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Thermal unwinding of simian virus 40 transcription complex DNA

(DNA topology/chromatin/gene regulation)

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ABSTRACT **Two long-standing questions in the control of eukaryotic gene expression have been how the structure of transcribing chromatin compares with that of nontranscribing chromatin and how chromatin structure differs among various eukaryotic organisms. We have addressed aspects of these two questions by characterizing the rotational flexibility of the DNA of the simian virus 40 (SV40) transcription complex. When transcription complex samples are incubated with topoisomerase at 0°C or 37°C, the DNA of the 37°C sample is unwound by 1.8 turns relative to that of the 0°C sample. This amount of unwinding is similar to that observed for bulk, untranscribed SV40 minichromosome DNA, indicating that the chromatin structure of a transcribed gene resembles that of a nontranscribed gene in the degree of constraint that it imposes on its DNA. However, this amount of unwinding differs substantially from the value observed for yeast plasmid chromatin DNA, suggesting that yeast chromatin differs significantly from mammalian chromatin in this fundamental property.**

Substantial progress has been made in describing structural details of bulk eukaryotic chromatin, but as yet little is known about the structural details of that minor fraction of chromatin that is transcribed (1–3). The work of Weintraub and Groudine (4) demonstrates that some structural difference exists because the enzyme DNase I digests the DNA of transcriptionally active chromatin more rapidly than that of transcriptionally inactive chromatin. This indicates that active chromatin has a more "open" or accessible structure, suggesting that the DNA–histone interactions may perhaps be less stable than those of inactive chromatin.

Recent results from studies of yeast plasmid chromatin (5, 6) would seen to support this view. The topology of the DNA of yeast plasmid chromatin was found to change with temperature in an amount that is ≈70% the value exhibited by bare DNA. This substantial rotational flexibility of the yeast chromatin DNA differs markedly from that of the DNA of the simian virus 40 (SV40) minichromosome (7, 8), which has been shown to unwind by only 25% [or ≈1.5 turns per 5243 base pairs (bp)] of the value for bare DNA. However, the bulk chromatin of SV40 is predominantly inactive transcriptionally: only ≈1% of the SV40 minichromosomes in an infected cell appear to be transcriptionally active (9). Yeast 2-μm plasmid chromatin, on the other hand, is thought for the most part to be transcriptionally active. Noting these functional differences, Saavedra and Huberman (5) have suggested that the relatively flexible structure that they observe in the yeast 2-μm minichromosome may be a fundamental feature of the transcriptionally active subclass of eukaryotic chromatin. By this proposal, the transcribed fraction of mammalian chromatin would be expected to exhibit the enhanced DNA flexibility characteristic of the yeast chromatin.

We have carried out experiments here that directly test this proposal by analyzing the topological properties of that fraction of SV40 minichromosomes that contain *in vivo*-initiated RNA polymerase molecules that are capable of run-on transcription *in vitro* (10). The ability to specifically analyze the topology of the transcription complex is important because, as mentioned above, the transcription complexes comprise only ≈1% of the bulk intracellular minichromosome population late in infection (9). Thus, the numerous topological characterizations of the bulk minichromosome DNA that have been reported (7, 11–13) do not necessarily describe features of the minor fraction of minichromosomes that are transcribed. The method we used previously (10) for this specific analysis involves labeling with radioactive UTP the nascent RNA of the transcription complex by extension *in vitro* of *in vivo*-initiated RNA polymerases. Here we use this method to quantitate the extent to which the transcription complex DNA unwinds upon an increase in temperature. The results indicate that the amount of unwinding in SV40 transcription complex DNA is very similar to that of the DNA of bulk SV40 chromatin but differs substantially from that of yeast plasmid chromatin.

MATERIALS AND METHODS

Cells, Virus, and Nuclear Extract Preparation. General methodologies for growing cells, virus infection, and *in vitro* extension incorporation of radioactive UTP into late viral transcription complexes are described by Petryniak and Lutter (10). The nuclear extract preparation was the modification of that of Llopis and Stark (14), which was described by Petryniak and Lutter (10), except that the final resuspension of nuclei was in TL buffer (137 mM NaCl/5.1 mM KCl/1 mM Na_2EDTA/0.2 mM phenylmethylsulfonyl fluoride/50 mM Tris·HCl, pH 7.9). This procedure extracts the majority of recoverable SV40-specific *in vitro* transcription activity from the nucleus (15). The activity represents transcription from all portions of the SV40 DNA (16, 40).

