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Cell Cycle Changes in *Physarum polycephalum* Histone H1 Phosphate: Relationship to Deoxyribonucleic Acid Binding and Chromosome Condensation†

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ABSTRACT: We have examined the relationship of phosphate content in histone H1 of *Physarum polycephalum* to mitotic chromosome condensation and affinity for deoxyribonucleic acid (DNA). H1 undergoes a series of posttranslational phosphorylations which increase its apparent molecular weight on $NaDodSO_4$–polyacrylamide gels. Our studies confirm the observation by Bradbury and co-workers [Bradbury, E. M., Inglis, R. J., Matthews, H. R., & Sarner, N. (1973) *Eur. J. Biochem. 33*, 131–139; Bradbury, E. M., Inglis, R. J., & Matthews, H. R. (1974) *Nature (London) 247*, 257–261] that the accumulation of phosphate in H1 increases markedly shortly before the onset of mitosis. However, we show in pulse–chase experiments with both $^{32}PO_4$ H1 and [^{14}C]lysine H1 that there is no significant dephosphorylation of the histone either during or shortly after mitosis, suggesting that nonspecific postmitotic dephosphorylation of H1 is not a prerequisite for chromosome decondensation. We also show that both phosphorylated and unphosphorylated forms of H1 bind with somewhat greater affinity to single-stranded DNA–cellulose than to native DNA–cellulose and that phosphorylation weakens the affinity of H1 to both forms of DNA–cellulose.

Chromosomal DNA in eucaryotes is compacted by at least three levels of organization. A wealth of data shows that the primary folding of the double helix is brought about by the nucleosomes (Kornberg, 1974; Woodcock, 1973; Olins & Olins, 1974). The chain of nucleosomes in turn is compacted to form a 200–300 Å thick chromatin fiber folded into loops secured by a set of nonhistone, scaffolding proteins (Laemmli et al., 1977; Adolph et al., 1977; Paulson & Laemmli, 1977). Although histone H1 is not part of the nucleosome, several lines of evidence suggest that H1 is involved in the assembly of the nucleosome into the chromatin fiber (Keller et al., 1977; Renz et al., 1977). In addition, the temporal correlation between H1 phosphorylation and the onset of mitosis has frequently been cited as suggesting a role for H1 in the condensation of the extended interphase chromatin fiber into the

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highly condensed mitotic form (Bradbury et al., 1974; Marks et al., 1973; Balhorn et al., 1975; Hohman et al., 1976; Gurley et al., 1978).

To understand how H1 functions in chromosome condensation, we have studied the DNA binding of H1 isolated at various points in the cell cycle. For this purpose we have chosen as a model system the acellular slime mold *Physarum polycephalum*. *Physarum* is advantageous for these studies since, in the plasmodial stage, the nuclei go through mitosis synchronously and divide without disrupting the nuclear membrane (Mohberg, 1975). In the course of these experiments we have also reexamined the cell cycle dependent phosphorylation of H1 and confirmed the findings of extensive premitotic phosphorylation of H1. However, we did not observe significant postmitotic dephosphorylation. This demonstrated that H1 dephosphorylation is not essential for postmitotic decondensation of *Physarum* chromosomes.

Materials and Methods

Culturing. The Colonia strain of *P. polycephalum*, a gift from Dr. C. E. Holt of Massachusetts Institute of Technology to Dr. A. Worcel, was used. Plasmodia were prepared as described by Mittermayer et al. (1965) by spreading the microplasmodial "paste" in a 3 cm diameter ring on filter paper (Schleicher & Schuell, No. 595) supported on a wire screen in a sterile 15 × 100 mm petri dish, underlaying with 16.5 mL of media, and growing in the dark at 28 °C. The first synchronous mitosis, determined by phase-contrast microscopy of ethanol-fixed smears of the plasmodium (Guttes et al., 1961), occurs in 6–7 h, and the next three divisions occur in 8.5–11-h intervals thereafter. DNA synthesis begins immediately after mitosis, lasts 3–4 h, and is followed by G_2-phase and then mitosis (Braun et al., 1965).

Media. The growth media were prepared as follows from a protocol obtained from the McArdle Laboratory of the University of Wisconsin: to 2 L of doubly distilled water we added 12 mL of 30% (w/v) KOH, 20 g of tryptone (Difco), 20 g of dextrose, 3.1 g of yeast extract (Difco), 4 g of KH_2PO_4, 8.4 g of citric acid hydrate, 0.15 g of $FeCl_2 \cdot 4H_2O$, 1.25 g of $MgSO_4 \cdot 7H_2O$, 0.18 g of $MnCl_2 \cdot 4H_2O$, 1.25 g of $CaCl_2 \cdot 2H_2O$, 0.7 mL of 10% (w/v) $ZnSO_4 \cdot 7H_2O$, and then about 3 mL more of 30% KOH to bring the final pH to 4.6. Before use, 0.25 mL of hematin (0.1% hemin in 100% NaOH) was added to each 100 mL of media. For continuous $^{32}PO_4$ labeling, the amount of KH_2PO_4 in the media was decreased by 80%, and 1 mCi of $^{32}PO_4$ (New England Nuclear) was added to each plate at planting. For $^{32}PO_4$ pulse labeling, the growth media contained no added potassium phosphate. For labeling histones, plasmodia were grown during S-phase in media without tryptone, supplemented with 50 μCi per plate of [^{3}H]lysine or [^{14}C]lysine (New England Nuclear).

