ISOLATION AND CHARACTERIZATION OF CHROMATIN FROM *NEUROSPORA CRASSA*

R. S. DWIVEDI, S. K. DUTTA, and D. P. BLOCH

From the Department of Botany and Cell Research Institute, The University of Texas, Austin, Texas 78712, and the Department of Botany, Howard University, Washington, D. C. 20001

ABSTRACT

Different preparations of chromatin isolated from mycelia of *Neurospora crassa* were analyzed for DNA-associated RNA and proteins. The UV absorption spectra, the ultrastructure of chromatin, and the amino acid composition of the acid-extractable proteins were studied. The protein:DNA ratios range from 1.5 to 2.8; the RNA:DNA ratios range from 0.5 to 1.24. UV absorption shows a macimum at 259 mμ and a minimum at 238–239 mμ. The E280/E260 ranges from 0.59 to 0.70. Electron microscopy reveals a fibrous structure with individual fibers of 120–150 A average diameter. Attempts were made to study the protein by polyacrylamide gel electrophoresis and amino acid analysis. The results indicate that *Neurospora* chromatin does not contain basic proteins comparable to calf thymus histone. The ratios of basic to acidic amino acids range from 0.93 to 1.19. On electrophoresis, no bands are seen whose positions correspond to those of histones. Staining for basic proteins with fast green or eosin Y at pH 8.2 also shows a negative reaction, suggesting the absence of histones.

Histones are found in the nuclei of most eukaryotic cells, a few notable exceptions being the protozoan dinoflagellate *Amphidinium* (Ris, 1962), the ctenophore *Beroë* (Cruft, 1966), some parasitic protozoans (Stewart and Beck, 1967), and the mature sperms of some crabs (Chevaillier, 1966; Vaughn et al., 1969). Bacteria, on the other hand, are probably devoid of histones. Although there have been numerous reports of histones in bacteria (Mirsky and Pollister, 1946; Palmade et al., 1958), most of these reports describe work done prior to the recognition of ribosomes as a possible source of basic proteins; more recent work in which pains have been taken to avoid contamination from this source has failed to reveal the presence of these proteins (Zubay and Watson, 1959; Wilkins and Zubay, 1959). The difference in the chromosome constitution of these two great classes of organisms has led to the speculation that histones perform a role or roles unique to eukaryotic cells, such as chromosome organization (Zubay, 1963; Cole, 1962), tissue differentiation (Stedman and Stedman, 1943), or gene regulation, perhaps of a type peculiar to eukaryotes (Bonner et al., 1968).

For these reasons, an attempt was made to determine whether histones exist in *Neurospora crassa*. This fungus, like all fungi, is eukaryotic. Its genome is comprised of discrete linkage units (Barrat et al., 1954) that behave morphologically like the organized chromosomes of most eukaryotes. Differentiation, however, is rudimentary, perhaps more akin to changes in bacteria than in higher organisms that exhibit complete and stable tissue differentiation. Little is known of the composition of fungal chromatin. Tonino and Rozijn (1966) have reported the presence of a weakly basic protein in yeast which they consider histone. Bloch (1966) reported a positive alkaline fast green reaction of the nuclei of the fruiting body of an unknown basidiomycete. The results of a recent study

of *Allomyces arbuscula* (Stumm and van Went, 1968), on the other hand, suggest the absence of histone in this fungus. To our knowledge, however, there have been no other reports of serious nature to characterize fungal chromatin with regard to presence or absence of histones. A preliminary report of some of the results presented here has been made earlier by Dwivedi et al. (1968) and Dutta and Dwivedi (1968).

MATERIALS AND METHODS

Neurospora crassa wild strain 74 A was used in these studies. Conidial cultures were maintained on *Neurospora* medium (Difco Laboratories, Detroit, Mich.) or complete medium with 1.5% agar (Vogel, 1956). Mycelia were grown in 4-liter flasks with continued aeration in Vogel's complete medium (Vogel, 1956) and were collected after 48–60 hr. The mycelia were stained through two layers of cheesecloth, were washed thoroughly in water, and finally washed in a chilled solution of sucrose-EDTA (0.5 M sucrose, 1 mM Na ethylenediaminetetraacetic acid (EDTA), 0.01 M Tris-HCl, pH 6.5). Unless otherwise stated, all the subsequent operations were carried out at 4°C. The fungal mat was squeezed dry by pressing between paper towels, chopped into small pieces, and finally ground gently with 2–3 times its weight of fine, acid-washed sea sand (Merck Chemical Div., Rahway N. J.) until a smooth paste was obtained. 5 volumes of sucrose-EDTA solution, pH 6.2, were added gradually; the mixture was stirred into a thick paste that was strained first through eight layers of cheesecloth and then through four layers of silk.

