O'Malley, B. W. (1975), *Biochemistry 14,* 69.
Sharp, P. A., Sugden, B., and Sambrook, J. (1973), *Biochemistry 12,* 3055.
Shatkin, A. J. (1962), *Biochim. Biophys. Acta 61,* 310.
Singer, H., and Penman, S. (1973), *J. Mol. Biol. 78,* 321.
Spector, D. H., and Baltimore, D. (1975), *J. Virol. 15,* 1418.
Weil, P. A., and Hampel, A. (1973), *Biochemistry 12,* 4361.
Weinberg, R., and Penman, S. (1968), *J. Mol. Biol. 38,* 289.
Wellauer, P. K., and Dawid, I. B. (1973), *Proc. Natl. Acad. Sci. U.S.A. 70,* 2827.
Willems, M., and Penman, S. (1966), *Virology 30,* 355.
Zimmerman, E. F., Heeter, M., and Darnell, J. E. (1963), *Virology 19,* 400.

Serological Analysis of the H3–H4 Histone Complex†

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ABSTRACT: The H3–H4 histone complex has been prepared by mild methods of salt extraction from calf thymus chromatin. Its composition was characterized electrophoretically and its size corresponded to that of a tetramer. It was studied serologically with antibodies to both acid-extracted histones and to the complex itself. In quantitative complement fixation reactions, antibodies to the H3–H4 histone complex reacted more strongly with the complex than with either acid-extracted H3 or H4 alone, or a 1:1 mixture of the two histones. Antibodies to isolated H3 or H4 reacted at least as effectively with the complex as with the individual histones; however, in both cases, 1:1 mixtures of the H3 and H4 were less reactive than either the homologous histone alone or the salt-extracted preparation. The results seen with all three antibody systems indicated that reactions of the salt-extracted complex were not simply the sum of reactions of the H3 and H4 components. This suggested that unique structural features are present in the tetramer. Antibodies to the H3–H4 complex or to acid-extracted H3 or H4 did not give complement fixation reactions with chromatin, but differed in reactivity with chromatin as measured by an absorption assay. Whereas antibodies induced by the H3–H4 complex were readily absorbed by insoluble chromatin, those induced by acid-extracted H3 or H4 were not. Both the electrophoretic and serological properties of the H3–H4 preparation remained stable during storage in 50 mM sodium acetate, pH 5, at −20 °C for 1 year and after repeated freezing and thawing.

In recent years, a great deal of attention has been focused on the role of histone–histone interactions in maintaining the structure of chromatin. Chromatin fragments prepared by nuclease digestion (Hewish and Burgoyne, 1973; Noll, 1974; Sahasrabuddhe and Van Holde, 1974) have been seen by electron microscopy to consist of a series of uniform beadlike structures (Olins and Olins, 1974). Kornberg (1974) proposed that these beads are the primary structural subunits of chromatin and are composed of an octamer of two each of histones H2A, H2B, H3, and H4, around which the DNA of chromatin is wrapped. This model further proposed that the structure consists of a unique $(H3)_2(H4)_2$ tetramer and a linear H2A–H2B oligomer (Kornberg, 1974).

The association of H3 and H4 in a tetramer or dimer–tetramer equilibrium has been characterized further (Roark et al., 1974; D'Anna and Isenberg, 1974; Sperling and Bustin, 1975; Thomas and Kornberg, 1975; Lewis, 1976a) and found to be the most strongly associating of several possible histone–histone interactions that can occur in solution (D'Anna and Isenberg, 1974; Sperling and Bustin, 1975). It provides the minimal requirement for association with DNA in a way that reconstitutes some aspects of native chromatin structure (Camerini-Otero et al., 1976). Further, the association prevents self-aggregation of the individual histone components (Rubin and Moudrianakis, 1975; Sperling and Bustin, 1975). This suggests there may be a unique structure for the H3–H4 complex and raises the question of whether the complex, as isolated from chromatin under mild conditions, has the same structure as the complex that is reconstituted from individual acid-extracted components. In this article, we report serological studies of this question. Immunizing rabbits with H3–H4 histone–RNA complexes (Stollar and Ward, 1970), we obtained antibodies to the H3–H4 complex, as did Mihalakis et al. (1976). With these antisera and antisera to the isolated H3 and H4, we have found that the H3–H4 complex isolated by the mild procedures of van der Westhuyzen and von Holt (1971) differs from a mixture of the two acid-extracted histones.