Preparation of Nuclei and Histones. Nuclei were prepared as described by Mohberg & Rusch (1971). Total histone was prepared by extracting a 20% Cl_3CCOOH precipitate of total nuclear extract with 0.02 N H_2SO_4 as described by Mohberg & Rusch (1969). To prepare H1 from the 0.02 N H_2SO_4 extract, we added Cl_3CCOOH to a final concentration of 5% and centrifuged the precipitate; we then brought the supernatant to 20% Cl_3CCOOH and centrifuged the precipitate containing H1 at 10000 rpm for 30 min in 15-mL Corex tubes in the JS13 swinging bucket rotor in a Beckman J21B centrifuge.

Preparation of H1 by DNA–Cellulose Chromatography. To prepare H1 by DNA–cellulose chromatography, we extracted the nuclei for 5 min with 10 volumes of cold 0.2 M $CaCl_2$ and 20 mM Tris (pH 7.5 with HCl) and centrifuged the mixture at 10000 rpm for 10 min in Corex tubes in the Beckman J21B centrifuge. The extract was desalted through a Sephadex G-25 column equilibrated to 20 mM Tris (pH 7.5 with HCl), 1 mM EDTA, and 0.05 M NaCl and then chromatographed on a single-stranded calf thymus DNA–cellulose column as described below.

DNA–Cellulose Chromatography. DNA–cellulose chromatography was performed as described by Alberts & Herrick (1971) in 1-mL columns containing 1 mg of native or denatured calf thymus DNA in 0.05 M NaCl, 20 mM Tris (pH 7.5 with HCl), and 1 mM EDTA. The sample was loaded by pumping at 2.5 mL/h and eluted at 2.5 mL/h with the same Tris–EDTA buffer containing 0.5 mg/mL dextran sulfate and then increasing concentrations of salt.

Disc Electrophoresis in $NaDodSO_4$. $NaDodSO_4$–polyacrylamide gel electrophoresis was performed as described by Laemmli (1970) in a slab gel apparatus. The resolution of low molecular weight histone bands was improved by decreasing the amount of *N',N'*-methylenebis(acrylamide) by one-half in 15% acrylamide gels as suggested by Weintraub & VanLente (1974). Gels were stained in a solution containing final concentrations of 0.1% Coomassie Blue, 50% methanol, and 10% acetic acid and destained by diffusion in a solution of 5% methanol and 10% acetic acid. Autoradiography of the stained slab gels was performed as described by Fairbanks et al. (1965).

Samples for electrophoresis were concentrated by precipitation at 0 °C by adding 100% Cl_3CCOOH (w/v) containing 0.5 mg/mL sodium deoxycholate (DOC) to a final concentration of 20% Cl_3CCOOH. The precipitate was rinsed with cold ethyl ether, dried in vacuo, and resuspended by boiling in a final sample buffer (0.125 M Tris-HCl, pH 6.8, 2% $NaDodSO_4$, 10% glycerol, and 0.001% bromophenol blue or phenol red). The sample was titrated to neutral pH by adding a few microliters of 0.2 N NaOH, reduced by addition of β-mercaptoethanol (final concentration 5%), and boiled for 2 min.

Amino Acid Analysis. The S-phase H1 bands (~200 μg each) were cut from a stained $NaDodSO_4$ gel and hydrolyzed for 20 h at 100 °C in 6 M HCl in thick-walled Pyrex tubes. The hydrolysate was clarified at 30000 rpm for 30 min at 4 °C in Oak Ridge tubes in a Type 40 rotor in the Beckman L3-50 ultracentrifuge, and the supernatant was dried in vacuo at 40 °C in a 50-mL round-bottom flask. The white powdery flakes were redissolved in 0.25 mL of 2 N NaOH and dried in vacuo at 40 °C. This process was repeated two more times. The powder was then dissolved in 0.25 mL of 2 N HCl and dried, and this process was repeated two more times. The amino acid composition of the hydrolysate was determined on a Beckman amino acid analyzer. A gel slice equal in size to that containing the H1 bands was subjected to the same analysis and its absorbance subtracted as background (Table I).