The filtrate was centrifuged at 2000 *g* for 30 min in an International centrifuge (International Equipment Co., Needham Heights, Mass.). The pellet, a crude preparation of nuclei, was purified according to the method of Munkres et al. (1966) with some modifications. The nuclear pellet was suspended in 10 volumes of 0.5 M sucrose, 5 mM $CaCl_2$, 2 mM Na-EDTA at pH 6.2. The broken hyphae and conidia were discarded as a hard pellet after centrifuging for 2 min at 5000 *g*. The fluffy layer was stirred in its supernatant, and the centrifugation was repeated two or three times. The fluffy nuclear pellet was finally resuspended in its own supernatant, and 10 ml of the suspension was layered over 5 ml of 1.65 M sucrose and centrifuged for 1 hr at 2200 *g*. The pellet (referred to as 2000 *g* pellet hereafter) represents a purified nuclear preparation. The yield of nuclei, however, is very low with this isolation procedure (see Table I). A large number of nuclei are retained in the 2000 *g* supernatant, most of which can be collected according to the method of Reich and Tsuda (1961), i.e., centrifuging the 2000 *g* supernatant for 30 min at 5000 *g*, resuspending the pellet, and recentrifuging it at 3000 *g* for a short time. In our experience such a nuclear pellet is always contaminated with mitochondria even when centrifuged through 1.65 M sucrose at 3000 *g* for 1 hr (referred to as 5000 *g* pellet hereafter), and consistently gives higher proportions of protein and RNA as compared to the 2000 *g* pellet (see Table II). The nuclear pellet was then suspended and stirred for 1½ hr in 10 volumes of saline-EDTA (0.08 M NaCl, 0.02 M Na-EDTA, pH 6.2), sedimented at 2000 *g*, and resuspended and stirred in fresh solution of saline-EDTA containing 0.01% Triton-X-100, for 15–20 min. By the end of this treatment most of the nuclei become lysed. The lysate was collected by centrifuging at 2000 *g* for 30 min. The pellet was resuspended in 5 volumes of Tris-EDTA (10 mM Tris, 10 mM Na-EDTA, pH adjusted to 7.8 with HCl) and homogenized vigorously in a glass homogenizer. Fresh Tris-EDTA solution was added to make a total of 10 volumes, and the mixture was stirred overnight in the cold room. Crude chromatin was obtained as a clear supernatant after centrifuging at 105,000 *g* for 1 hr. Chromatin was purified by 50% 1,4-dioxane precipitation, i.e., by adding equal volumes of this reagent to crude chromatin solution. Adjusting the pH to 5.0 gave equally satisfactory results. The dioxane precipitation was preferred over 0.15 N NaCl precipitation, since in very dilute solutions NaCl precipitation was not found to be very effective. The dioxane precipitate was washed with ethanol and redissolved in 0.2 mM Na-EDTA, pH 7.1. This solution was used for various tests as such or after further purification by 0.15 N NaCl precipitation, which works better at this stage as the chromatin solution is concentrated.

A Beckman DU spectrophotometer was used for ultraviolet absorption and colorimetric analyses. DNA was estimated with the diphenylamine reaction (Burton, 1956) as modified by Kupila et al. (1961), RNA with the orcinol procedure (Mejbaum, 1939), and total protein by the Lowry et al. (1951) method. In all chemical analyses, duplicate samples were taken, and standard curves were made for each run. Highly polymerized salmon sperm DNA and yeast RNA (both purchased from Calbiochem, Los Angeles, Calif.), and bovine serum albumen (Pentex, Inc., Kankakee, Ill.) were used as standards.

