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Aspects of Chromosomal Structure I. Circular Dichroism Studies*

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ABSTRACT: Circular dichroism spectroscopy was used to examine the chromosomal components of intact nuclei, broken nuclei, isolated chromatin, and residual chromatin complexes from which specific chromosomal proteins had been removed. The DNA component of intact nuclei displayed a positive ellipticity band at 264 nm characterizing a nonconservative DNA spectrum. Upon lysis of the nuclei the conservative DNA spectrum characteristic of DNA in aqueous solution was seen to reappear. The DNA region of the chromatin circular dichroism spectrum was little different from that of broken nuclei.

These observations are taken as evidence of a unique geometry for nuclear DNA due to specific DNA packaging in the nucleus. A specific change in DNA geometry within isolated chromatin indicated by circular dichroism spectroscopy is observed concomitant with the removal of a major portion of histone IV (f2a1) from chromatin, suggesting a DNA conformation determining role for this histone. The observed conformational change in the DNA component of chromatin shows a direct correlation with the RNA template activity of the chromatin. On the basis of this evidence histone IV is suggested to act as a general repressor of RNA synthesis through its conformational effect on the DNA template. The results of this study indicate that the conformation of DNA in the chromosomes of eucaryote cells is determined by both the nuclear environment of the chromosomal material and the specific interactions between the DNA and chromosomal protein components of the chromosome.

The structural study of eucaryote chromosomes represents a particularly difficult problem because of the large number of protein and nucleic acid molecules which constitute these genetic structures. The complexity of the problem is magnified by the structural interrelationships between these macromolecular components and the structural effects of the native milieu of chromosomal material. Because of the primary genetic role played by the DNA component of the chromosome, the particular geometry of DNA in the active chromosome is of central importance in studies of chromosomal structure. Studies of the optical rotatory properties of isolated complexes of nucleohistone or chromatin (Oriel, 1966; Tuan and Bonner, 1969; Permogorov *et al.*, 1970; Simpson and Sober, 1970; Shih and Fasman, 1970; Wilhelm *et al.*, 1970; Henson and Walker, 1970) suggest a change in conformation of the DNA due to interaction with chromosomal proteins. Conformational changes in DNA due to interaction with specific histone proteins (Fasman *et al.*, 1970; Shih and Fasman, 1970) and models for histones such as poly-L-lysine (Cohen and Kidson, 1968; Shapiro *et al.*, 1969) are indicated by changes in the rotatory properties of the DNA. Conversely, X-ray diffraction studies have concluded that the DNA configuration found in nucleohistones remains essentially the same as DNA in aqueous media (Wilkins *et al.*, 1959; Zubay and Wilkins, 1962, 1964; Pardon *et al.*, 1967). Although these combined studies provide valuable information regarding the effects of chromosomal protein on DNA structure in isolated or reconstituted DNA complexes, none attempt to take into account the role played by the nuclear packaging of chromosomes. The unusually high density of DNA in the interphase nucleus (Mirsky and Osawa, 1961) suggests that the nucleoprotein material of nuclei is closely packed in some unique manner. It would not seem unusual that this packaging could alter the protein–DNA interactions within the chromosome and affect the geometry of chromosomal DNA.

In the present study we wish to investigate the combined effects of chromosomal packaging within the nucleus and chromosomal protein interactions on the geometry of chromosomal DNA. Circular dichroism spectroscopy has been utilized to examine DNA geometry within the interphase nucleus, within isolated chromatin, and within residual chromatin complexes from which certain chromosomal proteins have been removed. The DNA template activity of the isolated chromatin and subchromatin fractions utilized in this structural study are presented.

Experimental Section

Materials. Rat thymus (male Sprague–Dawley) nuclei were prepared from fresh rat thymus glands by the method of Blobel and Potter (1966). The resulting nuclear preparation was washed into 0.01 M Tris buffer (pH 7.0) containing 0.0033 M $CaCl_2$ for circular dichroism spectroscopy.

Rat thymus chromatin was prepared from rat thymus (male Sprague–Dawley) glands frozen immediately after sacrificing the animals. As needed, these glands were thawed and the nuclei isolated and purified by the method of Blobel and Potter (1966). The isolation of chromatin from these purified nuclei has been described and is based on previous studies of

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several investigators (Paul and Gilmour, 1968; Dingman and Sporn, 1964; Commerford *et al.*, 1963). Briefly, purified nuclei were successively extracted three times with 50 volumes (w/v) of 0.08 M NaCl containing 0.02 M EDTA (pH 6.3) using a Teflon, hand-driven homogenizer, once with 0.35 M NaCl (Johns and Forrester, 1969), and twice with 0.01X SSC (0.0015 M NaCl–0.00015 M sodium citrate; pH 7.0). The homogenates are centrifuged for 10 min at 6000*g* after each extraction.

