

HISTONES OF GENETICALLY ACTIVE AND INACTIVE CHROMATIN

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ABSTRACT

It has frequently been proposed that a variation in the relative content of lysine-rich, moderately lysine-rich, and arginine-rich histones might provide a mechanism by which specific portions of the genome may be genetically regulated. This possibility was investigated by comparing the electrophoretic pattern of these three fractions in cells differing markedly in their content of genetically active and genetically inactive chromatin. Three models were used: heterochromatin versus euchromatin; metaphase cells versus interphase cells, and mature lymphocytes versus phytohemagglutinin-stimulated lymphocytes. In no case was there a significant difference in the histone patterns of these contrasting models. It is concluded that, although histones may act as a generalized repressor and structural component of chromatin, factors other than a variation in histone pattern may be responsible for repression or derepression of specific segments of the genome.

INTRODUCTION

One of the intriguing problems concerning the genetics of higher organisms is the mechanism by which large segments of the genome are genetically repressed. Recent studies have indicated that histones repress DNA-dependent RNA synthesis (1-3) and that lysine-rich and arginine-rich histones differ in their relative capacity to bind to DNA and to inhibit RNA synthesis (2-9). These interesting observations have led to the suggestion that the genetic activity of a segment of chromatin may depend on the type of histone with which it is associated. Despite the compelling nature of this suggestion, it is based on differential extraction procedures or on artificially produced DNA-histone complexes. A more physiological and logical approach to this possibility is the comparison of the histones of genetically inactive chromatin with those of genetically active chromatin. For this study, three systems were used which provide ready separation of these two forms of chromatin: (a) heterochromatin versus euchro-

matin; (b) metaphase versus interphase cells, and (c), mature versus phytohemagglutinin-stimulated lymphocytes.

The investigation of most of these systems required a technique which would allow the characterization of the histones of a very small amount of tissue. Murray (10) has recently demonstrated that, after the removal of cytoplasmic basic proteins at pH 2.75, histones may be separated into lysine-rich, moderately lysine-rich, and arginine-rich fractions by extraction at progressively lower pH's. The use of this technique in combination with polyacrylamide-gel electrophoresis allowed the characterization of the histones from as little as 0.05 ml of tissue.

MATERIALS AND METHODS

Acrylamide-Gel Electrophoresis

Acrylamide gel electrophoresis was done by an extensive modification of the technique of Reisfield et al. (11). The solutions were made up as follows:

solution A, KOH 3.0 g, urea 48 g, glacial acetic acid to pH 4.3, water to 100 ml; solution B, KOH 5.6 g, urea 48 g, TEMED N,N,N',N'-tetramethylethylenediamine (Eastman Kodak Co., Rochester, N. Y.) 0.46 ml, glacial acetic acid to pH 6.3, water to 100 ml; solution C, acrylamide 30.0 g, methylene bisacrylamide (BIS) 0.4 g, urea 48 g, water to 100 ml; solution D, acrylamide 10.0 g, BIS 2.5 g, urea 48 g, water to 100 ml; solution E, riboflavin 4.0 mg, urea 48 g, water to 100 ml; solution F, urea 48 g, acrylamide 15 g, water to 100 ml, buffer, glycine 27.8 g, water to 1000 ml, glacial acetic acid to pH 4.5. Small pore gel was prepared from solutions G and H; for solution G, 0.04 ml of TEMED was added to each milliliter of solution A and to one part of this were added two parts of solution C and one part of 8 M urea; for solution H, for a 15% gel, ammonium persulfate, 10 mg/ml, were added to four parts of solution F. Solutions G and H were mixed and 0.6 ml was added to disposable (upright) plastic tuberculin syringes the needle ends of which had been cut off. This was overlaid with distilled water and exposed to fluorescent light for 30 min. Large pore gel was prepared from one part solution B, two parts solution D, and one part solution E. Several drops of 0.1% pyronine Y were added to one-half of this preparation which was stored in the dark for later use as sample gel. An equal volume of 8 M urea was added to the other half, and this solution was layered over the small pore gel and polymerized for 20 min with fluorescent light. Equal volumes of the sample and sample gel were mixed and layered on the spacer gel. The buffer was diluted 1:5 and the gels were electrophoresed at 1.5 ma/tube for 3-6 hr.