Topoisomerase Treatment and Run-On Transcription Labeling. Incubation of nuclear extract with topoisomerase was carried out as follows: nuclear extract was diluted 1:4 with TE (10 mM Tris·HCl/1 mM Na_2EDTA, pH 7.9) and then concentrated to 1/4 the original volume using a Centricon 30 (Amicon). Final concentration was the extract of one plate (≈10^6 cells) in 0.050 ml. To this was added 8 units of wheat germ topoisomerase (Promega), after which the sample was incubated at the indicated temperature for the indicated time. Reactions were terminated by the addition of sodium lauryl sarcosinate (Sarkosyl; Sigma) to 0.5% and aurintricarboxylic acid (ATA; Sigma) to 0.2 mM. This treatment inhibits topoisomerase activity as well as removes the histones from the DNA (see data in Figs. 2 and 3; ref. 40) but leaves engaged RNA polymerase II in a form that is capable of further transcription elongation (17).

This ternary complex of DNA, RNA polymerase, and radioactive nascent RNA can then be analyzed directly for its topological characteristics by agarose gel electrophoresis

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Abbreviations: SV40, simian virus 40; ATA, aurintricarboxylic acid.

(18). Thus, after terminating the topoisomerase incubation, the sample was added to an *in vitro* extension reaction (10). Final concentrations in the extension reaction were 24 mM NaCl, 290 mM ammonium acetate, 3.8 mM $MnCl_2$, 1 mM KCl, 0.03 mM phenylmethylsulfonyl fluoride, 0.17 mM Na_2EDTA, 0.4% Sarkosyl, 0.16 mM ATA, 1.9 mM dithiothreitol, 0.51 mM ATP, 0.51 mM GTP, 0.51 mM CTP, 0.66 mCi of [^{32}P]UTP per ml (650 Ci/mmol; 1 Ci = 37 GBq; ICN), and 85 mM Tris·HCl (pH 7.9). Incubation was for 20 min at 30°C. After extension incubation, 1/10th vol of 100 mM Na_2EDTA was added, after which unincorporated nucleotides were removed from the sample with a Sephadex G50 spin column equilibrated with TE buffer (10 mM Tris·HCl/1 mm Na_2EDTA, pH 7.9) containing 0.2 mM ATA. Sample volume was then reduced severalfold by evaporation in a Savant Speed-Vac before electrophoresis. The sample was then fractionated by electrophoresis in an agarose gel (0.7% agarose; 30 cm; 18 hr; 2.6 V/cm) as described (10). After electrophoresis, the gel was dried and subjected to autoradiography with Kodak XAR film. Autoradiographs were scanned with an LKB Ultroscan densitometer. Bulk DNA distributions were determined by illuminating the ethidium bromide-stained gel on a transilluminator and photographing with Tri-X 4 × 5 film. The negative was then scanned with the LKB Ultroscan.

Determination of Peak Width and Center of Topoisomer Distribution. Multiple autoradiograph exposures of each sample were scanned with an LKB Ultroscan densitometer, and peak heights above baseline of individual topoisomers were measured. A Gaussian curve was then fitted to these point distributions by using a computer program supplied by M. Bina (7). This method provides an accurate determination of both the peak width at half height (Γ) (7) and the distribution center (mean) because all of the data points (individual topoisomer peaks) contribute to the determination. In practice, peak centers could be located with an accuracy of better than ±0.1 linking number units with this method for the actual autoradiographs shown in Fig. 2, while Γ values could be determined to an accuracy of about ±0.1 linking number units (estimated by repeated fits to the data sets of the channels shown).

RESULTS

Topoisomerase Can Relax Tension in the Viral Transcription Complex at 0°C. Methods were previously developed to analyze the topology of the DNA of the SV40 late transcription complex (10). This earlier study demonstrated that the transcription complex does not contain a substantial amount of unconstrained topological tension. In the current study, the previous methodology has been modified to allow analysis of the topology of the ternary transcription complex, a complex composed of DNA, RNA polymerase II, and nascent RNA labeled with ^{32}P *in vitro* (18). These ternary complexes are analyzed by electrophoresis in an agarose gel under conditions in which mobility reflects their DNA topology. When the initial labeling is carried out with [^{32}P]UTP, an autoradiograph of the dried gel reveals the mobility of the transcription complex DNA. Analysis of the ternary complex permits a more accurate quantitation of topology than was possible in the binary complex we used previously (10) because individual topoisomers can be visualized in the autoradiograph of the ternary complex gel (18, 19). A scheme of the procedure is shown in Fig. 1.