Histones were selectively removed from chromatin by salt dissociation. Isolated chromatin was first sedimented from the 0.01X SSC buffer by centrifugation at 30,000*g* for 20 min. The sediment was suspended thoroughly in a small volume of 0.01X SSC and an equal volume of solution containing twice the desired NaCl concentration in 0.02 M Tris (pH 7.0) buffer was added. The solutions were again thoroughly mixed by gentle homogenization (Teflon homogenizer) and centrifuged at 110,000*g* for 24 hr. The supernatants containing dissociated histone fractions as well as some of the nonhistone protein and RNA, were carefully decanted, acidified to 0.4 M H_2SO_4 (0°), and centrifuged. The histone-containing supernatant was dialyzed against deionized water and lyophilized. The recovered histones were analyzed by polyacrylamide gel electrophoresis. The sediment from the high-speed centrifugation containing the residual chromatin was first rinsed very gently with cold 0.01X SSC buffer and then resuspended thoroughly in the same buffer. Part of the sediment of each treatment was extracted with 0.4 M H_2SO_4 to remove all of the residual histones. The remaining proteins associated with the DNA represent the nonhistone protein fraction. Both of these protein fractions were quantitatively analyzed. The histones remaining with the DNA were also extracted, dialyzed against deionized water, lyophilized, and analyzed by polyacrylamide gel electrophoresis. Another portion of the residual chromatin was used as a template in *in vitro* RNA synthesis. A control of untreated chromatin homogenized in 0.01X SSC buffer was used in these template studies.

The chemical analyses of chromatin used in these studies (Spelsberg and Hnilica, 1971) are based on the work of Munro and Fleck (1967) and Webb and Lindstrom (1965). The histones represent those chromosomal proteins extracted with 0.4 N H_2SO_4 at 0°. The nonhistone proteins represent the residual chromosomal proteins remaining with the DNA after the acid extraction. All quantitative analyses were performed for protein by the method of Lowry *et al.* (1951) and for DNA by the diphenylamine reaction (Burton, 1956). Quantitative analyses of nuclear suspensions were also carried out by using the Lowry method for protein and the diphenylamine reaction for DNA. These analyses were augmented by counting techniques and the DNA and protein per rat thymus nucleus obtained was consistent with the reported value (Mirsky and Osawa, 1961).

Methods. To determine the efficiency of the treated chromatin preparations to serve as a template for RNA synthesis, highly purified rat spleen DNA or rat thymus chromatin (either native or devoid of histones) and bacterial RNA polymerase purified by DEAE-cellulose chromatography (Burgess, 1969) (specific activity 400 units/mg of protein from *Escherichia coli*) were employed. In each reaction mixture, 1 μg of purified DNA or 2–5 μg of chromatin (as DNA) was used as a template with 20–25 units of the enzyme. Each reaction also contained 10 μmoles of Tris buffer (pH 8.0), 2.5 μmoles of $MgCl_2$, 0.025 μmole of dithiothreitol, 0.16 μmole each of GTP, ATP, and CTP, 0.1 μmole of [^{14}C]UTP (1 μCi/μmole, Schwarz BioResearch, Orangeburg, N. Y.), 0.1 μmole of phosphate buffer (pH 7.5), 25 μmoles of KCl, and 0.025 μmole of EDTA all in 0.25 ml final volume. The assay mixtures were incubated at 37° for 10 min after which 1 ml of cold 10% trichloroacetic acid was added, the solutions passed through filters with a wash of 25 ml of cold 5% trichloroacetic acid containing 1% sodium pyrophosphate and 25 ml of cold absolute ethanol. The filters were dried and counted in a scintillation spectrometer.

Electrophoretic characterization of histones was carried out by performing polyacrylamide gel electrophoresis essentially by the techniques of Panyim and Chalkley (1969).

The circular dichroism experiments were carried out on the Cary Model 60 recording spectropolarimeter with a Model 6002 circular dichroism attachment. All experiments unless otherwise mentioned, were carried out at 25 ± 0.5° in 1-cm or 1-mm cells using an OD_{260} of no higher than 0.6. Mean residue ellipticity, $[\theta]$, is reported in (deg cm^2) per dmole of nucleotide residue.

The nuclear suspensions as well as a number of the chromatin preparations studied showed slight opalescence. Light scattering was observed in the absorption spectra of some of these solutions. However, the large circular dichroism changes observed could not be attributed entirely to such effects. Ultraviolet spectroscopy was carried out on a Cary Model 14.

