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Isolation and Characterization of Chromatin from the Cellular Slime Mold, *Dictyostelium discoideum*†

Thoru Pederson

ABSTRACT: A method is described for purifying chromatin from the cellular slime mold, *Dictyostelium discoideum,* starting with isolated nuclei. Reconstruction experiments indicate exceptionally low contamination levels of the purified chromatin by soluble cytoplasmic proteins and membrane phospholipid. The ultraviolet absorption spectrum and protein:DNA ratio of purified slime mold chromatin are similar to that for metazoan preparations, but the RNA content is higher. The nonhistone proteins are electrophoretically complex but somewhat less so than in chromatin from higher eukaryotes. When purified in the presence of the protease inhibitor phenylmethanesulfonyl fluoride, two of the major nonhistone chromatin proteins have molecular weights identical with mammalian actin and myosin. Together these two components account for about 35% of the nonhistone chromatin protein mass. Reconstruction experiments indicate that the actin- and myosin-like chromatin proteins are not attributable to contamination by *soluble* cytoplasmic proteins, but the possibility that they reside on the outer surfaces of nuclear envelope fragments or other cytoplasmic contaminants of the chromatin preparation has not been ruled out. This is also true for other reports of "chromatin-associated" contractile proteins. The chromatin purification method described here for *Dictyostelium* also permits simultaneous recovery of nucleoli that are free of adherent chromatin. These methods now make it possible to apply in vitro transcription technology to both ribosomal and nonribosomal segments of this simple eukaryotic genome.

The cellular slime mold, *Dictyostelium discoideum,* is an attractive eukaryotic system for studies on the relationship between differential gene expression and development. Exponentially growing *Dictyostelium* vegetative amoebae have an average nuclear DNA content of only 0.10 pg, and a nucleotide sequence complexity of approximately 3×10^{10} daltons, only about 11 times that of *Escherichia coli* (Firtel and Bonner, 1972). RNA-driven hybridization experiments indicate that a relatively large fraction of nonrepetitive DNA is expressed during the life history of *Dictyostelium* and that the developmental cycle is determined, at least in part, by differential gene transcription (Firtel, 1972; Firtel et al., 1973). Moreover, the biosynthesis of messenger RNA in *Dictyostelium* appears, at least superficially, to be a somewhat less complex process than in higher eukaryotes. Slime mold heterogeneous nuclear RNA is only slightly larger than polyribosomal messenger RNA, in contrast to the very large hnRNA[1] seen in many metazoa (Firtel and Lodish, 1973; Firtel and Pederson, 1975). Moreover, a much larger weight fraction of *Dictyostelium* hnRNA is converted to mRNA than in mammalian cells.

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[1] Abbreviations used are: hn- and mRNA, heterogeneous nuclear and messenger ribonucleic acid; Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid, $PhMeSO_2F$, phenylmethanesulfonyl fluoride; EDTA, ethylenediaminetetraacetic acid; Tris, tris(hydroxymethyl)aminomethane; DNase, deoxyribonuclease.

TABLE I: DNA Content:Cell Volume–Mass Relationships.

Cell	DNA content (pg)	Cell vol (μm^3)	DNA content/cell vol (pg/μm^3)	DNA content/cell mass[a] (μg/mg)
HeLa (G1)	14	718	1.90×10^{-2}	1.80×10^{-2}
Dictyostelium (G1)	0.06[b]	350	1.71×10^{-4}	1.67×10^{-4}
		Dictyostelium:HeLa = $1.67 \times 10^{-4}/1.80 \times 10^{-2} = 0.92\%$		

[a] Using a density of 1.051 g/cm^3 (Chinese hamster cells) for HeLa (Anderson et al., 1970) and a density of 1.019 g/cm^3 (*Amoeba proteus*) for *Dictyostelium* (Prescott, 1955). [b] Sussman and Rayner, 1971.

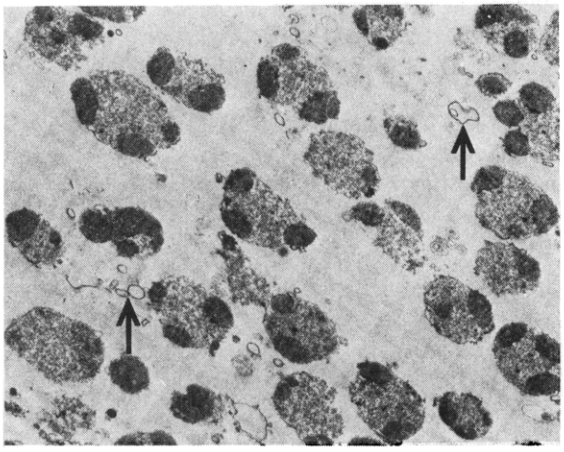

FIGURE 1: Electron micrograph of purified nuclei. Arrows indicate occasional membrane contaminants; ×5940.

