STUDIES ON NUCLEAR STRUCTURE AND
FUNCTION IN TETRAHYMENA PYRIFORMIS

III. Comparison of the Histones of
Macro- and Micronuclei by Quantitative
Polyacrylamide Gel Electrophoresis

MARTIN A. GOROVSKY

From the Whitman Laboratory, the Department of Biology, The University of Chicago, Chicago, Illinois 60637. Dr. Gorovsky's present address is Department of Biology, The University of Rochester, Rochester, New York 14627

ABSTRACT

Histones were extracted from isolated macro- and micronuclear fractions and from nucleohistone fibers which were prepared from the isolated macronuclear fraction. Analysis of these histones by polyacrylamide gel electrophoresis indicated that there are electrophoretic differences between the histones of macro- and micronuclei.

INTRODUCTION

It would seem to be desirable to study the mechanisms of control of gene activity in systems in which the differences in genetic activities are maximized, preferably by comparing situations in which DNA-dependent RNA synthesis is occurring at a high rate with those in which it is not occurring at all. It would also seem desirable to compare these "on" and "off" states in a single cell, where it is likely that the differential capacities for RNA synthesis actually reflect different properties of the loci themselves, since the cellular milieu is similar for both. Previously, it has been reported (Gorovsky and Woodard, 1969) that the micronucleus of Tetrahymena synthesizes and contains little, if any, cytochemically detectable RNA, while the macronucleus synthesizes and contains RNA in large amounts. Since the micronucleus gives rise to a new macronucleus during conjugation in Tetrahymena, macro- and micronuclei probably represent the same genetic information in extremely different states of activity—they are an "on-off" system of the type described above. Since histones have frequently been implicated as repressors of genetic activity in eukaryotic cells (Stedman and Stedman, 1950; for review of the properties of histones and their possible roles as repressors, see Bonner and Ts'o, 1964; Busch, 1965; Phillips, 1962; Bloch, 1966; Vendrely and Vendrely, 1966; Hnilica, 1967; and Gorovsky, 1968), we have studied the histones extracted from isolated macro- and micronuclei of Tetrahymena. This report describes differences...
between the histones of these two nuclei when compared by polyacrylamide gel electrophoresis.

**MATERIALS AND METHODS**

**Cells and Culture Conditions**

Log-phase cultures of *Tetrahymena pyriformis*, mating type I, variety 1 were used throughout this study. The methods of culturing and harvesting the cells have been described previously (Gorovsky, 1968; Gorovsky and Woodard, 1969).

**Isolation of Macro- and Micronuclei**

The methods for isolating macro- and micronuclear fractions have been described in detail elsewhere (Gorovsky, 1970).

**Isolation of Nucleohistone**

All operations were performed at 0-5°C. The macronuclear fraction was washed twice by centrifugation with 10 volumes of 0.15 M NaCl, pH 7.0, followed by two washes at pH 3.6 (Butler, 1964). Nucleohistone (NH) was extracted twice with 10 volumes of 2.0 M NaCl, pH 3.6. The salt-soluble extracts were diluted to a final salt concentration of 0.15 M with iced, distilled water, pH 3.6. A white, fibrous precipitate (NH fibers) formed almost immediately, and was sedimented by centrifugation 1 hr after dilution.

**Isolation of Histones**

Nuclear fractions were washed with saline as described above, and histones were extracted from nuclei or from isolated nucleohistone fibers by repeated extractions with 0.2 N H₂SO₄ (Fisher Certified Reagent). The solubilized histones were precipitated at −25° with 10–20 volumes of acetone for at least 18 hr, and then were washed with acetone-HCl (1000/1, v/v). Precipitated histones were dissolved in 4 M urea in 0.01 N HCl and stored at −25°C until used.

**Acrylamide Gel Electrophoresis**

Acrylamide gel electrophoresis was performed according to a modification (Leboy et al., 1964; see R. Low, 1968, for a complete description of this method) of the method of Reisfield et al. (1962), for basic proteins. The specific modifications which were used in this study are described in detail elsewhere (Gorovsky, 1968; See Discussion). Staining was done in 1% fast green in 7% acetic acid and densitometry was performed with a Joyce-Loebl III B microdensitometer as previously described (Gorovsky, 1968; Gorovsky, Carlson, and Rosenbaum, 1970).

**RESULTS**

Ultraviolet analysis of H₂SO₄ washes of macro- and micronuclei indicated that the extractions were 90–95% complete after three extractions, and that materials with similar spectrophotometric properties were removed from macro- and micronuclei during each extraction. Therefore, differences between the electrophoretic patterns of the pooled extracts from macro- and micro-
Figure 2: Quantitative microdensitometer tracings of the polyacrylamide gels in Fig. 1. Fig. 2 a, Histones extracted from the micronuclear fraction. Fig. 2 b, Histones extracted from nucleohistone fibers prepared from the macronuclear fraction. Fig. 2 c, Histones extracted from the macronuclear fraction. The vertical lines indicate the peaks and shoulders which were reproducibly observed in these studies.
nuclei (see below) probably did not result from different rates of solubilization of similar materials from the two nuclei or from the failure to extract completely the histones of either of the two nuclei.