Materials and Methods

Histone Isolation. Chromatin was prepared from fresh calf thymus (50 g) by the method of Busch (1968), and histones were then extracted by a modification of the procedure of van der Westhuyzen and von Holt (1971). All procedures were carried out at 4 °C. Briefly, histones were extracted from chromatin with 2.0 M NaCl–50 mM sodium acetate–50 mM sodium bisulfite. DNA was precipitated by the addition of protamine sulfate (Sigma, grade I, recrystallized two times from H_2O). The resulting supernatant, containing histones and excess protamine, was applied to a Sephadex G-50 column (8.5 × 100 cm) and eluted with 50 mM sodium acetate–50 mM

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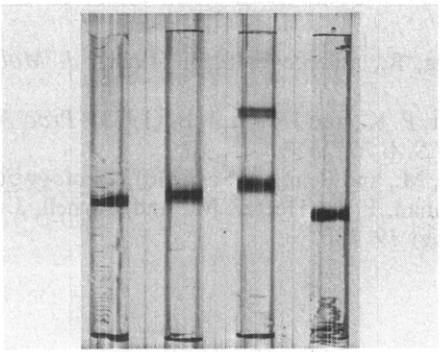

FIGURE 1: Polyacrylamide gel electrophoresis of acid-extracted histones. Samples of 50 μg of each histone were run in 15% polyacrylamide gels containing 2.5 M urea, according to Panyim and Chalkley (1969). Samples are (from left to right) H2A, H2B, H3, and H4. The H3 sample for this electrophoresis run was concentrated by lyophilization and oxidized H3 (top band in H3 gel) appeared. The amount of oxidized material was significantly reduced when the sample was concentrated for electrophoresis by dialysis.

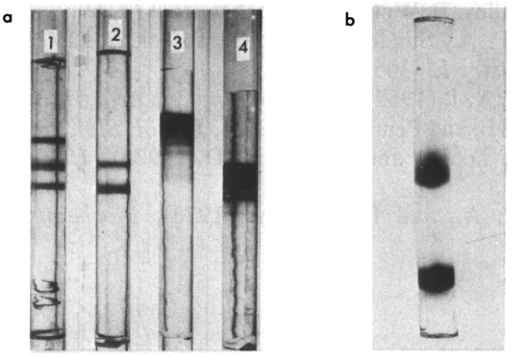

FIGURE 2: Electrophoretic characterization of the composition and purity of the H3–H4 complex. (a) Acid–urea gel system. 15% polyacrylamide gels containing 2.5 M urea were run according to Panyim and Chalkley (1969). Samples are (1) standard from lyophilized H1 + H3 + H4; (2) 25 μg of protein from the H3–H4 pellet following $(NH_4)_2SO_4$ treatment of the first Sephadex G-100 column peak; (3) 100 μg of protein from supernatant following $(NH_4)_2SO_4$ treatment of the first Sephadex G-100 column peak; (4) 200 μg of protein from the same sample as 2. (b) Triton–urea gel system (Alfageme et al., 1974). A sample of 1.3 mg of the H3–H4 complex was applied to a 1.2 × 11 cm gel of 7.5% polyacrylamide containing 8 M urea and 6 mM Triton X-100. Electrophoresis was run at 150 V for 5 h.

