THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 258, No. 22, Issue of November 25, pp. 13478-13485, 1983
Printed in U.S.A.

Structural Organization of the Meiotic Prophase Chromatin in the Rat Testis*

(Received for publication, April 12, 1983)

Basuthkar Jagadeeswar Rao‡§, Samir K. Brahmachari¶, and Manchanahalli R. Satyanarayana Rao‡

From the ‡Department of Biochemistry and the ¶Molecular Biophysics Unit, Indian Institute of Science, Bangalore 560012, India

Pachytene nuclei were isolated from rat testes by the unit gravity sedimentation technique and contained histone variants H1a, H1t, TH2A, TH2B, and X2 in addition to the somatic histones H1bde, H1c, H2A, H2B, H3, and H4. The basic organization of the pachytene chromatin namely the nucleosome repeat length and the accessibility to micrococcal nuclease, was similar to that of rat liver interphase chromatin. However, when digested by DNase I, the susceptibility of pachytene chromatin was 25% more than liver chromatin under identical conditions.

Nucleosome core particles were isolated from both liver and pachytene nuclei and were characterized for their DNA length and integrity of the nucleoprotein on low ionic strength nucleoprotein gels. While liver core particles contained all the somatic histones H2A, H2B, H3, and H4, in the pachytene core particles, histone variants TH2A, X2, and TH2B had replaced nearly 60% of the respective somatic histones. A comparison of the circular dichroism spectra obtained for pachytene and liver core particles indicated that the pachytene core particles were less compact than the liver core particles. Studies on the thermal denaturation properties of the two types of core particles revealed that the fraction of the pachytene core DNA melting at the premelting temperature region of 55–60 °C was significantly higher than that of the liver core DNA.

Mammalian spermatogenesis is characterized by several dramatic transitions in the nuclear basic proteins both during premeiotic and meiotic stages and during spermiogenesis (1). Several histone variants, namely H1t, TH2A, TH2B, X2, and TH3, start appearing in the primary spermatocytes of rat before the first meiotic division (2–8). Although the functional importance of these histone variants is not clear, it is generally believed that they facilitate the meiotic events. The major event that takes place during the meiotic prophase of the germ cells (pachytene stage) is the pairing of homologous chromosomes and genetic recombination through (*a*) single-stranded nicks created by meiotic specific endonuclease, (*b*) single-stranded exchange, and (*c*) DNA repair. All these phenomena are well regulated processes and are associated with nonrandomly selected and definite regions of the chromosomes (9).

* This work was supported by grants from the Indian Council of Medical Research, New Delhi. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

§ Senior Research Fellow of the Department of Atomic Energy, Government of India.

Recently, we had demonstrated that the histone variants are present as a part of the nucleosome type of organization of the chromatin of rat testis in that histone H1t is bound to the linker DNA while histones TH2A, TH2B, and X2 are present in the nucleosome core particles (10). Based on the premise that, whatever roles these histone variants do have in the meiotic events, they should be mediated through subtle structural alterations at the nucleosomal level, we have carried out a detailed study regarding the structural organization of meiotic prophase (pachytene) chromatin. For this purpose, we have purified pachytene stage nuclei from the total testis nuclear preparation by employing the STAPUT technique and studied its chromatin organization with respect to gross structural features like accessibility to micrococcal nuclease and DNase I, and nucleosomal repeat length. Further, we have studied physicochemical properties like circular dichroism and thermal denaturation patterns of isolated nucleosomal core particles from pachytene nuclei. These properties have been compared with those of rat liver nuclei which represent the interphase stage of the cell, since liver chromatin contains only the somatic histones. The results of these studies presented here indicate that the higher order structure of the pachytene chromatin is more loosened than the interphase chromatin and further that the nucleosome core particles from pachytene chromatin are also less compact than the liver core particles, probably mediated through the presence of histone variants TH2A and TH2B at the two ends of the core particle.

MATERIALS AND METHODS

Micrococcal nuclease, DNase I, PMSF,[1] acrylamide, *N,N'*-methylene bisacrylamide, TEMED, and Triton X-100 were purchased from Sigma Chemical Co., St. Louis, MO. Bio-Gel A-5m was obtained from Bio-Rad Laboratories, Richmond, CA. All other chemicals used in these studies were of reagent grade.

Male albino rats of this Institute strain weighing 160 ± 10 g were used in all the studies. Total nuclei were isolated from liver and testes according to the method described by Rao *et al.* (10).

Pachytene stage nuclei were prepared by employing the STAPUT technique described by Meistrich (11) with minor modifications. Briefly, the minced testes were subjected to four strokes of mild hand homogenization in 5 volumes of buffer A (10 mM Tris-HCl, pH 7.5, 5 mM $MgCl_2$, 1 mM $CaCl_2$, and 40 mM $NaHSO_3$) containing 0.34 M sucrose. The pellet obtained after centrifugation at 1,000 × *g* for 10 min in a Sorvall RC-5B refrigerated centrifuge was suspended in 10 volumes of 2.3 M sucrose in buffer A and subjected to centrifugation at 100,000 × *g* for 60 min in a Beckman model L5-50 ultracentrifuge. The pellet containing the purified nuclei was washed sequentially once each with 1 and 0.34 M sucrose in buffer A. The washed nuclear pellet was suspended gently in 0.5% sucrose in buffer B (10 mM

[1] The abbreviations used are: PMSF, phenylmethylsulfonyl fluoride; TEMED, *N,N,N',N'*-tetramethyulethylenediamine; SDS, sodium dodecyl sulfate; bp, base pair.

Tris-HCl, pH 7.4, 0.9% NaCl, 5 mM $MgCl_2$, 1 mM $CaCl_2$, 0.1 mM PMSF, and 0.1% Triton X-100 and loaded on a STAPUT chamber containing a gradient of 2 to 10% sucrose in buffer B. After A 10-h sedimentation at unit gravity at 4 °C, the individual bands of nuclei were collected separately and identified under a phase-contrast microscope. Generally, the yield of pachytene nuclei from 15 rats was in the range of 30 A_{260} units.

Digestion of Nuclei with Micrococcal Nuclease and Isolation of Nucleosome Core Particles—The conditions for the digestion of nuclei with micrococcal nuclease and subsequent isolation of nucleosome core particles were essentially the same as described by Rao *et al.* (10), except that the dimensions of the Bio-Gel A-5m column were smaller (1.6 × 55 cm). The peak fractions absorbing at 260 nm were pooled and concentrated against polyvinylpyrrolidone.

Digestion with DNase I—Nuclei obtained after the wash with buffer A containing 0.34 M sucrose were suspended in 50 mM Tris-HCl, pH 7.4, containing 25 mM KCl, 5 mM $MgCl_2$, 0.1 mM PMSF, and 0.1% Triton X-100 and centrifuged at 10,000 × *g* for 10 min. The resulting pellet was washed once with the digestion buffer (10 mM Tris-HCl, pH 7.4, containing 10 mM NaCl, 5 mM $MgCl_2$, 0.1 mM PMSF). Digestion was carried out in the same buffer at a nuclear concentration of 10 A_{260} units/ml (measured in 2 M NaCl and 5 M urea) with 3 μg/ml of DNase I at 37 °C (12). The kinetics of digestion was followed by measuring the amount of DNA that was soluble in 1 M perchloric acid and 1 M NaCl at 260 nm.