Results

Spectra of Nuclei and Isolated Chromatin. The circular dichroism spectrum of a dilute suspension of rat thymus nuclei (4.6×10^6 nuclei/ml) at pH 7.0 in 0.01 M Tris buffer containing 0.0033 M $CaCl_2$ is shown in Figure 1. Above 240 nm the spectrum shows a single positive band at 264 nm with $[\theta]_{264}$ +4800. Below 240 nm the spectrum shows two bands at 222.5 nm, with $[\theta]_{222.5}$ −18,500 and at 208.5 nm, with $[\theta]_{208.5}$ −21,500. The nuclear spectrum shows a crossover point at 243 nm. Nuclei in dilute buffer containing less than a minimal required amount of divalent cation (Mirsky and Osawa, 1961) will undergo lysis. By diluting nuclear suspensions to a calcium concentration of less than 2×10^{-4} M, followed by gentle homogenization, suspensions of broken nuclei (complete lysis of the suspended nuclei was confirmed by phase contrast microscopy) were prepared. The circular dichroism spectrum of a dilute suspension of broken rat thymus nuclei (4.6×10^6 nuclei/ml) at pH 7.0 and 0.01 M Tris buffer containing 0.0033 M $CaCl_2$ is also displayed in Figure 1. Above 240 nm the spectrum shows two bands; a positive band at 271 nm, with $[\theta]_{271}$ +4500 and a negative band at 245 nm, with $[\theta]_{245}$ +5200. Below 240 nm the circular dichroism spectrum of broken nuclei is characterized by two negative bands at 222.5 nm, with $[\theta]_{222.5}$ −38,000 and at 208 nm, with $[\theta]_{208}$ −41,000. A crossover point is observed in the circular dichroism spectrum of broken nuclei at 258 nm. Presented along with the nuclear spectra in Figure 1 is the circular dichroism spectrum of isolated rat thymus chromatin. The chromatin spectrum displays two bands above 240 nm; a positive band at 275 nm, with $[\theta]_{275}$ +5400, and a negative band at 244 nm, with $[\theta]_{244}$ −9000. Below 240 nm, the chromatin spectrum is characterized by two negative bands at 222.5 nm, with $[\theta]_{222.5}$ −32,500, and at 208 nm, with $[\theta]_{208}$ −35,000. The crossover point in the circular dichroism spectrum of isolated chromatin occurs at 257 nm.

A number of rather dramatic changes occur in the circular dichroism spectra of rat thymus nuclear material in going from intact nuclei through broken nuclei to isolated chromatin. Since the ellipticity displayed in these spectra above 240 nm

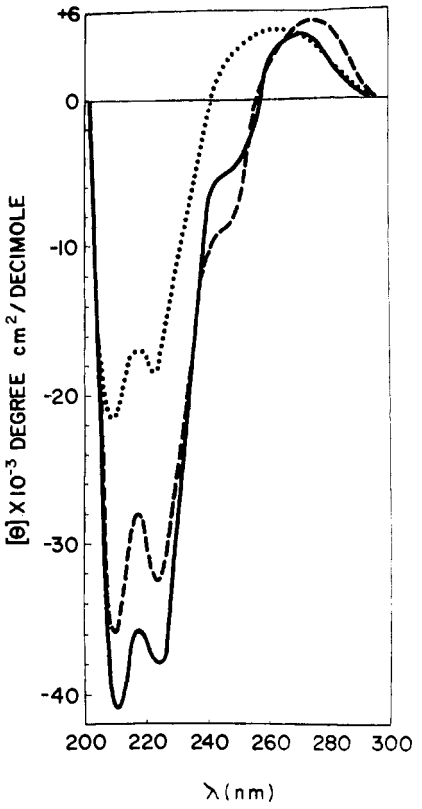

FIGURE 1: Circular dichroism spectra of rat thymus nuclear material. (dotted line) Rat thymus nuclei at pH 7.0 in 0.01 M Tris buffer containing 0.0033 M $CaCl_2$. Concentration of nuclei was 4.6×10^6 nuclei/ml. The protein to DNA ratio is 2.7 for nuclei and broken nuclei, and 1.75 for isolated chromatin. (solid line) Broken rat thymus nuclei at pH 7.0 in 0.01 M Tris buffer containing 0.0033 M $CaCl_2$. (dashed line) Isolated rat thymus chromatin at pH 7.0 in 0.01 M Tris buffer.