Attempts were made to extract basic proteins from chromatin according to the methods of Dingman and Sporn (1964) and Leboy et al. (1964) as described under *Results*. Polyacrylamide gel electrophoresis was done according to the method of Reisfeld et al. (1962). The small pore gel was made with 7.5% acrylamide. Ammonium persulfate, 0.28%, was made in 8 M urea of pH 4.5, adjusted with acetic acid. Electrophoresis was carried out at room temperature or at 4°C with a current of 4–6 ma/tube. Calf thymus histone was used as a standard in each run.

Electron Microscopy

A dilute solution of chromatin in 0.2 mM EDTA was spread on a clean air-water interface and was picked up on Butvar 98 (0.5% in chloroform)-coated grids. Alternately, the grids were floated on a drop of chromatin solution. Excess solution was removed by touching the sides of the grids to filter paper, and then the preparations were fixed in either 3% solution of glutaraldehyde or 0.2% osmium tetroxide, both in 0.01 M phosphate buffer pH 7.8, by floating the grids on the drops of these fixatives for 20–30 min. Following a brief rinse in water, the preparations were stained in 0.5% uranyl acetate for 15–20 min and subsequently examined and photographed in an RCA EMU 3D electron microscope.

RESULTS

The analytical data on the composition of *Neurospora* chromatin are presented in Tables I, II, and III. As compared to those of higher organisms, the relative amounts of RNA complexed with DNA are higher. The proteins show similar proportions. However, there is an important difference between the protein complex of *Neurospora* chromatin and that reported for the chromatin of higher organisms. In the latter, histones form an essential part of these constituent proteins; in *Neurospora*, histone is apparently lacking as is indicated by the lack of bands seen on electrophoresis in polyacrylamide gels at pH 4.5. For electrophoresis, extraction of basic proteins from chromatin was attempted by two methods.

(*a*) The first method employed was NaCl-HCl extraction according to Dingman and Sporn (1964). 3 volumes of *Neurospora* chromatin solution were mixed with 2 volumes of 5 N NaCl and allowed to stand for at least 1 hr. The solutions were then adjusted to 0.2 N with respect to HCl and set in the cold overnight.

(*b*) The alternate method was LiCl-urea extraction according to Leboy et al., (1964). 1.2 volumes of 4 M LiCl in 8 M urea were added to 1 volume of *Neurospora* chromatin solution and held overnight in an ice bath.

Both extractions were followed by a 10-min centrifugation at 20,000*g*. The protein solutions were obtained as clear supernatants, which were used for electrophoresis after they had been concentrated in a flash evaporator. The protein contents of supernatants as estimated by the Lowry et al. (1951) method are given in Table IV. It is clear from these data that only a small fraction of proteins are extracted by NaCl-HCl which preferentially extracts basic proteins (Dingman and Sporn, 1964). The yields are higher with LiCl-urea extraction, which is expected, since many other proteins besides basic proteins would be extracted by such reagents.

The amino acid composition of NaCl-HCl extracts from two separate experiments are presented in Table V. The overall composition of these proteins with a ratio of basic/acidic amino acids of approximately 1.1, suggests that these proteins are neutral rather than basic. Certainly these proteins

TABLE I

*Protein Content of Fractions Isolated from Neurospora crassa Mycelia**

No.	Fraction	Total protein	
		mg	%
1.	Filtered homogenate	477.00	100.00
2.	2000 *g* pellet	4.36	0.91
3.	5000 *g* pellet	7.18	1.51
4.	Chromatin isolated from 2	0.76	0.15
5.	Chromatin isolated from 3	3.15	0.66

*Results of one representative experiment. See text for the details of isolation procedure.

TABLE II

*Relative Amounts of DNA, RNA, and Protein in Neurospora crassa Chromatin Isolated from 2000 g and 5000 × g Pellets**

	No. of isolations							
	I		II		III		Mean	
Ratios	2000 *g*	5000 *g*	2000 *g*	5000 *g*	2000 *g*	5000 *g*	2000 *g*	5000 *g*
Protein/DNA	0.94	2.50	1.61	2.89	2.07	1.53	1.54	2.30
RNA/DNA	0.51	1.49	0.67	2.91	0.70	1.07	0.63	1.82

* See text for the details of isolation procedure.