Heterochromatin Versus Euchromatin

HUMAN XY CELLS VERSUS XXXXY CELLS: Fibroblasts from a normal male (XY), a normal female (XX), and an XXXXY individual were grown, in Rous bottles, in Eagle's medium with 10% fetal calf serum. When the cells became confluent, they were harvested with 0.25% trypsin. The trypsin was inactivated with fresh medium and the cells were washed three times with 0.15 N NaCl, 0.015 N Na citrate (SSC). 0.05 ml of packed cells in 1.0 ml of SSC was then sonicated for 15 sec and repeatedly washed with SSC until no trichloroacetic acid-precipitable material remained. The residue was then extracted with 0.25 N HCl buffered to pH 2.75 with 0.25 M Na₂HPO₄. In neither this nor any of the subsequent experiments did this fraction show a significant amount of basic protein. The residue was then extracted successively with 0.2 ml of 0.25 N HCl buffered to pH 1.7, 0.2 ml of 0.25 N HCl buffered to pH 1.2, and 0.2 ml of 0.25 N HCl at pH 0.6. These extracts were immediately electrophoresed in 15% polyacrylamide gel in 8 M urea at pH 4.5.

MALE VERSUS FEMALE MEALY BUGS: Dr. Spencer Brown in Berkeley very kindly provided two separate harvests of *Planococcus citri*. The first harvest consisted of 1,558 males and 925 females, and the second of 1,977 males and 1,632 females. During each collection period the bugs were frozen in SSC with dry ice. When the collection was complete, they were flown to Duarte. Here they were thawed, sonicated, and washed in SSC until no trichloroacetic acid-precipitable material remained. The waxy exoskeleton floated to the top of the SSC after centrifugation and was readily removed. The first harvest was extracted only at pH's 1.7 and 0.6. The second harvest was extracted once at pH's 1.7 and 1.2 and twice at pH 0.6.

XO VERSUS XYY *Drosophila*: Both larvae and adults were studied. The *Drosophila* were kindly supplied by Dr. William Kaplan. 200 XO and XYY third instar larvae were homogenized with a Teflon homogenizer, sonicated for 10 sec, and extracted as above. Electrophoresis was performed on the extracts of three separate experiments. For the study of adults, 2,900 XYY and 2,700 XO flies were homogenized in a Teflon homogenizer, sonicated for 15 sec, filtered through two layers of cheesecloth for removal of keratinized structures, thoroughly extracted with SSC and at pH 2.75, and extracted as above.

Metaphase Versus Interphase Cells

A heteroploid human amnion cell line (12) was grown, in Rous bottles, in Eagle's medium with 10% fetal calf serum. Mitotic cells were isolated by exposing the cultures to a calcium-free medium (Spinner's) containing 0.1 µg/ml of colchicine for varying periods of time and then by shaking the culture to remove cells in mitosis (13). The interphase cells remaining on the glass were removed with 0.25% trypsin and immediately washed in fresh medium containing calcium and calf serum for inactivation of the trypsin. In the mitotic fraction 75-96% of the cells were in mitosis, while in the interphase fraction 0.1-1.2% of the cells were in mitosis. For obtaining cells for histone electrophoresis, cultures were exposed to Spinner's medium containing colchicine for 5-8 hr. A packed cell volume of 0.05 ml of metaphase and interphase cells was extracted and electrophoresed as above. For one harvest, unfractionated histones were obtained by extraction at pH 0.6 only.

For investigation of histone synthesis during the G₂ period, cultures were incubated for 2.5, 5.0, and 7.5 hr in Spinner's medium with 0.1 µg/ml colchicine and 2.0 µc/ml of DL-lysine-4,5-³H (4 c/mmole, New England Nuclear Corp., Boston, Mass.), or L-arginine-³H (0.5 c/mmole, Schwarz Bio Research, Orangeburg, N.Y.). The isolated metaphase and interphase cells were extracted as for histone electrophoresis except that 1.0 ml of the buffered HCl solutions was

used for extraction and the residual protein remaining after acid extraction was solubilized in 1 N NaOH. Since some of the tritium of arginine-³H is incorporated into DNA, DNA was first removed from the residual material by two extractions with 5% trichloroacetic acid at 90°C for 10 min when this label was used. The SSC, pH 2.75, 1.7, 1.2, 0.6, and residual protein extracts were precipitated with 10 volumes of cold 20% trichloroacetic acid, filtered onto Bac-T-Flex filters (Schleicher & Schuell Co., Keene, N.H.) and washed with 10 ml of cold 5% trichloroacetic acid. The filters were dried, placed in vials with 10 ml of a scintillation fluid consisting of 4 g/L of PPO (2,5-diphenyloxazole) and 100 mg/L of POPOP (1,4-bis[2-(5-phenyloxazolyl)]-benzene) in toluene, and counted in a liquid scintillation counter. Each sample was corrected for quenching by the channels ratio method (14). The average counting efficiency was 10%.