Results

Cell Cycle Changes in the Mobility of H1 Histone. Physarum H1 histone was prepared either by acid extraction, according to Mohberg & Rusch (1969), or by salt extraction and purification by DNA–cellulose chromatography as described under Materials and Methods. On analysis of these samples by $NaDodSO_4$ gel electrophoresis, we see that the region of the gel containing H1 consists of two domains whose intensity and relative mobility vary in extracts prepared from nuclei at different stages of the cell cycle. Figure 1 shows the changes in the electrophoretic mobility of H1 prepared by acid extraction of nuclei from plasmodia at different times in the

Table I: Amino Acid Composition of H1 from *Physarum* and Other Sources[a]

amino acid	percentage				
	Physarum polycephalum				
	upper band	lower band	M3C	calf thymus	*N. crassa*
Lys	20.7	17.9	18.2	24.5	16.8
Ala	20.8	20.0	17.4	19.1	21.1
Pro	12.6	11.4	10.0	10.4	7.6
Ser	11.7	10.9	6.2	7.3	5.7
Thr	7.0	6.5	5.4	5.1	6.3
Glu	7.2	8.6	9.1	4.1	8.5
Asp	3.6	5.9	5.4	3.7	7.2
Gly	6.6	6.6	6.7	7.7	5.8
Arg	1.7	1.6	1.7	1.3	3.4
Leu	2.8	3.2	3.3	4.1	4.8
Ile	1.9	2.0	2.5	7.3	2.0
Val	1.7	2.7	3.3	4.1	4.9
Tyr	0.5	0.8	0.0	0.7	1.5
Phe	0.6	0.8	1.2	0.6	1.8
His	0.8	0.3	2.5	0.0	1.5
Met	0.0	0.0	0.0	0.0	1.2
Cys	0.0	0.0	0.0	0.0	0.4
Lys, Ala, Pro	54.1	49.3	45.6	54.0	45.5

[a] Early S-phase H1 was prepared as described in Figure 7, eluted from a preparative $NaDodSO_4$ gel, and analyzed for amino acid composition as described under Materials and Methods. Amino acid composition of H1 from calf thymus and the M3C strain of *Physarum* is from Mohberg & Rusch (1969). Composition of *N. crassa* H1 is from Goff (1976).

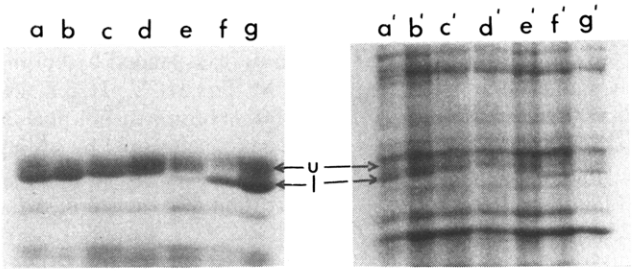

FIGURE 1: Cell cycle changes in H1 mobility in $NaDodSO_4$–polyacrylamide gels. Histones, prepared according to Mohberg & Rusch (1969) by acid extraction of nuclei from different cell cycle stages, were fractionated on an 8% polyacrylamide–$NaDodSO_4$ gel. The left panel shows the H1 bands in the acid extract. u, upper H1 band; l, lower H1 band. The right panel is the same region of a gel of intact nuclei. (a, a′) Late S; (b, b′) early G_2; (c, c′) mid G_2; (d, d′) early prophase; (e, e′) metaphase; (f, f′) early S; (g, g′) mid S.

cell cycle. In late S-phase (Figure 1, a) the H1 fraction consists of two domains containing a sharp lower band (called l) and a diffuse upper band (called u). The apparent molecular weight of the lower band increases during G_2 (Figure 1, b and c), approaching the lower edge of the upper band. Early in mitosis (Figure 1, d) we can see only the upper band, which is now intensely staining. After mitosis, as the plasmodium reenters S-phase (Figure 1, e–g), the lower band reappears, attains its greatest electrophoretic mobility, and is most clearly resolved from the upper band. The source of the mobility change is not generated during the course of the extraction of H1 from the nuclei since the mobility change can be seen in the gel pattern of unextracted, intact nuclei (Figure 1, a′–g′). Although the presence of nonhistone bands obscures the observation to some extent, the domain containing the upper H1 band(s) is evident as a dark zone between the lower H1 band and the next sharp band above it. The cell cycle dependent mobility change of the lower H1 band remains clear.

Comparison with Other H1 Histones. The well-known H1 histones typically have a molecular weight of about 21 000–23 000 (Elgin & Weintraub, 1975). However, the apparent molecular weight of the upper and lower H1 bands ranges from 51 000 to 54 000 on $NaDodSO_4$ gels calibrated with nonhistone protein standards. LeStourgeon & Rusch (1973) have also reported that the H1 of *P. polycephalum* has a similar, anomalously high, apparent molecular weight on $NaDodSO_4$ gels. Since histones do not move strictly according to their molecular weight on $NaDodSO_4$ gels (Cohen & Gotchel, 1971), we standardized the molecular weight curve with calf thymus histones H1 and H3, as suggested by Panyim & Chalkley (1971), and found a molecular weight for the *Physarum* H1 (lower band) of about 27 500 (not shown). By a similar method Jockusch & Walker (1974) determined that the molecular weight of H1 of the M3C subline of *P. polycephalum* is 24 500. Thus, *Physarum* H1 is similar in size to other well-characterized H1 histones.

