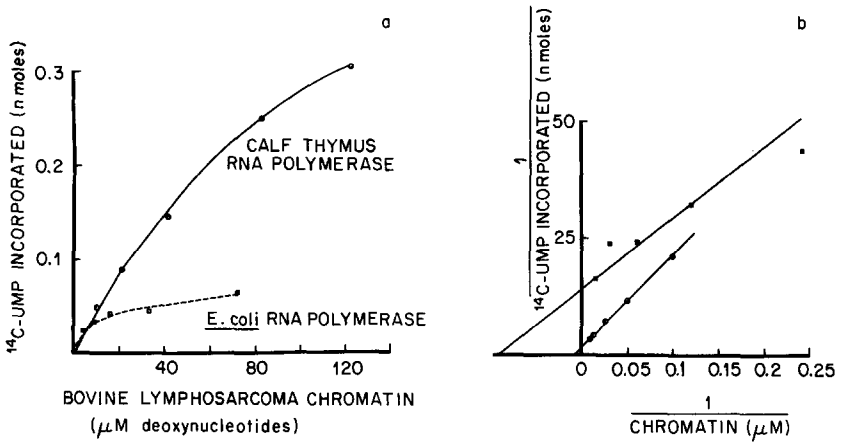

Figure 2. Transcription of bovine lymphosarcoma chromatin by calf thymus and E. coli RNA polymerases.
(a) Effect of varying template concentration. θ——θ calf thymus RNA polymerase, ▣ ---▣ E. coli RNA polymerase. Reaction conditions as in Figure 1, except that 0.62 units of calf thymus polymerase and 0.67 units of E. coli polymerase were used.
(b) Double reciprocal plot of (a). Using calf thymus polymerase (θ——θ) Km is 150 μmoles deoxynucleotides per liter, Vmax is 0.70 nmole UMP incorporated per 20 min. Using E. coli polymerase (▣——▣), Km is 11, Vmax is 0.07 nmole UMP incorporated per 10 min.

equal to that for DNA. Similar results were reported by Marushige and Bonner (1966) for E. coli RNA polymerase.

These differences are also observed with chromatin from bovine lymphosarcoma (Fig. 2). Using the same amounts of calf thymus or E. coli RNA polymerase, calf thymus enzyme is much more active in transcribing lymphosarcoma chromatin. With mammalian enzyme, the Vmax is similar to the Vmax using calf thymus DNA as template, whereas the Vmax for the same amount of E. coli RNA polymerase is one-tenth as much. The Km for lymphosarcoma chromatin using calf thymus RNA polymerase is about 3 times the Km for calf thymus DNA at this level of enzyme. The Km for lymphosarcoma chromatin with E. coli enzyme is approximately the same as for calf thymus DNA.

The apparent Km for DNA compared with chromatin in the RNA polymerase reaction probably reflects, as a first approximation, the number and

availability of binding sites. A higher Km for chromatin than for DNA using calf thymus RNA polymerase suggests that calf thymus enzyme binds to specific sites on chromatin and thus needs a higher concentration of chromatin to achieve a sufficient concentration of these specific sites. Calf thymus enzyme transcribes at all sites to which it binds, so the theoretical maximal velocity is the same for chromatin as for DNA.

E. coli RNA polymerase, having the same Km for DNA and chromatin, binds to the same number of sites on chromatin as on DNA. Its Vmax, however, is lower on chromatin than on DNA, suggesting (a) a large fraction of E. coli enzyme molecules bind to sites on chromatin at which they do not transcribe, or (b) the enzyme transcribes at all sites to which it binds on chromatin but less efficiently than on DNA. [Less efficient transcription could result from lack of re-initiation, a slower rate of nucleotide incorporation or "early quitters" (Bremer, 1970) on chromatin.] The hybridization competition data obtained in a number of laboratories (Paul and Gilmour, 1968; Smith, Church, and McCarthy, 1969; Bekhor, Kung, and Bonner, 1969; Huang and Huang, 1969), suggest that (a) is the case--bacterial RNA polymerase binds to many sites but transcribes from only some of them. The present study suggests that mammalian enzyme is less wasteful, binding only to those sites at which transcription occurs.