TABLE III

Analytical Data on Chromatin Isolated from Neurospora and Chicken

		Chicken‡	
	Neurospora crassa mycelia*	Embryo	Erythrocytes
DNA	23.30 μg§		
RNA	20.57 μg§		
Protein	29.00 μg§		
Protein/DNA	1.24	2.40	1.10
RNA/DNA	0.88	0.23	0.01
Maximum extinction in UV range	259 mμ	259 mμ	259 mμ
Extinction 280 mμ / Extinction 260 mμ	0.68	0.61	0.58

* Data from one representative experiment.

‡ From Dingman and Sporn (1964).

§ Results expressed as μg/100 g dry weight of fungus.

TABLE IV

Protein Content of NaCl-HCl- and LiCl-Urea–Extracted Fractions of Different Preparations of Nuclei and Chromatin*

No.	Fraction	Protein		
		Total	NaCl-HCl extractable	LiCl-urea extractable
		μg/ml	μg/ml	μg/ml
1.	Nuclei			
	a) 2000 *g*	706.25	11.25	160.25
	b) 5000 *g*	1293.00	13.00	380.00
2.	Chromatin from			
	a) 2000 *g*	82.50	11.00	57.00
	b) 5000 *g*	125.00	11.00	85.00

* Data presented here originate from two separate experiments, for nuclei and chromatin.

do not resemble histones of higher organisms.

Polyacrylamide gel electrophoresis shows no bands either from chromatin or from nuclei extracted with either NaCl-HCl or with LiCl-urea. The control gels (calf thymus histone) show typical bands.

The absence of histones is further indicated by cytochemical tests in *Neurospora crassa*. Staining with alkaline fast green (Alfert and Geshwind, 1953) or with alkaline fast green and eosin Y (Bloch, 1966), which are specific for basic proteins, show negative reactions. Cytoplasm, in some instances, does show a slight positive reaction, perhaps because of ribosomes which are so abundant in *Neurospora crassa*. Similar negative results were obtained when tissues were hydrolysed in saturated picric acid to help prevent the loss of labile histones (Bloch, 1966; Bloch and Hew, 1960), prior to staining with fast green or eosin Y. These observations are in agreement with Robinow and Bakerspiegel (1965), who have reported a negative alkaline fast green reaction in fungal nuclei. The common edible mushroom, *Agaricus* sp., gave similar negative results, suggesting the possible absence of histones in this higher fungus.

The Fine Structure of Neurospora crassa Chromatin

Fig. 1 shows an electron micrograph of chromatin isolated from *Neurospora crassa*. The fibrous structure shows an overall resemblance to the ultrastructure of interphase chromosomes reported in the literature (Gall, 1963; Ris, 1967; Wolfe, 1968; Wolfe and Grim, 1967). The diameter of individual fibers ranges from 125 to 250 A. Some thick fibers, with diameters up to 600 A, are also seen and are probably aggregates of thinner fibers.

TABLE V

*Amino Acid Composition of Protein Extracted by NaCl-HCl from N. crassa Chromatin**

Amino acid	I	II
	% μ mole	% μ mole
Lysine	10.92	8.89
Histidine	4.28	1.13
Arginine	6.79	7.77
Aspartic	10.01	9.03
Threonine	6.44	7.03
Serine	6.79	7.62
Glutamic	8.33	9.99
Proline	5.81	4.07
Glycine	8.97	13.67
Alanine	7.72	10.08
Cysteine	0.00	0.00
Valine	6.66	5.78
Methionine	1.67	0.62
Isoleucine	4.58	3.88
Leucine	5.89	6.24
Tyrosine	2.25	1.29
Phenylalanine	2.80	2.91
Basic/Acidic	1.19	0.93

* Chromatin preparation from 2000 *g* pellet extracted with NaCl-HCl as described in text. Authors are grateful to Dr. I. E. P. Taylor for the help with these analyses.

DISCUSSION

The chromatin of *Neurospora crassa* appears to be different from that of most other eukaryotes, in that it does not contain histones or, at least, proteins that behave like histones from the standpoint of their chemical properties. The possibility that basic proteins exist in a complex with DNA but are lost during the isolation procedure cannot be ruled out, although this seems improbable since the conditions of isolation, pH, and ionic concentration were such as to maintain the integrity of nucleohistone up until the point of acid extraction. The possibility that histones exist that cannot be dissociated from DNA by acid conditions, as is the case in mammalian sperm cells (Hendricks and Mayer, 1965), also seems unlikely, for protein that is extractable from *Neurospora crassa* chromatin whose protein to DNA ratio is similar to that of nucleohistone was not found to be basic.