We have also determined the amino acid compositions of both the upper and lower H1 bands eluted from one preparative $NaDodSO_4$–polyacrylamide gel and compared them with other H1s to verify their identity. Pulse–chase experiments reported below and peptide mapping (not shown) indicate that the two *Physarum* H1 bands have substantially the same polypeptide backbone. Thus, experimental uncertainty with this procedure is reflected in the variance between the H1 bands of about 10% in the mole fraction of most amino acids. Consideration of the overall amino acid composition shows, therefore, that both *Physarum* H1 bands are very similar in amino acid content to calf thymus H1 and less so to *Neuorospora crassa* H1.

Preparation of H1 by DNA–Cellulose Chromatography. To study the affinity of H1 for DNA and to avoid acid extraction, we developed a gentle method with nearly quantitative recovery of preparing *Physarum* H1 by DNA–cellulose chromatography of salt extracts of nuclei. Briefly, the method consists of extraction of nuclei with 0.2 M $CaCl_2$ and chromatography of the desalted extract on a DNA–cellulose column. To ensure that the binding of H1 is specific for DNA in the column, we chromatographed a sample of the extract through similarly prepared cellulose containing no DNA and found that H1 did not bind to this mock cellulose column. The various stages of purification were monitored by $NaDodSO_4$ gel electrophoresis and are shown in Figure 2. Note the enrichment of the H1 doublet in the 0.2 M $CaCl_2$ extract (Figure 2, c) and the elution of the purified H1 histone upper and lower bands with 0.65 M NaCl (Figure 2, h). Protein bands whose mobilities are intermediate to those of the H1 bands are eluted from the column at lower salt concentrations (Figure 2, f and g); however, these bands are not H1 as shown by differential labeling with both radioactive lysine and phosphate (see Figure 5).

Pulse Chase of New H1. The cell cycle dependent decrease in the mobility of the lower H1 band to the position of the upper H1 band suggests a possible precursor–product relationship. To substantiate this further, plasmodia were grown during early S-phase in media containing [^{3}H]lysine, in order to label newly synthesized H1, and then transferred to nonradioactive media. The fate of the new H1 was followed by preparing H1 from sections of the growing plasmodium at various times during the chase and examining it on an $NaDodSO_4$–polyacrylamide gel which was stained and then autoradiographed. Figure 3 shows in the upper panel the stained gels and in the lower panel the autoradiograph. Immediately after the pulse at mid S-phase, although both bands are visible in the stained gel (Figure 3, a), all the label is in the lower band (Figure 3, a′). Thus, the lower band is newly synthesized

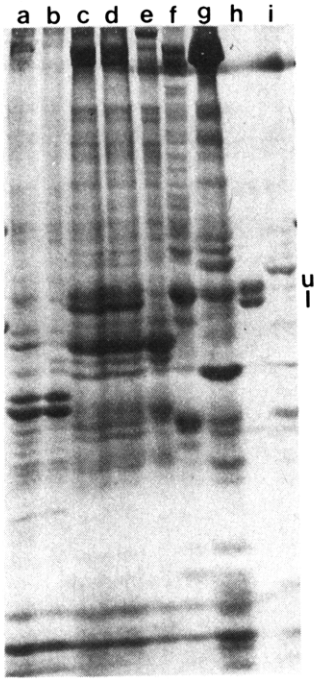

FIGURE 2: Preparation of H1 by DNA–cellulose chromatography. Nuclei from a mid S-phase plasmodium were extracted with 0.2 M $CaCl_2$, and the extract was desalted on a Sephadex G-25 column and then chromatographed on a single-stranded calf thymus DNA–cellulose column by salt elution as described under Materials and Methods. Fractions from the column were analyzed on a 12% polyacrylamide–$NaDodSO_4$ gel. u, upper H1 band; l, lower H1 band. (a) Nuclei, (b) extracted nuclei, (c) 0.2 M $CaCl_2$ extract, (d) desalted extract, (e) breakthrough, (f) 0.5 mg/mL sodium dextran sulfate cut, (g) 0.47 M NaCl cut, (h) 0.65 M NaCl cut, and (i) 2 M NaCl cut.

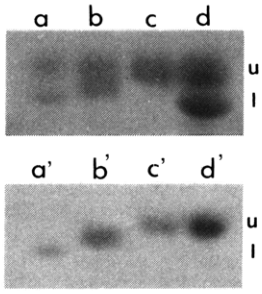

FIGURE 3: Pulse–chase of newly synthesized H1. A plasmodium was grown for 2 h at the beginning of the S-phase in tryptone-free medium containing 50 μCi/mL [^{14}C]lysine. The plasmodium was cut into quarters and nuclei were isolated from one quarter. The other sections were chased in complete, cold medium until G_2, very early prophase, or mid S-phase after the next mitosis. H1 was then prepared by DNA–cellulose chromatography and analyzed on an 8.5% polyacrylamide–$NaDodSO_4$ gel which was stained with 0.1% Coomassie Brilliant Blue (upper panel) and then autoradiographed (lower panel). u, upper H1 band; l, lower H1 band. (a, a′) Mid S-phase after pulse; (b, b′) early G_2; (c, c′) very early prophase; (d, d′) mid S-phase after next mitosis.