The analysis of differential gene expression in *Dictyostelium* would be greatly facilitated by the availability of purified, transcription-competent chromatin. Since a large fraction of the slime mold genome is transcribed in vivo, and since the production of mRNA in this organism appears to circumvent high molecular weight hnRNA, the use of purified *Dictyostelium* chromatin for in vitro transcription experiments promises to be particularly enlightening. In this paper, a method for purifying *Dictyostelium* chromatin is described, along with a partial characterization of its protein components.

The initial isolation of chromatin from an untested organism or tissue is often limited by the ability to obtain pure nuclear fractions or, as is becoming increasingly appreciated, the action of endogenous proteases. In addition, individual cell or tissue types, or their nuclei, can vary significantly in their responses to "standard" chromatin isolation techniques. In contrast, the principal limitation to the isolation of *Dictyostelium* chromatin has proven to be the organism's very small genome size and corresponding low chromatin content. The small genome of *Dictyostelium* translates into a particularly low chromatin content when considered on a cell mass basis. This is illustrated by the data in Table I, where the relationships between DNA content and cell size are compared for *Dictyostelium* and a typical mammalian tissue culture cell (HeLa). Per unit of cell mass, G1-phase *Dictyostelium* vegetative amoebae have a nuclear DNA content only 0.92% that of G1-phase HeLa cells.

Materials and Methods

Cell Culture. The axenic strain A-3 of *Dictyostelium discoideum* was grown at 22 °C in shaker cultures (120 rpm) on a medium containing, in grams per liter: Difco yeast extract, 5.0; Difco proteose peptone, 10.0; D-glucose, 10.0; 2-(*N*-morpholino)ethanesulfonic acid, 1.3, pH 6.5. Cultures were inoculated at approximately 10^4 cells/mL and harvested when the cell density had reached 5×10^5 to 5×10^6/mL. Under these conditions the cell doubling time is 9–12 h. Cultures were harvested in 600-mL portions by sedimentation at 1000*g* for 5 min in conical glass centrifuge bottles in a swing-out rotor. The packed cells were washed once in 50 vol of ice-cold 0.2% NaCl.

Cell Fractionation. Additional aspects of methodology are detailed in the Results section, while this section is confined to a description of the standard isolation technique that evolved as the result of this investigation. All operations were carried out at 2 °C unless otherwise noted. Lysis buffer is 5 mM magnesium acetate, 10% sucrose, 50 mM Hepes (pH 8.0), 1 mM phenylmethanesulfonyl fluoride ($PhMeSO_2F$), and 0.5% (v/v) Triton X-100. Buffer lacking the detergent and $PhMeSO_2F$ may be prepared in advance, autoclaved, and stored indefinitely at 4 °C. $PhMeSO_2F$ was added just before use from a 100 mM stock solution made up in 95% (v/v) 2-propanol. $PhMeSO_2F$ is toxic and should be handled and pipetted with proper precautions. Glassware containing it should be thoroughly rinsed to protect kitchen personnel. Washed cells were resuspended in cold lysis buffer at a concentration not exceeding 5×10^8 cells/mL. The cell suspension was immediately transferred to a prechilled tube and vortexed (at ambient room temperature) for 15 s. The lysate was placed in an ice-cold, tight Dounce homogenizer and subjected to 4–5 strokes of the pestle. The lysate was then centrifuged at 1500 rpm for 4 min in the Sorvall SS-34 rotor. This step pellets unlysed cells and some of the larger cytoplasmic skeletons (see Results and Figure 1). The supernatant was then sedimented at 4000 rpm (SS-34 rotor) to pellet nuclei. The crude nuclei were washed three times in lysis buffer lacking Triton (25 mL for each 10^{10} cells in the initial sample). After the third wash, the supernatant was water clear. The nuclei were resuspended in buffer 1, which is: 0.01 M KCl, 0.01 M magnesium acetate, 12% (w/v) sucrose, 1 mM $PhMeSO_2F$, and 0.03 M Hepes (pH 7.9), using 15 mL for each 10^{10} cells in the initial sample. Aliquots (15 mL) of the nuclear suspension were transferred to 1 × 3.5 in. polyallomer tubes (for the Beckman Spinco SW 27 rotor) in an ice–water bath and sonicated for 10 s at 40 W with a Branson Model W185 sonifier, using the "standard" microtip. This was usually sufficient to disrupt greater than 90% of the nuclei, as judged by phase-contrast microscopy. If necessary, another 10-s sonication was applied.

