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Purification and the Histones of *Dictyostelium discoideum* Chromatin†

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ABSTRACT: *Dictyostelium* chromatin has been purified from nuclei in high yield by differential centrifugation and nuclease cleaving. Its chemical composition has been assayed, and its histones have been analyzed by gel electrophoresis, peptide fingerprints, amino acid composition, and ion-exchange chromatography. The mass ratios of DNA/RNA/histone/nonhistone are 1.0:0.18:0.98:1.02. There are four histones including one unusual histone, H7, which is the most abundant histone in the slime mold. The H4-like protein is the most conserved protein, while the other histones show both similarities and differences with mammalian histones.

One of the goals of biology is to understand the molecular basis of gene regulation. For this purpose the components of the genetic apparatus have been dissected and studied. In eucaryotes the components are known, but their functions are not completely understood. The histones apparently convey a particular structure to the DNA (Felsenfeld, 1978). The nucleosomes of higher eucaryotes have two molecules each of four well-defined histones (Kornberg, 1974). Our purpose was to study the categories of *Dictyostelium* histones and their relationship to the structure and composition of its chromatin.

The simple life cycle of the slime mold makes it ideal for studying the relationship of gene expression to development. The synthesis of specific enzymes, membrane proteins, and mRNA is developmentally regulated (Sussman & Sussman, 1969; Siu et al., 1976; Ma & Firtel, 1978). The composition and structure of slime mold chromatin are similar to those in higher eucaryotes (Pederson, 1977; Bakke & Bonner, 1978). A rapid, unique method to purify chromatin is described here using limited nuclease digestions to free the chromatin from contaminants. We have found that the slime mold contains only four histones. One of these is very similar, but not identical, to H4. Another is present in nearly twice the amount of the H4-like protein and may replace both H2a and H2b. Therefore, the typical chromatin structure can be maintained by different basic proteins, except for H4. Its role is so vital that it must be carefully conserved.

Experimental Procedure

Organism and Growth Conditions. Dictyostelium discoideum, strain Ax-3, was grown at 22 °C on a rotary shaker in a sterile liquid medium termed HL5 by Cocucci & Sussman (1970). The cells have a generation time of ~12 h and were grown to a density of 8×10^6 cells/mL before harvesting. Cells were harvested in a large swinging bucket rotor at 700*g* in 1-L bottles and washed once with cold 0.4% NaCl.

Isolation of Nuclei and Chromatin. Nuclei were isolated by a modification of the method of Firtel & Lodish (1973). The harvested and washed cells were suspended at a density of 2×10^8 cells/mL in cold 0.37 M sucrose, 40 mM KCl, 20 mM phosphate, pH 7.6, 0.1 mM EGTA,[1] 1 mM PMSF, and 0.5% Nonidet P-40 (NP-40) with 1.0 mM $Mg(OAc)_2$ added after the EGTA is dissolved. This suspension was shaken by hand for 45 s to lyse the cells, and lysis was monitored by phase-contrast microscopy. The solution was then pelleted at 1000*g* for 5 min in a swinging bucket rotor. This procedure was repeated once more except that the $Mg(OAc)_2$ was increased to 3 mM, the pellet was resuspended with a glass Teflon homogenizer to shear off cytoplasmic material adhering to the nuclei, and the suspension was sedimented at 2000*g* for 5 min. The nuclei were further purified by resuspending with

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[1] Abbreviations used: EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N'*-tetraacetic acid; EDTA, ethylenediaminetetraacetic acid; PMSF, phenylmethanesulfonyl fluoride; Tris, tris(hydroxymethyl)aminomethane; GdmCl, guanidinium chloride; OAc, acetate; PCA, perchloric acid; NADH, nicotinamide adenine dinucleotide (reduced form); BSA, bovine serum albumin; IgG, immunoglobulin.

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the homogenizer in the same buffer but containing 1 M sucrose and 0.1% NP-40. They were centrifuged at 5000*g* for 10 min. At this point most of the nuclei were intact and only a trace of cytoplasmic contamination remained attached to them.

Next, chromatin was purified from these nuclei by a modification of the procedure of Bonner et al. (1968). The pellet was washed as follows: twice with 0.05 M NaCl, 5 mM EDTA, 5 mM EGTA, and 1 mM PMSF, pH 8 (SEEP buffer); twice with 10 mM Tris, 0.1 mM EGTA, and 1 mM PMSF, pH 8; once with 10 mM Tris and 1 mM PMSF, pH 8; and finally with the nuclease shearing buffer (5 mM Tris–acetate, pH 7.8, 20 mM ammonium acetate, 0.4 mM $CaCl_2$, 0.2 mM EDTA, and 1 mM PMSF) (Noll et al., 1975). This material is then cleaved with the nuclease to yield the chromatin.