sodium bisulfite, pH 5. The resultant histone-containing peak was concentrated by ultrafiltration on an Amicon PM-10 membrane and fractionated on a 4 × 80 cm Sephadex G-100 column, developed with 50 mM sodium acetate, pH 5. The omission of sodium bisulfite from this and all subsequent buffers was necessary in order to prevent significant pH changes, resulting from oxidation of the bisulfite (Geoghegan, 1974). In the absence of sodium bisulfite, histones were fractionated into (H1,H3–H4)- and (H2A–H2B)-containing peaks on Sephadex G-100. When the first peak was treated with 70% saturated ammonium sulfate, H3–H4 precipitated while H1 remained in solution. The precipitate was redissolved in 50 mM sodium acetate, pH 5, and dialyzed extensively against the same buffer prior to storage at −20 °C.

Acid-extracted histones for use as standards were prepared according to Johns (1964) and individual fractions were further purified on preparative scale (1.2 × 11 cm) polyacrylamide gels in the presence of 2.5 M urea, pH 2.7 (Panyim and Chalkley, 1969). When the resultant fractions were rerun on analytical gels (0.5 × 7.5 cm) under the same conditions, each histone was shown to be free of contamination by any other histone or histone fragment (Figure 1).

Polyacrylamide Gel Electrophoresis. Electrophoresis was performed according to the procedure of Panyim and Chalkley (1969) or with 8 M urea–6 mM Triton X-100 as described by Alfageme et al. (1974). Electrophoresis was also performed under the nondenaturing conditions of Lewis (1976b). These 10% polyacrylamide gels were run at 4 °C with 0.05 M sodium phosphate buffer, pH 7.

Protein Determination. Protein concentration was determined by either the microbiuret method of Itzhaki and Gill (1964) or the microprotein method of Schaffner and Weissman (1973). Lyophilized whole histone from calf thymus (Worthington Biochemical Corp.) was used as a standard at all times.

Molecular Weight Determination. The molecular weight of the H3–H4 complex was determined on a Bio-Gel P-100 column (1.7 × 100 cm) calibrated with ribonuclease, chymotrypsinogen, ovalbumin, and blue dextran as standards. The column was eluted at room temperature with 50 mM sodium acetate, pH 5.

Immunological Methods. Antiserum against ammonium sulfate precipitated H3–H4 was obtained by immunizing female New Zealand white rabbits with complexes of 500 μg of H3–H4 and 150 μg of yeast RNA (Sigma) according to Stollar and Ward (1970). The complexes were emulsified with an equal volume of complete Freunds adjuvant (Difco) and two injections were given, 14 days apart, at multiple intradermal sites. An intravenous booster without adjuvant was given 7 days after the second intradermal injection and each rabbit was bled 5 days later. The schedule of iv boosts and bleeds was repeated a total of four times, at monthly intervals.

Antisera against individual acid-extracted histones were previously prepared in our laboratory (Stollar and Ward, 1970). All sera were heat inactivated at 56 °C for 15 min.

Quantitative microcomplement fixation was performed according to Stollar (1977), with a buffer of 0.14 M NaCl, 0.01 M Tris,[1] pH 7.4, 0.5 mM Mg^{2+}, and 0.15 mM Ca^{2+} (Isotris-M) or the same buffer with 0.1% bovine serum albumin (Isotris–BSA). For complement fixation tests in which chromatin was used as an immunoadsorbent, the procedure of Goldblatt and Bustin (1975) was followed.

Mixing Experiments. Purified acid-extracted H3 and H4 were mixed in varying molar ratios in either H_2O or 10 M urea and then dialyzed extensively against the complement fixation buffer Isotris-M at 4 °C. Alternatively, histones H3 and H4 were simply mixed in the desired ratios in H_2O or Isotris-M, without dialysis.

