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Studies on the Structure of Metaphase and Interphase Chromatin of Chinese Hamster Cells by Circular Dichroism and Thermal Denaturation†

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ABSTRACT: As a step toward understanding the molecular basis of chromosome changes during mitosis, deoxyribonucleoprotein was isolated from Chinese hamster cell cultures arrested at metaphase with vinblastine (metaphase chromatin) and compared to chromatin of interphase cells. Metaphase chromatin possesses a histone complement similar to interphase chromatin; however, some difference in nonhistone protein is evident by polyacrylamide gel electrophoresis. Thermal denaturation profiles of metaphase and interphase chromatin in either 2.5×10^{-4} M EDTA (pH 8.0) or in medium containing 5 M urea, 0.01 M NaCl, and 0.001 M Tris (pH 7.0) are very similar. Metaphase and interphase chromatin in the latter medium demonstrate recognizable thermal transitions of DNA at 72 and 65° due to association of histones which stabilize the DNA double helix, and a third transition at about 54° due to DNA that is not covered by histone. Circular dichroism (CD) spectra of metaphase and interphase chromatin are similar. Both show positive CD bands at 277 nm which are 45% in magnitude of that of isolated DNA or of chromatin in 0.5% sodium dodecyl sulfate in which chromosomal proteins are dissociated from DNA. The CD spectral alteration of chromatin DNA is partially reversed to that of isolated DNA in the presence of urea (5 M urea, 0.01 M NaCl, and 0.001 M Tris, pH 7.0), even though there is no histone dissociation as revealed by gel electrophoresis. Thus, DNA conformational changes appear to result from the organization of nucleohistone into a complex structure such as supercoil model which is unfolded in urea solution. The increased histone phosphorylation in metaphase chromatin that is observed in the native complex does not affect CD changes. The present study suggests that an essentially similar elementary structure of nucleohistone persists throughout the interphase and metaphase of the cell cycle, and thereby ensures that the same chromatin structure is passed down to the identical daughter cells.

Chromosomes of eukaryotic cells are complexes of DNA with basic histone, acidic nonhistone protein, and some RNA. During the process of mitosis, the dispersed chromatin of interphase cells is gradually condensed to a metaphase chromosome which possesses a characteristic morphology.[1] The present study is an attempt to understand the molecular basis of this cellular process.

Procedures for isolation of metaphase chromosomes in quantities large enough for biochemical and biophysical studies have been developed (Salzman *et al.*, 1966; Mendelsohn *et al.*, 1968; Cantor and Hearst, 1966; Maio and Schildkraut, 1967; Huberman and Attardi, 1966), and some structural studies by physicochemical methods were reported (Cantor and Hearst, 1970). There are problems in studies to determine the properties of metaphase chromosomes due to their large particle size and possible cellular contamination, *e.g.*, of the isolated chromosomes by ribosomes. In this paper, the elementary deoxyribonucleoprotein complex was extracted from cells arrested at metaphase by vinblastine and from interphase cells and they were studied in order to reveal whether there is an altered structure of elementary nucleohistone during the process of mitosis. However, the superstructure characteristic of chromosome morphological appearance is not preserved in the present isolation procedure. The methods employed to probe the structure are circular dichroism and thermal denaturation. Circular dichroism studies of chromatin of interphase cells (Shih and Fasman, 1970; Permogorov *et al.*, 1970; Simpson and Sober, 1970; Wilhelm *et al.*, 1970; Henson and Walker, 1970; Wagner and Spelsberg, 1971; Matsuyama *et al.*, 1971), and DNA–histone complexes (Shih and Fasman, 1971, 1972; Fasman *et al.*, 1970; Olins and Olins, 1971; Gottesfeld *et al.*, 1972; Wagner and Vandegrift, 1972) demonstrated altered spectra of DNA due to association with histones which suggest conformational changes of DNA. Chromatin and DNA–histone complexes also exhibit characteristic melting profiles indicating stabilization of the DNA double-helical structure by histone binding (Shih and Bonner, 1970; Li and Bonner, 1971; Smart and Bonner, 1971; Ansevin *et al.*, 1971).

Materials and Methods

Materials. A clonal line of Chinese hamster cells (V79-589FR) was grown in Tricine-buffered Eagle's 2 medium supplemented with 10% fetal calf serum as previously described (Lake *et al.*, 1972). Metaphase cells were harvested after 4-hr exposure to 0.1 μg/ml of vinblastine sulfate (Eli Lilly, Indianapolis, Ind.) by selective detachment. The re-

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[1] The term, metaphase chromatin, refers to deoxyribonucleoprotein isolated from metaphase cells.