Nuclease Cleaving. The chromatin was suspended in the nuclease buffer at 50 A_{260} units/mL (determined in 0.1 N NaOH) and cleaved with staphylococcal nuclease (Worthington, micrococcal, EC 3.1.4.7) for 1 min at 23 °C by using 1 unit of enzyme per 3 A_{260} units of chromatin. The reaction was halted by adding EGTA to 1 mM and cooling to 0 °C. The insoluble material was then removed by sedimentation at 10000*g* for 10 min. The insoluble chromatin was resuspended, and $CaCl_2$ was added to compensate for the EGTA remaining in the volume of the pellet. It was cleaved again for 4 min with nuclease at 1 unit/4 A_{260} units of chromatin. The reaction was stopped and the insoluble material pelleted and discarded. The supernatants contained two fractions (first and second, respectively) of chromatin. Although it was usually used at this stage for purification of proteins or DNA, the chromatin can be further purified by pelleting at 100000*g* for 90 min.

Assays for Chemical Composition. The method of Burton (1956) was used to determine the DNA content of cleaved chromatin. Aliquots were made 0.3 N in KOH, digested for 2 h at 37 °C, and precipitated by titrating to pH 1 with 3 N $HClO_4$ to determine the RNA content. The supernatant was assayed for RNA by the orcinol method (Dische & Schwartz, 1937). Basic proteins were extracted with 0.4 N H_2SO_4. The samples were stirred for 30 min and then sedimented at 100000*g* for 2 h. The supernatants were dialyzed against two or three changes of 0.08 N NaOH and assayed by the method of Lowry et al. (1951). The pellet was assayed by the same method for acidic proteins after being dissolved in 0.08 N NaOH and dialyzed. Total proteins were assayed by either this method or the method of Brumhall et al. (1969). Hexose was assayed by the anthrone method of Hassid & Abraham (1957).

Ion-Exchange Chromatography. The procedure for separating the histones on Bio-Rex 70 with a guanidinium chloride (GdmCl) gradient has been described by Bonner et al. (1968).

Gel Electrophoresis. Basic proteins were analyzed on several gel systems, including an acid–urea system (Panyim & Chalkley, 1969), a high pH system (Panyim & Chalkley, 1971), and a neutral pH system (Panyim & Chalkley, 1971). The proteins prepared by acid extraction (above) were either dialyzed extensively against 1% acetic acid and lyophilized or precipitated with 4 volumes of ethanol at −20 °C for at least 12 h. Calf histone, BSA, cytochrome *c*, ovalbumin, and IgG were used as standards in some of these systems.

Peptide Mapping. Proteins were radioiodinated by the chloramine-T method (Greenwood et al., 1963) either in solution before electrophoresis or in gel slices. Tryptic and chymotryptic fragments from gel slices were mapped by the method of Elder et al. (1977). A total of 4×10^4 cpm was applied to each thin-layer chromatography plate, and autoradiography was performed at −70 °C for 18 h.

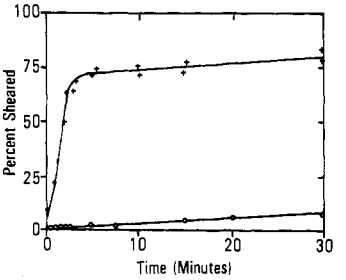

FIGURE 1: Nuclease shearing of *Dictyostelium* chromatin. (+) Fragments of chromatin soluble in a low ionic strength buffer were produced by nuclease shearing. The chromatin was labeled with [^{3}H]thymidine and then digested with staphylococcal nuclease. The reaction was stopped by adding EGTA to 1 mM and cooling to 0 °C. The samples were sedimented at 10000*g* for 10 min, and the supernatants were adjusted to 1% $NaDodSO_4$ and counted in a Beckman liquid scintillation counter using Aquasol 2 scintillation fluid (Amersham/Searle). Total radioactivity was determined by adjusting an aliquot of totally digested chromatin to 1% $NaDodSO_4$ and counting. (○) Only a small percentage of the DNA was rendered acid soluble during the digestion. The chromatin was sheared as above, but the aliquots were precipitated with 7% perchloric acid and sedimented. The supernatants were again counted after neutralization. Total radioactivity was determined by digesting aliquots in 7% PCA at 70 °C for 15 min and counting after neutralization.

Amino Acid Composition. Gels were electrophoretically destained, and their individual protein and blank bands were sliced out. These were weighed, lyophilized, and hydrolyzed with 6 N HCl at 110 °C for 24, 48, and 72 h. The amino acid composition was determined on a Beckman Model 121M analyzer. Corrections were made for serine, threonine, alanine, and isoleucine.

Results

The purification procedure has three successive sections: isolation of intact nuclei; washing the chromatin from lysed nuclei; and cleaving the chromatin to a soluble size.