The cytochemical observations further support the view that histones are absent in this organism. The tests are sensitive both to such labile basic proteins as protamines and to such unextractable ones as the "basic keratins" of mammalian sperm nuclei.

If histones are indeed absent, then these proteins must not be essential either for condensation or for the structural integrity of chromosomes, since nuclear division in *Neurospora* shows a pattern similar to that of mitotic division in higher organisms (Somers et al., 1960). Even if the division in *Neurospora* is not typically mitotic as some workers contend (Robinow and Bakerspiegel, 1965; Bakerspiegel, 1959), there is no doubt that the chromosomes go through progressive condensation prior to the actual division cycle. It is possible that in *Neurospora crassa* neutral proteins may play the role attributed to histones in higher organisms, or the neutral proteins may play similar roles in both groups, the histones of higher organisms having some function that has no place in *Neurospora* cells.

The absence of histones in fungi would place these organisms in a class by themselves, different both from other eukaryotes and from bacterial cells whose relatively simple genophore remains uncondensed and is not separated from cytoplasm by means of a nuclear membrane. Perhaps a clue to the reason for the difference between *Neurospora* and other eukaryotes lies in a comparison of the mechanisms of gene regulation in pro- and eukaryotes. Regulation as exemplified by the adaptive enzymes of bacteria (and yeast, a fungus) is highly plastic. Changes are reversible and transitory. Enzyme adaptation doubtlessly plays a role in differentiation in higher eukaryotes; however, change in potential for induction also plays an important role here (Stern and Hotta, 1963). Stability manifested by such phenomena as paramutation (Brink, 1958), heterochromatization (Ohno et al., 1959; Taylor, 1960; Lyon, 1961), and phase changes (Brink, 1958) indicates that gene expression in higher forms is subject to factors whose effects persist long beyond the time that they are brought into play. Some bacteria do exhibit metastable gene changes (phase variation in *Salmonella*, Lederberg and Iino, 1956), and so do perhaps some fungi (e.g., barrage phenomenon, Ephrussi, 1953). Nevertheless, the eukaryotes and prokaryotes by and large differ in the degree to which flexible and stable mechanisms play a role, and what is known of gene regulation in the fungi would seem to place them with the lower order of life. Multicellularity, incidentally, is not a prerequisite for function of histones. *Tetrahymena* (Alfert