The rotational flexibility of pure double-helical DNA can be assessed by examining the topological change that occurs in closed circular DNA when it is incubated at two different temperatures in the presence of topoisomerase (20, 21). This flexibility can also be determined for DNA in nucleoprotein complexes such as the SV40 minichromosome (7, 11–13).

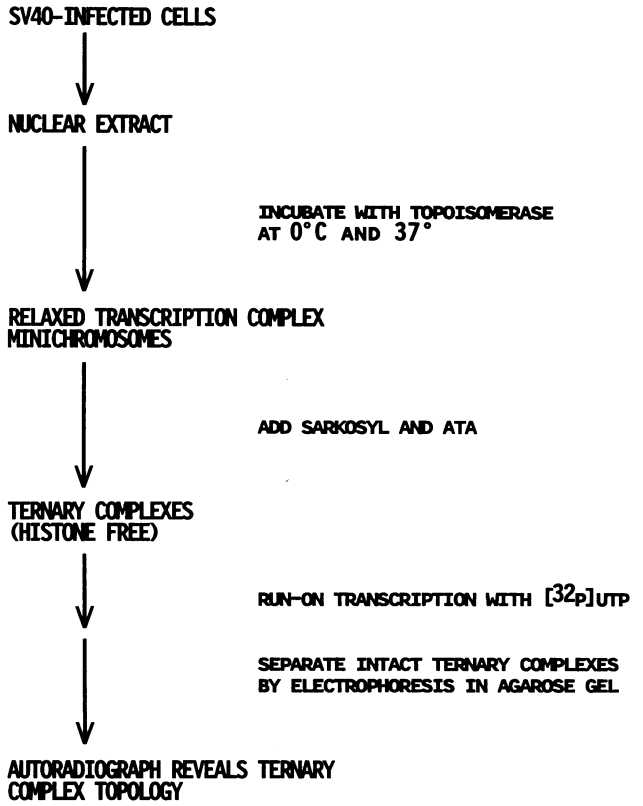

FIG. 1. Scheme for analysis of the temperature dependence of the topology of the DNA of the viral transcription complex.

Here this thermal unwinding approach has been used in conjunction with the transcription complex labeling methodology to characterize the DNA flexibility of the SV40 transcription complex. The experiment involves preparing a nuclear extract from SV40-infected cells and incubating samples of this extract at 0°C or 37°C in the presence of added topoisomerase, after which the topological difference between the transcription complexes in the two samples is quantitated by analyzing the autoradiograph of the agarose gel upon which the labeled ternary complexes were separated (see Fig. 1).

The results of such an experiment are shown in Fig. 2. Several features of the results should be noted at the outset. First, the discrete banding of the topoisomers in the autoradiograph demonstrates that the length of the RNA·DNA duplex in the complex does not vary by much more than 1 bp among different members within the transcription complex population [the length of the duplex has been reported to be ≈20 bp (19)]. This feature is important because, as noted above, it allows accurate determination of the center of the distribution.

Scans of the channels on the autoradiograph reveal a second structural feature of the transcription complex: the width of the topoisomer distribution of the transcription complex (e.g., see Fig. 2, lanes C and D) is considerably narrower than that of the bulk intracellular minichromosome DNA (Hirt extracted; lane A), indicating that the population of the transcription complexes is structurally more uniform than that of the total intracellular minichromosome population. The breadth of the distribution can be most accurately quantitated by fitting the individual topoisomer peak heights to a Gaussian distribution (see above; ref. 7). An example of this is shown in Fig. 2 (*Lower Left*), where the data points for lane C can be seen to fit quite well to a Gaussian distribution with a half-height width of 4.8 turns (linking number units). Thus, the data here show that the transcription complex