Isolation of Intact Nuclei. This is complicated by a high lipid/DNA ratio in the rather small nuclei, the presence of nucleases and proteases released from the lysosomes, and the high polysaccharide content of slime mold cells. The lipid/DNA ratio is reduced by lysing the cells with a nonionic detergent like Nonident (NP-40; Shell, Inc.). This strips off the plasma membrane and at least part of the outer nuclear membrane (Hancock, 1974). Common nuclease activities were inhibited by using EGTA and a pH of 8. Phenylmethanesulfonyl fluoride (PMSF) was added to inhibit serine proteases (Chong et al., 1974). When less than 1 mM PMSF was used, degradation products from protease activity appeared on gels of both histones and nonhistones.

Nuclease Cleaving and Characterization of Chromatin. Mechanical shearing cannot separate polysaccharides and nonchromosomal proteins from chromatin and also disrupts the native structure of the chromatin (Noll et al., 1975). When low concentrations of staphylococcal nuclease (Worthington) are used, long pieces of chromatin are generated that are soluble in low ionic strength buffer but not in perchloric acid (Figure 1). Between 80 and 90% of the DNA is released, while only 5–10% of the polysaccharide and nonchromosomal protein is released. The remaining pellet is 50% of the original mass before cleaving and 30% of the original alkaline OD_{260} units. The pellet is 70% protein, 25% polysaccharide, and 5% DNA.

The chromatin may be cleaved either once completely or twice in partial successive stages. If the insoluble, crude

Table I: Chemical Composition of *Dictyostelium* Chromatin

fraction	% total DNA	ratio DNA	RNA	histone	non-histone[e]
total chromatin[a]		1	0.18 ± 0.1	0.98 ± 0.093	1.02 ± 0.27
first[b] fraction	3	1		1.06 ± 0.11[d]	10.3
second[c] fraction	75–80	1		1.02 ± 0.014	1.01

[a] The data for total chromatin are compiled from preparations which were separated into first and second fractions and from ones which were cleaved once totally. [b] The crude chromatin is treated with staphylococcal nuclease for 1 min at 22 °C and centrifuged. The supernatant is the first fraction. [c] The first pellet was treated again for 4 min, centrifuged, and washed. The supernatant is the second fraction. [d] This ratio is calculated after subtracting the contribution from nonchromosomal protein in the acid extraction of the first fraction. This can be done from an analysis of a gel scan of these proteins, providing one assumes equal molar staining of the protein bands. In this case, the histones make up 38% of the scan. It is likely that the basic histones bind more than an equal amount of strain. Therefore, 1.06 is an upper limit to the histone/DNA ratio. [e] A major difference in the chemical composition of these two fractions occurs in their nonhistone/DNA ratios. For the first fraction the observed ratio is 11.1, while for the second fraction it is 1.08. Some of the nonhistones are loosely bound to the chromatin of the first fraction and are extracted with the histones by acid. These may be contaminants and may give the high ratio. The two fractions contain 7.4 and 6.6% contamination, respectively, according to the ^{14}C-labeled protein experiment. Subtracting this contribution would alter the protein ratios to give the ones reported above.

chromatin is exposed briefly to the nuclease, centrifuged to remove the supernatant, and cleaved again, the chromatin can be separated into two fractions. The first nuclease treatment releases ~3% of the DNA into the supernatant. The nucleic acid in this fraction has a hyperchromicity of 13%, indicating that there may be single-stranded RNA present. The second cleaved fraction (75–80%) has a hyperchromicity of 28.7%. The length of 95% of the DNA in both fractions is greater than 1000 bp (base pairs) on electrophoresis gels (data not shown). From these results, fraction 2 is more representative of total chromatin. The ultraviolet absorption spectrum of this chromatin has a A_{230}/A_{260} ratio greater than that found in higher eucaryotes, but otherwise it is similar.

The chemical compositions of total cleaved chromatin and the two separated fractions are shown in Table I. The histone/DNA ratio is 0.98 for the entire genome, and it is adjusted for a slightly greater (12%) reactivity of histones in the Lowry protein reaction. The nonhistone/DNA ratio is 1.02 for the entire genome, but there is a difference in the nonhistone content of the first and second cleaved fractions (Table I). These results agree with those of Pederson (1977) for the total protein and RNA content of slime mold chromatin.

Assaying the Purity of the Chromatin. The purity of this chromatin has been assayed by the following four methods: the specific activity of a cytoplasmic enzyme, the absence of mitochondrial DNA, contamination from ^{14}C-labeled proteins, and the quantity of the RNA isolated with the chromatin. A typical cytoplasmic enzyme is lactate dehydrogenase. The data for the activity of lactate dehydrogenase in the whole-cell lysate and in the purified chromatin indicate that less than 0.1% of the total activity is isolated with the chromatin. This is equivalent to a 14-fold reduction in specific activity (see upper section of Table II).