H1. Later in the cell cycle, the label progressively moves toward the upper band (Figure 3, compare b and c to b′ and c′). Concomitantly, the staining intensity of the upper band increases as a result of the contribution of the new H1 to the total mass of the upper band.

When the plasmodium passes into the next S-phase, new, unlabeled H1 again appears at the position of the lower band (Figure 3, d and d′). There is no decrease in either the staining or the radioactive-labeling intensity of the upper band in the sample taken in S-phase (Figure 3, d and d′). Moreover, no lysine-labeled H1 shifts back to the position of the lower band (Figure 3, d′). This experiment substantiates that the H1

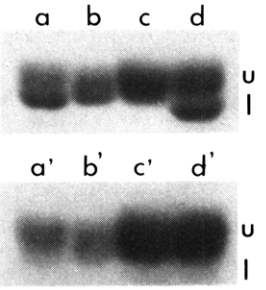

FIGURE 4: Cell cycle changes in H1 phosphate. H1, prepared by DNA–cellulose chromatography, from plasmodia continuously grown in low phosphate media containing $^{32}PO_4$, was analyzed on an 8.5% polyacrylamide–$NaDodSO_4$ gel which was stained with 0.1% Coomassie Brilliant Blue (upper panel) and then autoradiographed (lower panel). u, upper H1 band; l, lower H1 band. Time of nuclei isolation: (a, a′) mid S-phase II; (b, b′) early G_2 II; (c, c′) very early prophase II; (d, d′) early S-phase III.

histone synthesized in S-phase (lower band) is converted in G_2-phase to a polypeptide which migrates at the upper band position. No labeled lysine shifts back to the lower band position following mitosis, suggesting that the mobility change observed in late G_2 is not reversed.

Source of Mobility Change. To identify the cause of the mobility change, we analyzed H1 isolated at different times in the cell cycle from plasmodia which had been continuously grown in media containing $^{32}PO_4$ on an $NaDodSO_4$–polyacrylamide gel (Figure 4) which was stained (upper panel) and autoradiographed (lower panel). Figure 4 shows the $^{32}PO_4$ label is in the upper band at all stages of the cell cycle. However, there is a large increase in both staining intensity and $^{32}PO_4$ content of the upper band between early G_2 and very early prophase (Figure 4, compare b and c with b′ and c′). This also coincides with the migration of newly synthesized H1 into the upper band and indicates that the large increase in H1 phosphate content just prior to mitosis is largely due to the phosphorylation of new H1. Important is the finding that after mitosis, the phosphate content of the upper band is maintained into the next S-phase (Figure 4, d and d′). This finding was unexpected, since experiments by Bradbury et al. (1973) indicated dephosphorylation of *Physarum* H1 after mitosis. Moreover, we found that the maintenance of the phosphate content of H1 following mitosis is very reproducible.

To evaluate the stability of the H1 phosphate, we followed the fate of phosphate-labeled H1 through two successive mitoses. We pulse labeled a growing plasmodium in the first synchronous S-phase (SI) with [^{3}H]lysine, to label newly synthesized H1, and with $^{32}PO_4$ during G_2 phase. The labeled plasmodium was transferred to unlabeled media at the beginning of prophase. The fate of the labeled H1 was followed through two consecutive divisions by preparing samples at prophase (Figure 5, a and a′), in the following S-phase (SII) (Figure 5, b and b′) and prophase (Figure 5, c and c′), and then again in the subsequent S-phase (SIII) (Figure 5, d and d′). This experiment shows that the H1 histone pulse labeled with $^{32}PO_4$ (upper band) remains labeled through at least two divisions. Note again, by comparison of the autoradiogram and the gel, the absence of the $^{32}PO_4$ label in the H1 newly synthesized (lower band) in SII and SIII (Figure 5, b, b′ and d, d′).