Chromatin Isolation. All sucrose solutions contained 1 mM $PhMeSO_2F$. Portions (8 mL) of the disrupted nuclei were layered on 25 mL of 30% (w/v) sucrose in buffer 2 (0.01 M KCl, 0.01 M magnesium acetate, and 0.03 M Hepes (pH 7.9)), and were centrifuged at 5000 rpm for 20 min in a Spinco SW 27 rotor (4 °C). This pellets nucleoli, while chromatin and nucleoplasmic ribonucleoprotein particles band at the 30% sucrose interface as a visible opalescent zone. The postnucleolar

supernatant was removed and layered on discontinuous gradients in SW 27 tubes consisting of 6 mL of 60% (w/v) sucrose under 10 mL of 45% (w/v) sucrose, both in buffer 2. In cases where smaller amounts of material were being isolated, the discontinuous gradients were made up in SW 41 tubes by overlaying 2 mL of 60% sucrose with 3.5 mL of 45% sucrose. The tubes were then filled with postnucleolar supernatant and centrifuged at 26 000 rpm for 7.5 h (SW 27) or at 40 000 rpm for 3.5 h (SW 41). Under both conditions, the chromatin sediments through the 60% sucrose cushion and pellets, while the 55S nucleoplasmic ribonucleoprotein particles (Firtel and Pederson, 1975) and membrane fragments do not.

Analytical Methods. Ultraviolet spectral data on purified chromatin were recorded in buffer 3, which contains 0.10 M NaCl, 0.01 M EDTA, and 0.01 M Tris-HCl (pH 8.0). DNA, RNA, and protein were determined by the diphenylamine, orcinol, and Lowry methods, respectively. For electrophoresis of proteins, chromatin in buffer 3 containing 1 mM PhMe-SO_2F was made 1% in sodium dodecyl sulfate–0.1% 2-mercaptoethanol–0.01 M sodium phosphate buffer (pH 7.0). Electrophoresis was in 7.5-cm cylindrical gels containing 7.5% polyacrylamide, as detailed previously (Bhorjee and Pederson, 1973). Other experimental conditions and analytical details are given in the table and figure legends.

Results

Cell Lysis and Isolation of Nuclei. Lysates of *Dictyostelium* vegetative amoebae were found to contain potent DNase activity. Buffers antagonistic to pancreatic and lysosomal DNases (e.g., EDTA, pH 8.0) did not support nuclear integrity. Although the DNases of *Dictyostelium* and their subcellular localization were not systematically investigated, they appear to be principally cytoplasmic. By rapidly sedimenting the nuclei out of lysates, damage by these cytoplasmic DNases was effectively eliminated, and chromatin containing DNA of high molecular weight (10^7 to $>10^8$, single-strand molecular weight) could be routinely prepared. A variety of detergents were explored to lyse cells for nuclear isolation. Cemusol (2%, v/v) (Melle-Bezons) is used to prepare crude *Dictyostelium* nuclei for studies of RNA metabolism (Firtel and Lodish, 1973), but in our hands this detergent produced a relatively low yield of nuclei. In addition, we have recently been unable to secure this detergent from its manufacturer. Nonidet P-40 (Shell Chemical Co.) at concentrations of 0.2–0.5% (v/v) produced high yields of nuclei heavily contaminated by "rims" of cytoplasm limited by largely intact plasma membranes. The liquid dishwashing detergents Joy and Ivory (Proctor and Gamble), used at concentrations of 0.5% (v/v), led to partial nuclear lysis. The most effective detergent for cell lysis without nuclear disruption proved to be Triton X-100 (Rohm and Haas) at a concentration of 0.5% (v/v). In a buffer containing 5 mM magnesium acetate, 10% (w/v) sucrose, and 50 mM Hepes (pH 8.0), this concentration of Triton routinely lysed over 98% of the cells in 5 min at 4 °C. Lysis was less effective when the cell concentration exceeded 5×10^8 cells/mL and therefore a standard concentration of 10^8 cells/mL was adopted. "Lysis" appears to consist of early release of nuclei followed by the persistence of cytoplasmic "skeletons". (In contrast, it is of interest that mammalian tissue culture cells exposed to similar concentrations of Triton are literally "lysed" in that phase-contrast microscopy reveals no intact structures other than nuclei.) The rate of lysis of *Dictyostelium* vegetative amoebae is temperature dependent. Although rapid at 4 °C (2–4 min = 90% lysis), it proceeds even faster at 20 °C. However, in view of the potent cytoplasmic DNase activities (vide supra), elevated temperatures should be avoided, especially since lysis is so effective at 4 °C.

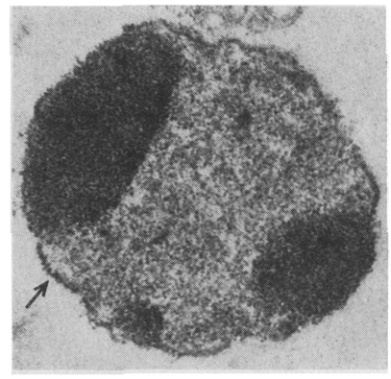

FIGURE 2: Representative electron micrograph of a purified nucleus. Arrow indicates a relatively intact region of the nuclear envelope; ×19 800.