Circular Dichroism Measurements—Nucleosome core particles at a concentration of 1 A_{260} unit/ml isolated from liver and pachytene nuclei were dialyzed overnight against 1 mM sodium cacodylate containing 0.1 mM disodium salt of EDTA neutralized to pH 7.4 and 0.1 mM PMSF. Circular dichroism spectra were recorded in a Jasco J-20 spectropolarimeter between 220 and 320 nm. The recording was done using a 0.5-cm cuvette at a scale of 0.002°/cm. The spectra were recorded both at 2 and 25 °C and there was no difference in the spectra obtained at these two temperatures. To get the spectra of free DNA, SDS was added to core particle preparation to a final concentration of 0.4% and incubated at room temperature for 2 h, after which the spectra were recorded as described above.

Thermal Denaturation of Core Particles—For this purpose, nucleosome core particles were dialyzed overnight against either 0.1 mM sodium cacodylate or 1 mM sodium cacodylate containing 0.1 mM PMSF and 0.1 mM Na_2EDTA. After dialysis, the samples were checked for their purity by UV absorption spectra. The melting studies of samples containing core particles at a concentration of 1 A_{260} unit/ml was carried out in a Shimadzu model 210 A spectrophotometer with jacket attachment around the cuvette holders for water circulation. The rate of increase in the temperature was 1 °C/2 min using an external circulating water bath and the absorbance at 260 nm was recorded at 0.5 °C intervals. The measurement of temperature was done by a precalibrated thermocouple. A derivative plot ($\Delta H/\Delta T$) for the hyperchromicity was obtained by the use of the 3-point average method with the help of a programmed calculator.

Extraction of Acid-soluble Proteins and Polyacrylamide Gel Electrophoresis of the Extracted Proteins—The acid-soluble proteins were extracted from either total nuclei or nucleosome core particles according to the method of Platz *et al.* (13) and were analyzed on two-dimensional polyacrylamide gel electrophoresis as described by Alfageme *et al.* (14) and Rao *et al.* (10) in which the first dimension was carried out on a 15% gel in the presence of acid/urea while the second dimension was carried out on a 12% gel in the presence of acid/urea and 0.4% Triton X-100 (Method 1). For the analysis of histone H1 subspecies, the two-dimensional gel electrophoresis system described by Seyedin and Kistler (6) was employed in which the first dimension was carried out on a 15% gel in the presence of acid/urea while the second dimension was carried out on 10% gel in the presence of 0.1% SDS (Method 2).

Proteins were stained either with 0.3% amido black or 0.2% Coomassie brilliant blue in methanol:acetic acid:water (43:7:50) and destained with the same solvent.

Analysis of Nucleosome Core Proteins on SDS-Polyacrylamide Gels—Total proteins from 1.5 A_{260} units of nucleosome core particles were precipitated with 25% trichloroacetic acid (final concentration, w/v) and were subjected to SDS–10% polyacrylamide gel electrophoresis according to method of Laemmli (15). After the electrophoresis, the proteins were stained with 0.3% Coomassie brilliant blue as described above.

Analysis of DNA Fragments—Electrophoretic analysis of the DNA fragments on nondenaturing 6% polyacrylamide gels was carried out according to the method of Maniatis *et al.* (16) with modifications as described by Creusot and Christman (17) using either an endonuclease *Hae*III digest of pBR322 DNA or *Hind*III digest of PM2 DNA as the size markers.

Electrophoretic analysis of the core nucleoprotein particle was done in a low ionic strength gel system as described by Olins *et al.* (18).

DNA fragments were visualized in the gels after staining with 1 μg/ml of ethidium bromide in water and observing under ultraviolet light, while the proteins in the nucleoprotein gel were stained with amido black and destained as described above.

RESULTS

The purity of the pachytene nuclei isolated by the STAPUT technique which has been used for all the studies reported here was greater than 85% as judged by the phase-contrast microscope as shown in Fig. 1. The morphological integrity of these nuclei is well maintained and typical chromosome bivalents generated by homologous chromosome pairing can be seen in most of these nuclei.

Fig. 2*A* shows the electrophoretic pattern of the acid-soluble proteins of pachytene nuclei using the two-dimensional polyacrylamide gel electrophoresis system (Method 1). For comparative purposes, the electrophoretic pattern of the acid-soluble proteins isolated from rat liver nuclei is shown in Fig. 2*B*. It is clear that pachytene nuclei contain the variant histones TH1, TH2A, TH2B, and X2 which are absent in liver nuclei. Histones H1 and TH1 were further resolved by carrying out the second dimension in SDS-polyacrylamide gel electrophoresis as described in Method 2. As can be seen from Fig. 2, *C* and *D*, histone TH1 was resolved into two polypeptides, namely H1a and H1t, both of which were absent in liver nuclei. On the other hand, histone H1 was resolved into two spots, H1bde and H1c. A quantitative analysis of the various histone variants present in pachytene nuclei was done by eluting the stain from the individual spots with acidified dimethyl sulfoxide (19) and measuring the absorption at 600 nm. it was found that the individual percentages of histone TH2A and histone TH2B were 40 and 66% of total histone H2A and histone H2B, respectively, in the pachytene nuclei. The level of protein X2 which is another variant of histone H2A (7) and present in liver nuclei also is increased 3–4-fold in pachytene nuclei. In these nuclei, the relative contents of histone H1a and H1t were 47 and 18.5% of the total histone H1. The relative contents of H1bde and histones H1C and $H1^0$ were of the order of 55, 37, and 8%, respectively, of the total histone H1 in liver nuclei. Thus, the increase in the

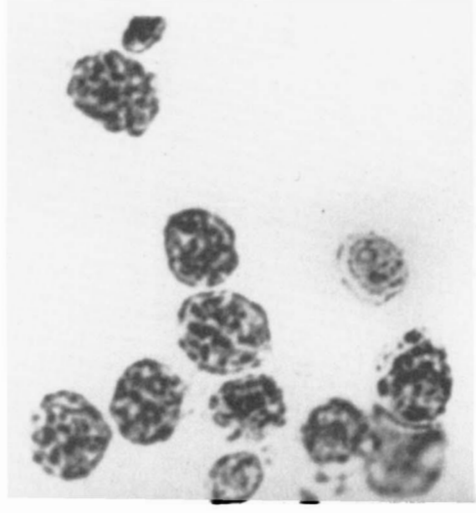

FIG. 1. **Phase-contrast photomicrograph of isolated pachytene nuclei from rat testes.** × 800.

content of histones H1a and H1t in the pachytene nuclei was mostly at the expense of histone H1bde and to a lesser extent this was true in histone H1c.