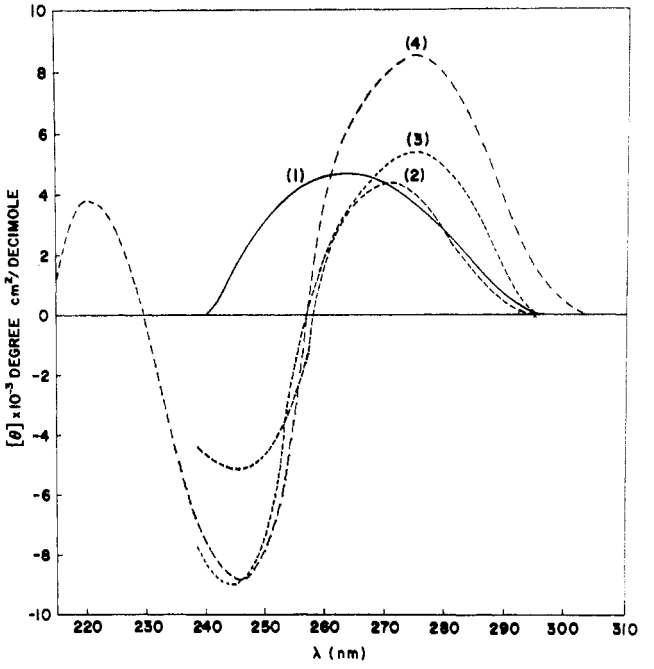

FIGURE 2: Circular dichroism spectrum of purified DNA and the DNA region of the circular dichroism spectra of rat thymus nuclei, broken rat thymus nuclei and rat thymus chromatin. Solvent conditions; pH 7.0 in 0.01 M Tris buffer. Nuclear and broken nuclear suspensions contain 0.0033 M $CaCl_2$. (1) Intact rat thymus nuclei, (2) broken rat thymus nuclei, (3) isolated rat thymus chromatin, and (4) purified rat thymus DNA.

is almost exclusively due to the DNA component of this nuclear material, this region of the spectrum is of particular interest. Figure 2 contrasts the portions of the circular dichroism spectrum of intact nuclei, broken nuclei, and isolated chromatin above 240 nm with the circular dichroism spectrum of purified DNA [prepared as described by Fasman *et al.* (1970)]. Purified DNA exhibits a typical conservative spectrum (Brahms and Mommaerts, 1964). There is a positive band at 275 nm with $[\theta]_{275}$ −8500 and a negative band at 246 nm with $[\theta]_{246}$ −8800; the crossover point is at 257 nm. At lower wavelength there is another positive band at 220 nm with $[\theta]_{220}$ 3800. The DNA portion of the nuclear circular dichroism spectrum (Figure 2) exhibits a completely nonconservative spectrum (Tinoco, 1968) with a maximum ellipticity near the crossover point for the conservative spectrum of purified DNA. After lysis of the nuclei, the nuclear DNA spectrum again takes on the conservative character of purified DNA although the magnitudes of the ellipticities of the positive and negative bands are considerably diminished in comparison to purified DNA. The positive ellipticity band at 271 nm arising from the DNA portion of broken nuclei is also slightly blue shifted from the corresponding band in purified DNA. The circular dichroism spectrum of the DNA region of isolated chromatin (Figure 2) is markedly similar to the purified DNA spectrum in terms of the positions of the positive and negative ellipticity bands. The intensity of the 275-nm bands remains somewhat reduced in comparison to the purified DNA spectrum.

The nonconservative spectrum characterizing the DNA component of intact nuclei indicates a helical geometry for DNA which is considerably altered from that of purified DNA in aqueous media. The circular dichroism spectrum of the DNA component of isolated nuclei also indicates that the geometry of nuclear DNA is quite different from that of the DNA components of broken nuclei and isolated chromatin. The decreased ellipticity of the 275-nm band of chromatin which characterizes the major difference between the DNA region of the circular dichroism spectrum of isolated chromatin and the spectrum of purified DNA is consistent with the observations of Simpson and Sober (1970) as well as those of Permogorov *et al.* (1970). The decreased ellipticity of this band may be the result of protein–DNA interactions within the chromatin complex resulting in a gemoetry for the DNA of chromatin which is somewhat different from that of purified DNA.

The suspensions of both intact and broken nuceli displayed some light scattering (OD_{460} 0.04) which accompanies quasi-particulate material. Although the light-scattering character of this solution may have altered their circular dichroism spectrum in some small manner, it seems extremely unlikely that this phenomena could explain the major changes observed between the spectra from nuclear suspensions and those from broken nuclear preparations and isolated chromatin solutions. Although Urry and Ji (1968) have suggested that damping and red shifts of circular dichroism bands may occur as a result of light scattering, their calculations would not explain changes in crossover wavelength or band sign, such as are observed in the systems studied here.

The region of the nuclear spectrum below 240 nm (Figure 1), where optical activity from both the DNA and protein components of nuclei contribute to the circular dichroism

TABLE I: Chemical Compositions[a] and Template Efficiencies of Treated Rat Thymus Chromatins.