There were no marked differences in the ultraviolet-absorption spectra of histones extracted from macronuclei, micronuclei, or from nucleohistone fibers, and these extracts had properties similar to those of calf thymus histones (Nutritional Biochemical Corp., Cleveland, Ohio). *Tetrahymena* histones, like calf thymus histones, were poor in aromatic amino acids as indicated by low absorption in the region of 275–280 m\(\mu\). The *Tetrahymena* histone fractions showed slightly more absorption in the region of 260 m\(\mu\) than the calf thymus preparation, but nucleic acid contamination of the *Tetrahymena* histones was calculated to be less than 5%.

Fig. 1 shows typical polyacrylamide gels of histones extracted from the micronuclear fraction, the macronuclear fraction and from nucleohistone fibers derived from macronuclei. Densitometer tracings of these gels are seen in Fig. 2. No marked differences exist between the electrophoretic patterns of histones isolated from the macronuclear fraction or from nucleohistone fibers which were derived from this fraction. However, both of these patterns differ quantitatively from that of the micronuclear histones (Figs. 1 and 2). Examination of the tracings also reveals that any band which was a major fraction in micronuclear histones was found either as a major fraction, a shoulder or an asymmetry in histones extracted from macronuclei (or nucleohistone fibers), and vice-versa. These homologies were also seen in densitometer tracings of split gels containing macronuclear histones on one side, and micronuclear histones on the other, and are to be expected since each nuclear fraction contained some nuclei of the other type (Gorovsky, 1968, and Gorovsky, 1970).

**DISCUSSION**

**Methodological Variables**

To demonstrate that the electrophoretic differences between the histones of macro- and micronuclear histones were not due to differential extraction of the two nuclei. The electrophoretic patterns which were obtained were independent of the concentrations of protein which were applied to the gels, and the differences between macro- and micronuclear histones could be demonstrated in gels containing 10 or 15% acrylamide, polymerized either with riboflavin or with persulfate in the multiple, urea-containing gel system of Leboy et al. (1964) as well as in gels containing 20% acrylamide in the single gel system of Johns (1967).

Since acid extracts of nuclei may contain ribosomal proteins as contaminants (Bonner et al., 1968; Cohn and Simson, 1963), histones were also isolated from nucleohistone fibers prepared from the isolated macronuclear fraction. Histones extracted from these fibers showed less contamination by faint, slow moving ribosomal proteins (Fig. 1), but showed little or no differences in the major electrophoretic fractions when compared to histone extracted directly from macronuclei. Moreover, proteins isolated from *Tetrahymena* ribosomes by the method of Leboy et al. (1964) had electrophoretic mobilities which were slower than all but the slowest moving fraction of micronuclear histones.

**Histone Heterogeneity in Tetrahymena**

Eleven reproducible peaks or shoulders have been consistently resolved in our studies of macro- and micronuclei of *Tetrahymena* (Fig. 2). This number agrees well with recent reports that there are on the order of 5–15 electrophoretically distinct histone fractions in calf thymus (Johns, 1967; Panyim and Chalkley, 1969) and pea tissues (Fambrough et al., 1968). Therefore, recent studies on animal, plant, and protozoan materials have all demonstrated approximately the same, low degree of histone heterogeneity.

**Macro- and Micronuclear Histones**

The histones extracted from macro- or micronuclear fractions contain the same electrophoretic fractions, but these fractions appear to be present in different amounts in the two types of nuclei. Preliminary quantitative analyses of densitometer tracings of macro- and micronuclear histones suggest that the electrophoretic fractions which are enriched in a given fraction may, in fact, be specific to that fraction (see Gorovsky, 1968, for
a quantitative comparison of macro- and micro-
nuclear histones). However, a final determination
as to whether these electrophoretic differences
between macro- and micronuclear histones
actually are due to macro- and micronucleus-
specific histones will require purer preparations
of micronuclei than are currently available,
coupled with better resolution in acrylamide
gels. It is also interesting to note that many of
the electrophoretic fractions, most notably the
most prominent band, appear to be present in the
same or similar amounts in both nuclei.

The Role of Histones in Nuclear Structure
and Function

A number of mechanisms have been proposed
whereby histones might influence the structure
of chromatin and play a role in the control of
RNA synthesis (Allfrey and Mirsky, 1963; Allfrey
et al., 1964; Bonner et al., 1968; Frenster, 1963;
Huang et al., 1964; Huang and Bonner, 1965;
Pogo et al., 1968). In a number of these mechan-
isms, it might be expected that the histones
isolated from condensed, inactive chromatin
would have different electrophoretic properties
than histones from more extended, active chroma-
tin. Although there is presently little information
as to the underlying molecular basis for the dif-
ferences between the electrophoretic patterns of
macro- and micronuclear histones, it should be
possible to isolate and to characterize these
histones, and hopefully to establish the role of
histones in maintaining the marked morphological
and functional differences between macro- and
micronuclei.