Electron microscopy revealed that small fragments of cytoplasm remain associated with some of the nuclei as they are released from lysing cells. It was found that this material could be removed from the nuclei by the hydrodynamic shear generated with 4–5 strokes in a tight Dounce-type homogenizer, and this step was therefore incorporated into the standard procedure. Homogenization per se, without prior detergent lysis, does not disrupt *Dictyostelium* vegetative amoebae.

After three successive washes in buffer lacking detergent, the nuclei appeared to be relatively clean by phase-contrast miscroscopy. The yield was 75–80%. The remaining nuclei were lost in the wash supernatants. The cleanest nuclei were obtained from exponential cultures having cell densities of 2×10^5–2×10^6/mL. At higher densities (such as 5–10×10^6/mL), nuclear purity fell off sharply, although good purity could be obtained in these cases by prolonged washing, with corresponding decreases in yield (to 45–55%). Figure 1 is an electron micrograph of a typical field of washed nuclei. The only nonnuclear materials apparent are occasional membrane vesicles (indicated by arrows). Although this degree of membrane contamination could compromise metabolic experiments with purified nuclei, for analytical work (i.e., on a mass basis), it appears to be quite low. Figure 2 illustrates a typical isolated nucleus at higher magnification. The gross ultrastructural texture is very similar to nuclei seen in thin sections of intact cells fixed and stained similarly (see, for example, Figure 2.7 of Loomis, 1975). Although Triton causes some disruption of normal nuclear envelope integrity, many areas appear to be intact (arrow, Figure 2).

Purification of Chromatin from Isolated Nuclei. Clean nuclei obtained as above were disrupted by very brief sonication in buffer 1 as detailed under Materials and Methods. Nuclear disruption was monitored by phase-contrast microscopy and was judged complete when less than 5% of the original nuclei remained intact. Under these conditions the nucleoli are not disrupted and can be visualized in phase-contrast microscopy as bright, roughly spherical bodies with a high degree of Brownian movement. Eight-milliliter portions of the sonicate were layered on 30% sucrose and centrifuged at 4500*g* for 20 min as detailed under Materials and Methods. The conditions selected were determined empirically so that nucleoli were quantitatively pelleted. Figure 3 is an electron micrograph of the resuspended pellet. The nucleoli retain the highly granular texture they display in thin sections of isolated nuclei (compare with Figure 2) and are largely free of adherent material. The small amount of fragmented nucleoli and other contaminants

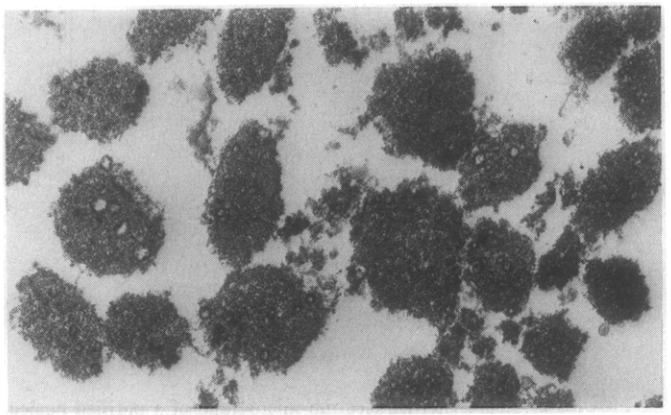

FIGURE 3: Nucleoli obtained by sedimentation of nuclear homogenate through 30% sucrose (see text); × 14 850.

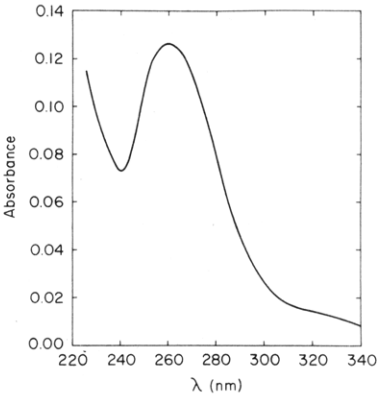

FIGURE 4: Typical ultraviolet absorption spectrum of purified *Dictyostelium* chromatin. The solvent was 0.1 M NaCl–0.01 M EDTA–0.01 M Tris-HCl (pH 8.0) (20 °C). See additional data in Table III.