Chromatin Treatments	mg of Protein/mg of DNA		%		
	Histone	Nonhistone Protein	% Histone Remaining	Nonhistone Remaining	% Open Template
1. 0.01 M Tris-HCl, pH 7.0	0.93	0.42	100	100	7.0
2. 0.5 M NaCl + 0.1 M Tris-HCl, pH 7.0	0.76	0.32	82	76	10.0
3. 1.0 M NaCl + 0.01 M Tris-HCl, pH 7.0	0.50	0.28	54	67	26.0
4. 1.3 M NaCl + 0.01 M Tris-HCl, pH 7.0	0.36	0.26	39	62	29.0
5. 1.6 M NaCl + 0.01 M Tris-HCl, pH 7.0	0.22	0.22	24	52	45.0
6. 2.0 M NaCl + 0.01 M Tris-HCl, pH 7.0	0.15	0.19	16	45	55.0
Pure DNA	0	0	0	0	100.0

[a] Histones were extracted from chromatin with 0.4 N H_2SO_4; the residual material, pelleted by centrifugation, was dissolved in 0.1 N NaOH. The histone solutions were neutralized with 5.0 N NaOH and both types of proteins chemically quantitated. DNA was selectively extracted by hydrolysis in 0.3 N $HClO_4$, 30 min, 90°, and chemically analyzed.

spectrum, appears to be very similar to a simple helical protein spectrum in terms of the spectral shape and the positions of the ellipticity bands although the magnitude of the ellipticity of the two bands is much reduced from the expected ellipticity of a helical protein (Greenfield and Fasman, 1969). The reduced ellipticity may be due to contributions from the DNA component of nuclei in the low-wavelength region of the spectrum or from other effects. Upon nuclear lysis the shape of the low-wavelength region of the nuclear spectrum (Figure 1) remains unchanged but the ellipticity of both of the low-wavelength bands is increased about twofold. Similarly, the low-wavelength region of the chromatin spectrum (Figure 1) differs from the low-wavelength region of the nuclear and broken nuclear spectra only in terms of the magnitude of the ellipticity of the two negative circular dichroism bands. The differences displayed between the lower wavelength region of the nuclear and broken nuclear spectra suggest the possibility that changes in protein geometry may accompany nuclear lysis, but several other explanations for these differences are equally plausible. The differences may be due to changes in the rotatory properties of DNA in the region of the spectrum below 240 nm or absorption flattening resulting from the particulate nature of intact nuclei (Urry and Ji, 1968). The latter explanation seems least likely because the measured light scattering from intact and broken nuclei was not appreciably different. Since the protein composition of isolated chromatin is different from that of intact nuclei, direct comparison of the protein regions of the circular dichroism spectra of these genetic structures would be misleading.

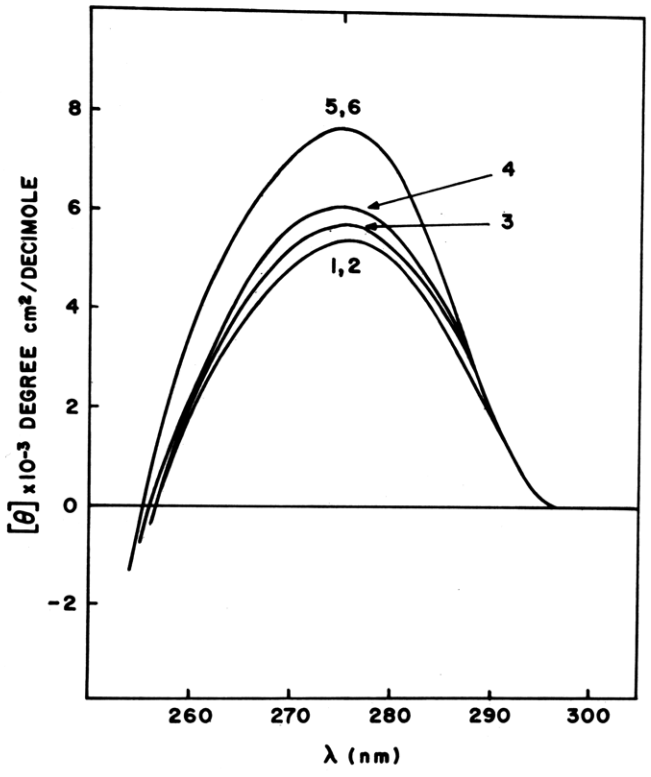

FIGURE 3: Circular dichroism spectra of rat thymus chromatin and rat thymus chromatin from which specific histone fractions have been removed. Solvent conditions; pH 7.0 in 0.01 M Tris buffer. For a description of the chemical composition of chromatin 1–6 refer to Tables I and II.