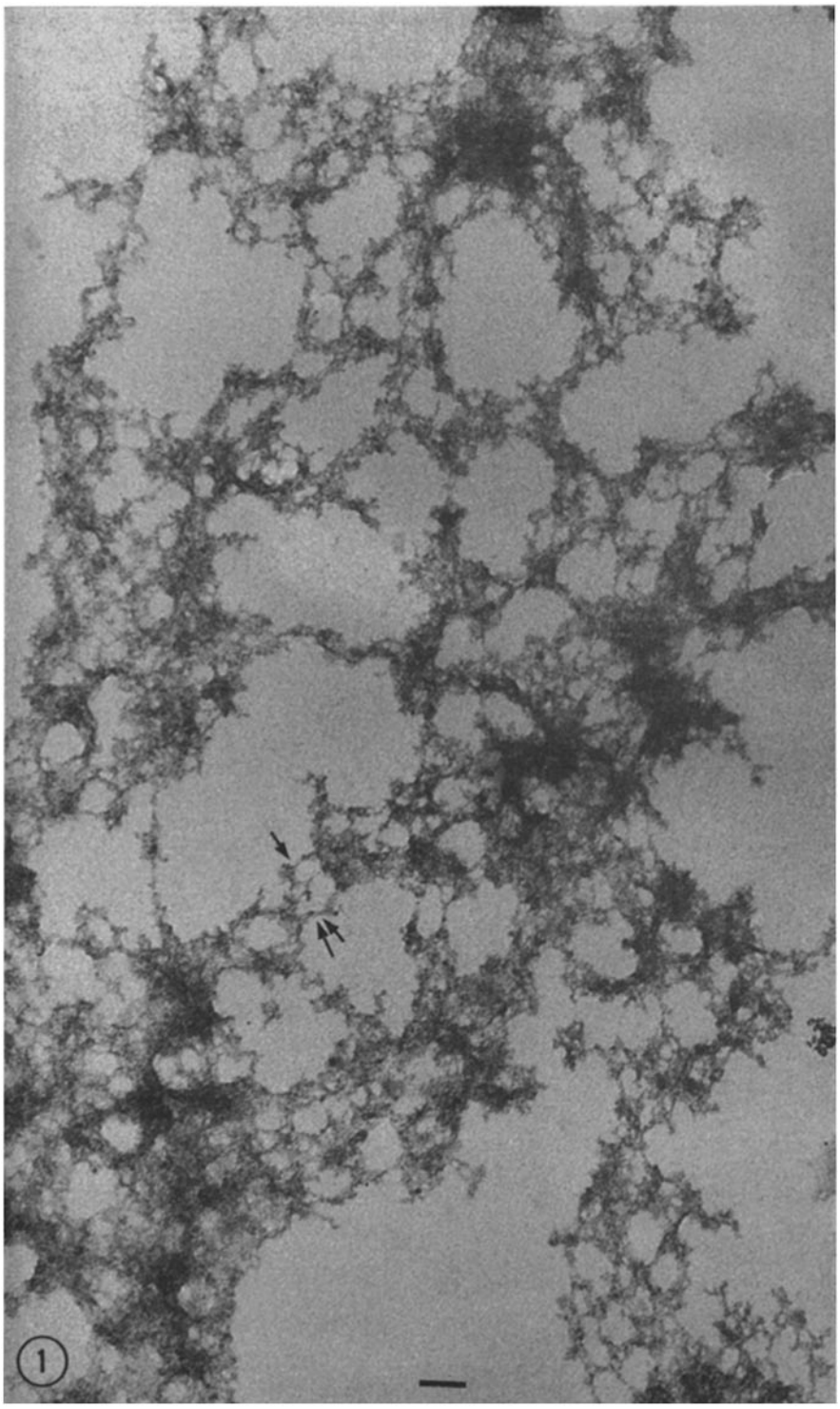

FIGURE 1 Electron micrograph of *Neurospora crassa* chromatin. Specimen prepared by spreading a dilute solution of chromatin on water-air interface. Osmium tetroxide fixation. Arrows, single and double, point to 125-A and 250-A fibers, respectively. Bar indicates 0.1 μ × 63,000.

and Goldstein, 1955) and trypanosomes (Stewart and Beck, 1967; Steinert, 1965) contain histones.

The authors thank Dr. A. Zweidler for many discussions and useful suggestions, Dr. W. G. Whaley and his staff for the use of equipment and facilities for electron microscopy, and Dr. I. E. P. Taylor for the amino acid analyses.

This study was supported by United States Public Health Service grants GM 09654, 2K03 GM 04253, and National Science Foundation grant GB 6051 to Dr. D. P. Bloch, and National Science Foundation grant GY 3894 to Dr. S. K. Dutta.

Received for publication 17 March 1969.

Note Added in Proof: Chevaillier (*Compt. Rend. Ser. D.* 1969. **268**:599.) has recently demonstrated, using cytochemical techniques, the presence of histones in the catenophore, *Beroë*.

REFERENCES

ALFERT, M., and I. I. GESHWIND. 1953. A selective staining method for the basic proteins of cell nuclei. *Proc. Nat. Acad. Sci. U.S.A.* **39**:991.

ALFERT, M., and N. O. GOLDSTEIN. 1955. Cytochemical properties of nucleoproteins in *Tetrahymena pyriformis*; a difference in protein composition between macro- and micronuclei. *J. Exp. Zool.* **130**:403.

BAKERSPIEGEL, A. 1959. The structure and manner of division of the nuclei in the vegetative mycelium of *Neurospora crassa*. *Amer. J. Bot.* **46**:180.

BARRAT, R. W., D. NEWMEYER, D. D. PERKINS, and L. GARNJOBST. 1954. Map construction in *Neurospora crassa*. *Advan. Genet.* **6**:1.

BLOCH, D. P. 1966. Histone differentiation and nuclear activity. *Chromosoma.* **19**:317.