For the determination of the timing of DNA synthesis two different sets of cultures were labeled with 0.1 μ Ci/ml of thymidine-¹⁴C (25 mCi/mMole, New England Nuclear Corp.). For the 2.5 hr interval, cells were continuously labeled in the Spinner's with colchicine and harvested at 2.5 hr. For the 5.0 hr interval, the cells were labeled for 2.5 hr then chased with an excess of cold thymidine in Spinner's with colchicine, and harvested after an additional 2.5 hr. For the 7.5 hr interval, the cells were labeled for 2.5 hr, chased with excess cold thymidine in Eagle's medium for 2.5 hr, then in Spinners with colchicine, and collected after an additional 2.5 hr. The cells were processed as for the histones, and the residual fraction was counted.

Mature Versus Phytohemagglutinin-Stimulated Lymphocytes

200 ml of heparinized whole blood were allowed to settle in sterile tubes, and the buffy coat was intermittently removed over a period of 2-4 hr. The leukocyte-rich plasma was passed over a 1 X 45 cm column of siliconized 1 mm glass beads to remove the polymorphonuclear leukocytes (15). The column was flushed with an equal volume of NCTC 199 (Hyland Laboratories, Los Angeles, Calif.), and the eluate was diluted with an additional two volumes of NCTC 199. Stained smears of these cells showed them to consist of 96-98% mature small lymphocytes. This cell suspension was divided into one- and two-third portions. 0.1 ml of a 1:10 dilution of phytohemagglutinin P (Difco Laboratories, Detroit, Mich.) was added to each 5.0 ml of the small portion. Both portions were incubated at 37°C for 92 hr. At the end of incubation the cells with phytohemagglutinin consisted of 83-85% transformed lymphocytes, 13-15% mature lymphocytes, and 2% polymorphonuclear neutrophils. The cells without phytohemagglutinin consisted of 95-97% mature lymphocytes, 2-4%

polymorphonuclear neutrophils, and 1% transformed lymphocytes. An equal number of cells from each portion was sonicated, and the histones were extracted and electrophoresed as above.

Limitations of the Extraction Technique

The advantage of extraction on the basis of pH is that very small quantities of tissue may be used. The separation is probably not comparable to that obtained with larger amounts of protein and the use of column chromatography. However, the question investigated in this study is whether there are differences in the electrophoretic pattern of the various extracts. Some cross-contamination of lysine-rich, moderately lysine-rich, and arginine-rich histones and possible extraction of acidic proteins would not be critical if the patterns for tissues containing genetically inactive and genetically active chromatin proved to be identical.

RESULTS

Heterochromatin Versus Euchromatin

Heterochromatin is characterized by three features: (a) dark staining throughout interphase (16); (b) genetic inactivity (17-18), and (c) replication of DNA late in the S period (19-20). It may be classified into facultative and constitutive heterochromatin (21). Facultative heterochromatin occurs on only one of the two homologous chromosomes. The inactive X chromosome forming the heterochromatic sex chromatin body in humans and the heterochromatic haploid set of chromosomes in the male mealy bug are examples of facultative heterochromatin (Fig. 1). Constitutive heterochromatin occurs on both homologous chromosomes. The heterochromatic areas of both X chromosomes in the female *Drosophila* is an example of constitutive heterochromatin. The following examples of heterochromatin were studied.

HUMANS XY CELLS VERSUS XXXXY CELLS (FACULTATIVE HETEROCHROMATIN): In a human diploid cell the X chromosome constitutes 2.5% of the total DNA (22). None of the DNA of an XY cell would occur as heterochromatic X chromatin, while approximately 7.5% of the DNA of an XXXXY cell would have such a form. A total of five different collections of XY, XX, and XXXXY cells were examined. In no case was there a significant difference in the electrophoretic pattern or relative amount of histones in the different fractions. Because of the relatively small difference in amount of heterochromatin between XY and XXXXY cells, these results did not con-