Twenty-eight percent of the whole-cell DNA is mitochondrial (Firtel & Bonner, 1972). When whole-cell DNA

Table II: Contamination of Chromatin

assay	sp act.	contamination (%)[a]
lactate dehydrogenase activity[b]		
homogenate	69.5 units/μg	100.0
total chromatin	5.0 units/μg	7.2
binding of labeled crude cytoplasm[c]		
homogenate	0.76 cpm/μg	100.0
first chromatin fraction	0.19 cpm/μg	25.0
second chromatin fraction	0.11 cpm/μg	14.5
binding of labeled pure cytoplasm[c]		
homogenate	3.32 cpm/μg	100.0
first chromatin fraction	0.247 cpm/μg	7.4
second chromatin fraction	0.218 cpm/μg	6.6

[a] Contamination is the percent of the total protein which can be attributed to nonchromosomal origin. [b] This enzyme is assayed by the oxidation of NADH to NAD$^+$. One unit is equivalent to 1 μmol of NADH oxidized per min. [c] The specific activity of the protein is determined for the labeled homogenate and for chromatin prepared after mixing unlabeled nuclei with a labeled supernatant. The proteins are precipitated with hot trichloroacetic acid. Contamination is equivalent to 100 times the specific activity of the fraction divided by the specific activity of the homogenate.

is sheared with *Eco*RI restriction enzyme, three distinct mitochondrial DNA bands are present on gels (Firtel et al., 1976). Nuclear DNA isolated by the procedure described here does not contain any visible mitochondrial bands.

Contamination was also determined by using ^{14}C-labeled proteins. In this experiment one batch of cells was grown with ^{14}C-labeled amino acids and one without. The nuclei were isolated from both as usual. No precautions were taken to further purify the supernatant. The labeled supernatant was then mixed with the unlabeled nuclei for 10 min at 0–4 °C. The nuclei were next pelleted, and chromatin was prepared as usual and cleaved with the nuclease. From the middle section of Table II it is apparent that there was considerable contamination of the chromatin fraction with cytoplasmic material. Chromosomal material from lysed nuclei and mitochondria was precipitated from the crude supernatant by 0.2 M NaCl and centrifuged at 20000*g* for 30 min. When this purified supernatant was mixed with unlabeled nuclei, the contamination was reduced to 7% (lower section of Table II).

The final assay of purity is the quantity of RNA isolated with the chromatin. Large amounts of RNA, especially rRNA, would indicate contamination. The RNA/DNA ratio of the chromatin is only 0.18, and it compares well to the ratios in other eucaryotes (Rizzo, 1976; Elgin & Bonner, 1970; Pederson, 1977).

Histones Analyzed on Acrylamide Gels. The basic proteins or histones of *Dictyostelium* have been extracted in acid and analyzed on three gel electrophoresis systems: acidic, neutral, and basic. A low pH (3.2) urea gel is shown in Figure 2. Two of the five bands coelectrophorese with mammalian H1 and H3. Near the position of H1 there is a doublet, which will be called H7 and its modified form. Peptide maps of these two bands (see below) indicate that they are identical. The final slime mold band lies between H2a and H4, but there is no exact equivalence between it and a mammalian histone on these gels.

As the pH shifts to neutral and basic the gel pattern changes, reflecting the effects of charge on migration. The neutral gels are shown in Figure 3. The H1-like protein migrates faster than mammalian H1, but the H3-like protein still comigrates with H3. It forms a doublet at pH 7.6, but is a single band at pH 10 (data not shown). This may indicate a secondary modification of the protein. Histone H7 elec-

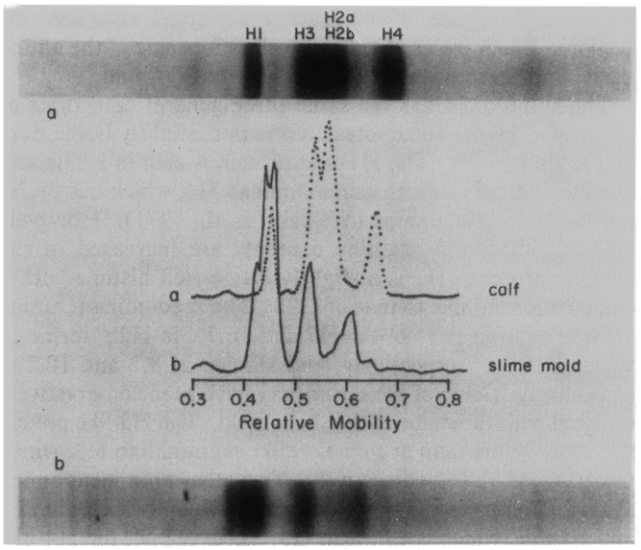

FIGURE 2: pH 3.2, acid–urea gels of histones. (a) Calf histone gel with its associated scan (···). The histones are labeled above their bands. Histones H2a and H2b appear as a single band, although they can be resolved into two bands on longer gels. (b) *Dictyostelium* histone gel with its associated scan (—). The relative mobility was on a scale of 0.0 to 1.0. The portions of the gels not shown in the scan have almost zero background. The bands lie opposite their peaks in the scans.