TABLE II: Estimate of Cytoplasmic Protein Contamination.[a]

	cpm/mg
Sp act. of cytoplasm	47 900
Sp act. of chromatin	140
Mass ratio of cytoplasmic protein to chromatin protein	140/47 900 = 0.292%

[a] Two 600-mL cultures were resuspended at 5.7 × 10^6 cells/mL in 600 mL of medium lacking proteose-peptone and yeast extract and containing 125 μCi of [^{14}C]leucine, 100 μCi of ^{14}C-labeled reconstituted protein hydrolysate, 150 μCi of [^{14}C]arginine, and 125 μCi of [^{14}C]lysine (total isotope concentration = 0.83 μCi/mL). Under these conditions, protein synthesis is depressed due to the absence of bulk peptides and amino acids from the medium, but the radioactive amino acids are "loaded" into the cells. After 1 h, the cells were resuspended in 1200 mL of complete medium whereupon they rapidly resume protein synthesis and growth. After 4 h, the cells were harvested and fractionated into nuclei and cytoplasm (see Materials and Methods). The cytoplasmic fraction was then used as a buffer to fractionate an equal mass of unlabeled cells, from which chromatin was purified. Aliquots of the second cytoplasmic fraction and the purified chromatin were removed for measurement of acid-insoluble radioactivity and total protein.

can be almost entirely removed by recentrifuging the nucleoli through 30% sucrose. The round vacuolar structures seen in many of the isolated nucleoli (Figure 3) are also present in thin sections of isolated nuclei or intact cells and are therefore apparently not generated by nuclear isolation or sonication. Similar vacuoles are also often seen in nucleoli of the ciliate *Tetrahymena* (M. Gorovsky, personal communication).

The post-nucleolar supernatant obtained as above and containing 85–95% of the initial nuclear DNA was sedimented on 45%:60% discontinuous sucrose gradients as detailed under Materials and Methods. The chromatin (measured as DNA) was recovered as an ivory-yellow pellet. A white opalescent band was also routinely observed at the 45%:60% sucrose interface. Although this latter fraction contained some DNA (about 15% of the total), further compositional analysis revealed a much higher protein:DNA ratio than the pelleted chromatin as well as the presence of membrane-derived material. All of the data reported here concern the chromatin that pellets through 60% sucrose.

Extent of Chromatin Contamination by Cytoplasmic Protein. The extent of contamination of the purified chromatin by soluble cytoplasmic proteins was investigated as follows. Vegetative amoebae were grown for 5 h in the presence of a mixture of ^{14}C-labeled amino acids and a cytoplasmic fraction was prepared. This was then used as a "buffer" to lyse an equal number of unlabeled cells. Based on the specific activities of the resulting cytoplasmic and chromatin preparations, cytoplasmic protein contamination was less than 0.30% of the total chromatin protein mass (Table II). This contrasts with a value of 2.0% when a similar experiment is performed with HeLa cell chromatin (Bhorjee and Pederson, 1972) and values as high as 30% when chromatin is isolated directly from lysed whole cells (e.g., see Hill et al., 1971). However, it is emphasized that these reconstruction experiments have a potential defect, in that the initial cytoplasmic fraction may be partially (or conceivably completely) depleted of proteins that enter nuclei during cell fractionation and bind nonspecifically to chromatin. The values obtained must therefore be taken as minimum estimates. Conversely, the values observed may include the proper nuclear entrance during cell homogenization of those proteins destined for specific assembly into replicating chromatin (e.g., histones). To this extent, the observed values may actually be overestimates of true (i.e., nonspecific) contamination.

Extent of Chromatin Contamination by Membrane. Vegetative amoebae were grown for 17 h (approximately 1.5 cell generations) in the presence of [2-^{3}H]glycerol. Eighty percent of the trichloroacetic acid insoluble ^{3}H radioactivity incorporated into whole cells was extractable in cold acetone under conditions where lipid is selectively solubilized. The chromatin fraction contained only 0.01% of the total cell [^{3}H]glycerol. However, this result refers only to the phospholipid components of membranes, and the possibility of chromatin contamination by membrane proteins per se is not excluded. This latter possibility is examined in a subsequent section.

Composition. A typical ultraviolet absorption spectrum of purified *Dictyostelium* chromatin is shown in Figure 4, and data from four separate preparations are given in Table III, along with data for purified HeLa cell chromatin for comparison. The average A_{260}:A_{280} ratios for *Dictyostelium* and HeLa chromatin are 1.44 and 1.70, respectively, suggesting a higher protein content in the former, which was confirmed by chemical analysis as shown below. Chemical analysis of purified *Dictyostelium* chromatin gave a protein:DNA ratio of 2.32, which lies well within the range observed for chromatin preparations from most metazoan organisms (1.75 to 2.50). However, the RNA content (RNA:DNA = 0.30) was somewhat higher than usually observed (RNA:DNA = 0.10 for most metazoan preparations). Since a greater proportion of the slime mold genome is engaged in transcription than in most

TABLE III: Spectral Data.