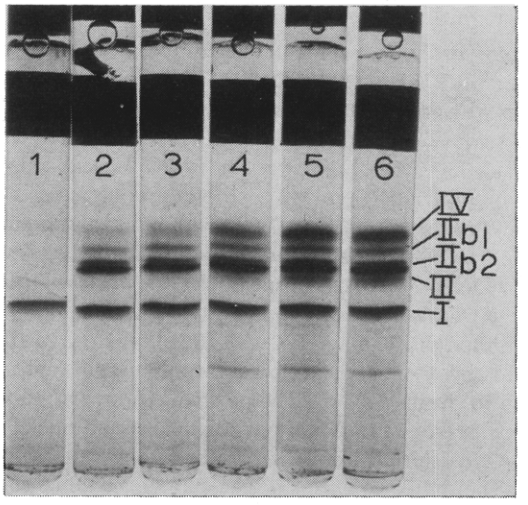

FIGURE 4: Polyacrylamide gel electrophoretic patterns of histones extracted from chromatin with: column 1 = 0.5 M NaCl, column 2 = 1.0 M NaCl, column 3 = 1.3 M NaCl, column 4 = 1.6 M NaCl, column 5 = 2.0 M NaCl. Column 6 is total histone extracted with 0.4 N H_2SO_4. The direction of migration is from bottom (anode) to top (cathode).

Spectra of Chromatin and Residual Chromatin Complexes from Which Certain Chromosomal Proteins Have Been Removed. Circular dichroism spectra above 250 nm of rat thymus chromatin and several residual chromatin complexes from which specific chromosomal protein fractions have been removed are shown in Figure 3. The chemical composition and template activity for RNA synthesis of each of the chromatin complexes shown in Figure 3 are presented in Table I. The DNA component of rat thymus chromatin has a positive ellipticity maximum at 275 nm of +5400 (deg cm²)/dmole. The residual chromatin resulting from the treatment of whole chromatin with 0.5 M NaCl (see Tables I and II and Figure 4 for chemical composition) has a circular dichroism spectrum above 250 nm which is indistingishable from whole chromatin (Figure 3). The residual chromatins resulting from treatment of whole chromation with 1.0 and 1.3 M NaCl (see Tables I and II and Figure 4 for chemical composition) have a maximum ellipticity at 275 nm 6 and 12%, respectively, greater than whole chromatin (Figure 3). The residual chromatin resulting from treatment of whole chromatin with 1.6 M NaCl (see Tables I and II and Figure 4 for chemical composition) displays a circular dichroism spectrum with a band at 275 nm which is both 40% more intense than whole chromatin and of almost the same intensity as purified DNA. The residual chromatin resulting from the treatment of whole chromatin with 2.0 M NaCl displays a circular dichroism spectrum above 250 nm which is identical with that of the 1.6 M NaCl residual chromatin. The results of Figure 3 are most interesting when observed in conjunction with the chemical composition of the residual chromatins. In Figure 4 polyacrylamide gels are presented showing the electrophoretic separation of histones extracted from the residual chromatin preparations studied. Although the residual chromatins resulting from 0.5, 1.0, and 1.3 M NaCl displayed ellipticity at 275 nm which were somewhat greater than the ellipticity of whole chromatin at this wavelength, it is the residual chromatin produced by 1.6 M NaCl treatment which results in by far the most dramatic change in the 275-nm band. Inspection of Figure 4 and Table II indicate clearly that treatment with 1.3 M NaCl causes the dissociation of numerous histone fractions from chromatin, but not histone IV (f2a1) or histone III (f3). A major portion of histone IV is not dissociated from chromatin until treatment with 1.6 M NaCl. Treatment of whole chromatin with 2.0 M NaCl results in the removal of many histone fractions including most of histone IV (Figure 4). The results of the circular dichroism spectra (Figure 3) of residual chromatins of known chemical composition (Figure 4 and Table II) clearly indicates that the removal of a major portion of histone IV along with some histone III (f3) from rat thymus chromatin results in a conformational change in the DNA component of chromatin. This conformational change is of particular biological significance because the template activities (Table I) of the residual chromatins studied are directly related to the magnitude of the 275-nm band in the circular dichroism spectrum of the chromatins. The smallest change in the per cent open template of chromatin (7% → 10%) is observed upon treatment of the chromatin with 0.5 M NaCl. No change in the observed circular dichroism band at 275 nm is observed due to treatment of the chromatin with 0.5 M NaCl. By far the largest change in the per cent open template of chromatin (29% → 45%) is observed between 1.3 M NaCl residual chromatin and 1.6 M NaCl residual chromatin. The largest change in the chromatin circular dichroism band at 275 nm (28% increase) is observed between the 1.3 M NaCl residual chromatin and the 1.6 M NaCl residual chromatin.

TABLE II: Quantitation of the Histone Fractions Remaining with the Residual Chromatin after the Various Salt Treatments.[a]

Fraction	M NaCl in Extraction Buffer (%)					
	0.0 M	0.5 M	1.0 M	1.3 M	1.6 M	2.0 M
I (f1)	100	5	0	0	0	0
III (f3)	100	100	90	66	43	6
IIb2 (f2b)	100	100	16	8	0	0
IIb1 (f2a2)	100	100	53	11	0	0
IV (f2a1)	100	100	85	76	33	4

[a] The values represent the per cent of each fraction using as 100% the amount of each fraction measured in untreated chromatin. The quantitation of the histone fractions remaining with the DNA after the salt treatments was performed by scanning the gels in a microdensitometer (Gilford, Model 2000, gel scanner).