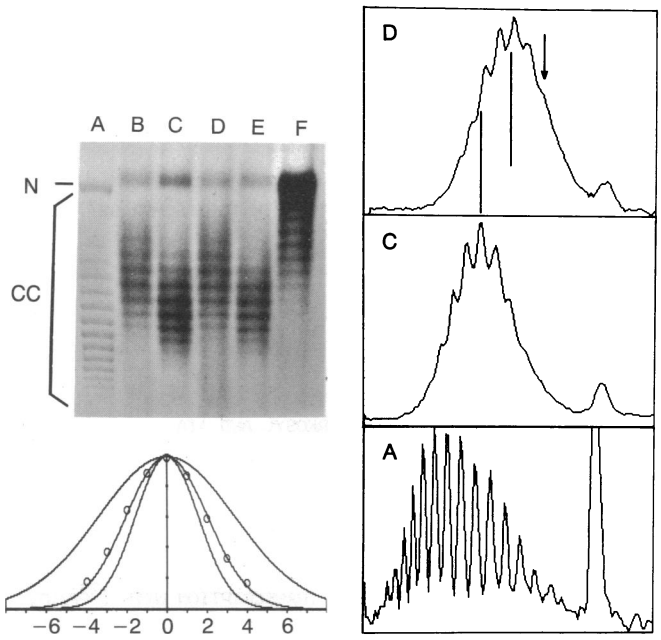

FIG. 2. Topoisomer distributions of the ternary transcription complexes. Nuclear extract samples were incubated at 0°C or 37°C with added wheat germ topoisomerase, after which the sample was processed (see Fig. 1). These samples containing deproteinized bulk DNA but intact labeled ternary transcription complexes (DNA, engaged RNA polymerase, and ^{32}P-labeled nascent RNA) were then fractionated on an agarose gel containing 0.030 mM chloroquine. This level of chloroquine causes each of the DNA samples to migrate as a symmetrical distribution of positively supercoiled topoisomers. (*Upper Left*) Autoradiograph of the agarose gel, with the ternary complexes being visualized in lanes B–F. Lanes: A, [^{14}C]thymidine-labeled, Hirt-extracted SV40 DNA standard. The following lanes represent samples of nuclear extract that were incubated at: B, 37°C for 130 min; C, 0°C for 2.5 hr; D, 37°C for 40 min; E, 0°C for 18 hr; F, O°C for 18 hr, in the presence of chloroquine (1 mg/ml). *N*, mobility of the nicked circular DNA in lane A; CC, mobilities of the closed circular DNA topoisomers. Note that the mobility of the nicked ternary complex (lanes B–F) is less than that of the nicked bare DNA (lane A); this "band shift" is most likely due to the RNA polymerase present on the ternary complex. (*Right*) Densitometric traces of lanes A (Hirt standard), C (0°C; 18 hr), and D (37°; 40 min). (*Lower Left*) Gaussian curve (mean, −0.1; Γ, 4.8) fitted to the topoisomer peak heights from the densitometer scan of lane C (○, middle curve). Also shown for comparison are curves of the average distribution of Hirt-extracted DNA (outer curve; Γ, 8.0; cf. Table 1) and bare SV40 DNA (inner curve; Γ, 3.6; cf. Table 1).

DNA topoisomer distribution is Gaussian, as has been shown previously for the bare DNA distribution (20, 21), the free minichromosome DNA distribution (7), and the bulk intracellular (Hirt) DNA distribution (7, 11). The Gaussian fitting method has been used to quantitate the half-height width (Γ) of transcription complex samples as well as Hirt extract DNA samples, and the results are tabulated in Table 1 in the column Γ. From these data, it is clear that the transcription complex DNA distribution is substantially narrower (Γ = 4.8–5.2) than that of either the total intracellular (Hirt) SV40 DNA (Γ = 8.0) or the bulk minichromosome DNA (Γ = 6.4–7.2). This indicates that the transcription complex population is structurally more uniform than these other minichromosome populations.

One requirement in the design of the experiment shown in Fig. 2 is the need to establish that the added wheat germ topoisomerase is capable of relaxing tension in the viral transcription complex at 0°C if such tension is present. This was demonstrated in a control experiment in which chloroquine was added to a sample of nuclear extract during the topoisomerase incubation at 0°C. The intercalation of chloroquine into the transcription complex DNA will unwind the DNA duplex (produce negative twist) and induce opposite and equivalent positive supercoiling (positive writhe) in the transcription complex minichromosome. If the topoisomerase is active at 0°C on the transcription complex minichromosome, this induced unconstrained positive supercoiling will be relaxed, causing the transcription complex DNA to acquire a more negative linking number.