Absorbance ratio	*Dictyostelium*					HeLa
	Expt 1	Expt 2	Expt 3	Expt 4	Av	
260:240	1.74	1.60	1.72	1.67	1.68	1.40
260:280	1.51	1.46	1.41	1.40	1.44	1.70
320:260	0.12	0.10	0.09	0.12	0.10	0.03

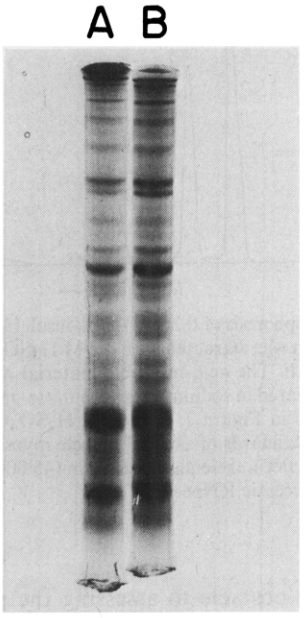

FIGURE 5: Sodium dodecyl sulfate–polyacrylamide gel electrophoresis of *Dictyostelium* chromatin proteins. Chromatin purified on two separate occasions was dissociated in sodium dodecyl sulfate–mercaptoethanol and electrophoresed in 7.5% polyacrylamide gels containing 0.1% sodium dodecyl sulfate for 6.5 h in a field of 10 V/cm. Direction of electrophoretic migration is from top to bottom.

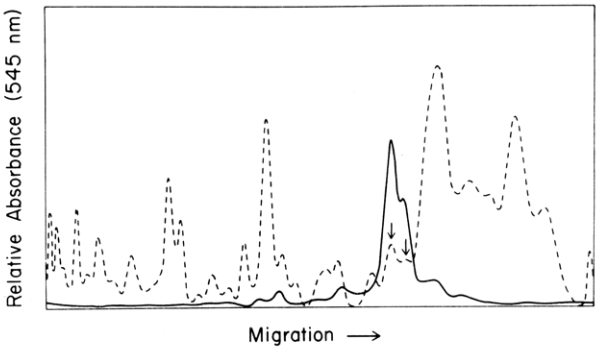

FIGURE 6: Electrophoretic comparison of *Dictyostelium* plasma membrane proteins and chromatin proteins.

higher eukaryotic cells or tissues that have been similarly examined, it is possible that its somewhat elevated content of RNA reflects nascent transcripts. It is also noteworthy that an identical RNA:DNA ratio (0.3) has been reported for chromatin from the plasmodial slime mold, *Physarum* (Mohberg and Rusch, 1970).

Electrophoretic Analysis of Chromatin Proteins. Figure 5 illustrates the sodium dodecyl sulfate–polyacrylamide gel electrophoretic distribution of proteins from two preparations of *Dictyostelium* chromatin purified on separate occasions (1 month apart), and demonstrates the high reproducibility routinely obtained. Comparison with patterns for HeLa cell chromatin proteins electrophoresed under identical conditions (Bhorjee and Pederson, 1972, 1973) reveals that *Dictyostelium* chromatin proteins have a somewhat lower analytical complexity. In particular, the *Dictyostelium* pattern is dominated by five major components against a backdrop of approximately 25 minor but reproducible bands.

As described earlier, [^{3}H]glycerol labeling experiments indicated that the purified chromatin preparation contained less than 0.01 of a percent of the total membrane phospholipid present in the initial cell lysate. To examine possible membrane contamination from the standpoint of protein components, a highly purified *Dictyostelium* plasma membrane preparation was electrophoresed in parallel with chromatin proteins (Figure 6). The membrane preparation (solid curve, Figure 6) contained two major proteins of 26 000 and 29 000 mol wt. The chromatin preparation also contained components at these molecular weights (dashed curve, Figure 6). If it is assumed that the 26 000 and 29 000 mol wt chromatin protein components represent plasma membrane contamination, the extent of this contamination can be estimated to be less than 3% of the total chromatin protein mass. The true extent of plasma membrane contamination could of course be even lower since it is not at all certain that the 26 000 and 29 000 mol wt chromatin protein components are the same polypeptide species as those of the plasma membrane standard.