The autoradiogram indicates a slow turnover of radiophosphate during this chase. To quantitate this turnover, we have measured the ratio of [^{3}H]lysine to $^{32}PO_4$ in the upper band. We find that only about half of the $^{32}PO_4$ label is lost from the H1 upper band between SII and SIII with no abrupt loss through mitosis. These experiments indicate a gradual,

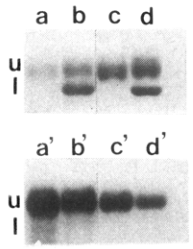

FIGURE 5: Pulse–chase of H1 phosphate. Plasmodia were grown in S-phase in tryptone-free media containing 20 μCi/mL [^{3}H]lysine and then in G_2-phase in low phosphate medium containing 100 μCi/mL $^{32}PO_4$. The plasmodium was sectioned and transferred to complete, cold medium. H1 was prepared from sections at different times, analyzed on an 8.5% polyacrylamide–NaDodSO$_4$ gel, stained with 0.1% Coomassie Brilliant Blue (upper panel), and then autoradiographed (lower panel). u, upper H1 band; l, lower H1 band. (a, a′) Late G_2 I; (b, b′) mid S-phase II; (c, c′) late G_2 II; (d, d′) mid S-phase III.

slow phosphate turnover in contrast to the observations by others (Bradbury et al., 1973; Gurley et al., 1978) who report a rapid dephosphorylation of H1 around mitosis.

Chromatography of H1 on Native and Denatured DNA–Cellulose. We have shown above that both H1 histone bands are eluted from a DNA–cellulose column containing single-stranded DNA at a salt concentration of 0.65 M NaCl. The differences in the salt concentrations of the elution steps in the experiment described in Figure 2 are too large to demonstrate a possible difference in affinity for DNA of the upper and lower H1 bands. Therefore, a salt extract of nuclei isolated from S-phase plasmodia containing both the upper and lower H1 bands was chromatographed on both single-stranded and double-stranded DNA–cellulose to study the effects of phosphorylation on the binding of H1 to DNA. Figure 6 shows that the upper, phosphorylated band is eluted at a lower salt concentration than the unphosphorylated band from both single- and double-stranded DNA–cellulose (in Figure 6, compare d–f with k and l). On single-stranded DNA–cellulose the upper, phosphorylated band remains bound at 0.5 M NaCl but is eluted with 0.575 M NaCl. The lower band, however, is not eluted until 0.65 M NaCl. On native DNA–cellulose both bands are eluted at lower salt concentration, the phosphorylated, upper band at 0.5 M NaCl and the lower band at 0.575 M NaCl. Phosphorylation, therefore, decreases the affinity of H1 for DNA so that under the same ionic conditions, the phosphorylated, mitotic form of the histone may be less tightly bound to the DNA than the unphosphorylated H1. Furthermore, both forms of H1 bind with greater affinity to single-stranded DNA than to double-stranded DNA.

Effect of Phosphatase. In order to determine if phosphorylation was the only posttranslational modification of H1, we enzymatically dephosphorylated the upper band, isolated by DNA–cellulose chromatography, with *Escherichia coli* alkaline phosphatase and compared its electrophoretic mobility with the newly synthesized lower band (Figure 7). The autoradiogram in the lower panel shows that all H1 phosphate was removed by the phosphatase, while the stained gel in the upper panel shows that the dephosphorylated upper band migrates near the lower edge of the untreated upper band. Thus, the apparent molecular weight of the upper band is decreased by dephosphorylation. However, the apparent molecular weight of the dephosphorylated upper band is still greater than that of the newly synthesized H1. Therefore, some other posttranslational modification which we have not attempted to further characterize is, in addition to phosphate, contributing to the difference in the apparent molecular weights of the H1 bands.