Table 1. Topological parameters

Sample	Γ*	Mean†	$L_{37} - L_0$
VTC			
37°C, 40 min	5.2	−1.8	
37°C, 130 min	5.2	−1.7	−1.8 ± 0.1
0°C, 2.5 hr	4.8	−0.1	
0°C, 18 hr	4.8	0.0	
Bulk minichromosome			
37°C, 40 min	7.0	−1.8	
37°C, 130 min	7.2	−1.9	−1.4 ± 0.1
0°C, 2.5 hr	6.4	0.45	
0°C, 18 hr	7.0	0.45	
Nuclear extract minichromosome	5.3 ± 0.3		−1.5 ± 0.7
Yeast plasmid chromatin‡			−4.0
Total Hirt DNA	8.0 ± 0.25		
Bare SV40 DNA	3.6		−5.93

Viral transcription complex (VTC) values were determined by fitting Gaussian curves to data from samples in Fig. 3 (lanes B–E). Bulk minichromosome values were determined by fitting Gaussian curves to data from samples in Fig. 2 (lanes B–E). Nuclear extract minichromosome values are from ref. 7. Total Hirt DNA values were determined by fitting Gaussian curves to data from densitometric scans of six samples. Bare SV40 DNA values were calculated from data in refs. 20, 21, and 39.

*Defined, as in ref. 7, as full width at half height, in linking number units.

†The topoisomer of maximum abundance in Figs. 2 (lane C) and 3 (lane C) is arbitrarily assigned a value of 0.

‡Linkage change that would occur if SV40 DNA were unwound by an amount equivalent to that which occurs in yeast chromatin with a 37°C increase in temperature (5, 6) (see text for calculation).

A comparison of Fig. 2 (lane E) (0°C, no chloroquine) with lane F (0°C, with chloroquine) shows that such is in fact the case. The agarose gel here contains 0.03 mM chloroquine, so the ternary transcription complex topoisomers visualized here are positively supercoiled—i.e., lower mobility corresponds to lower linking number. It can be seen that the mean mobility of the topoisomer distribution in the chloroquine-treated sample (lane F) is less than that of the untreated sample (lane E), demonstrating that the linking number of the DNA of the intact transcription complex has indeed become more negative as expected. An additional control to establish this fact involved incubating a sample at 37°C for 40 min, then shifting to 0°C for 18 hr; the mean of the topoisomer distribution was identical to that of a sample that had been incubated at 0°C for the whole time period, demonstrating by using the temperature shift (see below) as the perturbant instead of chloroquine (cf. ref. 22.) that the topoisomerase is active at 0°C on the transcription complex (data not shown).

Another feature that should be noted in Fig. 2 (lane E) is that topoisomerase treatment in the absence of chloroquine did not result in the migration of the radioactivity at the position of relaxed DNA. This result demonstrates that the SV40 transcription complex does not contain a substantial amount of unconstrained topological tension, a finding that confirms our previous results (10) by using an alternative system of analysis.

Having established the methodology for locating accurately the center of the topoisomer distribution as well as the fact that the added topoisomerase can relax unconstrained

tension in the intact transcription complex at 0°C, the amount of topological change that occurs in the complex when it is incubated with topoisomerase at 0°C and 37°C was assessed. The results are shown in Fig. 2 (lanes B–E) and are tabulated in Table 1. It can be seen that the difference between the samples relaxed at 0°C (lanes C and E) and 37°C (lanes B and D) is -1.8 ± 0.1 turns—i.e., the 37°C sample is unwound by 1.8 turns relative to the 0°C sample. Values for the mean of the distribution for the two time points are essentially the same at a given temperature, indicating that the relaxation has reached completion at the shorter of the two time points for both temperatures. A similar value was obtained when the binary complex (i.e., the RNase-treated RNA·DNA hybrid from the transcription complex, as described in ref. 10) was analyzed, although the accuracy was limited by the lack of the internal topoisomer ladder to serve as a scale, as mentioned above (data not shown). The value of -1.8 ± 0.1 is less than half the value of -4.0 (Table 1), which the yeast result would suggest for the SV40 transcription complex [SV40 DNA is 5243 bp, and the 6318-bp yeast 2-μm plasmid was found to unwind by 5.0 turns over 38°C (5), so 5.0 turns $\times$ (5243/6318) $\times$ (37/38) = 4.04 turns of unwinding expected for SV40]. The magnitude of this difference is illustrated by the location of the vertical arrow in the densitometer scan of Fig. 2 (*Right*, panel D); this arrow indicates where the center of the 37°C sample distribution would be if it were unwound by 4 turns relative to the 0°C sample.