To better resolve high molecular weight components, the samples used in Figure 5 were electrophoresed longer. As shown in Figure 7A, this resolved a major high molecular weight component with an electrophoretic mobility identical with rabbit skeletal muscle myosin run in a parallel gel (Figure 7B). In addition, another major chromatin protein coelectrophoresed with authentic muscle actin run in parallel. Additional analyses demonstrated that this coelectrophoretic behavior of the two chromatin proteins with muscle myosin and actin was maintained under a variety of polyacrylamide concentrations and migration distances. Using several independent molecular weight standards, the myosin- and actin-like chromatin proteins (arrows in Figure 7A) were estimated to have molecular weights of 210 000 and 45 000, respectively. From densitometric scans of gels such as shown in Figure 7A, the combined myosin- and actin-like components were estimated to account for approxmately 17% of the total chromatin protein mass, and 34% of the protein greater than 30 000 (which as shown below constitutes the acidic, nonhistone protein complement). From the relative intensities of the 210 000 and 45 000 molecular weight components (Figure 7A), it was estimated that there is a sevenfold molar excess of the actin-like protein over the myosin-like component. Of course, all of these considerations assume a constant dye stoichiometry for the chromatin proteins, for which we have no direct evidence. However, autoradiographic analysis of ^{14}C amino acid-labeled HeLa chromatin proteins after electrophoresis reveals a very good correlation between radioactivity per band and dye per band for the nonhistone proteins (T. Pederson, unpublished

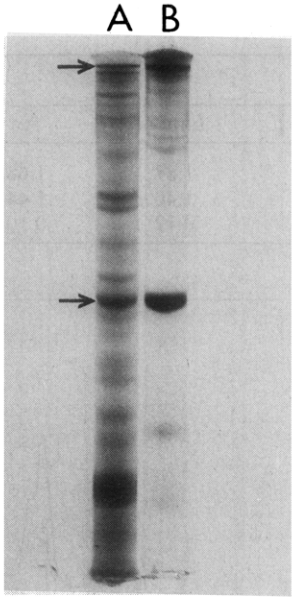

FIGURE 7: Resolution of myosin- and actin-like chromatin proteins. Purified chromatin was dissociated in sodium dodecyl sulfate–mercaptoethanol and electrophoresed as in Figure 5 except for 7.5 h (field = 10 V/cm): (gel A) chromatin proteins; (gel B) standards of skeletal muscle myosin and actin run in parallel. Arrows indicate myosin- and actin-like chromatin proteins.

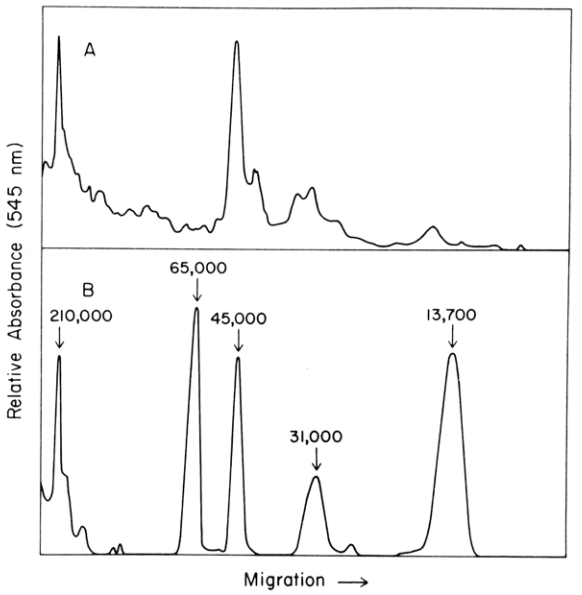

FIGURE 8: Electrophoresis of 0.2 M H_2SO_4-insoluble chromatin proteins. Purified chromatin was extracted with 0.2 M H_2SO_4 at 4 °C in the presence of $PhMeSO_2F$. The acid-insoluble material was collected by centrifugation, dissociated in sodium dodecyl sulfate–mercaptoethanol, and electrophoresed as in Figure 7: (panel A) H_2SO_4-insoluble chromatin proteins; (gel B) standards of skeletal muscle myosin (210 000), bovine serum albumin (68 000), skeletal muscle actin (45 000), pancreatic DNase (31 000), and pancreatic RNase (13 700).

results), suggesting that at least the HeLa nonhistone chromatin proteins have nearly constant dye binding per unit mass.

The results discussed above (Figures 5–7) were obtained with total chromatin proteins. Electrophoretic analysis of the 0.2 M H_2SO_4-insoluble chromatin proteins revealed a substantial enrichment of the myosin- and actin-like proteins over other *nonhistone* polypeptide components greater than 25 000 mol wt. This is shown in Figure 8. This enrichment leads to a combined myosin- and actin-like protein content of 47% of the total nonhistone protein (H_2SO_4 insoluble). The remaining nonhistones present in total chromatin are not recovered in the 0.2 M H_2SO_4-soluble fraction, raising the possibility of a $PhMeSO_2F$-resistant acid protease in *Dictyostelium* chromatin that preferentially attacks the nonmyosin and nonactin acidic proteins.