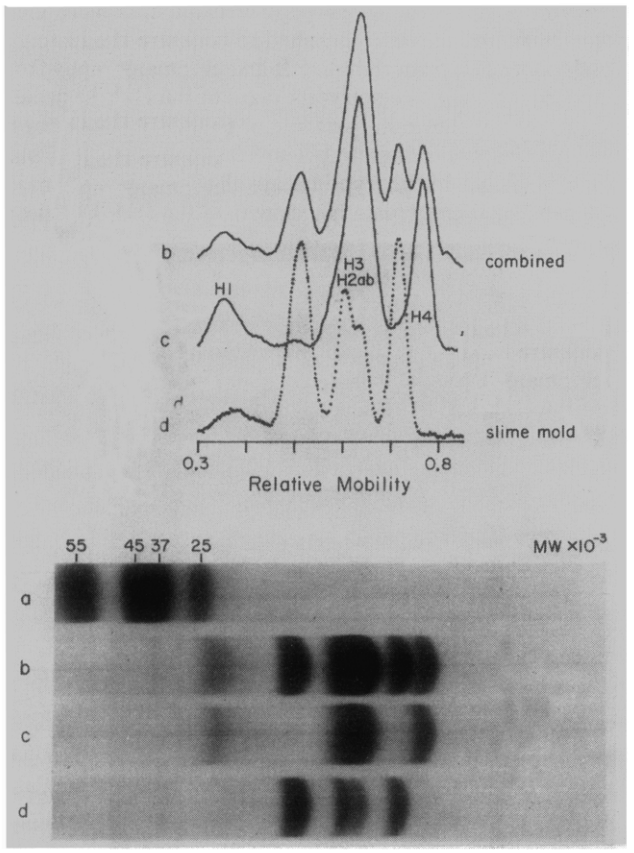

FIGURE 3: pH 7.6, $NaDodSO_4$–urea gels of histones. (a) Molecular weight markers were used to determine the size of any nonhistone contaminants in the histones. They were IgG (55 000 and 25 000 daltons for the heavy and light chains of human), ovalbumin (45 000), and alcohol dehydrogenase (yeast, 37 000). They did not migrate accurately with respect to histones because of their vastly different isoelectric points. No scan is shown of this gel. (b) A scan and gel of a mixture of calf and *Dictyostelium* histones illustrate their alignment (—). (c) A scan and gel of calf histones. The scan is labeled with the names of the peaks. Histones H3, H2a, and H2b have migrated as a single broad peak (—). (d) A scan and gel of *Dictyostelium* histones (···).

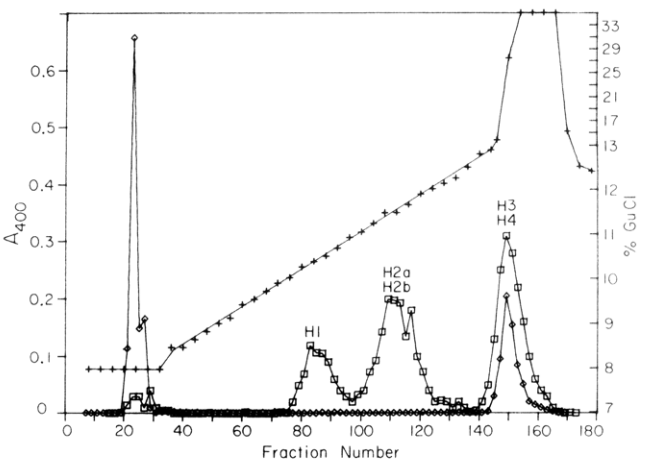

FIGURE 4: Ion-exchange chromatography profile of histones. The histones were applied to the column in 8% GdmCl. An 8–13% GdmCl gradient was used to elute some of the histones. Then a 35% GdmCl step eluted the remaining histones. The samples were precipitated with 1.1 M trichloroacetic acid for 15 min and read at 400 Å. (+) GdmCl percentage; (□) calf histones; and (◊) *Dictyostelium* histones. The calf histone peaks are labeled.

trophoreses between H1 and H3 and is the most heavily stained band in the slime mold. The fourth slime mold band is found between H2a and H4 again at pH 7.6, but it overlaps with H4 at pH 10. The neutral and basic gels contain $NaDodSO_4$, so that the molecular weight of the slime mold histones can be determined by comparison with mammalian histones (Fasman et al., 1977; Elgin & Weintraub, 1975; Panyim & Chalkley, 1971). Slime mold H1-like protein has a molecular weight of 20 000, H7 is 15 800, the H3-like protein is 14 900, and the H4-like protein is 12 000. The corresponding molecular weights of mammalian histones are 22 130 for H1, 15 324 for H3, 14 004 for H2a, 13 774 for H2b, and 11 282 for H4.

A computer was used to analyze the scans and fit Gaussian curves to them (Wallace et al., 1977). On the assumption that histones stain equally with respect to mass (Fambrough et al., 1968), the weight fraction of each histone may be estimated from the scans. On this basis, the weight fraction of the H1-like protein is 0.1, H7 is 0.4, the H3-like protein is 0.26, and the H4-like protein is 0.24.