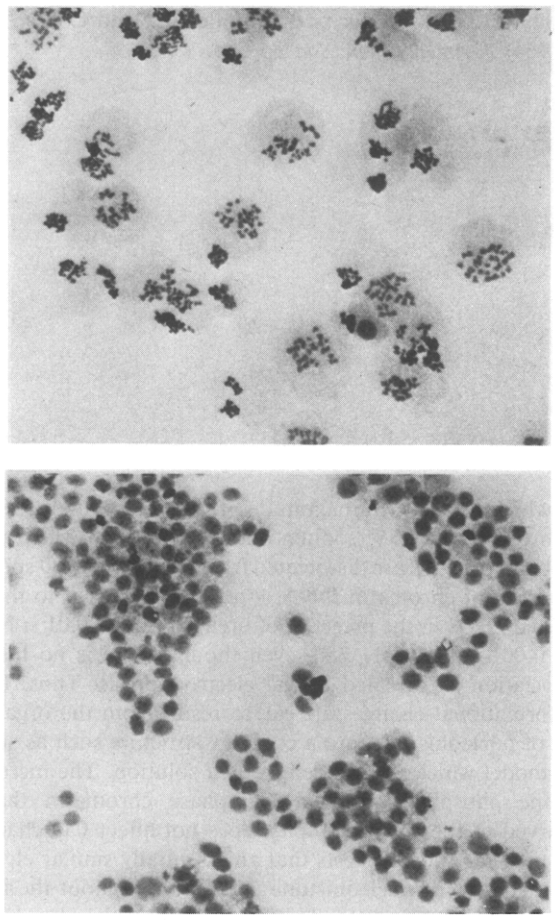

FIGURE 1: Photomicrographs of metaphase (upper) and interphase (lower) cells. Cells were separated from a random population of Chinese hamster cell culture. Cells were partially swollen in hypotonic buffer and stained with Giemsa stain after acetic acid–methanol fixation.

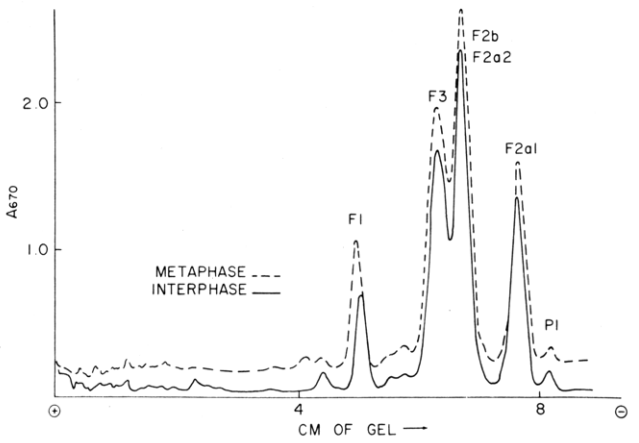

FIGURE 2: Gel electropherograms of acid-soluble proteins from metaphase and interphase chromatin. Proteins were extracted with 0.25 N HCl from metaphase and interphase chromatin of which CD spectra had been measured (Figure 4). Electrophoresis was performed on urea–acetic acid–gels (6.25 M urea–0.9 M acetic acid (pH 2.7)–15% polyacrylamide gel), 0.6 × 10 cm with constant current of 2.5 mA for 8 hr. The gels stained with Amido Schwarz were traced with Gilford spectrophotometer. The metaphase sample was 88 μg of protein and the interphase sample, 70 μg of protein.

maining interphase cells were then scraped from the glass with 2-mm glass beads.

Cells were washed in 20 volumes of Tris-buffered isotonic saline at 0° and then swollen for 10 min in reticulocyte standard buffer (RSB), 0.01 M NaCl–0.01 M Tris-HCl (pH 7.6)–0.0015 M $MgCl_2$, diluted 1:1 with distilled water. After Dounce homogenization the nuclear fraction was collected at 2000g for 10 min and washed twice in RSB. Nuclei were washed once in 0.01 M Tris-HCl (pH 7.6)–0.001 M EDTA and the chromatin was further extracted by homogenization at 12,000 rpm in a Teflon-pestled homogenizer. The homogenate was clarified at 1000g for 10 min and the supernatant chromatin pelleted at 17,000 rpm in a SS-34 Sorvall head.

DNA was isolated by dissolving chromatin of interphase cells in 4 M CsCl. The solution was centrifuged in a Spinco Model L ultracentrifuge at 40,000 rpm in a SW 65 rotor (2°) for 20 hr. The bottom portion of the tubes containing DNA was dialyzed against 0.05 M Tris (pH 7.9) and treated with 0.1 mg/ml of RNase A (Worthington Biochemical Corp., heated in a boiling-water bath for 10 min) at 37° for 30 min. The solution was then repeatedly extracted with phenol in 0.1% sodium dodecyl sulfate, 0.15 M NaCl, and 0.05 M Tris (pH 7.9) until there was no protein interphase. DNA in the aqueous phase was exhaustively dialyzed against the desired solvent for experiments.

Urea used for thermal denaturation and CD measurement was obtained from Allied Chemical, Morristown, N. J. (Reagent ACS Code 2407).

Circular dichroism (CD) spectra were measured with a Cary 60 recording spectropolarimeter with a Model 6001 CD attachment. Spectra were obtained under a nitrogen atmosphere in quartz cells of 0.5- or 1-cm path length at room temperature unless otherwise specified. The concentration of samples was about one OD_{260}. The instrument was set for a slit program of 1.5 nm and spectra were normally obtained with a time constant of 3 and scanning speed below 5, and dynode voltage not over 400 V. Mean residue ellipticity, $[\theta]$, is reported in (deg cm^2) dmol^{-1} on the basis of DNA residue concentration (Shih and Fasman, 1970).