Results

Characterization of the H3–H4 Complex. The first peak from the Sephadex G-100 column contained histones H1, H3, and H4, but no H2A or H2B, as tested by polyacrylamide gel electrophoresis with an acid–urea gel system. In the next preparative step, the separation of H3 and H4 from H1 by precipitation with 70% saturated ammonium sulfate was complete; even when an analytical gel was heavily loaded, the H3–H4 precipitate was found to be free of H1 (Figure 2a). In a more extreme test, when a sample of 1.3 mg was examined on a 1.2 × 11 cm gel run with Triton X-100 and urea, only the H3 and H4 bands were seen (Figure 2b).

While the analyses performed with acid–urea or Triton–urea gels demonstrated the presence of only H3 and H4 in the

[1] Abbreviations used are: Tris, 2-amino-2-hydroxymethyl-1,3-propanediol; BSA, bovine serum albumin.

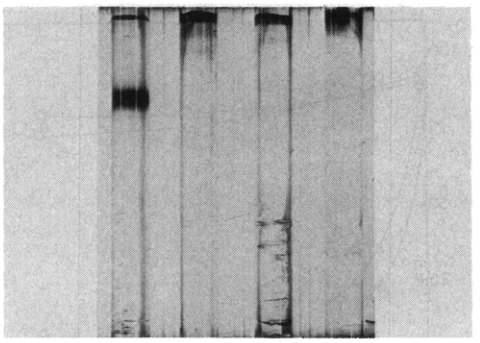

FIGURE 3: Nondenaturing polyacrylamide gels according to Lewis (1976b), run at 100 V, 16 h, 4 °C. From left to right, samples are H3–H4 complex, acid-extracted H3, acid-extracted H4, and acid-extracted H3 + H4 (1:1).

TABLE I: Reaction of H3–H4 Histone Complex with Antihistone Sera.

Antiserum	Maximum % C Fixation	
	H3–H4 complex	Homologous histone
Anti-H1 (1/6000)	0	72
Anti-H2A (1/1800)	6	56
Anti-H2B (1/1800)	0	86
Anti-H3 (1/9000)	75	26
Anti-H4 (1/21000)	85	88

TABLE II: Comparison of the Reactivity of Histones H3 and H4 Mixed in Various Ratios with that of Isolated Histone Complexes.

	Maximum % C Fixation	
	Anti-H3 (1/4500)	Anti-H4 (1/15000)
H3	89	
H3:H4, 2:1	75	17
H3:H4, 1:1	43	32
H3:H4, 1:2	26	35
H4		53
H3–H4 complex	90	72

salt-extracted preparation, analysis with a nondenaturing gel system was used to test whether the components were present as a complex. Under these conditions, the H3–H4 migrated as a single discrete band, while either isolated H3 or H4 or a mixture of the two histones in a 1:1 molar ratio aggregated and remained at the top of the gel (Figure 3).

That H3 and H4 were in fact present as a complex was further demonstrated by running the complex on a Bio-Gel P-100 column in 50 mM sodium acetate, pH 5, in comparison with known standards. The complex eluted with an apparent molecular weight of 48 000, which is consistent with the composition $(H3)_2(H4)_2$.

Serological Reactions with Antibodies to Isolated Histone Components. The purity of the H3–H4 preparation was further demonstrated serologically. When antisera to individual acid-extracted histones were used at concentrations that gave strong homologous complement fixation reactions, the H3–H4 preparation reacted only with anti-H3 and anti-H4 sera (Table I). With both sera, the H3–H4 complex reacted as well as or better than the isolated histone antigens (Table I; Figure 4). The salt-extracted complex, however, was different than a simple mixture of acid-extracted H3 and H4. With both the