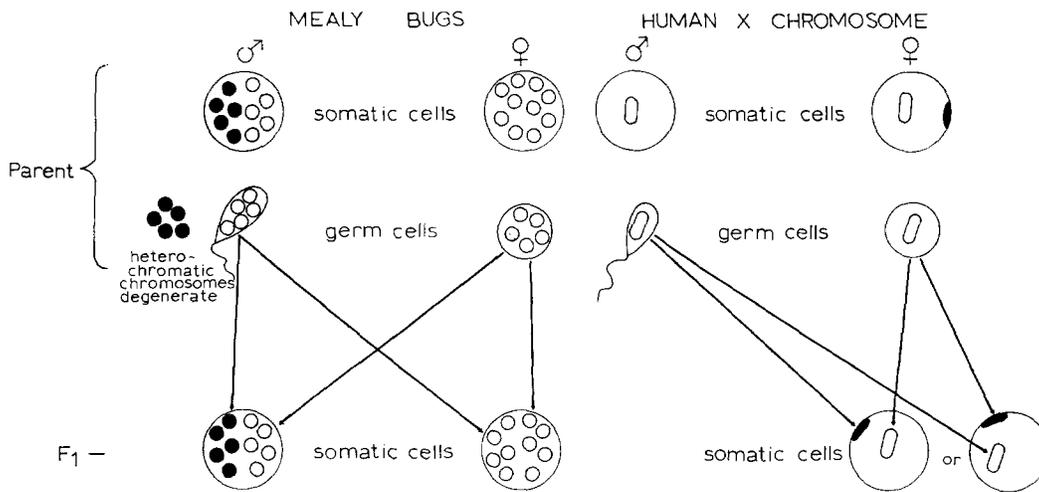


FIGURE 1 Facultative heterochromatin in mealy bugs and man. In the mealy bug (*Planococcus citri*), the paternal set of chromosomes becomes heterochromatic in F₁ males, while both paternal and maternal sets remain euchromatic in females. During spermiogenesis the heterochromatic set of chromosomes degenerates. In man, either the paternal or maternal X chromosome may become heterochromatic, forming the sex chromatin body in female cells.

vincingly rule out a difference in histone pattern between euchromatin and heterochromatin. A model with a greater difference between cells with and without heterochromatin was required.

MALE VERSUS FEMALE MEALY BUGS (FACULTATIVE HETEROCHROMATIN): Such a model was ideally provided by a scale insect, *Planococcus citri*. In the male of this species one entire haploid set of chromatin occurs as heterochromatin, while in the female both haploid sets are euchromatic (18).

Electrophoresis of the histones extracted from the second harvest of mealy bugs is shown in Fig. 2. The patterns for males and females were virtually identical for each extract. The relative concentration of protein in the different extracts was quantitated with a Canalco microdensitometer (Canal Industries, Bethesda, Md.) and the area under each curve was cut out and weighed. The results shown in Table I indicate the similarity of the histones from the males and females.¹ There was also no difference in the electrophoretic

¹ In the first harvest the more rapidly migrating bands in the pH 0.6 extract were heavier in the males than in the females. This harvest, however, was extracted only at pH 1.7 and pH 0.6. In the more completely fractionated second harvest all extracts were virtually identical.

pattern of the SSC extract between males and females at pH 4.5 (Fig. 2) or 8.6.

XO VERSUS XYY *Drosophila* (CONSTITUTIVE HETEROCHROMATIN): The choice of which homologous chromosome is to be genetically inactivated is a random one in facultative heterochromatin, and the inactivation does not usually occur until the gastrula stage of embryogenesis. Since there is no such random choice for constitutive heterochromatin, its greater permanence might be the result of a different basic mechanism of genetic inactivation. Thus the failure to find any difference between the histone patterns of facultative heterochromatin and euchromatin does not prove that such differences do not exist for constitutive heterochromatin. For an analysis of constitutive heterochromatin, the histone patterns of XO and XYY *Drosophila melanogaster* were studied. In XO flies 21.1% of the DNA occurs as heterochromatin, while in XYY flies 38.9% occurs as heterochromatin (23). There were no significant differences in either the patterns or relative amounts of histone in the three different extracts of either larvae or adults.

Metaphase Cells Versus Interphase Cells

As proliferating cells prepare for mitosis, the chromatin becomes condensed and dark-staining.