The author would like to thank Dr. H. Swift for con-
tinued encouragement and advice and for the use of
facilities provided him under Grant HD 1242. This
work is based on a dissertation submitted in partial
fulfillment of the requirements for the degree of
Doctor of Philosophy in the Department of Biology,
The University of Chicago, Chicago, Illinois. It was
supported by National Institutes of Health Grants
HD 174 and GM 20890.

Received for publication 4 March 1970, and in revised form
28 July 1970.

REFERENCES

Allfrey, V. G., and A. E. Mirsky. 1963. Mechan-
isms of synthesis and control of protein and ribo-
nucleic acid synthesis in the cell nucleus. Cold
Spring Harbor Symp. Quant. Biol. 28:247.

Allfrey, V. G., R. Faulkner, and A. E. Mirsky.
1964. Acetylation and methylation of histones and
their possible role in the regulation of RNA synthe-
sis. Proc. Nat. Acad. Sci. U. S. A. 51:786.

BLOCH, D. P. 1966. Cytosol chemistry of the histones.
Protoplasmatologia. 5th.

Bonner, J., and P. O. P. Ts'o, editors. 1964. The
Nucleohistones. Holden-Day, Inc., San Francisco.

Bonner, J., M. E. Dahmus, D. Fambrough, R. C.
Huang, K. Marushige, and D. Y. H. Tuan. 1968.
The biology of isolated chromatin. Science (Wash-
ington). 159:47.

Busch, H. 1965. Histones and Other Nuclear Pro-
teins. Academic Press, Inc., New York.

Butler, J. A. V. 1964. Fractionation and charac-
teristics of histones. In The Nucleohistones. J. Bonner
and P. Ts'o, editors. Holden-Day, San Fran-
cisco, 36.

Cohn, P., and P. Simson. 1963. Basic and other pro-
teins in microsomes of rat liver. Biochem. J. 88:206.

Fambrough, D. M., F. Fujimara, and J. Bonner.
1968. Quantitative distribution of histone compo-
nents in the pea plant. Biochem. 7:575.

Frenster, J. H. 1965. Mechanisms of repression and
de-repression within interphase chromatin. In
Vitro. 1:78.

Gorovsky, M. A. 1968. Biochemical and cytochemi-
ical studies on histones and nucleic acids of Dro-
 sophila polytene chromosomes and Tetrahymena mac-
ronuclei and micronuclei. Ph.D. Thesis, University
of Chicago, Chicago, Ill.

Gorovsky, M. A. 1970. Studies on nuclear structure
and function in Tetrahymena pyriformis. II. Isolation
of micro- and macronuclei. 47:519.

Gorovsky, M. A., K. Carlson, and J. L. Rosen-
baum. 1970. Simple method for quantitative den-
sitometry of polyacrylamide gels using fast green.
Anal. Biochem. 35:359.

Gorovsky, M. A., and J. Woodard. 1969. Studies
on nuclear structure and function in Tetrahymena
pyriformis. I. RNA synthesis in macro- and micro-
nuclei. J. Cell Biol. 42:573.

Hnilica, L. S. 1967. Proteins of the cell nucleus. In
Progress in Nucleic Acid Research and Molecular
Biology. J. Davidson and W. Cohn, editors. Aca-
demic Press Inc., New York. 7:25.

Huang, R. C., and J. Bonner. 1965. Histone-bound
RNA, a component of native nucleohistone. Proc.
Nat. Acad. Sci. U. S. A. 54:960.

Huang, R. C., J. Bonner, and K. Murray. 1964.
Physical and biological properties of soluble nu-
cleohistones. J. Mol. Biol. 8:54.

Johns, E. W. 1967. The electrophoresis of histones in
polyacrylamide gel and their quantitative deter-
mination. Biochem. J. 104:78.
LEBOY, P. S., E. C. COX, and J. G. FLAKS. 1964. The chromosomal site specifying a ribosomal protein in E. coli. Proc. Nat. Acad. Sci. U. S. A. 52:1367.

LOW, R. 1968. Characteristics of mammalian ribosomal proteins. Ph.D. Thesis, University of Chicago, Chicago, Ill.

PANYIM, S., and R. CHALKLEY. 1969. The heterogeneity of histones. I. A quantitative analysis of calf thymus histones in very long polyacrylamide gels. Biochem. 8:3972.

PHILLIPS, D. M. P. 1962. The histones. Progr. Biophys. Chem. 12:211.

POGO, B. G. T., A. O. POGO, V. G. ALLFREY, and A. E. MIRSKY. 1968. Changing patterns of histone acetylation and RNA synthesis in regeneration of the liver. Proc. Nat. Acad. Sci. U. S. A. 59:1337.

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

STEDMAN, E., and E. STEDMAN. 1930. Cell specificity of histones. Nature (London). 166:780.

VENDRELY, R., and C. VENDRELY. 1966. Biochemistry of histones and protamines. Protoplasmatologia. 58.