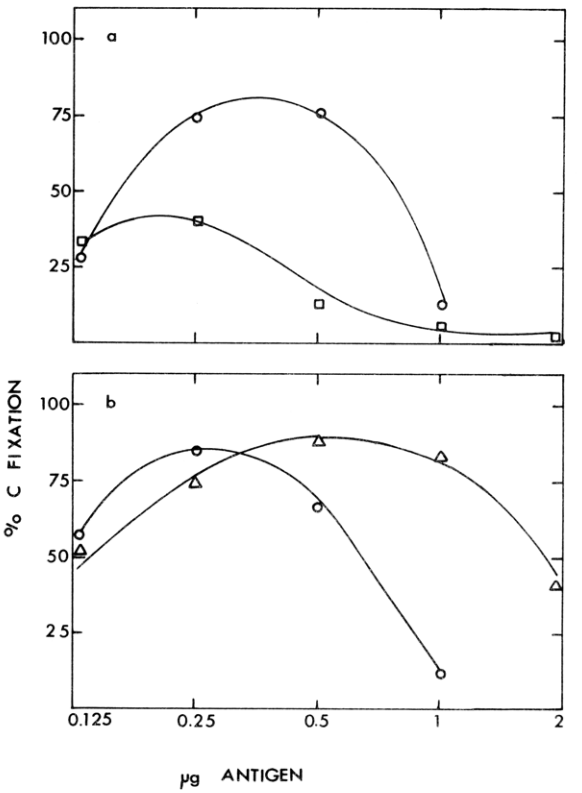

FIGURE 4: Complement fixation reaction between H3–H4 complex and antihistone sera. (a) Anti-H3 1/9000; H3–H4 complex (○), acid-extracted H3 (□). (b) Anti-H4 1/9000; H3–H4 complex (○), acid-extracted H4 (△).

anti-H3 and anti-H4 systems, addition of the second histone inhibited the homologous reaction. The inhibition increased as the amount of the second histone was increased, and was extensive in a 1:1 mixture (Table II). The same inhibition was observed whether the H3 and H4 were simply mixed in the complement fixation buffer, mixed in H_2O and dialyzed against the buffer, or mixed in 10 M urea and then dialyzed stepwise into buffer. The more effective reactivity of the isolated H3–H4 complex as compared to these histone mixtures was consistent with the concept that there is a unique structural relationship between the H3 and H4 in the complex. This was tested further with antiserum to the complex itself.

Reactions with Antiserum to the Salt-Extracted H3–H4 Complex. Antiserum induced by the H3–H4 complex reacted strongly with the immunogen and clearly distinguished between the complexes and the individual components. Complement fixation reactivity with the complexes persisted at a 1/9000 serum dilution. Histone H3 did not react with this serum dilution, but was the only acid-extracted component that reacted with serum diluted 1/3000. The H4 reacted with a 1/900 serum dilution, while H1, H2A, and H2B were not reactive even with this amount of serum.

The serum also distinguished between the isolated complexes and mixtures of acid-extracted H3 and H4. When several samples with varying proportions of H3 and H4 were tested, these mixtures showed a reactivity close to that of H3 alone (Figure 5). At this dilution, the H3–H4 complex showed virtually complete complement fixation with a wide range of antigen concentration, and it was still reactive with a sevenfold higher serum dilution, at which the artificial mixtures were not detectably reactive at all.

The more effective serological reactivity of the salt-extracted H3–H4 was a stable property and persisted through a year of

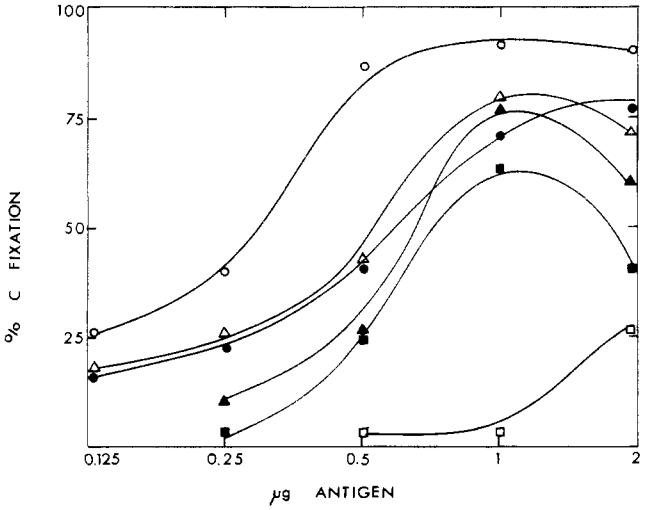

FIGURE 5: Complement fixation reactions between anticomplex serum and various mixtures of histones H3 and H4. Serum was diluted 1/1200 and antigens were H3–H4 complex (○); acid-extracted H3 (△); acid-extracted H4 (□); H3:H4, 2:1 (●); H3:H4, 1:1 (▲); and H3:H4, 1:2 (■).