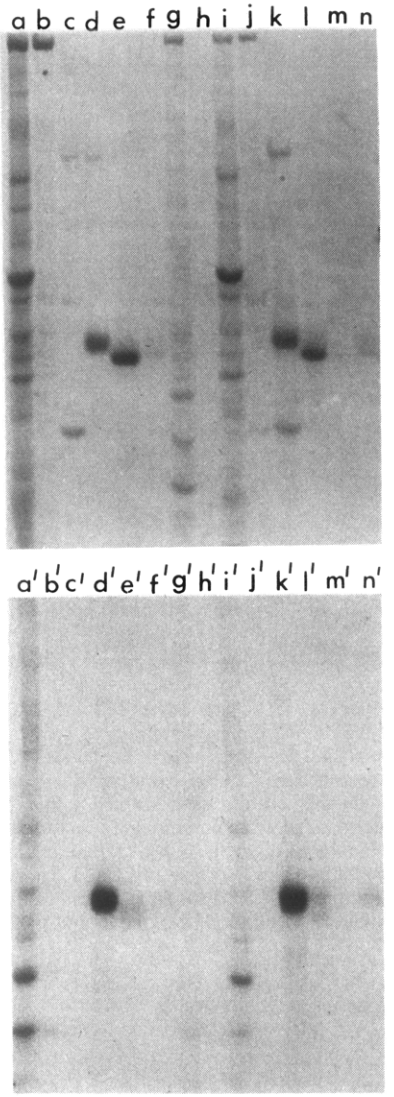

FIGURE 6: Chromatography of H1 on single-stranded and double-stranded DNA–cellulose. S-phase nuclei from plasmodia grown in media containing $^{32}PO_4$ were extracted with 0.2 M $CaCl_2$. The extract was dialyzed to 0.02 M Tris (pH 7.5 with HCl), 0.05 M NaCl, and 1 mM EDTA and chromatographed on single-stranded or double-stranded DNA–cellulose as described under Materials and Methods. Samples were analyzed on a 9% polyacrylamide–NaDodSO$_4$ gel stained with 0.1% Coomassie Brilliant Blue (upper panel) and autoradiographed (lower panel). (a–f) Single-stranded column; (g–n) double-stranded column. (a, a′, i, i′) 0.5 mg/mL dextran sulfate cut; (b, b′, j, j′) 0.4 M NaCl cut; (c, c′, k, k′) 0.5 M NaCl cut; (d, d′, l, l′) 0.575 M NaCl cut; (e, e′, m, m′) 0.65 M NaCl cut; (f, f′, n, n′) 2 M NaCl cut; (g) breakthrough; (h) 0.05 M NaCl wash.

Discussion

Our studies show that histone H1 of *P. polycephalum* is synthesized during S-phase and then undergoes posttranslational modifications which increase its apparent molecular weight on NaDodSO$_4$–polyacrylamide gels. The ability to clearly resolve new and mature H1 by NaDodSO$_4$ gel electrophoresis permits a careful analysis of the fate of this protein around the cell cycle. We were thus able to demonstrate a precursor–product relationship between the lower, newly synthesized H1 and the upper, mature H1 by pulse–chase experiments with [^{14}C]lysine and by comparison of the amino acid composition of the two bands. A major modification was identified by radiophosphate incorporation which can be removed by alkaline phosphatase. Dephosphorylation with alkaline phosphatase decreased the apparent molecular weight of the mature, phosphorylated H1 to nearly that of the newly

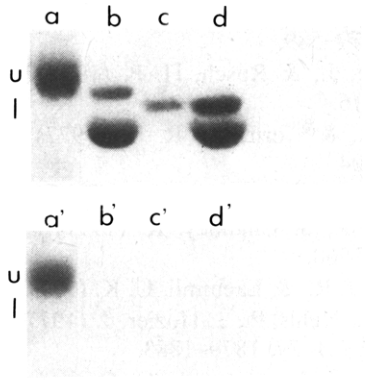

FIGURE 7: Effect of alkaline phosphatase on electrophoretic mobility of H1. H1 upper and lower bands were prepared by DNA–cellulose chromatography, as described in Figure 6, from S-phase plasmodia grown in media containing $^{32}PO_4$. Fractions containing the separate H1 bands were dialyzed to 0.06 M Tris (pH 8.2 with HCl) and 0.1 mM $ZnCl_2$ and incubated 9 h at 37 °C with an equimolar amount of *E. coli* alkaline phosphatase or with no added enzyme. Samples were analyzed on one 8.1% polyacrylamide–$NaDodSO_4$ gel stained with 0.1% Coomassie Brilliant Blue (upper panel) and autoradiographed (lower panel). (a, a′) Upper band; (b, b′) alkaline phosphatase-treated upper band (the lower band is the phosphatase); (c, c′) lower band; (d, d′) alkaline phosphatase-treated lower band.

synthesized H1. However, a slight difference in apparent molecular weight persists between the newly synthesized H1 and the in vitro dephosphorylated H1 and is indicative of a further modification.

The accumulation of phosphate on H1 was shown to increase markedly in late G_2, confirming the observations by Bradbury et al. (1973, 1974). Our experiments, however, are in marked contrast to those of Bradbury et al. in regard to dephosphorylation. They observe a rapid removal of H1 phosphate commencing 20 min before metaphase and continuing into S-phase. Our experiments show no marked dephosphorylation of H1 in this same time period. This was shown first by the maintenance of most of the radiophosphate in the mature H1 band through mitosis. The stability of the phosphate was further substantiated by following the fate of radiophosphate, which had been incorporated into H1 during G_2, through two successive mitoses. During this prolonged chase we observe only a gradual loss of radiophosphate at about 50% per generation. The same conclusion is drawn from experiments in which the backbone of the histone was labeled with [^{14}C]lysine. Unmodified new H1 and maturing H1 were identified by gel electrophoresis. However, we observe no decrease in the apparent molecular weight of the lysine-labeled upper band around mitosis which would be a consequence of dephosphorylation.

The cause of the variance between Bradbury et al. (1973, 1974) and our observation is not clear. Activation of endogenous phosphatase during H1 preparation could lead to dephosphorylation. Alternatively, the method of H1 preparation may affect the recovery of the most highly phosphorylated H1 species. This latter mechanism is suggested by experiments in which we used either salt extraction combined with DNA–cellulose chromatography or acid extraction to prepare H1. On some occasions we noted that the recovery of the most highly phosphorylated H1 species near mitosis is poorer from acid-extracted nuclei than from salt-extracted nuclei. In addition, a strain difference might cause variation, since our experiments are conducted with the homothallic Colonia strain, whereas their experiments use strain (a + i).