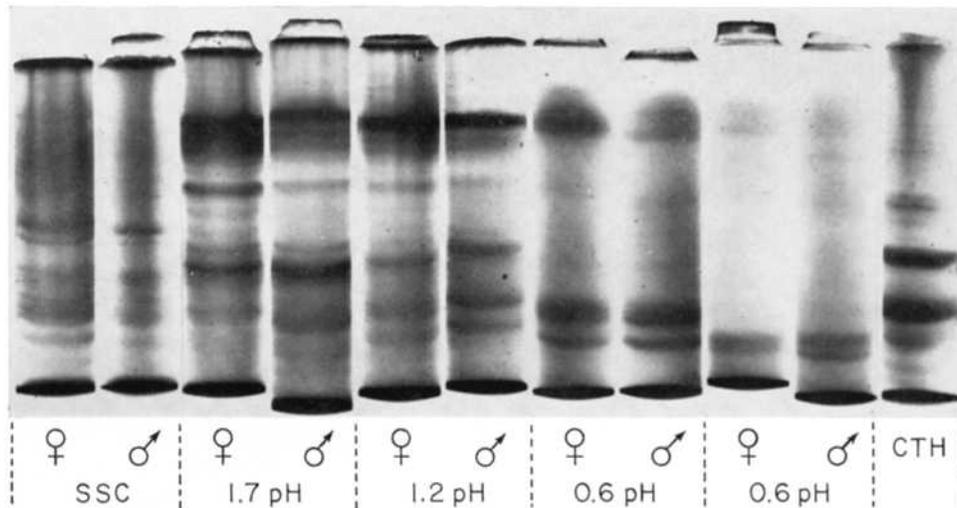


FIGURE 2 Polyacrylamide-gel electrophoresis of male and female mealy bug histones. *SSC*, 0.15 *N* NaCl, 0.015 *N* Na citrate extract; 1.7 pH, 1.2 pH, 0.6 pH: successive histone extracts at decreasing pH; *CTH*, calf thymus histone, 50 μ g.

At metaphase the chromosomes take on a distinctive shape and become genetically inactive, as is indicated by their failure to synthesize RNA (24). Protein synthesis is also suppressed as a result of disaggregation of polyribosomes (25), coating of ribosomes with a trypsin-sensitive material (26), and inhibition of RNA synthesis. Little is known about the mechanism by which chromosomes contract and become genetically inactive during mitosis. The proposed role of histones in chromosome coiling (9, 27, 28) and their ability to inhibit RNA synthesis suggest that they may be involved in the process of metaphase contraction and inactivation of chromosomes.

For investigation of the role of histones in genetic inactivation during mitosis, histone extracts of metaphase cells were compared, by acrylamide-gel electrophoresis, to those of interphase cells. In addition, cell cultures labeled with lysine- ^3H or arginine- ^3H were utilized to investigate whether there is any histone synthesis during the G_2 period in preparation for mitosis. Fig. 3 shows the results of electrophoresis of the unfractionated histones, and Fig. 4 shows the electrophoresis of the fractionated histones. Four separate experiments failed to show any consistent difference between the histone patterns of metaphase and interphase cells. The relative concentration of histone in the different extracts is shown in Table I.

The result of a typical experiment with lysine-

TABLE I
Quantitation by Densitometry of the
Percentage of Histone

	pH of extract		
	1.7	1.2	0.6
Mealy bugs			
Female	40.2	40.4	19.4
Male	41.6	39.3	19.1
Metaphase-interphase cells			
Metaphase	34.8	49.2	16.0
Interphase	31.2	52.7	16.1
Mature-PHA lymphocytes			
Mature Lymphocytes	43.6	34.9	21.5
PHA Stimulated	47.2	33.4	19.4

^3H is shown in Table II. Arginine- ^3H gave similar results. The average results of four separate experiments, in terms of the ratio of metaphase-to-interphase counts, are shown in Fig. 5. The small amount of histone synthesis that took place during the G_2 period (2.5 hr) could be explained as contamination of the metaphase fraction by interphase cells. As the S period was partially (5.0 hr) and then more completely entered (7.5 hr), the ratio of counts in metaphase cells to counts in interphase cells rose rapidly. This coupling of DNA and his-