Restriction of binding sites for mammalian enzyme on chromatin compared with DNA does not appear to result in significant differences in the base composition of RNA synthesized (Table 1). To illustrate the sensitivity of the enzyme in transcribing templates varying in base composition, results are also shown for RNA transcribed from M. luteus DNA. RNA transcribed from this DNA differs markedly in base composition from that transcribed from calf thymus DNA.[1] While this excludes preferential transcription of

[1]The percent GC (deoxyguanosine and deoxycytosine) of M. luteus DNA is 72% (Lee, et al., 1956) and the percent GC of calf thymus DNA is 44% (Lehman, et al., 1958). The % GC of RNA synthesized thus does not reflect the % GC of DNA template, and the sum of the purines usually does not equal the sum of the pyrimidines. While this is consistent with asymmetric transcription, further study is needed, including a rigid examination of the purity of the nucleoside triphosphates used.

Table 1. Nucleotide Composition of RNA Synthesized *in vitro*.

A. Calf Thymus RNA Polymerase

Template	Determinations	UMP	AMP	CMP	GMP
Calf Thymus DNA	8	1.00	0.92 ± 0.10	1.15 ± 0.10	1.20 ± 0.08
Calf Thymus Chromatin	4	1.00	0.95 ± 0.03	1.09 ± 0.13	1.23 ± 0.13
M. luteus DNA	1	1.00	0.70	2.46	3.22

B. *E. coli* RNA Polymerase

Template	Determinations	UMP	AMP	CMP	GMP
Calf Thymus DNA	3	1.00	1.32 ± .03	1.10 ± .14	1.29 ± .25
Calf Thymus Chromatin	3	1.00	1.31 ± .002	0.97 ± .002	1.12 ± .005
M. luteus DNA	2	1.00	1.15 ± .01	2.86 ± .21	3.54 ± .12

Conditions for RNA synthesis were as described in the legend for Figure 1, except that the concentration of each of the 4 nucleoside triphosphates was 160 μM. One nucleoside triphosphate was labelled in each of four reaction mixtures. The specific activities of the ^{14}C-labelled triphosphates were (cpm X 10^6/μmole): UTP, 3.4 - 4.5; ATP, 2.0; CTP, 4.7 - 5.7; GTP, 3.0 - 4.1. With calf thymus polymerase, reaction mixtures contained (μmoles/liter deoxynucleotides): calf thymus DNA, 120; sheared calf thymus chromatin, 600; or *M. luteus* DNA, 140. With *E. coli* polymerase reaction mixtures contained (μ moles/liter deoxynucleotides): calf thymus DNA, 120; sheared calf thymus chromatin, 40; or *M. luteus* DNA 140. The amounts of enzyme used varied slightly in the experiments. In a typical experiment incorporation of UMP with calf thymus DNA was: calf thymus enzyme 0.24 nmole, *E. coli* enzyme 0.73 nmole. (Incorporation with calf thymus DNA equals units of enzyme.) Incorporation of UMP with chromatin as template was 60% of incorporation with calf thymus DNA using calf thymus enzyme, and 13% using *E. coli* enzyme. Incorporation of UMP with *M. luteus* DNA was 0.28 nmole using calf thymus enzyme, 0.14 nmole using *E. coli*. Incorporation of labeled nucleotide in the absence of template has been subtracted. In a typical experiment using calf thymus enzyme these were (nmole) GMP, 0.013; AMP, 0.015; CMP, 0.019; GMP, 0.020; for *E. coli* enzyme these were (nmole) UMP, 0.007; AMP, 0.024; CMP, 0.010; GMP, 0.010. Values are reported relative to incorporation of UMP, which is set at 1.00. The relative rate of incorporation of the four nucleoside triphosphates reflects the relative base composition of the RNA synthesized (Hurwitz, *et al.*, 1962).

RNA containing an unusual amount of one or more bases such as poly A or ribosomal RNA, more sophisticated analysis is required to establish qualitative differences between RNA transcribed from DNA and chromatin as well as to establish whether RNA transcribed *in vitro* from chromatin is similar to RNA transcribed *in vivo*.

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