After establishing the purity and the histone composition of pachytene nuclei, digestion studies with micrococcal nuclease were carried out in order to understand gross chromatin organization in these meiotic prophase nuclei. The kinetics of digestion of pachytene nuclei and liver nuclei with micrococcal nuclease under identical conditions, shown in Fig. 3*A*, indicates that the rate and the extent of digestion (40%) are identical in both kinds of nuclei, suggesting that accessibility of these types of nuclei to micrococcal nuclease is similar. When the DNA of the EDTA-solubilized nuclease digests of both pachytene and liver nuclei obtained at similar digestion points were analyzed on polyacrylamide gel electrophoresis, the pattern of which is shown in Fig. 3*B*, both types of nuclei were revealed to yield similar DNA fragments starting from mononucleosomes to higher oligomers. This similarity was observed both at 6 and 22% digestion. The densitometric scans of the negative of the DNA gel photograph as shown in Fig. 3*C* clearly show that the peaks corresponding to various oligomers of nucleosomes generated from both pachytene and liver nuclei are perfectly superimposable, thus suggesting that the pachytene chromatin has the same nucleosomal repeat length as that of liver chromatin, namely 196 base pairs (20).

On the other hand, a comparison of the kinetics of digestion of pachytene nuclei and liver nuclei with DNase I revealed significant differences. The results of DNase I digestion studies shown in Fig. 4 reveal that, although the initial rate of digestion was similar in these two types of nuclei, the extent of digestion was significantly different. While a limit digest of 55% of acid solubility of the total DNA was obtained in the case of liver nuclei, a near limit digestion was obtained at 80% acid solubility in the case of pachytene nuclei. It can also be seen that even at 80 min the digestion was still in the increasing phase although the rate of digestion at this time of digestion was much lower than the initial rates of digestion. This kind of differential sensitivity of pachytene nuclei toward micrococcal nuclease and DNase I is very analogous to the situation observed with hyperacetylated chromatin. For

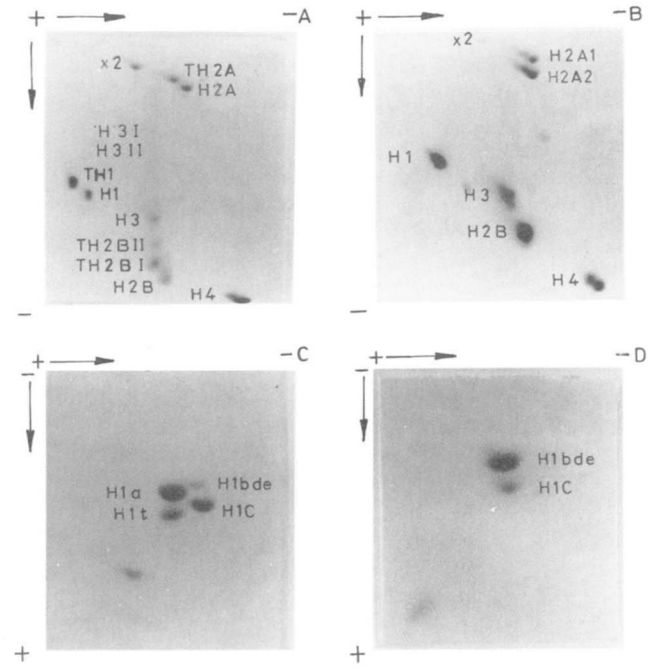

FIG. 2. **Analysis of acid-soluble proteins on two-dimensional polyacrylamide gel electrophoresis.** Acid-soluble proteins from (*A*) pachytene nuclei of rat testes and (*B*) rat liver nuclei were analyzed in the first dimension on an acid/urea gel and in the second dimension on acid/urea/Triton X-100 gels. Histone H1 species present in acid-soluble proteins of (*C*) pachytene nuclei and (*D*) rat liver nuclei were analyzed in the first dimension on an acid/urea gel and in the second dimension on SDS-polyacrylamide gels.

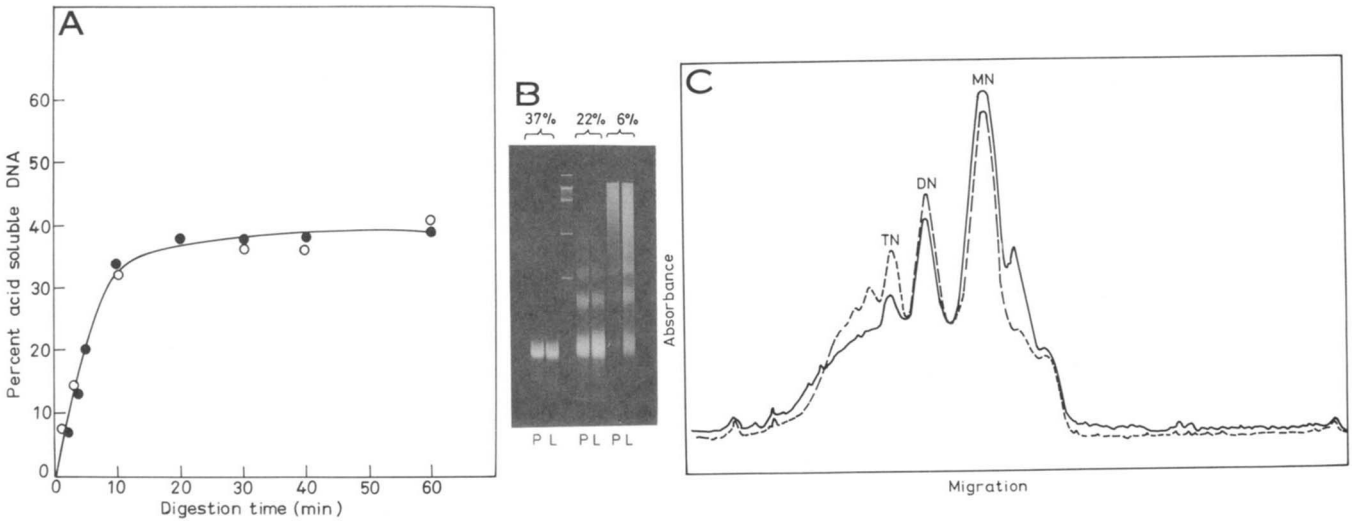

FIG. 3. **Micrococcal nuclease digestion of pachytene and liver nuclei.** *A*, kinetics of release of acid-soluble DNA. Both pachytene and liver nuclei were digested at a concentration of 10 A_{260} units of nuclei/ml with 1 unit of micrococal nuclease (Sigma)/ml. Aliquots were taken at different time intervals in 1 M NaCl, 1 M perchloric acid and the acid-soluble DNA was measured at 260 nm. ○, pachytene nuclei; ●, liver nulcei. *B*, Analysis of DNA in the nuclease digest on polyacrylamide gels. The micrococcal nuclease digests obtained at 6, 22, and 37% digestion from pachytene and liver nuclei were made 1% and 0.2 M with respect to SDS and NaCl, respectively. After treatment with 5 μg of pronase/100 μg of DNA for 2 h at 37 °C, they were extracted with an equal volume of phenol and chloroform (1:1) and the DNA in the aqueous phase was precipitated with 2 volumes of ethanol. The DNAs in the various fractions were analyzed on 3.5% polyacrylamide gels according to the method described by Maniatis *et al.* (16) using endonuclease *Hind*III digests of PM2 DNA as size markers. The bands were visualized under ultraviolet light after staining with 1 μg/ml of ethidium bromide. *P*, pachytene; *L*, liver. *C*, densitometric scans of the negative of *B* obtained using a Joyce-Loebl microdensitometer. – – –, pachytene; ——, liver. *MN*, *DN*, and *TN*, mono-, di-, and trinucleosomes.