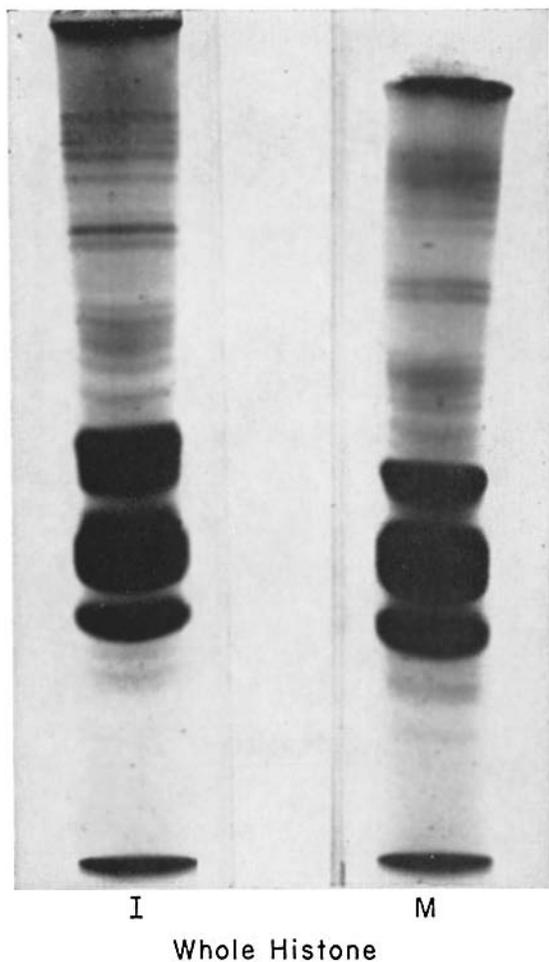


FIGURE 3 Polyacrylamide gel electrophoresis of a 0.25 N HCl whole histone extract of interphase (I) and metaphase (M) human amnion cells.

tone syntheses is in agreement with previous microspectrophotometric (29-31), radioautographic (31-33), and biochemical (34-38) studies. There was no evidence for the synthesis of any specific histone during the G_2 period.

There has been very little investigation into the question whether residual nuclear proteins are also coupled with DNA synthesis. The results shown in Fig. 5 suggest that, when the total nuclear residual fraction is studied, a portion of it is synthesized independently of DNA synthesis, since there was a significant incorporation of labeled amino acid during the G_2 period; a portion is dependent on DNA synthesis since the M/I ratio increased markedly as the collection entered the

S period. More detailed studies of this are in progress.

Mature Versus Phytohemagglutinin-Stimulated Lymphocytes

The mature circulating human lymphocyte has a small, compact nucleus with densely staining chromatin. It is a relatively dormant cell which does not divide or undergo DNA replication and which synthesizes only small amounts of RNA and protein. When exposed to phytohemagglutinin, an extract of the kidney bean *Phaseolus vulgaris*, the mature lymphocyte dedifferentiates and becomes transformed to a metabolically active lymphoblastic cell which undergoes cell division, actively synthesizes DNA, RNA, and protein, and possesses a large nucleus with diffuse chromatin and prominent nucleoli (39-41). This system provides a model for the study of the histones of two contrasting states: precursor cell versus differentiated mature cell, and condensed pycnotic chromatin versus extended active chromatin.

In each of three separate experiments there was no significant difference between the histone patterns of mature and phytohemagglutinin-stimulated lymphocytes (Fig. 6). The relative concentration of histones in the different extracts is shown in Table I. The absence of any variation in the histone patterns of immature lymphoblasts and the relatively differentiated mature lymphocytes is consistent with other studies which have shown no change in histone patterns during differentiation (42-44). Pogo et al. (45) have shown, however, that there is an increase in acetylation of basic proteins in lymphocytes exposed to phytohemagglutinin.

DISCUSSION

The relatively small number of different histone molecules (46) makes it unlikely that histones, in the absence of ancillary help (47, 48), are involved in the regulation of individual genes. Histone molecules, however, are of sufficient diversity that they may be involved in the genetic inactivation of a large number of unrelated genes, such as that which occurs in heterochromatin, metaphase cells, and mature lymphocytes. Frenster (49) has shown that the histone/DNA ratio and electrophoretic patterns of histones of condensed calf-thymus chromatin are the same as those of extended chromatin. Similar findings have been reported with rat liver