Thermal Denaturation. Absorbance melting profiles were measured with a Gilford multiple sample recording spectrophotometer Model 2000. Samples contained in stoppered cuvettes of 1-cm light path were degassed under reduced pressure for 5 min prior to sealing, and heated linearly at a rate of 0.5–1°/min with a Haake temperature regulator. Thermal denaturation of DNA was monitored by absorption changes at 260 nm and hyperchromicity (H_{260}) relative to absorption at room temperature was calculated degree by degree. The derivative melting profiles were calculated by H_{260} increment per degree. The minor effects of light scattering were corrected by extrapolation of optical density from 360 to 320 nm according to the relation, $\log OD = -a \log \lambda + c$. Turbidity changes during melting were monitored at 320 nm with a separate sample under the same experimental conditions.

Chemical Analysis and Concentration Determination. DNA concentration of chromatin was determined by the diphenylamine method employing calf thymus DNA as standard (type 1, highly polymerized, Sigma Chemical Co., St. Louis, Mo.) (Burton, 1956), or by uv absorption at 260 nm in 0.1% sodium dodecyl sulfate (Matheson Coleman and Bell, E. Rutherford, N. J.) and 0.15 M NaCl, a procedure which eliminates turbidity. An ϵ_p of 6800 at 260 nm was used to calculate the nucleotide residue concentration and an E_{mg} of 22 was used for chemical composition. Protein was deter-

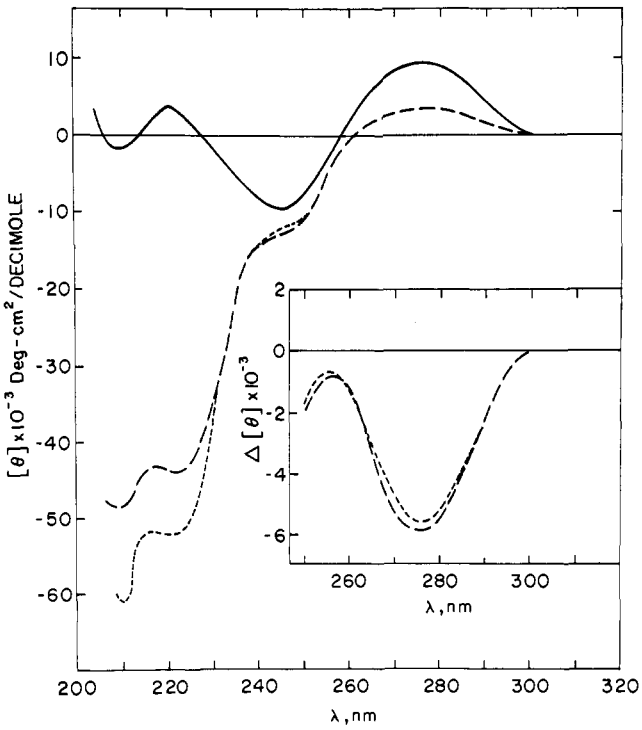

FIGURE 3: Circular dichroism spectra of metaphase and interphase chromatin. CD spectra were measured at DNA residue concentration of 1.41×10^{-4} M for metaphase chromatin (———) and of 1.44×10^{-4} M for interphase chromatin (– – – –) at wavelength region above 250 nm, and one-half of the concentration below 250 nm. [θ] was calculated on the basis of DNA residue concentration. Difference spectra shown in the inset ($\Delta[\theta] = [\theta]^{Dnp} - [\theta]^{DNA}$) were calculated by substracting CD of pure DNA measured under similar conditions from that of chromatin. The solvent was 0.01 M NaCl–0.001 M Tris (pH 7.6) and optical path length, 1 cm. The ratio of protein content of interphase to that of metaphase chromatin of this sample is 1.25 and is consistent with the ratio of $[\theta]_{222}^{interphase}/[\theta]_{222}^{metaphase} = 1.20$ and $[\theta]_{210}^{interphase}/[\theta]_{210}^{metaphase} = 1.25$.