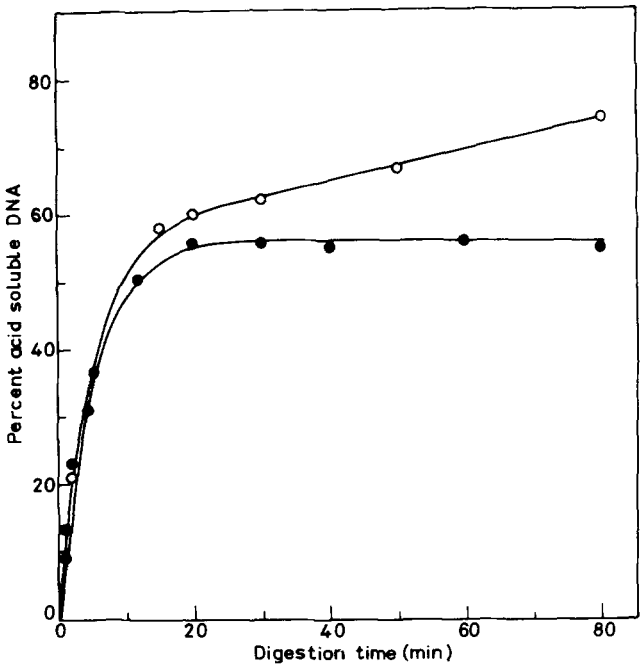

FIG. 4. **Kinetics of digestion of pachytene and liver nuclei with DNase I.** Pachytene and liver nuclei at a concentration of 10 A_{260} units/ml were digested with 3 μg/ml of DNase I. The percentage of acid-soluble DNA after different time intervals was measured as described in Fig. 3*A*. ○, pachytene; ●, liver.

example, Simpson (21) and Perry and Chalkely (22) have shown that although the basic repeating unit and digestibility with micrococcal nuclease of hyperacetylated chromatin obtained from butyrate-treated cells is similar to the chromatin from untreated cells, the hyperacetylated chromatin is digested with DNase I at a higher rate than the nonacetylated chromatin. In fact, there is evidence that histones H3 and H4 are acetylated in pachytene nuclei (23) and hence may explain their higher digestibility with DNase I. The mechanism by which acetylation of histones H3 and H4 influences the higher order structure of chromatin making it more accessible to DNase I is not clear at present. It is very likely that, since the acetylation sites on histones H3 and H4 are in the NH_2-terminal ends (24), the NH_2-terminal tails may take part in maintaining the higher order structure. Such a possibility is strengthened by the observations of Allan *et al.* (25) who have shown that reconstituted chromatin containing trypsinized histones H3 and H4 in which the NH_2-terminal tails have been removed failed to yield the solenoid structure even in the presence of histone H1 under optimal conditions necessary for the formation of 30-nm filament.

Physicochemical Studies of Isolated Nucleosome Core Particles from Pachytene and Liver Nuclei—Since 60% of histone H2A and 60% of histone H2B are replaced by the histone variants TH2A, X2, and TH2B and also since these variant histones have been shown to be present in nucleosome core particles isolated from total rat testes (10), it is quite logical to assume that these histone variants must influence the physicochemical properties of the core particles. In order to evaluate whether histone variants do influence the structure of the core particle, we have investigated the structure of pachytene core particles using circular dichroism and thermal denaturation techniques. The nucleosome core particles isolated from liver nuclei which contain only somatic histones H2A, H2B, H3, and H4 were used as the reference standard.

The nucleosome core particles were isolated from both rat liver nuclei and pachytene nuclei as described earlier and were characterized for their integrity by their DNA length (Fig. 5*A*) and their integrity as a nucleoprotein particle (Fig. 5*B*). It is clear that both the core particle preparations had a DNA length of 152 ± 6 bp and were also intact as judged by their mobility in the low ionic strength gel wherein the particle could be stained by both ethidium bromide and amido black. The various histone types present in the core particle preparations of liver and pachytene nuclei were ascertained by analyzing the acid-soluble proteins by two-dimensional polyacrylamide gel electrophoresis (Method 1, Fig. 6, *A* and *B*). It is clear that core particle preparations from pachytene nuclei had the complements of histone variants TH2A, TH2B, and X2, while the core particles from both liver nuclei and pachytene nuclei lacked histone H1.[2] Fig. 6*C* shows the pattern of total nucleosome core proteins on SDS-polyacrylamide gels and it is evident that these nucleosome particles are devoid of any nonhistone proteins and contain mainly the core histones.

The circular dichroism spectra of the nucleosome core particles from the two types of nuclei are shown in Fig. 7. The spectrum obtained after adding 0.4% SDS to core particles is also shown. Treatment with SDS disrupts the histone-DNA interactions and, hence, the resulting spectra should reflect the spectra of free DNA and free histones. The main feature of the circular dichroic spectra of core particles is that the positive molar ellipticity observed at 275 nm for free DNA (core particle + SDS) of 6000 degree-cm^2 dmol^{-1} is greatly reduced for DNA in the core particle. In the case of liver core particles, two positive maxima are observed, one at 276 nm having a molar ellipticity of 400 degree-cm^2 dmol^{-1} and the other at 282 nm having a molar ellipticity of 900 degree-cm^2 dmol^{-1}. Further, the crossover point for free DNA which is 257 nm is shifted to 270 nm in the case of liver core particles. In addition to these positive bands, negative ellipticities are observed for liver core particles at 222 nm (−34,000 degree-cm^2 dmol^{-1}) and at 292 nm (−400 degree-cm^2 dmol^{-1}). The band at 222 nm is contributed by the histones while the band at 292 nm is characteristic of the nulceosome core particles. These results for liver core particles are in general agreement with the results of Cowman and Fasman (26) obtained for the chicken erythrocyte core particles. There have been several hypotheses proposed for the decrease in the ellipticity observed for DNA in the core particle at 275 nm when compared to free DNA. Maestre and Reich (27) have proposed that the decrease in positive ellipticity results from a scattering resonance phenomenon arising out of side to side packing of DNA molecules on the core histones without changing the secondary structure of the DNA. On the other hand, Baase and Johnson (28) have interpreted this decrease as due to the change in the winding angle of the DNA helix when it is wrapped around the histone octamer.

A comparison in the circular spectra obtained for liver core particles and the pachytene core particles (Fig. 7) reveals several striking differences, especially in the high wavelength region. This region of the spectra has been expanded and illustrated in the *inset* to Fig. 7. It can be seen that the two positive bands observed with the liver core particles at 275 and 282 nm were shifted to 270 and 280 nm in the case of pachytene core particles. Further, the molar ellipticities at these two positive bands (θ_{270} = 540 degree-cm^2 dmol^{-1} and θ_{280} = 1100 degree-cm^2 dmol^{-1}) were higher than the corresponding molar ellipticities for the liver core particles. In

[2] It can be seen in Fig. 6*B* that histones H2B and TH2B of pachytene core particles are split into two spots. We have consistently observed splitting of these two histones when the electrophoresis in the second dimension was carried out for a long time and the reasons for such splitting are not clear at present.