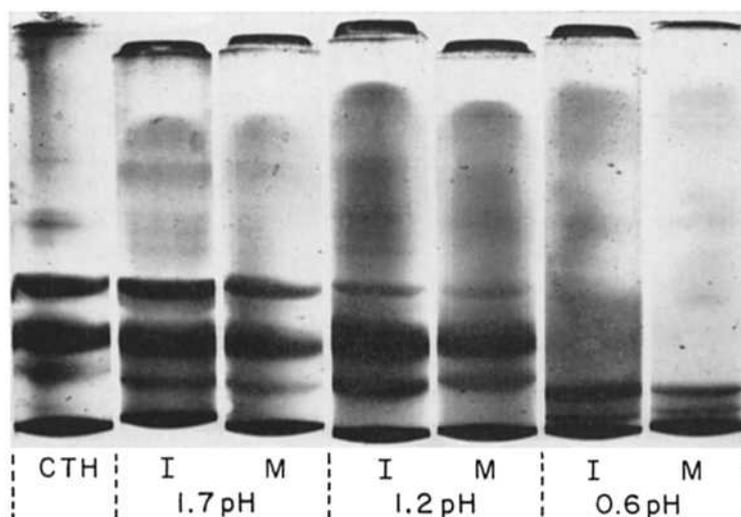


FIGURE 4 Polyacrylamide-gel electrophoresis of the pH 1.7, 1.2, and 0.6 extracts of interphase (I) and metaphase (M) human amnion cells.

(50). These reports did not exclude the possibility that heterochromatin might result from the association of DNA with a specific histone fraction since the condensed chromatin fraction studied cannot be equated with true heterochromatin (51). However, the results of the present extension of these studies to include true heterochromatin, as well as metaphase chromatin, and condensed chromatin of mature lymphocytes suggests that, even in those states involving repression of large groups of unrelated genes, the condensed chromosome coiling and genetic inactivation is probably due to factors other than the specific association of DNA with a lysine-rich, arginine-rich, or other specific histone fraction. In addition, the failure to find a unique histone pattern in the heterochromatin-rich male mealy bugs indicates that a variation in the ratio of the different histone fractions cannot be invoked as the cause of delayed DNA replication typical of heterochromatin.

An additional model of some interest is that of nuclei compared to nucleoli. In terms of equivalent amounts of DNA, nucleoli are considerably more active in RNA synthesis than nuclei, and they produce a distinct pattern high in 35 and 45S and low in 18S RNA. Yet here again the histones of these two chromatin fractions are the same, as is indicated by starch-gel electrophoresis and amino acid composition (52). Dingman and Sporn have also shown that the histone/DNA ratios are the same in tissues differing markedly in their capacity

for RNA synthesis (53), and Gorovskiy and Woodward have shown that there is no difference in histone/DNA ratio, and probably no difference between the types of histones in genetically active puffs and nonpuff bands in *Drosophila virilis* (54).

Several studies have implicated histones as an important factor in chromosome coiling. Izawa et al. (27) have demonstrated the retraction of the loops of lampbrush chromosomes in the newt after addition of arginine-rich histone, and Littau et al. (9) have shown by electron microscopy the uncoiling of condensed chromatin of calf thymus nuclei after the selective removal of lysine-rich histones. The present finding that the histones of several different types of condensed chromatins are similar to those of extended chromatins and the finding of Sheridan and Stern (55) that no change in the histone pattern is associated with chromosome coiling during meiosis in plants do not necessarily conflict with this idea. These findings, however, suggest that, although histones may be a necessary condition for chromosome coiling, they are not the primary cause.

Recent studies by Fambrough and Bonner (56) have demonstrated a striking similarity between plant and animal histones, and Palau and Butler (57) have demonstrated a similarity between trout-liver histones and calf-thymus histones. These similarities imply a severe restriction on the permissible variation of histone and amino acid sequence despite marked evolutionary changes in

TABLE II
*Lysine-³H Labeling of Metaphase and Interphase Cells**
 Results of a Typical Experiment

Time, hr	2.5	5.0	7.5
Metaphase cells in mitotic fraction, %	94	94	96
Different Extracts			
	<i>cpm</i>	<i>cpm</i>	<i>cpm</i>
SSC			
Metaphase	19,300	10,042	47,600
Interphase	58,200	25,600	107,020
M/I	0.33	0.39	0.45
2.75 pH			
Metaphase	20	62	386
Interphase	181	334	725
M/I	0.11	0.19	0.53
1.7 pH			
Metaphase	31	102	810
Interphase	206	482	1,209
M/I	0.15	0.21	0.67
1.2 pH			
Metaphase	23	71	555
Interphase	154	515	1,281
M/I	0.15	0.14	0.43
0.6 pH			
Metaphase	25	71	562
Interphase	131	419	1,031
M/I	0.19	0.17	0.55
Residual Protein			
Metaphase	490	840	6,060
Interphase	930	2,018	6,350
M/I	0.53	0.42	0.96

* For each time interval the packed cell volume of metaphase cells equals that of interphase cells.

the DNA nucleotide sequences. These similarities would be unlikely if histones are capable of selectively combining with and repressing specific DNA sites.