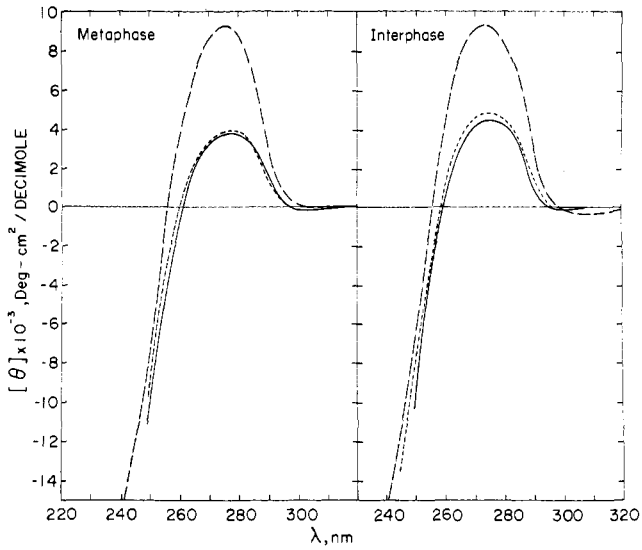

FIGURE 4: Circular dichroism of freshly prepared chromatin without exposure to room temperature. In order to minimize enzymatic dephosphorylation, CD spectra were measured for both metaphase and interphase chromatin prepared within 20 hr (kept in the cold) in jacketed cell at 5.2° (——). The spectra were essentially similar to those exposed and measured at room temperature for 4–6 hr (– – – –). The solvent was 0.01 M NaCl–0.001 M Tris (pH 7.6) and sample DNA concentration, 2.16×10^{-4} M for metaphase chromatin and 2.60×10^{-4} M for interphase chromatin with optical pathlength of 0.5 cm. CD spectra were also measured at room temperature by addition of 0.5% (w/v) sodium dodecyl sulfate (———) which causes reversal of the spectra to that of pure DNA.

mined by the method of Lowry (Lowry *et al.*, 1951) employing bovine serum albumin (Sigma Chemical Corp., St. Louis, Mo.) as standard after DNA was solubilized by boiling-water bath for 15 min in 10% trichloroacetic acid and the protein precipitate was washed with ethanol. RNA was determined by the orcinol reaction using an alkaline hydrolysate (0.3 N KOH, 37° for 18 hr) employing ATP as standard (Schwarz/Mann, Orangeburg, N. Y.) (Dische and Schwartz, 1937).

Results

Isolated Chromatin of Metaphase and Interphase Cells. More than 95% of the cells that were arrested with vinblastine and then selectively detached were in metaphase as noted on Giemsa-stained slides (Figure 1). In comparative studies, the interphase cells from the same culture were also collected. These interphase cells had been exposed to the same treatment as the metaphase cells. Isolated metaphase chromosomes contain more RNA and protein than does isolated chromatin of interphase cells (Salzman *et al.*, 1966). However, the amount and relative proportions of different histone molecular species are similar (Comings, 1967; Hancock, 1969; Sadgopal and Bonner, 1970). The present method of chromatin isolation yields preparations both from metaphase and interphase cells of almost the same composition. The average chemical composition of chromatin from metaphase cells in terms of weight ratio is DNA to protein to RNA of 1.0 to 2.2 to 0.01, and chromatin from interphase cells, 1.0 to 2.3 to 0.03. The entire histone complement of chromatin isolated from metaphase and interphase cells is similar as revealed by urea–acetic acid–gel electrophoresis (Figure 2). However, differences in nonhistone protein are noted, and histone phosphorylation is much more pronounced in metaphase cells (Lake *et al.*, 1972). Specifically, metaphase cells demonstrate increased incorporation of ^{32}P into histone I (f1) and increased f1 histone phosphokinase activity in metaphase chromatin (Lake, 1972; Lake and Salzman, 1972).

Circular Dichroism. In Figure 3 are seen CD spectra of chromatin isolated both from metaphase and interphase cells. There is a positive CD band centered at 277 nm, a negative shoulder at 245 nm and two large negative bands at 222 and 210 nm. The positive CD band of DNA at 275 nm is diminished in magnitude in chromatin both of metaphase and interphase cells. The mean residue ellipticity, [θ], of chromatin, based on its DNA concentration, is 45% that of pure DNA, and is quite similar in both metaphase and interphase chromatin. However, a slightly higher [θ] is consistently observed for interphase chromatin than for that of metaphase. The type of spectral changes in this wavelength region is best seen in the difference spectra obtained by substracting the spectrum of pure DNA from that of chromatin as shown in inset of Figure 3. There is a negative peak centered at 275 nm, which is very similar to that induced by histone I (f1) in the reconstituted DNA complexes (Shih and Fasman, 1972). The two large negative bands at 222 and 210 nm are characteristic of those of polypeptide in an α-helical conformation (Timasheff and Gorbunoff, 1967). The difference in $[\theta]_{222}$ and $[\theta]_{210}$ of metaphase and interphase chromatin seen in Figure 3 can be accounted for by slight difference in pro-