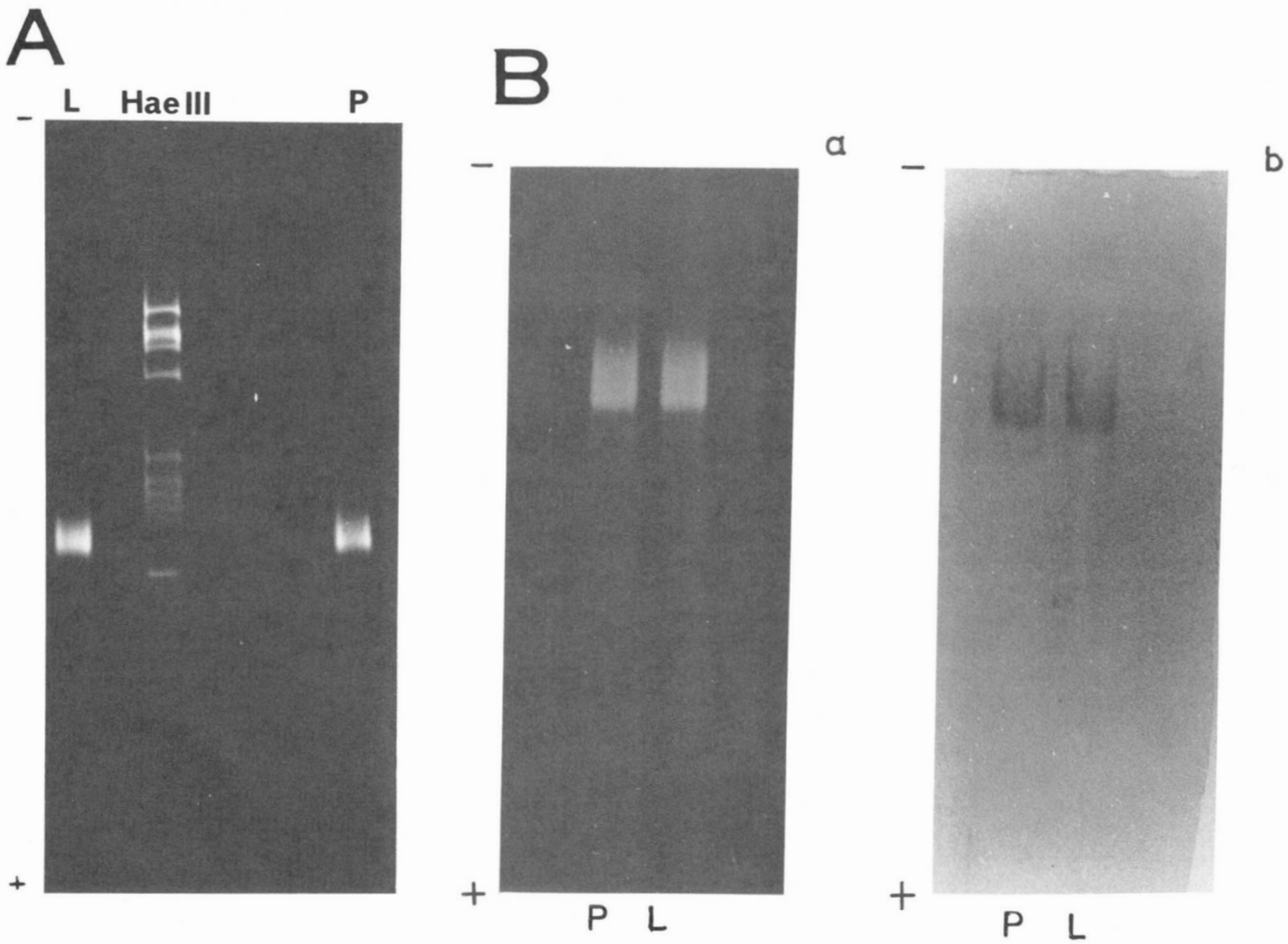

FIG. 5. **Analysis of pachytene and liver nucleosome core particle DNA.** *A*, pachytene and liver core particles were made 1% with respect to Sarkosyl and analyzed on 6% polyacrylamide gels according to the method described by Creusot and Christman (17). *HaeIII*, endonuclease digest of pBR322 DNA; *P*, pachytene core particle DNA; *L*, liver core particle DNA. *B*, integrity of the pachytene and liver core particles. pachytene (*P*) and liver (*L*) core particles were analyzed on 5% polyacrylamide gels under low ionic strength buffer conditions as described by Olins *et al.* (18). *a*, core particles stained with ethidium bromide; *b*, core particles stained for protein with amido black.

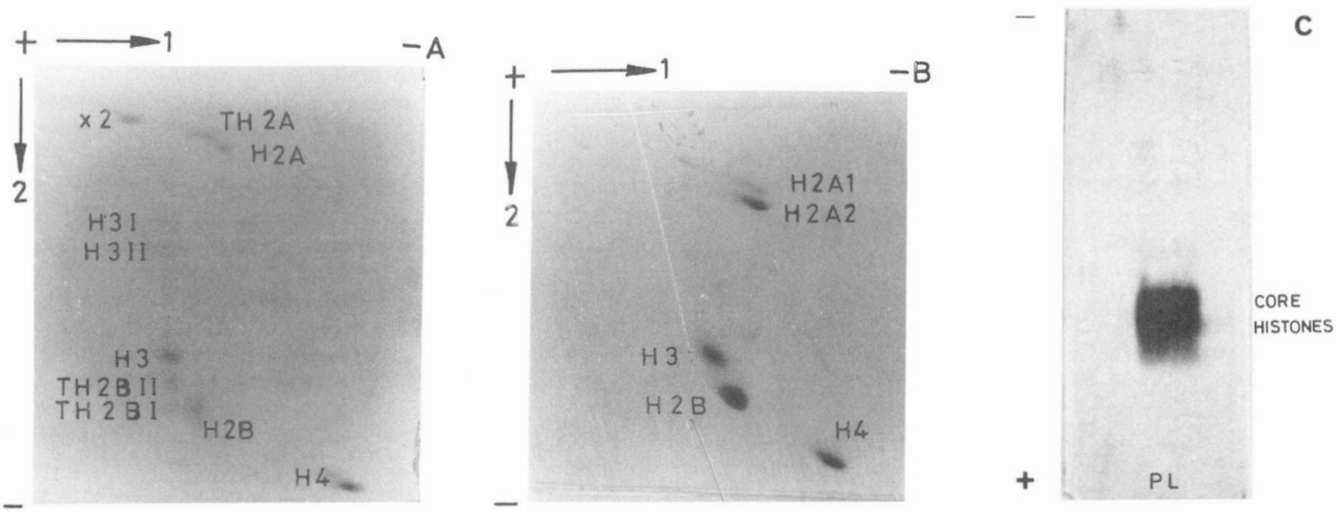

FIG. 6. **Analysis of acid-soluble proteins and total proteins of nucleosome core particles on polyacrylamide gels.** Acid-soluble proteins from (*A*) pachytene core particles and (*B*) liver core particles were analyzed in the first dimension on an acid/urea gel and in the second dimension on an acid/urea/Triton X-100 gel as described under "Materials and Methods." *C*, SDS-polyacrylamide gel electrophoresis. The total proteins from nucleosome core particles were precipitated with trichloroacetic acid and were analyzed on one-dimensional 10% polyacrylamide gels containing 0.1% SDS according to the method of Laemmli (15). *P*, pachytene; *L*, liver.