Numerous studies have suggested that the total number of different histone molecules is relatively limited (46, 56, 58, 59). However, until more detailed studies of the chemical structure of the histones are completed, the possibility cannot be considered closed that there is sufficient heterogeneity secondary to minor variations in structure to allow some degree of specific repression. With the exception of this reservation, the apparently limited number of different histone molecules, the similarity between histone patterns and content of genetically active chromatin and genetically inactive chromatin, the absence of significant variation

from tissue to tissue, and the similarity between plant and animal histones, all tend to emphasize a role for histones as an essential, structural element of chromatin and to de-emphasize a specific regulatory function other than that of a generalized repressor. The necessary specificity for repression or derepression may come from acidic proteins (60), RNA (48), hormones, secondary alteration of histone structure by acetylation or phosphorylation (47), or other factors.

This work was supported by Grant HD 02487 from the U.S.P.H.S. and General Research Support Grant, City of Hope Medical Center.

The technical assistance of Mr. Patric Clapshaw is greatly appreciated.

Received for publication 10 July 1967; revision accepted 25 August 1967

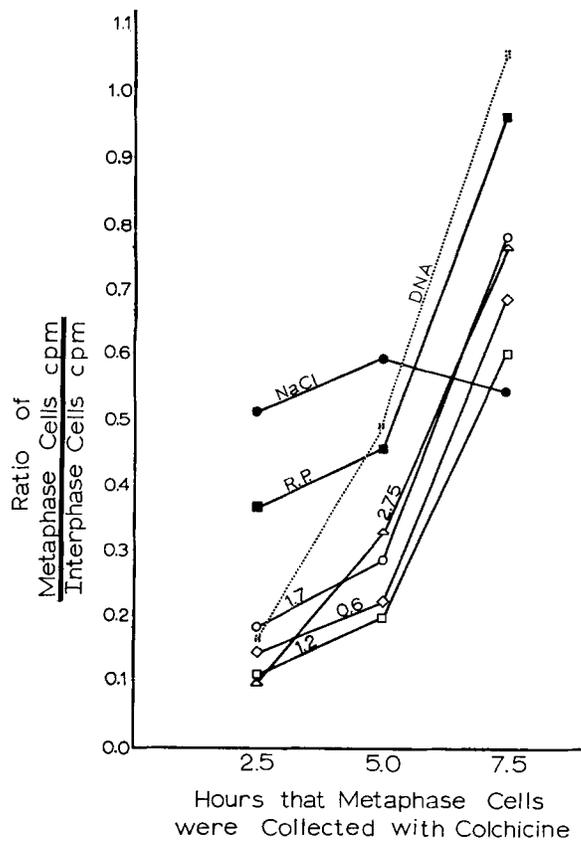


FIGURE 5 Summary of the results of four experiments on the incorporation of lysine-³H or arginine-³H into metaphase and interphase human amnion cells (see text). The results are expressed as the ratio of counts in the metaphase cells to the counts in the interphase cells. The DNA was labeled with thymidine-¹⁴C.

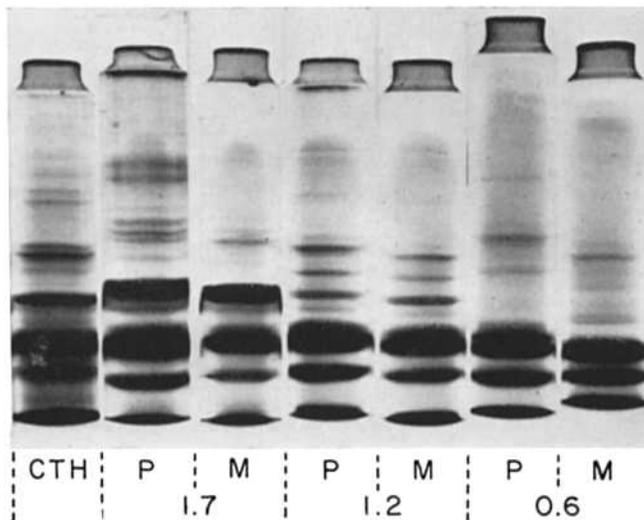


FIGURE 6 Polyacrylamide-gel electrophoresis of the 1.7, 1.2, and 0.6 pH extracts of phytohemagglutinin-stimulated human lymphocytes (*P*) and mature nonstimulated lymphocytes (*M*). *CTH*, calf thymus histones.

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