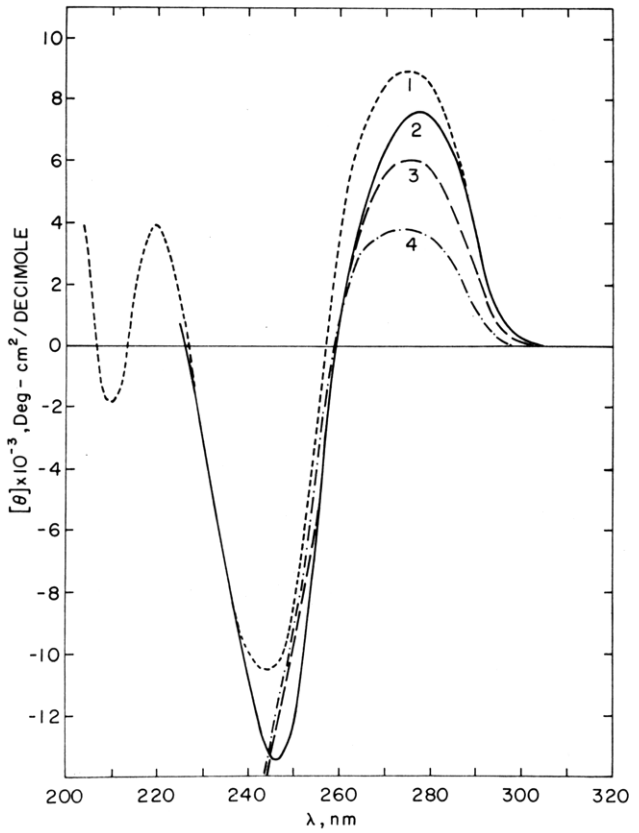

FIGURE 5: Partial reversal of circular dichroism of chromatin to that of isolated DNA in the presence of 5 M urea. The basic medium was 0.01 M NaCl and 0.001 M Tris (pH 7.0). Interphase chromatin (3) and isolated DNA(2) in urea was prepared by dropwise addition of an equal volume of 10 M urea in the basic medium to the samples under vigorous stirring. Control experiments were run for chromatin (4) and DNA (1) by the addition of an equal volume of the basic medium. The final DNA concentrations were 1.78×10^{-4} M for DNA and 1.40×10^{-4} M for chromatin, and the optical path length was 1.0 cm.

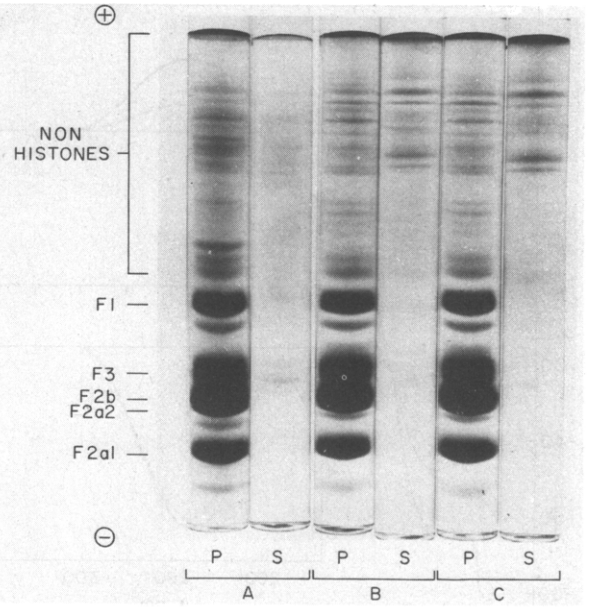

FIGURE 6: Electropherograms of chromatin proteins in pellet (P) or supernatant (S) fractions after treatment with 5 M urea. To 2 ml of Chinese hamster interphase cell chromatin at 2 OD_{260} in 0.02 M NaCl–0.002 M Tris-HCl was added either 2 ml of H_2O or 2 ml of 10 M urea in H_2O. After 2-hr gentle stirring at 0 or 21°, the nucleoprotein was pelleted by centrifugation for 18 hr at 40,000 rpm in a SW-50L rotor. Proteins were extracted from each pellet with 0.25 N HCl, precipitated with 25% trichloroacetic acid, and washed in acid–acetone and acetone. Proteins were precipitated from the supernatant fractions with 25% trichloroacetic acid and washed as above. Electrophoresis of samples from equivalent volumes of starting material was on 15% urea–acetic acid–acrylamide gels (Panyim and Chalkley, 1968) after reduction with 0.1 M β-mercaptoethanol. (A) Control, no urea; (B) 5 M urea, 0°; (C) 5 M urea, 21°.

tein content. Therefore the α-helix content of chromosomal proteins of both metaphase and interphase chromatin appears to be very similar. It is well documented that there are various side chain modifications of histones, *e.g.*, phosphorylation, acetylation, and methylation. The biological function of such modifications is still not clear (DeLange and Smith, 1971). It has been suggested that certain biological functions of the histones might be modulated by side chain modifications. It was reported that phosphorylated histone I (f1) causes less CD changes when complexed with DNA than does nonphosphorylated histone (Adler *et al.*, 1971). Histones of metaphase chromatin, especially histone I (f1), shows more pronounced phosphorylation than histones extracted from interphase chromatin. We have studied the effect of histone phosphorylation on CD of the isolated native complexes. The positive CD band at 277 nm (Figure 3) has essentially no contribution from CD of protein peptide bonds and permits monitoring the CD of DNA. It shows no change due to augmented phosphorylation of histone I (f1) in metaphase chromatin. However, it is observed by gel electrophoresis that considerable histone dephosphorylation occurs due to phosphatase activity associated with chromatin during the course of CD measurement at room temperature. Experiments were designed to measure the CD spectra of freshly prepared chromatin at 5.2° in jacketed cells. In Figure 4 is seen the CD spectra of the positive CD band at 277 nm under various conditions. There is essentially no change in CD of the sample measured at 5° and after exposure of samples to room temperature for several hours both in metaphase and interphase chromatin. Preservation of the phosphorylated histone I (f1) after CD measurement has been confirmed. Therefore, it can be concluded that increased histone phosphorylation of metaphase chromatin that exists in the native complexes does not cause a CD change of DNA.