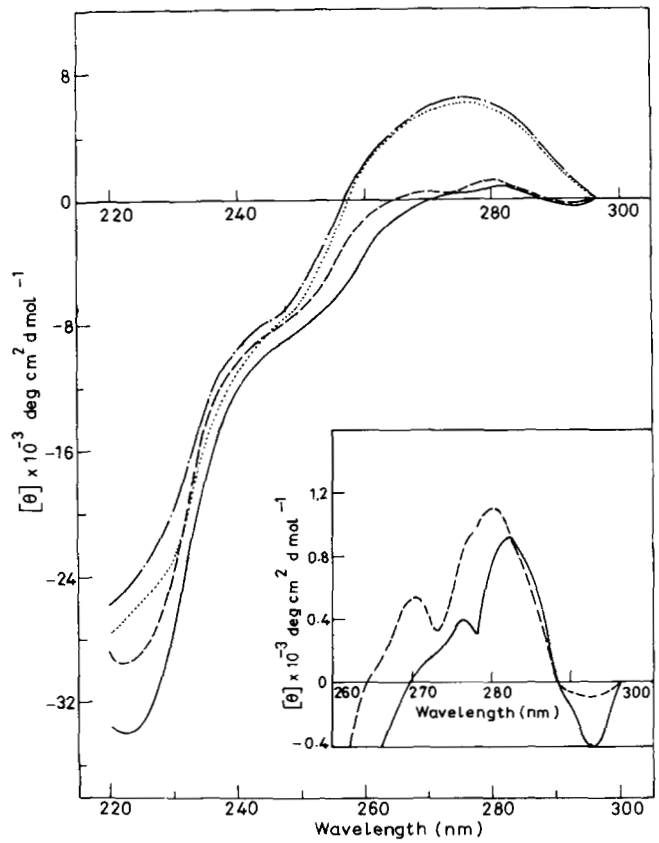

FIG. 7. **Circular dichroism spectra of pachytene and liver core particles (1 A_{260} unit/ml in 1 mM sodium cacodylate containing 0.1 mM Na_2EDTA, pH 7.4, and 0.1 mM PMSF) were recorded between 220 and 320 nm in a Jasco spectropolarimeter.** The *inset* gives the spectra obtained between 260 and 320 nm on an expanded scale. - - -, pachytene core particles; ——, liver core particles; -·-, pachytene core particles + 0.4% SDS; ·····, liver core particles + 0.4% SDS. CD spectra shown here are representative of a typical experiment. Similar spectra were obtained for several batches of preparations of pachytene and liver core particles.

addition to this increase in positive ellipticities, the crossover point also was shifted to 264 nm. Another interesting difference in the circular dichroism spectra between pachytene and liver core particles is the decrease in the negative molar ellipticity at 292 nm in pachytene core particles (−100 degree-cm^2 dmol^{-1}) when compared to liver core particle (−400 degree-cm^2 dmol^{-1}). It is not clear which parameters contribute toward this negative band at 292 nm although Cowman and Fasman (26) suggested that it is related to the compactness of the core particle. The negative molar ellipticity observed at 222 nm was also lowered in the case of pachytene core particles (−29,000 degree-cm^2 dmol^{-1}) as compared to the liver core particles (−34,000 degree-cm^2 dmol^{-1}), thereby indicating that the extent of α-helical conformation adopted by the histone core in pachytene nucleosome is less than that in the liver nucleosomes (29). It is also clear from Fig. 7 that, once the histone-DNA complex was dissociated by the addition of 0.4% SDS, the circular dichroism spectra obtained were nearly identical for liver and pachytene core particles.

In order to further understand the structural differences between liver and pachytene core particles, thermal denaturation studies were carried out and the derivative plots ($\Delta H/\Delta T$) of these results are presented in Fig. 8, *A* and *B*. With both the core particle preparations, the total hyperchromicity obtained was 36%. However, the melting of pachytene core DNA in the premelting region, namely between 55 and 60 °C, was higher than the liver core DNA. Similar higher melting of the pachytene core DNA was observed even in 1 mM sodium cacodylate and 0.1 mM EDTA (Fig. 8*B*). It can be seen that the core particles melt more sharply in 0.1 mM sodium cacodylate than in 1 mM sodium cacodylate. The temperatures at which main transition and premelting transition occur (71 ± 1 °C and 57 ± 1 °C in 0.1 mM sodium cacodylate) are shifted to 77 ± 1 °C and 60 ± 1 °C in 1 mM sodium cacodylate. The results are in good agreement with those obtained by Weischet *et al.* (30) for chicken erythrocyte core particles. However, at both the ionic strengths, the relative amount of DNA melting at the premelting region was higher in the case of pachytene core particles. In 0.1 mM cacodylate buffer, 50 bp of a total 146 bp of pachytene core DNA melted in the premelting

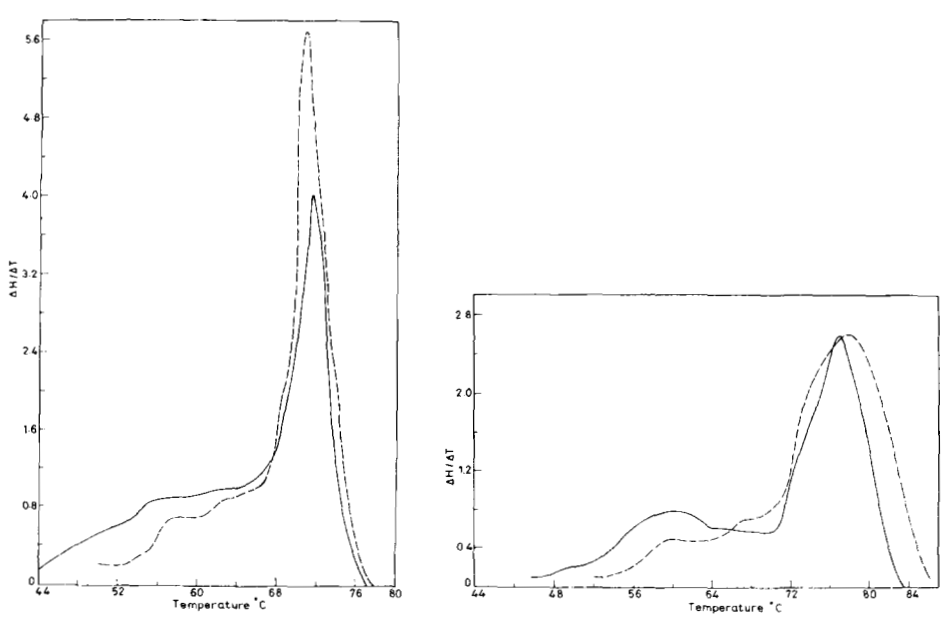

FIG. 8. **Thermal denaturation of core particles from pachytene and liver nulcei.** Melting of core particles was carried out (*A*) in 0.1 mM sodium cacodylate buffer, pH 7.1, containing 0.1 mM Na_2EDTA and (*B*) in 1 mM sodium cacodylate buffer, pH 7.1, containing 0.1 mM Na_2EDTA. The rate of increase in the temperaure was 1 °C/2 min and the absorbance at 260 nm was recorded at 0.5 °C intervals. Derivative plot ($\Delta H/\Delta T$) for the thermal denaturation profile was obtained by the use of the 3-point average method. ——, pachytene core particles; - - -, liver core particles.