Discussion

The double-stranded DNA helix, unlike some protein helical structures, does not have a fixed geometry. Depending on environmental conditions the DNA helix has been observed to take on three unique geometries, the A, B, and C form (Marvin *et al.*, 1961; Langridge *et al.*, 1960; Fuller *et al.*, 1965). Langridge *et al.* (1960) have determined by X-ray diffraction the molecular configuration of DNA in the B form, the form which is thought to exist in aqueous media (Brahms and Mommaerts, 1964; Maestre and Tunis Schneider, 1969). In the B conformation the bases are stacked almost parallel to one another (5° angle of twist) and are almost perpendicular to the helix (2° tilt). The circular dichroism spectrum of DNA in aqueous media has been characterized by Bush and Brahms (1967) as conservative, consisting of a positive and negative band above and below the absorption maximim of DNA, respectively, with a crossover point near this maximum. In the terms of Bush and Brahms (1967), a totally nonconservative nucleic acid spectrum would display only one circular dichroism band with maximum ellipticity at the absorption maximum of the polymer. Circular dichroism spectra of DNA in the presence of high salt (Maestre and Tunis Schneider, 1969), organic solvents (Green and Mahler, 1968; Brahms and Mommaerts, 1964), and a number of polycationic species (Cohen and Kidson, 1968; Shapiro *et al.*, 1969; Fasman *et al.*, 1970) display considerable nonconservative character. The circular dichroism spectrum of double-stranded RNA (Brahms and Mommaerts, 1964) is also nonconservative.

Theoretical calculations of the rotatory strength of polynucleotides based upon electronic interactions between the transition moments and polarizabilities of adjacent bases have been carried out by Johnson and Tinoco (1969) for DNA and RNA. The results of these calculations indicate that the conservative circular dichroism spectrum observed for DNA is a result of base stacking perpendicular to the helix axis. The nonconservative circular dichroism spectrum of RNA is attributed to the large tilt of the RNA bases with respect to the helix axis.

Several observations have been made in the study reported herein. These observations may be summarized as follows: (1) the totally nonconservative character of the DNA region

of the circular dichroism spectrum of intact nuclei; (2) the transition of the DNA spectrum to conservative upon the lysis of nuclei or the isolation of chromatin from nuclei; (3) the sharp increase in the ellipticity of the 275-nm band in the circular dichroism spectrum of isolated chromatin upon the removal of a significant fraction of histone IV (f2a1); (4) the direct correlation between the template activity of residual chromatin preparations and the magnitude of their 275-nm circular dichroism band.

All of these observations relate to the geometry of DNA in eucaryote genetic material. The first two observations taken in the light of the theoretical work of Johnson and Tinoco (1969) suggest that the bases of DNA within the nucleus may be tilted with respect to the helix axis to an exaggerated extent and that removal of chromosomal material from the nucleus allows the DNA component of this material to return to a conformation much closer to the DNA B-form helix. The suggested base tilt in nuclear DNA may be only one aspect of the geometry of nuclear DNA, an aspect to which circular dichroism spectroscopy is unusually sensitive. It has been suggested that the packaging of DNA in the nucleus may involve super complexes of nucleoprotein (Fasman *et al.*, 1970) in which the DNA component is supercoiled (Zubay, 1964; Pardon *et al.*, 1967; Anderson and Moudrianakis, 1969; H. J. Li and J. Bonner, to be published 1970; Bradbury and Crane-Robinson, 1970). In order for the relatively rigid DNA helical structure to be accommodated in such super complexes it may be necessary to alter several of the parameters of this structure. It is not surprising that these alterations might lead to changes in the circular dichroism spectrum of DNA.