In Figure 4 it is also seen that addition of 0.5% sodium dodecyl sulfate, which dissociates protein from DNA, restores the positive CD band to that of pure DNA both in metaphase and interphase chromatin as previously reported with calf thymus chromatin (Shih and Fasman, 1970). While it is clear that CD changes of DNA are due to association with chromosomal proteins, the nature of the conformational changes of DNA in chromatin remains to be determined. Studies on reconstituted complexes of DNA and histones demonstrated profound CD changes of DNA and the changes seem to be associated with specific aggregation or superstructure of the complexes. The complexes formed by salt-gradient dialysis to 0.015 M NaCl in the presence of urea, however, exhibit no CD alteration of DNA due to histone binding (Shih and Fasman, 1971, 1972; Olins, 1969; Li *et al.*, 1971). The CD spectrum of chromatin was therefore measured in the presence of 5 M urea at low ionic strength. These conditions destroy the superstructure due to weak interactions, such as hydrophobic and hydrogen bonding, but do not dissociate the bound histone. As seen in Figure 5, the CD spectrum of DNA in the presence of urea is slightly different than

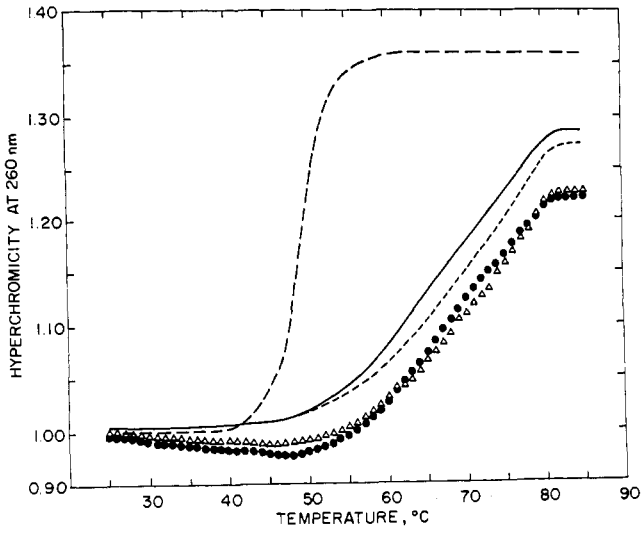

FIGURE 7: Thermal denaturation profiles of metaphase and interphase chromatin. The samples were dialyzed exhaustively against 2.5 $\times$ 10^{-4} M EDTA (pH 8.0). The sample concentrations (light scattering corrected as below) were metaphase chromatin (●), A_{260} = 0.653; interphase chromatin (Δ), A_{260} = 0.655; and isolated DNA (———), A_{260} = 1.147. Turbidity changes in the course of heating were monitored at 320 nm of separate samples at the same heating rate. The scattering contribution at 260 nm was calculated degree by degree by extrapolation of scattering from 320- to 360-nm region according to the relation, log OD = $-a$ log λ + C. The slope, a, was the same before and after heating. The corrected profiles were shown for metaphase chromatin (——) and interphase chromatin (– – – –).

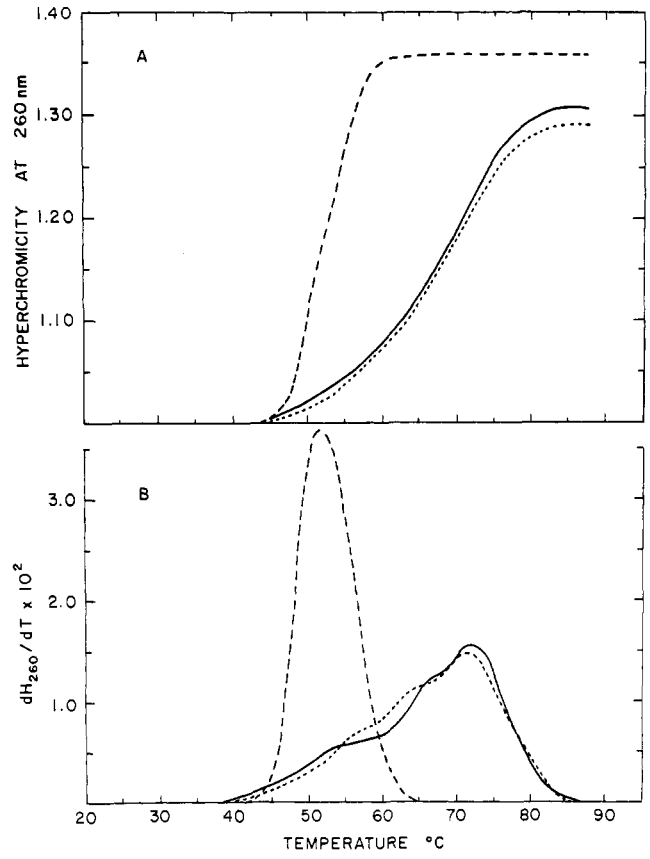

FIGURE 8: Thermal denaturation profiles of metaphase and interphase chromatin in urea solution. Samples were prepared in the same manner as described in Figure 5. The final composition of the medium was 5 M urea–0.01 M NaCl–0.001 M Tris (pH 7.0). The sample concentrations were (1) DNA (———), A_{260} = 1.300, (2) metaphase chromatin (———), A_{260} = 0.720, and (3) interphase chromatin (– – – –), A_{260} = 0.793. Turbidity changes in the course of heating were less than 2% and were not corrected. The derivative curves calculated degree by degree for H_{260} increment per degree were presented in the lower panel.