Histones Analyzed by Ion-Exchange Chromatography. To confirm further the identity of slime mold histones, we analyzed them on a Bio-Rex 70 column using a guanidinium chloride (GdmCl) gradient. Li & Bonner (1971) have found that the histones can be separated into species apparently by basic charge. The slime mold histones are also separated into species by this method. Samples were centrifuged before chromatography to remove aggregates. Their pattern of elution from this column is compared to that of mammalian histones in Figure 4. This profile compares two separate runs, but labeled mammalian histones gave similar results when run in parallel with slime mold histones. No slime mold proteins cochromatograph with either H1, H2a, or H2b. Instead, they elute with the unbound and with the tightly bound proteins. The peaks were pooled and run on pH 7.6 electrophoresis gels. The results are shown in Figure 5. The slime mold H1-like protein is not bound to the column in 8% GdmCl. This may simply be due to its lower molecular weight and less basic composition (see below) than mammalian H1. A small amount of the other histones also elutes with the H1-like protein. This is probably due to overloading of the column and proteolysis of the histones. The remaining slime mold histones are tightly bound and only eluted by 35% GdmCl.

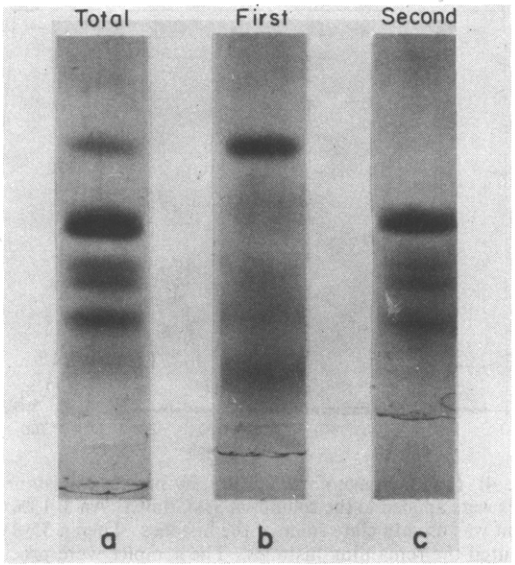

FIGURE 5: *Dictyostelium* histone peaks from ion-exchange column. The peaks were pooled, precipitated with trichloroacetic acid, washed with acetone, dissolved in sample buffer, and run on pH 7.6, $NaDodSO_4$–urea gels. (a) Total *Dictyostelium* histones; (b) first peak from the ion-exchange column; and (c) second peak from the column.

This supports the previous results, which indicated that the slime mold has both H3- and H4-like histones. Finally, the unusual H7 is tightly bound, indicating that it is more basic than either mammalian H1, H2a, or H2b.

Amino Acid Composition. Table III lists the amino acid composition of slime mold histones. Digestion time points at 24, 48, and 72 h were averaged except for threonine and serine, which were extrapolated to zero time of digestion, and isoleucine and alanine, which reached a plateau after long digestions. Blank pieces of gel gave values for some of the amino acids, and these were subtracted as a background.

The slime mold has the same three general categories of histones as higher eucaryotes: lysine rich, slightly lysine rich, and arginine rich. The H1-like protein is rich in lysine and alanine, but not as much as mammalian H1, which has 28.7% lysine and 25.1% alanine (Wilhelm et al., 1971). However, both histidine and arginine contents are increased in the H1-like protein. H7 is a slightly lysine-rich histone with a composition similar to that of H2b. The predominant amino acids are lysine at 15.6% in H7 and 16.7% in H2b, serine at 9.8 and 10.9%, respectively, and alanine at 8.2 and 10.2%, respectively. Some of the serine may have been conservatively replaced with threonine in the slime mold. The H3-like protein has more lysine than arginine, unlike mammalian H3, which has 10.1 and 13.6%, respectively. Two other prominent amino acids for comparison are glutamic acid, which is 10.2% in H3, and alanine, which is 13.5% in H3. As expected, the H4-like protein has more arginine than lysine and a high glycine content. It has a composition very similar to that of mammalian H4.

When rat histones were analyzed by this method, they gave values very close to those published (Wilhelm et al., 1971). The major amino acids were less than 1 mol % different, giving an error between 5 and 10%.