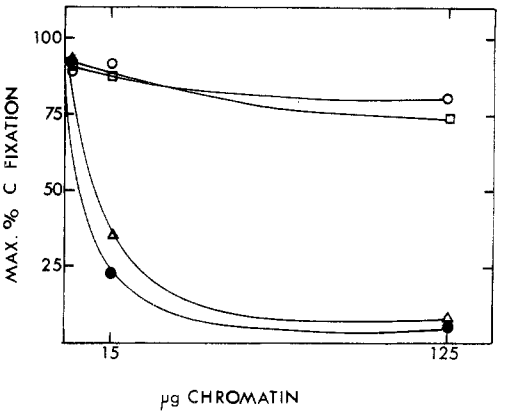

FIGURE 6: Absorption of antihistone antibodies by chromatin. Maximal complement fixation of untreated sera and sera absorbed with 15 or 125 μg of chromatin, when sera were tested with their homologous antigens. Sera of varying potency were adjusted by dilution before absorption so that similar levels of reactivity were present in each case (Goldblatt and Bustin, 1975). Sera were: anti-H1 (△); anti-H3 (□); anti-H4 (○); and anticomplex (●).

sample storage at −20 °C and through several freeze-thaw cycles. The electrophoretic properties described above were also stable under these conditions.

Chromatin Studies. When chromatin was used as an antigen in complement fixation reactions involving anti-H3, anti-H4, or anticomplex sera, no reaction was detected at antiserum dilutions that gave 100% complement fixation with the homologous antigens. It remained possible that some antigenic determinants were in fact accessible in chromatin, but that their number or distribution did not allow the aggregation required for complement fixation. To test this possibility, the immunoadsorption technique of Goldblatt and Bustin (1975) was used. Sera were absorbed with chromatin and retested in complement fixation against homologous histone antigen. While 125 μg of chromatin did not remove significant amounts of antibody from either anti-H3 or anti-H4 serum, as little as 15 μg effectively removed antibody to the H3–H4 complex (Figure 6). The reactive determinants of the complex were thus as accessible as the H1 histone which, as had been previously observed by Goldblatt and Bustin (1975), was also effective in absorbing homologous antibody.

Discussion

The main findings of these experiments are that serological differences between individual or mixed acid-extracted histones H3 and H4 and the salt-extracted H3–H4 complex can be seen with either the antihistone or anticomplex serum system. This conclusion requires characterization of the purity of the reagents involved; through the use of polyacrylamide gel electrophoresis under denaturing conditions, with heavily loaded gels, we have shown that the H3–H4 complex and the isolated H3 and H4 were free of contamination by any other histones or histone complexes. The behavior of salt-extracted H3–H4 on Sephadex G-100, where the histone components not only comigrate but also elute with the larger histone H1, indicates a specific interaction between the two histones. This interaction is further demonstrated by the appearance of a single band on nondenaturing polyacrylamide gels and by the behavior of the H3–H4 complex on a Bio-Gel P-100 column.

Some aspects of the structure of histone H3 or H4 are not drastically altered in the H3–H4 complex as compared to the acid-extracted forms. Both anti-H3 and anti-H4 sera react well with the respective histone components as they exist in the complex. That this reactivity is greater than that of the homologous systems may be due to the presence of all the major antigenic determinants in a favorable conformation, without self-aggregation. With the anticomplex serum, reactivity of acid-extracted H3 is much greater than that of acid-extracted H4, but neither of these antigens reacts as well as does the homologous system. This is parallel to the results of Mihalakis et al. (1976), who showed that, while antiserum prepared against acid-extracted H1 was unable to see differences between the acid- and salt-extracted antigen, antiserum prepared against salt-extracted H1 was able to detect the differences.