In addition to determining the topological change of the DNA in the transcription complex from the autoradiograph of the gel as described above, the topological change of the bulk minichromosome DNA can also be determined in the same experiment from a photograph of the gel stained with ethidium bromide. Such a photograph is shown in Fig. 3. Here it can be seen that the treatment with topoisomerase at 0°C (Fig. 3, lanes C and E) and 37°C (lanes B and D) has also resulted in a topological change in the bulk minichromosome population. Examples of densitometric scans of the negative of the photograph for lanes B and C are shown in Fig. 3 (*Right*). Again, the center of the topoisomer distribution of a sample was precisely located by measuring the peak heights of each individual topoisomer in a scan and fitting a Gaussian curve to the distribution of points. Such an analysis of the scans of lanes B–E revealed a difference of -1.4 ± 0.1 turns (i.e., $L_{37} - L_0 = -1.4$) between samples relaxed at 0°C and 37°C (Table 1). It should be pointed out that this value is a moderate underestimate of the degree of flexibility for the bulk free minichromosomes, since there is likely to be a contribution from the presence of disrupted virions (23); these in turn have a lower degree of thermal rotational flexibility (7). Ambrose *et al.* (7) found a similar value (-1.5 ± 0.7 turns) for wild-type SV40 minichromosomes isolated in nuclear extract {from their Table 1, [M(33°C,wt776,+topo)] − [M(0°,wt776,+topo)] = $(-0.8 \pm 0.4) - (0.5 \pm 0.1) = -1.3 \pm 0.6$ turns, which when corrected for a difference of 37°C instead of 33°C gives -1.5 ± 0.7 turns}. Thus, the values for the degree of thermally induced rotational flexibility of the transcription complex DNA and bulk minichromosome DNA agree quite well, indicating that the ability of the chromatin structure to constrain the DNA is very similar for both types of complex.

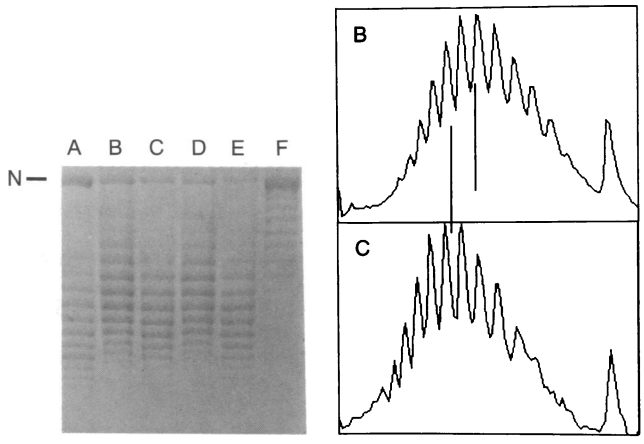

FIG. 3. Analysis of topology of DNA from bulk SV40 minichromosomes. (*Left*) Negative of a photograph of the ethidium bromide-stained gel from Fig. 2. Here the DNA from bulk SV40 minichromosomes is visualized. Lanes are labeled the same as in Fig. 2. N, mobility of the nicked circular DNA. (*Right*) Densitometer scans of the photographic negative of lanes B and C. Vertical lines indicate approximate centers of the respective distributions; exact centers of the distributions were determined by the Gaussian fitting procedure.