Peptide Maps. Iodinated peptide fragments were electrophoresed and chromatographed to compare the histones. Electrophoresis is from right to left and chromatography from bottom to top. The chymotrypsin maps of the H4-like protein and rat H4 are shown in parts a–c of Figure 6. Four out of seven major spots comigrate (Figure 6c), and the others only deviate slightly. In the trypsin maps three out of four major spots partially overlap (data not shown). These results indicate

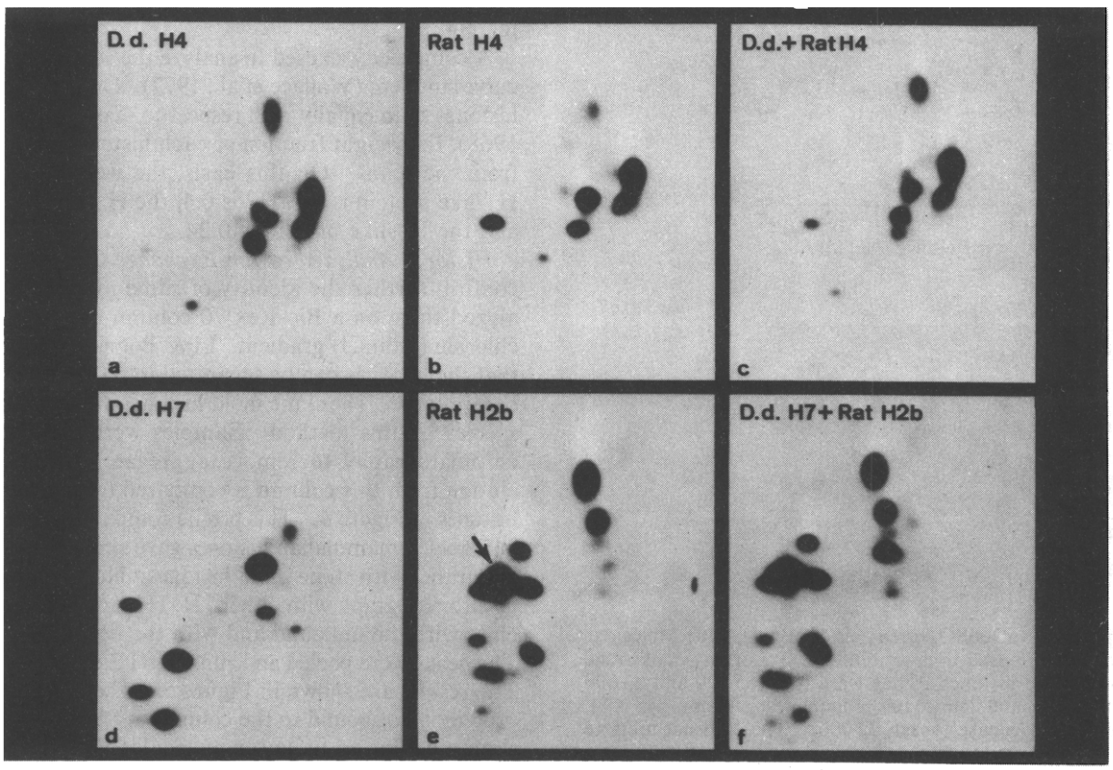

FIGURE 6: Peptide maps of histones. Labeled gel bands were excised, digested with chymotrypsin, and eluted. A total of 4×10^4 cpm was spotted near the lower right-hand corner of the thin-layer chromatography plate. Electrophoresis was carried out in the horizontal dimension and chromatography in the vertical dimension. (a) *Dictyostelium* H4-like protein; (b) rat H4; (c) 3×10^4 cpm of *Dictyostelium* H4-like protein plus 1×10^4 cpm of rat H_4; (d) *Dictyostelium* H7; (e) rat H2b (the arrow indicates a contaminating H3 spot; this is the only major H3 spot); and (f) 1×10^4 cpm of H7 plus 3×10^4 cpm of rat H2b.

Table III: Amino Acid Composition of *Dictyostelium* Histones

amino acid	H1-like protein	H7	H3-like protein	H4-like protein
Lys	16.0	15.6	10.3	9.9
His	1.6	2.3	2.6	2.7
Arg	3.2	5.0	9.6	12.3
Asp	7.0	8.8	6.8	7.1
Thr	10.0	11.5	7.3	8.6
Ser	7.5	9.5	6.0	4.6
Glu	8.0	9.5	11.2	7.4
Pro	8.1	3.6	4.8	3.3
Gly	4.6	6.3	5.1	11.8
Ala	15.5	8.0	10.2	8.0
Val	4.2	4.0	6.2	7.5
Met	1.0	1.3	1.0	0.9
Ile	3.6	5.7	6.4	5.6
Leu	5.5	4.6	7.3	4.7
Tyr	2.5	1.8	1.9	3.2
Phe	1.7	2.5	3.3	2.4

that the proteins are highly related.

The other histone-like proteins have only a few spots in common with the rat histones. Parts d–f of Figure 6 show a comparison of *Dictyostelium* H7 and rat H2b. Three spots on the left sides of the plates overlap, while the others only migrate in the same general area. The arrow in Figure 6e points to a large spot due to contaminating rat H3 which overlaps with H2b on the gels. This spot comigrates with a group of spots in the H3-like protein (data not shown). The H1-like protein contains no corresponding fragments in any rat histone.