BLOCH, D. P. 1966. Cytochemistry of histones. *Protoplasmatol Handb. Protoplasmaforch.* **5** (3):1.

BLOCH, D. P., and H. Y. C. HEW. 1960. Schedule of spermatogenesis in the pulmonate snail, *Helix aspersa*, with special reference to histone transition. *J. Biophys. Biochem. Cytol.* **7**:515.

BONNER, J., M. E. DAHMUS, D. FAMBROUGH, R. C. HUANG, K. MARUSHIGE, and D. Y. H. TUAN. 1968. Biology of isolated chromatin. *Science* (*Washington*). **159**:47.

BRINK, R. A. 1958. Paramutation at the R locus in maize. *Cold Spring. Harbor. Symp. Quant. Biol.* **23**: 379.

BURTON, K. 1956. A study of the conditions and mechanism of diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. *Biochem. J.* **62**:315.

CHEVAILLIER, P. 1966. Contribution à l'étude du complexe ADN-histone dans le spermatozoïde du pagure *Eupagurus bernhardus* L. (crustacé décapode). *J. Microsc.* **5**:739.

COLE, A. 1962. A molecular model for biological contractility: Implications in chromosome structure and function. *Nature* (*London*). **196**:211.

CRUFT, H. J. 1966. The absence of histone from the ctenophore (*Beroë cucumis*). *Biochem. J.* **101**:36 p.

DINGMAN, C. W., and M. B. SPORN. 1964. Studies on Chromatin. I. Isolation and characterization of nuclear complexes of deoxyribonucleic acid, ribonucleic acid, and protein from embryonic and adult tissues of the chicken. *J. Biol. Chem.* **239**:3483.

DUTTA, S. K., and R. S. DWIVEDI. 1968. Studies on chromatin isolated from *Neurospora crassa*. *Proc. XII. Int. Congr. Genet.* 139. (Abstr.).

DWIVEDI, R. S., S. K. DUTTA, I. E. P. TAYLOR, and D. P. BLOCH. 1968. Studies on chromatin and basic proteins isolated from *Neurospora crassa*. *Genetics.* **60**:174. (Abstr.).

EPHRUSSI, B. 1953. Nucleocytoplasmic relations in microorganisms: Their bearing on cell heredity and differentiation. Clarendon Press, Oxford.

GALL, J. G. 1963. Chromosome fibers from an interphase nucleus. *Science* (*Washington*). **139**:120.

HENRICKS, D. M., and D. T. MAYER. 1965. Isolation and characterization of a basic keratin-like protein from mammalian spermatozoa. *Exp. Cell Res.* **40**: 402.

KUPILA, S., A. M. BRYAN, and H. STERN. 1961. Extractability of DNA and its determination in tissues of higher plants. *Plant Physiol.* **36**:212.

LEBOY, P. S., E. C. COX, and J. G. FLAKS. 1964. The chromosomal site specifying a ribosomal protein in *Escherichia coli*. *Proc. Nat. Acad. Sci. U.S.A.* **52**:1367.

LEDERBERG, J., and T. IINO. 1956. Phase variation in *Salmonella*. *Genetics.* **41**:743.

LOWRY, O. H., N. J. ROSEBROUGH, A. L. FARR, and R. J. RANDALL. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**:265.

LYON, M. F. 1961. Gene action in the X-chromosome of the mouse (*Mus musculus* L.). *Nature* (*London*). **190**:732.

MEJBAUM, W. 1939. Über die Bestimmung kleiner Pentosemengen insbesondere in Derivaten der Adenylsäure. *Z. Physiol. Chem.* **258**:117.

MIRSKY, A. E., and A. W. POLLISTER. 1946. Chromosin, a desoxyribose nucleoprotein complex of the cell nucleus. *J. Gen. Physiol.* **30**:117.

MUNKRES, K. D., M. N. MUNKRES, P. GREENE, S. HEDMAN, B. J. ANDREWS, G. HOLLAND, and D. O. WOODWARD. 1966. Isolation of subcellular fractions of *Neurospora* mycelia. *Neurospora Newsletter.* **9**:14.

OHNO, S., W. D. KAPLAN, and H. KINOSITA. 1959.

Formation of the sex chromatin by a single X-chromosome in liver cells of *Rattus norvegicus*. *Exp. Cell Res.* **18**:415.