More striking is the difference between the H3–H4 complex and an equimolar mixture prepared from the two acid-extracted components. While association does occur between the isolated histones, it is apparently in a way that masks the antigenic determinants as seen with either the anti-H3 or anti-H4 serum. In a similar way, use of the anticomplex serum indicates that none of the synthetic mixtures was able to reconstitute the more native structure of the salt-extracted complex. These results also indicate that the stronger reactivity of the complex does not represent a simple summation of reactions with separate H3 and H4 components.

These observations have been extended in a preliminary way to an investigation of the structure of histones in chromatin. In an absorption assay, only the anticomplex antibodies and not the antibodies to isolated histones were reactive with chromatin. This further indicates that antibodies to the H3–H4 complex react with determinants that are distinct from those expressed on the acid-extracted histones and that may depend on the native structure of the complex.

A reagent such as the antiserum prepared against the salt-extracted H3–H4 complex will be useful for further study of histone–histone organization in chromatin, as well as for determining whether conditions can be designed to allow successful reconstitution of native H3–H4 from isolated histone components.

References

Alfageme, C. R., Zweidler, A., Mahowald, A., and Cohen, L. H. (1974), *J. Biol. Chem. 249,* 3729.

Busch, H. (1968), *Methods Enzymol. 12,* 68.

Camerini-Otero, R. D., Sollner-Webb, B., and Felsenfeld, G. (1976), *Cell 8,* 333.
D'Anna, J. A., and Isenberg, I. (1974), *Biochemistry 13,* 4992.
Geoghegan, T. E. (1974), Ph.D. Thesis, Pennsylvania State University.
Goldblatt, D., and Bustin, M. (1975), *Biochemistry 14,* 1689.
Hewish, D. R., and Burgoyne, L. A. (1973), *Biochem. Biophys. Res. Commun. 52,* 504.
Itzhaki, R. F., and Gill, D. M. (1964), *Anal. Biochem. 9,* 401.
Johns, E. W. (1964), *Biochem. J. 92,* 55.
Kornberg, R. D. (1974), *Science 184,* 868.
Lewis, P. N. (1976a), *Biochem. Biophys. Res. Commun. 68,* 329.
Lewis, P. N. (1976b), *Can. J. Biochem. 54,* 641.
Mihalakis, N., Miller, O. J., and Erlanger, B. F. (1976), *Science 192,* 469.
Noll, M. (1974), *Nature (London) 251,* 249.
Olins, A. L., and Olins, D. E. (1974), *Science 183,* 330.
Panyim, S., and Chalkley, R. (1969), *Arch. Biochem. Biophys. 130,* 337.
Roark, D. E., Geoghegan, T. E., and Keller, G. H. (1974), *Biochem. Biophys. Res. Commun. 59,* 542.
Rubin, R. L., and Moudrianakis, E. N. (1975), *Biochemistry 14,* 1718.
Sahasrabuddhe, C. G., and Van Holde, K. E. (1974), *J. Biol. Chem. 249,* 152.
Schaffner, W., and Weissman, C. (1973), *Anal. Biochem. 26,* 502.
Sperling, R., and Bustin, M. (1975), *Biochemistry 14,* 3322.
Stollar, B. D. (1977), *Methods Cell Biol. 9* (in press).
Stollar, B. D., and Ward, M. (1970), *J. Biol. Chem. 245,* 1261.
Thomas, J. O., and Kornberg, R. D. (1975), *Proc. Natl. Acad. Sci. U.S.A. 72,* 2626.
van der Westhuyzen, D. R., and von Holt, C. (1971), *FEBS Lett. 14,* 333.

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.