Discussion

Chromatin Isolation. Although it is sometimes possible to obtain chromatin preparations of good purity from lysates of whole cells or tissues, it is preferable to employ isolated nuclei as a starting material. The latter approach is not without problems either but, as expected, the extent of cytoplasmic protein contamination is considerably lower. For example, we showed that HeLa cell chromatin purified from nuclei has a cytoplasmic protein contamination amounting to only 2% of the total chromatin mass (Bhorjee and Pederson, 1972). The results presented here for *Dictyostelium* indicate that an even smaller fraction (0.3%) of the chromatin protein is attributable to cytoplasmic protein contamination. In contrast, procedures employing whole cells or tissues lead to levels of chromatin contamination by cytoplasmic proteins as high as 30% (see, e.g., Hill et al., 1971). Unfortunately, as discussed under Results, the reconstruction experiments used to arrive at these contamination estimates are themselves not totally satisfactory.

The primary obstacle to assessing the purity of a given chromatin preparation stems from our poor understanding of the macromolecular components that "belong" there and those that do not. For example, it is presumed that an ideal chromatin preparation should lack nuclear membrane, finished transcripts of heterogeneous nuclear RNA (and associated protein), and ribosomal precursor particles. Unfortunately, these sorts of suppositions have no solid empirical footing. Heterogeneous nuclear RNA–ribonucleoprotein particles (hnRNP) are a case in point. We have previously argued (Bhorjee and Pederson, 1973; Pederson, 1974a,b) that, except for nascent hnRNP, these particles should be absent from an "ideal" chromatin preparation, and we have presented methods that accomplish this arbitrary goal. However, there is no convincing evidence arguing against the possibility that hnRNA molecules spend their entire nuclear residence time anchored to chromatin and, if this is true, our methods have merely succeeded in detaching them. A related uncertainty is the physical reality of the conceptual entity termed "nucleoplasm". Moreover, the apparent existence of a structural matrix in eukaryotic nuclei (Berezny and Coffey, 1975) could further compromise a meaningful distinction between chromatin and nonchromatin elements. Because of these difficulties, the purity of chromatin is evaluated by "state-of-the-art" criteria, which must (or at least should) be recognized as arbitrary. These include sedimentation through 60% sucrose,[2] ultraviolet absorption spectra, and chemical composition. By such criteria, *Dictyostelium* chromatin isolated as described

[2] Upon tissue and organelle disruption, chromatin remains as the most highly sedimentable macromolecular constituent from most cells, which constitutes the basis for chromatin isolation procedures that employ whole cells or tissues as starting materials. The property also serves as a reasonably sound diagnostic feature of chromatin prepared from isolated nuclei.

here resembles preparations from metazoa. The RNA content is somewhat higher than generally observed, but this is the most variable of the chromatin compositional parameters that are routinely measured (Dingman and Sporn, 1964). Chromatin from the acellular (plasmodial) slime mold, *Physarum* (considered to be only a distant relative of the cellular slime molds), has been reported to have a similarly high RNA content (Mohberg and Rusch, 1970). However, the significance of this latter value is somewhat uncertain since the chromatin protein:DNA ratio in this case was reported to be 10, as compared to the more conventional value of 2.3 reported here for *Dictyostelium*.

Actin- and Myosin-Like Proteins. Actin- and myosin-like proteins have been reported in *Physarum* nuclei (LeSturgeon et al., 1975) and rat liver chromatin (Douvas et al., 1975). In the former report, but to a lesser extent in the latter, the evidence constituted a reasonable case that these proteins were authentic, muscle-like, actin and myosin. *Physarum* displays acytokinetic mitosis and an intranuclear spindle. If contractile proteins are associated with tubulin in spindle fibers and participate in the mitotic process, the existence of intranuclear actin and myosin in this creature is not particularly surprising. As reported here, two of the major nonhistone proteins that purify with *Dictyostelium* chromatin coelectrophorese precisely with standards of authentic muscle actin and myosin. However, in none of these cases has the nuclear localization of these proteins been rigorously established. Moreover, in all of the nonmuscle cells examined so far by immunofluorescent probes, actin and myosin are predominantly cytoplasmic, and direct biochemical analysis also reveals large cytoplasmic depots of actin and myosin in *Dictyostelium* (Clarke and Spudich, 1974; Spudich, 1974). Of course these results do not demonstrate an exclusive cytoplasmic localization, but they do suggest that observations of nuclear actin and myosin should be interpreted with caution. Although the actin- and myosin-like protein contents of *Dictyostelium* chromatin reported here exceed by two orders of magnitude the observed level of cytoplasmic protein contamination, the reconstruction experiments aimed at establishing the extent of contamination deal only with soluble proteins. If large amounts of *Dictyostelium* cytoplasmic actin and myosin are anchored or adhere to the outer face of the nuclear envelope, only a small copurification of nuclear membrane vesicles with chromatin would be needed to generate the observed results. Therefore, before testing further the similarity of these chromatin proteins to authentic actin and myosin, which we view as virtually certain, we are proceeding to map the intracellular distribution of actin and myosin in situ using immunological probes at the electron microscope level.

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