The second two observations reported herein relate to both the geometry of DNA within isolated chromatin and the template activity of this material. Although the DNA region of the circular dichroism spectrum of rat thymus chromatin appears similar to the conservative spectrum of purified DNA, the 275-nm band of the chromatin spectrum is considerably reduced in magnitude as compared to the same band in the purified DNA spectrum (Figures 1 and 2). This difference between the DNA region of the circular dichroism spectrum of chromatin and the spectrum of purified DNA has been suggested to be indicative of a conformational difference between purified DNA and the DNA component of isolated chromatin (Simpson and Sober, 1970; Shih and Fasman, 1970; Wilhelm *et al.*, 1970; Henson and Walker, 1970). Simpson and Sober (1970) have studied calf liver chromatin and the effects of salt concentration on the magnitude of the 275-nm band in the circular dichroism spectrum of this chromatin preparation. An increase in the magnitude of the 275-nm band of the circular dichroism spectrum of calf liver chromatin was observed at salt concentrations believed to cause the dissociation of the slightly lysine-rich histone faction (Simpson and Sober, 1970). On the basis of these observations Simpson and Sober (1970) have suggested that the slightly lysine-rich histones are responsible for the unique conformation of DNA in isolated liver chromatin. Calf thymus chromatin has been studied by three independent groups using the method of circular dichroism spectroscopy. Shih and Fasman (1970) have measured the circular dichroism spectrum of calf thymus chromatin at 0.14 M NaF in the presence and absence of 0.1% sodium dodecyl sulfate. The results of this experiment indicate convincingly that the altered circular dichroism spectrum of DNA in calf thymus chromatin is due to its association with chromosomal proteins (Shih and Fasman, 1970). Wilhelm *et al.* (1970) and Henson and Walker (1970) have independently studied nucleoprotein fractions prepared by salt fractionation from calf thymus chromatin. The results of both of these studies suggest that the F2 and F3 histone fractions are responsible for the unique circular dichroism spectrum of the DNA component of chromatin.

The work of Simpson and Sober (1970) as well as that of Wilhelm *et al.* (1970) and Henson and Walker (1970) suggests that histone fractions other than histone IV (f2a1) are responsible for the circular dichroism spectrum of the DNA component of chromatin. The results of the circular dichroism study (Figure 3) of residual chromatin complexes of known chemical composition (Table I and II and Figure 4) reported herein suggest that histone IV–DNA interactions are to a large extent responsible for the unique conformation of DNA in isolated thymus chromatin. The addition of salt to solutions of chromatin may well result in effects upon chromatin other than the dissociation of certain histone species. The interaction of histone IV with DNA, for example, has been shown to result in a variety of histone conformations dependent upon salt concentrations all of which are well below the salt concentration necessary to cause the dissociation of histone IV from DNA (Wagner, 1970; T. E. Wagner and V. Vandegrift, unpublished data, 1971). Therefore, treatment of chromatin with salt concentrations sufficient to cause the dissociation of the slightly lysine-rich histones would also alter histone IV–DNA interactions significantly and might well result in the change in the 275-nm band of the chromatin spectrum observed by Simpson and Sober (1970). The discrepancy between our results and those of Wilhelm *et al.* (1970) and Henson and Walker (1970) are more difficult to explain. The results reported herein, unlike those of Wilhelm *et al.* (1970) and Henson and Walker (1970), are supported by detailed chemical analysis of the nucleoprotein fractions studied (Figure 4 and Tables I and II). The degree of dissociation of the various histone fractions from chromatin by increasing levels of NaCl reported herein agree with previous reports using similar techniques (Spelsberg and Hnilica, 1971) but differ in some respects, *e.g.*, the removal of histone III, to other reports using different procedures (Henson and Walker, 1970; Ohlenbusch *et al.*, 1967). This discrepancy is not readily explained by the use of different methods for preparing chromatin. One possible cause for these differences may be that histones, especially the arginine-rich histones, tend to aggregate at higher ionic strengths and may sediment with the residual chromatin during centrifugation. Another explanation for the differences observed in the degree of dissociation of the histone fractions from DNA may be the method of quantitating the histones, *e.g.*, the acrylamide gel system utilized, the resolution of the various fractions, etc. In some reports (Henson and Walker, 1970) the polyacrylamide gel system failed to resolve three of the five primary histone fractions; in addition, the relative amounts of histone fractions removed at various salt treatments were not discernible. Consequently, the exact quantitative estimation of the histones extracted would be difficult with some of these histone fractions. The gel system used in these experiments resolved the five major fractions. The discrepancies reported for the selective extraction of histones from chromatin by various salt treatments cannot be explained at present. Our results, however, closely agree with those of Ohlenbusch *et al.* (1967) except in the case of histone III (f3).

Further studies of intact chromatin as well as complexes of DNA with the purified histone species thought to be important in determining chromatin DNA conformation may be

required in order to firmly establish those factors determining DNA conformation in isolated chromatin. At present, substantial data suggest that histone IV is of major importance in determining the unique conformation of DNA in isolated rat thymus chromatin. The per cent of available template for RNA synthesis displayed by six chromatin preparations was measured (Table I) and found to be related to the magnitude of the ellipticity of the 275-nm band in the circular dichroism spectrum of these chromatin fractions. This observation suggests that the template activity of the DNA component of chromatin depends on its conformational state. This conclusion together with the observation that histone IV appears to have a major role in determining DNA conformation is isolated chromatin strongly suggests that histone IV may have a special function in the repression and derepression of genetic information in eucaryote genetic systems.

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