in its absence, although thermal denaturation studies indicate that DNA is mostly native. The positive CD band at 275 nm is slightly decreased and shifted to the red; the negative band at 245 nm is increased and is also slightly shifted to the red. However, on the contrary in urea solution the positive CD band at 277 nm of chromatin is increased, but also slightly red shifted and approaching the spectrum of DNA in urea. Therefore, it appears that the changes of DNA conformation in chromatin are partially restored to that of isolated DNA in urea solution. We have further investigated whether the partial reversal of conformation of chromatin DNA in urea to that of pure DNA is due to histone dissociation or unfolding of the superstructure of nucleohistone. Chromatin was dissolved in 5 M urea–0.01 M NaCl–0.001 M Tris (pH 7.6), the same conditions that have been used for CD measurements. DNA with its associated proteins was pelleted by high-speed centrifugation (100,000g for 18 hr in the cold). The dissociated protein in the supernatant fluid and protein extracted from the pellet were analyzed by gel electrophoresis as shown in Figure 6. There is essentially no histone dissociated from chromatin in 5 M urea. However, some dissociation of nonhistone protein by urea can be noted. Therefore, it is unlikely that the reversal of the CD spectrum of chromatin DNA in urea is due to complete dissociation of histone from DNA.

Thermal Denaturation. Chromatin of interphase cells possesses a characteristic thermal denaturation profile due to histone binding. The melting profile of metaphase chromatin was studied in comparison to that of interphase chromatin in order to obtain further structural information. Figure 7 shows mleting profiles in 2.5 $\times$ 10^{-4} M EDTA (pH 8.0). Since chromatin prepared by the present procedure shows some light scattering, the turbidity changes were also monitored during the course of heating by changes in optical density at 320 nm of a spearate sample heated at the same rate. The turbidity contribution to the absorption at 260 nm was corrected by extrapolation from 320 nm. As seen in Figure 7, both the corrected and uncorrected melting profiles of metaphase and interphase chromatin are generally quite similar. The T_m of the major transition at about 76°, however, is 5° lower than that reported by other workers performed in the same solvent (Li and Bonner, 1971; Smart and Bonner, 1971). More accurate melting profiles were measured in 5 M urea, 0.01 M NaCl, and 0.001 M Tris (pH 7.0) in which chromatin is less turbid and shows negligible turbidity changes in the course of heating. Figure 8A illustrates the melting profiles and their derivative curves in 5 M urea. As seen most clearly in the derivative plot (Figure 8B), there are at least three recognizable thermal transitions. The major peak at 72° and the shoulder at about 65° appear to be the result of histone stabilization of DNA helical structure, and another shoulder at about 54° which is very close to that of pure DNA may represent melting of free DNA stretches or regions bound by nonhistone proteins (Ansevin *et al.*, 1971; Li and Bonner, 1971; Smart and Bonner, 1971; Shih and Bonner, 1970). The melting profiles of both metaphase and interphase chromatin are essentially similar and the slight differences as seen in Figure 8 are within

experimental error. Therefore, it is concluded that by these criteria the binding and distribution of histone in chromatin DNA appear to be well conserved at metaphase during mitosis.

Discussion

The present study reveals that there is a similar elementary structure of chromatin which persists throughout interphase and metaphase during the cell cycle. This is surprising considering the very obvious morphological and functional changes that occur in the genetic material during mitosis. Chromatin is condensed to metaphase chromosomes and gene activity is largely shut off at metaphase. There is a high degree of conservation of histone during growth and mitosis, and chromosome condensation at mitosis appears to occur with little changes of the histone complement (Comings, 1967; Hancock, 1969; Sadgopal and Bonner, 1970). This is also in accord with our gel electropherograms as seen in Figure 2. The similarity of the thermal denaturation profiles of metaphase and interphase chromatin further suggests that DNA–histone interaction and maybe even histone distribution in chromatin DNA are conserved during mitosis.