PALMADE, C., M. R. CHEVAILLIER, A. KNOBLOCH, and R. VENDRELY. 1958. Isolement d'une deoxyribonucleohistone à partir d'*Escherichia coli*. *Compt. Rend. Ser. D.* **246**:2534.

REICH, E., and S. TSUDA. 1961. Isolation of nuclei of *Neurospora crassa*. *Biochim. Biophys. Acta.* **53**:574.

REISFELD, R. A., U. J. LEWIS, and D. E. WILLIAMS. 1962 Disc electrophoresis of basic proteins and peptides on polyacrylamide gels. *Nature* (*London*). **195**:281.

RIS, H. 1962. Interpretation of ultrastructure in the cell nucleus. *In* Interpretation of Ultrastructure. R. J. C. HARRIS, editor. Academic Press Inc., New York. **1**:69.

RIS, H. 1967. Ultrastructure of the animal chromosome. *In* Regulation of Nucleic Acid and Protein Biosynthesis. V. V. Koningsberger and L. Bosch, editors. American Elsevier Publishing Co., Inc., New York. **10**:11.

ROBINOW, C. F., and A. BAKERSPIEGEL. 1965. Somatic nuclei and forms of mitosis in the fungi. *In* The Fungi. E. C. Ainsworth and A. S. Sussman, editors. Academic Press Inc., New York. **1**:119.

SOMERS, C. E., R. P. WAGNER, and T. C. HSU. 1960. Mitosis in vegetative nuclei of *Neurospora crassa*. *Genetics.* **45**:801.

STEDMAN, E., and E. STEDMAN. 1943. Probable function of histone as a regulator of mitosis. *Nature* (*London*). **152**:556.

STEINERT, M., 1965. L'absence d'histone dans le kinétonucléus des trypanosomes. *Exp. Cell Res.* **39**:69.

STERN, H., and Y. HOTTA. 1963. Regulated synthesis of RNA and protein in the control of cell division. *In* Meristems and Differentiation. *Brookhaven Symp. Biol. No.* **16**. 59.

STEWART, J. M., and J. S. BECK. 1967. Distribution of the DNA and the DNA-histone antigens in the nuclei of free-living and parasitic Sarcomastigophora. *Protozool.* **14**:225.

STUMM, C., and J. L. VAN WENT. 1968. Histone bei dem Phycomyceten *Allomyces arbuscula* (Butl.). *Experientia* (*Basel*). **24**:1112.

TAYLOR, J. H. 1960. Asynchronous duplication of chromosomes in cultured cells of Chinese hamster. *J. Biophys. Biochem. Cytol.* **7**:455.

TONINO, G. H. M., T. H. ROZIJN. 1966. On the occurence of histones in yeast. *Biochim. Biophys. Acta.* **124**:427.

VAUGHN, J.C., J. CHAITOFF, R. DELEON, C. GARLAND, and L. THOMSON. 1969. Changing nuclear histone patterns during development. II. Isolation and partial characterization of "decapodine" from sperm cells of the crab *Emerita analoga*. *Exp. Cell Res.* **54**:362.

VOGEL, H. J. 1956. A convenient growth medium for *Neurospora* (Medium N). *Microbial. Genet. Bull.* **13**: 42.

WILKINS, M. H. F., and G. ZUBAY. 1959. The absence of histone in the bacterium *Escherichia coli* II. X-ray diffraction of nucleoprotein extract. *J. Biophys. Biochem. Cytol.* **5**:55.

WOLFE, S. 1968. The effect of prefixation on the diameter of chromosome fibers isolated by the Langmuir trough critical point method. *J. Cell Biol.* **37**:610.

WOLFE, S., and J. N. GRIMM. 1967. The relationship of isolated chromosome fibers to the fibers of the embedded nucleus. *J. Ultrastrvct. Res.* **19**:382.

ZUBAY, G. 1963. The molecular basis of differentiation. In Meristems and Differentiation. *Brookhaven Symp. Biol.* No. 16. 170.

ZUBAY, G., and M. R. WATSON. 1959. The absence of histone in the bacterium *Escherichia coli*. I. Preparation and analysis of nucleoprotein extract. *J. Biophys. Biochem. Cytol.* **5**:51.