Discussion

Before discussing the character of slime mold histones and the results of other research on this topic, we would like to deal with the problems of defining purified chromatin. There is no universal criterion for the chemical composition of purified nuclei and chromatin. Obviously, the chemical composition would vary with organism, cell type, and stage in the cell growth cycles. However, upper limits for an acceptable composition can be established from the large amount of available data (Bonner et al., 1968; Stein & Borun, 1972; Elgin & Bonner, 1970; Itzhaki & Cooper, 1973). We suggest that the following mass ratios should be an upper limit for purified chromatin: RNA/DNA ≤ 0.3 and protein/DNA ≤ 3.0. The DNA should account for $\sim$70% or greater of the UV absorbance at 260 nm. The procedure followed in this paper does yield purified chromatin by the above criteria. The RNA/DNA ratio for *Dictyostelium* chromatin is fairly high as compared to that of other eucaryotes, but a larger fraction of the genome is also being expressed (Firtel, 1972).

The contamination in this preparation has been evaluated by several methods, one of which involved mixing labeled cytoplasmic proteins with unlabeled nuclei. Addition of the total 5000*g* supernatant to the nuclei yielded an overestimate of the contamination due to protein contributed from lysed nuclei and mitochondria (Table II, middle section). Escaped nucleoproteins were removed by making the supernatant 0.2 M in NaCl and centrifuging at 20000*g*. Testing with the purified supernatant gave a more accurate estimate of the contamination (Table II, lower section).

The preparation is complicated by the presence of many degradative enzymes, which must be inhibited. If less than 1 mM PMSF is used during the isolation, degradation of the histones can be detected on gels. Nonserine proteases may also be present. The action of these proteases has a twofold effect: proteins are degraded and stretches of DNA are exposed to nuclease attack (Bakke & Bonner, 1978).

One unique aspect of this preparation is solubilizing the chromatin specifically by cleaving with a nuclease. This allows the polysaccharides and cytoskeleton to pellet, while the chromatin remains in the supernatant.

On the basis of the results presented here, *Dictyostelium* has four histones. The H4-like protein is the most conserved according to its peptide map and amino acid composition. Identical spots on a peptide map have the same compositions for that peptide, and the maps are a sensitive means of comparing proteins. Apparently, H4 has a very specific function in all organisms. The H4-like protein has a slightly higher molecular weight than mammalian H4, on pH 7.6 gels, but this difference almost disappears on pH 10 gels, where the effects of the high basic charge are diminished. Minor bands appear above and below the H4-like protein, and these have peptide maps which are identical with the H4-like protein. Therefore, these bands represent both modified and degraded species of it.

The H3-like protein forms a doublet only on the pH 7.6 gels. If this is actually two polypeptides, the most likely candidates, besides H3, would be H2a or H2b. In this case, one band should chromatograph differently on Bio-Rex 70. Instead, they both elute with mammalian H3. Therefore, the H3-like protein must also be modified. Its amino acid composition has slightly more lysine than arginine. These compositions should be interpreted with caution because the acrylamide gives a background (which has been subtracted) and contaminating peptides may comigrate with the proteins.

The most unusual slime mold histone is H7. It has a higher molecular weight than any mammalian histone except H1 and is slightly lysine rich like the H2 histones. H7 forms a doublet on the pH 3.2 gels, but both of these bands have identical peptide maps, indicating that only one protein species is present. It is more numerous than any other histone-like protein in the chromatin with a mass almost equaling the combined masses of the H3- and H4-like proteins on the basis of gel staining. Since the four nucleosomal histones are generally found in equimolar ratios in higher eucaryotes (Garrard et al., 1974; Olins et al., 1976; Kornberg, 1974), H7 may replace both H2a and H2b in the slime mold. Although H7 does not have a double molar ratio on these gels, this kind of determination is always liable to errors due to differential extraction and proteolysis. Calculations based on the molecular weights of the slime mold histones indicate that four molecules of H7 and two molecules each of the H3- and H4-like proteins would equal the mass of 187 base pairs of DNA in the monomer repeat length (Bakke & Bonner, 1978).

Charlesworth & Parish (1975) and Coukell & Walker (1973) have isolated basic proteins from slime mold nuclei, and both laboratories report more than four histone bands. Our peptide maps indicate that several extra bands are either modified or degraded histone species. Approximately 80% of the area under the gel scans of Charlesworth and Parish lies in bands corresponding to those reported here. Coukell and Walker found a histone/DNA ratio of 1.06, which is almost identical with our results.

An interesting implication for chromatin structure arises from the histone composition of *Dictyostelium* and other lower eucaryotes (Mohberg & Rusch, 1969). The histones approximately equal the DNA in mass, as they do in higher eucaryotes. Since the fraction of DNA being expressed as RNA is several-fold higher in the lower eucaryotes (Firtel, 1972) and since there is no decrease in the histone mass ratio, histone must be present in regions of the chromatin that are being transcribed. Therefore, the primary role of histones may

be structural and the differences between transcribed and nontranscribed regions of the chromatin may be due mainly to differences in this structure.

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