The CD spectral changes of DNA as seen in the difference spectra shown in the inset of Figure 3 belong to the same spectral family induced by histone I (f1) (Shih and Fasman, 1972) or poly(L-lysine) (Shapiro *et al.*, 1969) but to a much less extent. The common feature of these spectra is a negative peak centered at about 275 nm which is also quite similar to the CD spectrum of a ψ structure of DNA driven into a compact state by excluded volume interactions as described by Jordan *et al.* (1972). The CD spectrum of chromatin DNA in this wavelength region is also similar to CD of a DNA lithium salt film presumably in C-form conformation (Tunis-Schneider and Maestre, 1970). The spectral changes of chromatin DNA appear to be associated with a higher order structure rather than primary histone binding. It is seen that in the presence of urea, partial restoration of the CD changes of chromatin DNA to that of isolated DNA is obtained (Figure 5). Although some nonhistone proteins are dissociated in 5 M urea, histones which interact with DNA largely by ionic bonds are still bound to DNA at an ionic strength of 0.01 M NaCl as seen in denaturation profiles of Figure 8 and gel electropherograms of protein in the supernatant and pellet after high-speed sedimentation (Figure 6). This is in accord with the finding of Kleiman and Huang (1972). However, we cannot exclude that under these conditions there is no partial alteration of DNA–histone interactions involving hydrophobic and hydrogen bonding. It has been speculated that nucleohistone may assume a supercoil structure with a diameter of 10 nm and a pitch of 12 nm suggested by X-ray diffraction (Pardon *et al.*, 1967) and electron microscopy. In the presence of urea, the supercoil structure may be unfolded as indicated by (1) increased viscosity (Bartley and Chalkley, 1968), (2) visualization under the electron microscope (Georgiev, 1969), and (3) the present observation of partial reversal of altered CD spectrum of chromatin DNA. It is likely that the altered CD spectrum of chromatin DNA results from the conformational changes of DNA induced by supercoil formation in nucleohistone. The essentially similar CD spectra of metaphase and interphase chromatin indicate similar supercoil structure exists in interphase and metaphase. This is consistent with the observation by electron microscopy that a supercoil with the same dimension exists in critical point dried and thin-sectioned metaphase chromosome, although it appears to be coiled to a further higher order structure of diameter of about 25 nm (Lampert, 1971).

In conclusion, the evidence presented in this paper supports the notion that the first-order structure (histone binding and distribution in chromatin DNA) and the second-order structure (supercoil of nucleohistone) are preserved during mitosis. This insures that chromatin of an interphase cell can be passed down to the identical daughter cells without a requirement for a major redistribution of histone binding in the process of mitosis, even though there are drastic morphological and functional changes of the genetic material at metaphase. Since structure is the basis of function, this also implies that the same specific functional state of the chromatin of an interphase cell (*e.g.*, specific template function of chromatin) imposed by specific histone binding to DNA can be transferred to the identical daughter cells. At metaphase, the nucleohistone fiber may be further folded into a third-order structure (coiled supercoil) (Lampert, 1971), and aggregated into distinct bodies of metaphase chromosomes. The larger quantity and different species of nonhistone protein and histone modifications may be involved in this particular morphological and functional change of the genetic material, but these may be lost or not observed by the present method of chromatin extraction and methods of structure investigation.

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Occurrence and Properties of a Chromatin-Associated F1-Histone Phosphokinase in Mitotic Chinese Hamster Cells†

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ABSTRACT: A chromatin-associated phosphokinase (ATP: histone phosphotransferase) activity having high specificity for F1 (lysine-rich) histone has been detected in mitotic (M) Chinese hamster cells. This activity, which is cyclic 3′,5′-adenosine monophosphate independent and elevated 6- to 10-fold in specific activity over that found in interphase (I) cell chromatin, is shown to account for an electrophoretic heterogeniety observed in F1 histone of mitotic cells. Control experiments have excluded the possibility that the differences in phosphokinase activity assayed in M and I chromatin are due to differential phosphatase, adenosine triphosphatase, or protease activities. F1-phosphokinase specific activity is highest in chromatin, but is also elevated in other mitotic cell fractions. This bound activity can be dissociated along with the non-histone proteins by treatment of chromatin with 0.35 M NaCl without loss of activity. Since phosphokinase activity decays with long metaphase-arrest times and in the presence of cycloheximide, it is suggested that F1 phosphokinase exists as an unstable enzyme especially active during a period of the cell cycle proximal to or during mitosis. The biological significance of this augmented phosphorylation and its relationship to cell cycle and mitotic events remain undefined.

Specific phosphorylation and dephosphorylation of histone F1, F2a2, and F3 fractions has been studied in a variety of mammalian tissues (Delange and Smith, 1971; Langan, 1971) to determine if protein modification reactions modulate gene activity by derepression. Recent comparisons have been made between phosphorylation in normal and regenerating liver (Gutierrez-Cernosek and Hnilica, 1971; Balhorn *et al.*, 1971) and between stationary and synchronously growing cultured cells (Balhorn *et al.*, 1972a,b). Their findings indicate that histone phosphorylation occurs coincident with cell growth but that it is not necessarily coupled with histone biosynthesis.

F1 (lysine-rich) and F2a2 (moderately lysine-rich) histone fractions are significantly phosphorylated in cultured animal cells. This has been shown for Ehrlich ascites (Sherod *et al.*, 1970), Chinese hamster ovary (Gurley and Walters, 1971), and HTC hepatoma cells (Balhorn *et al.*, 1972a). Sherod *et al.* (1970) and Balhorn *et al.* (1971, 1972a,b) have determined that an electrophoretic heterogeneity of F1 bands and phosphorylation of F1 molecules are coincident with the DNA synthetic (S) phase of the cell cycle.

Gurley and Walters (1971), however, have noted that Chinese hamster cell F1 phosphorylation is sensitive to X

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