DISCUSSION

The degree of constraint imposed by chromatin upon DNA has been studied previously by analysis of temperature-induced topological changes in the DNA of both native (5, 6, 11, 12) and reconstituted (24, 25) chromatin. This study carries out such an analysis on transcriptionally active chromatin—i.e., chromatin containing *in vivo*-initiated RNA polymerases that are capable of transcription run-on *in vitro*. The results (Figs. 2 and 3; Table 1) indicate that the degree of thermal rotational flexibility of the DNA of the SV40 transcription complex (-1.8 turns) is quite similar to that of bulk SV40 chromatin (-1.4 turns), especially considering the fact that the bulk total value is most likely an underestimate of the bulk free minichromosome value (7) (see above). Thus, transcribed and nontranscribed chromatins apparently differ very little in their ability to resist thermally induced rotation of their respective DNAs. This similarity in such a fundamental property in turn suggests that many of the features of the detailed structure available for bulk nucleosomes, such as the features of the crystal structure of the nucleosome core (26, 27), may apply directly to the transcribed nucleosome as well. Two qualifications should be pointed out, however. First, the chromatin DNA unwinds by 25–30% of the value of bare DNA, and this approximates the percentage of DNA that comprises nucleosome linker. Thus, the effect seen may reflect primarily unwinding of the linker DNA, meaning that DNA–histone interactions in the nucleosome core could still differ and not be detected by this particular method. It should also be noted that the SV40 late gene is not transcribed at as high a rate as some other genes—e.g., those driven by an enhancer. Thus, other mammalian genes may exhibit different degrees of constraint of DNA rotational flexibility.

The results here are also relevant to a second feature of transcription complex structure. In our previous study (10), the results of a direct analysis of RNase-trimmed RNA·DNA hybrids from the transcription complex demonstrated that the complex contains no substantial unconstrained tension. The results here, involving analysis of the ternary complex rather than the binary complex, confirm these earlier findings: the transcription complex contains a substantial level of supercoiling but these supercoils are not unconstrained (topoisomerase relaxable); instead they are constrained, and in an amount that is consistent with their being the result of typical nucleosomal organization.

The findings here (Table 1) indicate that both bulk SV40 chromatin as well as the transcription complex subpopulation exhibit DNA rotational flexibility, which is only $\approx$45% or less that of the DNA of yeast plasmid chromatin (5, 6). This substantial difference in such a fundamental property of the chromatin indicates that yeast chromatin structure differs significantly from both bulk as well as transcription complex mammalian chromatin. Other differences between yeast and

mammalian chromatin have been reported. For example, in contrast to mammalian chromatin, yeast chromatin exhibits no significant difference in the DNase I sensitivity of transcriptionally active and inactive regions (28). Furthermore, isolated yeast mononucleosomes tend not to be stable (29, 30), while those of higher eurkaryotes are sufficiently stable to allow them to be crystallized (26). One reported biochemical difference between the yeast and mammalian chromatins that could conceivably explain the difference in structural flexibility is the fact that histone H1 may be absent in yeast (31). Histone H1 has been implicated in condensation and constraint of mammalian chromatin (32), but removal of histone H1 does not result in an increase in rotational flexibility in the DNA of bulk SV40 minichromosomes (12). Even so, while histone H1 has been reported to be present in bulk SV40 chromatin (1), it is not yet known whether the SV40 transcription complex contains histone H1, and therefore the SV40 transcription complex may not in fact differ from yeast chromatin in this respect. Besides histone H1, yeast and mammalian chromatins are also known to differ in their core histones, both in primary sequence as well as postsynthetic modifications such as extent of acetylation (33–35). Finally, yeast DNA lacks 5-methylcytosine (36), a postsynthetic modification that is common in mammalian DNA.

Whatever the biochemical differences between yeast chromatin and mammalian transcribing chromatin, our results indicate that the structures assembled by those components are significantly different. Previously, in the absence of direct data on transcribing mammalian chromatin, the enhanced flexibility of yeast bulk chromatin was proposed to be representative of the mammalian transcribing class (5, 6) and therefore not necessarily anomalous with respect to one particular class of mammalian chromatin. Indeed, it was argued (37) that unconstrained topological tension, one of the properties thought to be characteristic of transcribing eukaryotic chromatin (38) and absent from bulk yeast 2-μm chromatin (5), might be found in the minor fraction of yeast 2-μm chromatin that is transcribed. In contrast, our results indicate that yeast chromatin structure differs not only from the bulk mammalian chromatin but from transcribing mammalian chromatin as well. This finding raises important questions about the extension of conclusions drawn from yeast chromatin studies to mammalian chromatin structure. At the very least, such findings will need to be confirmed directly in a mammalian chromatin system.

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