TABLE I

Summary of the melting properties of pachytene and liver core particles

	T_m	Hyperchromicity	No. bp melting of 146 total DNA bp
	°C	% *total*	
1.0 mM sodium cacodylate			
Premelting transition			
Pachytene	60 ± 1	34	50
Liver	60 ± 1	20	28
Main transition			
Pachytene	77	66	95
Liver	78	80	117
0.1 mM sodium cacodylate			
Premelting transition			
Pachytene	56 ± 1	45	65
Liver	58 ± 1	28	41
Main transition			
Pachytene	71	55	80
Liver	72	72	104

region as compared to 28 bp of liver core DNA. On the other hand, in 1 mM cacodylate buffer, the fractions of DNA melting in the premelting region were 65 and 41 bp in pachytene and liver core particles, respectively. The increase in the fraction of DNA melting in the premelting region of pachytene core particles is accompanied by a reciprocal decrease in the amount of DNA melting at the main transition temperature. The various parameters that are deduced from the derivative plots are summarized in Table I.

DISCUSSION

Meiotic crossing over involving genetic recombination is one of the major biochemical events occurring during the meiotic prophase of mammalian spermatogenesis. There has been very little information regarding the biochemistry of meiosis in general and that in mammals in particular. Most of our present understanding of the biochemistry of meiosis is derived from the extensive studies of Stern and his coworkers using *Lilium* as the model system, and they have also extended their studies to the mouse system on some specific aspects of recombination. Stern and Hotta (31) have proposed a general model for the mechanism of meiotic crossing over involving (*a*) single-stranded nicks caused by a specific meiotic endonuclease, (*b*) single-stranded DNA exchange, and (*c*) DNA repair. However, this model does not take into account the structural features of the chromatin that may influence the entire process of meiotic crossing over. Since it is now a well established fact that DNA in the eukaryotic cells is organized into nucleosomes, the fundamental unit of chromatin, it is essential to understand the organizational features of meiotic chromatin and also the structural parameters of nucleosome core particles derived from meiotic chromatin.

At about the same time that Kornberg (32) proposed the nucleosome model for the structure of eukaryotic chromatin, several groups of workers had shown that, during spermatogenesis in the rat, histone variants start appearing just before the meiotic stage of germ cell differentiation (2, 8). It was generally assumed that the appearance of these histone variants somehow facilitates the meiotic events. Since, at present, histones are believed to be purely structural proteins of the chromatin, whatever functions these histone variants do have in the meiotic events, they should be mediated through alterations in the structure of the chromatin. With this view in mind, we have studied both gross structural properties of pachytene chromatin as well as the fine physicochemical properties of isolated pachytene core particles.

The results presented in this paper on the gross structural features of pachytene chromatin have shown that the basic parameters of chromatin organization like (*a*) the rate and extent of digestion by micrococcal nuclease and (*b*) the nucleosomal repeat length of 196 bp are identical with those of interphase chromatin. Even though the pachytene chromatin has two histone H1 variants, namely histones H1a and H1t, the size of the linker DNA is not altered. There have been reports that the type of histone H1 present does influence the size of the linker DNA in chicken erythrocytes (33), sea urchin sperm (34), and *Neurospora crassa* (35). Although, we have found in these studies that nucleosome repeat length of the pachytene chromatin containing H1t to 24% is not altered, it is still possible that some localized regions of pachytene chromatin containing predominantly histone H1t may have an altered nucleosome repeat length.

A more interesting observation made with respect to gross structural features of pachytene chromatin is its digestibility with DNase I. Under identical conditions of digestion, pachytene chromatin was 25% more accessible to DNase I than the interphase chromatin. The DNase I probe has been extensively used to study the higher order structure of chromatin. The fact that pachytene chromatin was more accessible than the interphase chromatin suggests a loosened higher order structure for pachytene chromatin. Interestingly, such a difference in the accessibility to DNase I was not observed when the digestion rate was compared in the case of isolated nucleosome core particles from liver and pachytene nuclei (data not given). These results are very similar to that obtained with acetylated chromatin (21, 22). As already mentioned, acetylation of histones H3 and H4 has been demonstrated in pachytene nuclei (23). However, these workers have implicated acetylation of histones as facilitating their replacement by more basic proteins during the process of spermiogenesis. Another likely possibility is that acetylation of histones in the pachytene nuclei influences the higher order structure of chromatin so as to facilitate pairing of homologous chromosomes.

Another aspect of the present study is the finer structural details of the nucleosome core particles isolated from liver and pachytene nuclei with respect to DNA conformation and compactness of the core particles as measured by the circular dichroism as well as the nature of histone-DNA interactions as measured by thermal denaturation. The results obtained from circular dichroism studies clearly indicate that the compaction of DNA around the histone octamer in the pachytene core particle is not as extensive as one observes with the liver core particle as evidenced by (*a*) shift in the crossover point toward free DNA, (*b*) increase in the positive molar ellipticity, again more toward free DNA, and (*c*) decrease in the negative molar ellipticity obtained at 282 nm. The lesser compactness of the pachytene core particle as compared to liver core particle is also substantiated by the thermal denaturation studies which showed that the fraction of pachytene core DNA melting at premelting temperature of 55–60 °C was significantly higher than that of liver core DNA. The DNA that melts at the premelting temperature is believed to be due to the two ends of the core DNA. According to the model proposed by Schick *et al.* (36) which is shown in Fig. 9 for the topological distribution of histone contacts with the core

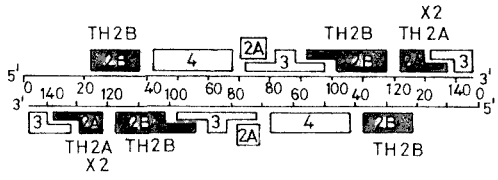

FIG. 9. **Mirzabekov's model (36) for the histone-DNA contact sites in the nucleosome core particle.** The histone variants TH2A, X2, and TH2B which replace partially the somatic histones H2A and H2B and might occupy similar topological positions in the core particle are also shown.