Our observations are consistent with the suggestion by Bradbury et al. (1974) and Gurley et al. (1975) that histone H1 phosphorylation may be involved in some way in the process of chromosome condensation. However, our evidence that H1 is not significantly dephosphorylated following metaphase demonstrates that the reversal of this modification is not a prerequisite for decondensation in *Physarum*. A similar conclusion may be drawn from experiments by Tanphaichitr et al. (1976), who showed that chemical inhibition of postmitotic dephosphorylation in cultured baby hamster cells does not interfere with chromosome decondensation.

Our studies on the salt elution pattern of H1 and DNA–cellulose show that binding is a function of the extent of H1 phosphorylation and whether the DNA is single-stranded or double-stranded. Both phosphorylated and unphosphorylated H1 bind with slightly higher affinity to denatured DNA than to native DNA. Moreover, increases in the phosphate content of H1 reduce its affinity for both forms of DNA. This latter observation is supported by experiments by Fasy et al. (1979), who showed that H1 phosphorylated in vitro is eluted from native DNA–Sephadex at slightly lower salt concentrations than the main H1 peak. Phosphorylated H1 has also been shown to bind more distributively (i.e., less cooperatively) to DNA than unphosphorylated H1 (Knippers et al., 1978). In this regard it is interesting that *Physarum* H1 is eluted from DNA–cellulose at about the same salt concentration which dissociates calf thymus H1 from nucleohistone (Bradbury et al., 1975). Since H1 binds to otherwise naked DNA between nucleosomes (Varshavsky et al., 1976; Noll & Kornberg, 1977), the electrostatic affinity between H1 and the DNA in the column may approximate biologically relevant interactions. Perhaps the effect of phosphorylation is to weaken the binding between H1 and internucleosomal DNA to allow new, less cooperative interactions to form. These changes in H1–H1 interactions along the nucleohistone fiber may then affect the packing of nucleosomes and promote condensation of the fiber.

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Antibodies Specific for Histone H2b Fragments 1–58 and 63–125 in Antisera to H2b and to the Fragments: Probes for Histone Evolution†

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ABSTRACT: Rabbit antibodies induced by H2b–RNA complexes reacted with intact H2b and with fragments 1–58 and 63–125 produced by cleavage of H2b with cyanogen bromide. Complement fixation titers for a typical serum were 5400 with H2b, 4300 with 63–125, and 2300 with 1–58. Affinity chromatography revealed that 0.9 as much anti-H2b antibody was isolated with (63–125)–Sepharose as with H2b–Sepharose, whereas only 0.1 as much was isolated with (1–58)–Sepharose. The antibodies isolated from the fragment–Sepharose columns reacted with the corresponding fragment and intact H2b in both complement fixation assays and in a solid-phase radioimmunoassay. The distribution of anti-H2b antibodies primarily to the 63–125 region may explain the inability of anti-H2b antisera to distinguish between *Drosophila* and calf H2b, which differ mainly in the amino-terminal portion of the molecule. Antibodies were then induced by mixtures of RNA with the separated fragments. Early anti-(1–58) antisera gave complement fixation reactions with H2b but stronger reactions with the fragment; late sera fixed complement only with the fragment. Both early and late anti-(63–125) antisera reacted with H2b better than with the immunogen 63–125 itself. Like anti-H2b, the anti-(63–125) antiserum measured little or no difference between *Drosophila* and calf H2b. Anti-(1–58) antiserum detected a large difference between these histones. Cross-reactions of H2b from other species with the antifragment antisera were also consistent with degrees of sequence homology in the corresponding regions of the molecule.

Antihistone antibodies have been useful for comparative studies of histones and for exploring the organization of histones in chromatin (Stollar, 1978; Bustin, 1978). In comparative studies, serological cross-reactions have usually been consistent with the extent of evolutionary change in amino acid sequences. Anti-H4 antibodies gave nearly identical complement fixation reactions with H4 samples from a wide range of vertebrate and invertebrate species (Stollar & Ward, 1970), whereas anti-H1 antisera revealed differences among H1 subfractions from one tissue and among H1 samples from much less divergent species (Bustin & Stollar, 1973a). With surprising specificity, anti-H2a sera were able to distinguish between two mouse H2a variants that differ in only two amino acids (Blankstein et al., 1977). In the case of H2b, on the other hand, specific antibodies showed little or no difference between H2b's as different as those of calf and *Drosophila* (Bustin et al., 1977) even though there are extensive sequence differences in the amino-terminal regions of these molecules (Elgin et al., 1979).

For both comparative studies and for the use of antibodies as probes for chromatin organization, it is important to know whether the recognition of different parts of the molecule is random and uniform or whether certain parts of the molecule are preferentially detected by the antibodies. This article describes experiments that approach this question with anti-H2b antibodies. Calf H2b has two methionines, at positions 59 and 62. This allows cleavage of the molecule into almost equal halves by cyanogen bromide (Johns et al., 1972). With

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