DNA, histones H2A and H2B are supposed to interact with the two ends of the core DNA. This model has been recently confirmed by Nelson *et al.* (37). The only difference between pachytene and liver core particles is the presence of histone variants TH2A, X2, and TH2B in the pachytene core particles. These variants account for almost 50 to 60% of the total histones H2A and H2B in pachytene core particles. Thus, it is very likely that the lesser compactness of the pachytene core particles is probably due to weaker DNA-histone interaction of the histone variants which might occupy similar topological positions as histones H2A and H2B in the nucleosome core particle. The loss of stability is also reflected in the higher percentage of premelting of the DNA duplexes in the pachytene core particle. The other possibility, namely acetylation of histones as the contributing factor for the altered physicochemical properties of the pachytene core particles, can be ruled out since Reczek *et al.* (38) and Yau *et al.* (39) have clearly shown that the circular dichroism spectra and the thermal denaturation profile of core particles containing hyperacetylated histones are nearly identical with those of core particles containing nonacetylated histones.

Recently, Trostle-Weige *et al.* (7) have shown that rat testes nuclei contain a histone H3 variant termed TH3 that may be present in our pachytene core particle preparations. However, we cannot ascertain at present the influence of TH3 on the physicochemical properties of pachytene core particles observed here.

Thus, the pachytene chromatin appears to be less compact both at the higher order level as well as at the nucleosome core particle level. At present, we can only speculate on the biological implications of a less compact structure in the meiotic crossing over process. Such a structure may influence either (*a*) pairing of homologous chromosomes and/or (*b*) single strand exchange between the two homologous chromosomes at the sites of recombination. From a purely structural point of view, it would be of great interest to see: (*a*) whether the somatic histones H2A and H2B and the histone variants TH2A and TH2B are intermixed with each other in a single core particle or separate core particles exist that contain only histones H2A and H2B or histone variants TH2A and TH2B and (*b*) if they exist as separate entities, are they localized or clustered in specific regions of chromatin, namely sites of recombination? Further work in this direction should greatly help us in understanding the physiological significance of the presence of histone variants in the meiotic prophase chromatin.

REFERENCES

1. Meistrich, M. L., Brock, W. A., Grimes, S. R., Jr., Platz, R. D., and Hnilica, L. S. (1978) *Fed. Proc.* **37,** 2522–2525
2. Kumaroo, K. K., Jahnke, G., and Irvin, J. L. (1975) *Arch. Biochem. Biophys.* **168,** 413–424
3. Platz, R. D., Grimes, S. R., Jr., Meistrich, M. L., and Hnilica, L. S. (1975) *J. Biol. Chem.* **250,** 5791–5800
4. Kumaroo, K. K., and Irvin, J. L. (1980) *Biochem. Biophys. Res. Commun.* **94,** 49–54
5. Shires, A., Carpenter, M. P., and Chalkely, R. (1976) *J. Biol. Chem.* **251,** 4155–4158
6. Seyedin, S. M., and Kistler, W. S. (1980) *J. Biol. Chem.* **255,** 5949–5954
7. Trostle-Weige, P. K., Meistrich, M. L., Brock, W. A., Nishioka, K., and Bremer, J. W. (1982) *J. Biol. Chem.* **257,** 5560–5567
8. Branson, R. E., Grimes, S. R., Jr., Yonuschot, G., and Irvin, J. L. (1975) *Arch. Biochem. Biophys.* **168,** 403–412
9. Stern, H., and Hotta, Y. (1977) *Phil. Trans. R. Soc. Lond. B* **277,** 277–294
10. Rao, M. R. S., Rao, B. J., and Ganguly, J. (1982) *Biochem. J.* **205,** 15–21
11. Meistrich, M. L. (1977) *Methods Cell Biol.* **15,** 16–54
12. Garel, A., Zolan, M., and Axel, R. (1977) *Proc. Natl. Acad. Sci. U. S. A.* **74,** 4867–4871
13. Platz, R. D., Meistrich, M. L., and Grimes, S. R., Jr. (1977) *Methods Cell Biol.* **16,** 297–316
14. Alfageme, C. R., Zweidler, A., Mahowald, A., and Cohen, L. H. (1974) *J. Biol. Chem.* **249,** 3729–3736
15. Laemmli, U. K. (1970) *Nature (Lond.)* **227,** 680–685
16. Maniatis, T., Andrea, J., and Van deSande, H. (1975) *Biochemistry* **14,** 3787–3794
17. Creusot, F., and Christman, J. K. (1980) *Anal. Biochem.* **103,** 343–349
18. Olins, A. L., Carlson, R. D., Wright, E. B., and Olins, D. E. (1976) *Nucleic Acids Res.* **3,** 3271–3291
19. Chiu, M. L., and Irvin, J. L. (1980) *Anal. Biochem.* **109,** 102–108
20. Compton, J. L., Bellard, M., and Chambon, P. (1976) *Proc. Natl. Acad. Sci. U. S. A.* **73,** 4382–4386
21. Simpson, R. T. (1978) *Cell* **13,** 691–699
22. Perry, M., and Chalkley, R. (1981) *J. Biol. Chem.* **256,** 3313–3318
23. Grimes, S. R., Jr., Chae, C. B., and Irvin, J. L. (1975) *Arch. Biochem. Biophys.* **168,** 425–435
24. Isenberg, I. (1979) *Annu. Rev. Biochem.* **48,** 159–191
25. Allan, J., Harbourne, N., Rau, D. C., and Gould, H. (1982) *J. Cell Biol.* **93,** 285–297
26. Cowman, M. K., and Fasman, G. D. (1978) *Proc. Natl. Acad. Sci. U. S. A.* **75,** 4759–4763
27. Maestre, M. F., and Reich, C. (1980) *Biochemistry* **19,** 5214–5223
28. Baase, W. A., and Johnson, W. C., Jr. (1979) *Nucleic Acids Res.* **6,** 797–813
29. Thomas, G. J., Jr., Prescott, B., and Olins, D. E. (1977) *Science (Wash. D. C.)* **197,** 385–387
30. Weischet, W. O., Tatchell, K., Van Holde, K. E., and Klump, H. (1978) *Nucleic Acids Res.* **5,** 139–160
31. Stern, H., and Hotta, Y. (1978) *Annu. Rev. Plant Physiol.* **29,** 415–436
32. Kornberg, R. D. (1974) *Science (Wash. D. C.)* **184,** 868–871
33. Weintraub, H. (1978) *Nucleic Acids Res.* **5,** 1179–1188
34. Simpson, R. T., and Bergman, L. W. (1980) *J. Biol. Chem.* **255,** 10702–10709
35. Noll, M. (1976) *Cell* **8,** 349–356
36. Schick, V. V., Belyavsky, A. V., Bavykin, S. G., and Mirzabekov, A. D. (1980) *J. Mol. Biol.* **139,** 491–517
37. Nelson, D. A., Mencke, A. J., Chambers, S. A., Osterhof, D. K., and Rill, R. J. (1982) *Biochemistry* **21,** 4350–4362
38. Reczek, P. R., Weissman, D., Huvos, P. E., and Fasman, G. D. (1982) *Biochemistry* **21,** 993–1002
39. Yau, P., Thorne, A. W., Imai, B. S., Mathews, H. R., and Bradbury, E. M. (1982) *Eur. J. Biochem.* **129,** 281–288