# STUDIES ON NUCLEAR STRUCTURE AND FUNCTION IN *TETRAHYMENA PYRIFORMIS*

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

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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[1]-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

[1] Abbreviations: DNA, deoxyribonucleic acid; RNA, ribonucleic acid; NH, nucleohistone; UV, ultraviolet; PCA, perchloric acid; TCA, trichloroacetic acid; I.D., inner diameter; REM, relative electrophoretic mobility; O.D., optical density.

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 I 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 $H_2SO_4$ (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 $H_2SO_4$ 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-

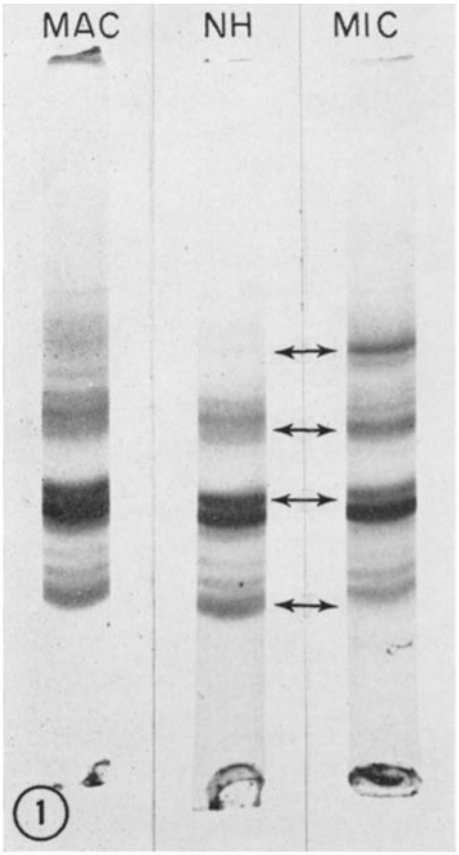


FIGURE 1 Polyacrylamide gels of histones extracted from the macronuclear fraction (*MAC*), from nucleohistone fibers prepared from the macronuclear fraction (*NH*), and from the micronuclear fraction (*MIC*). The small, slow moving bands at the top of the gels containing macronuclear and micronuclear histones are emphasized in this photograph owing to the nonlinearity of the photographic process. Fig. 2 is a quantitative representation of these gels, and indicates that these bands are actually present in small amounts. The arrows indicate the regions in which visible differences occur between the gels containing macronuclear (*NH*) and micronuclear histones.

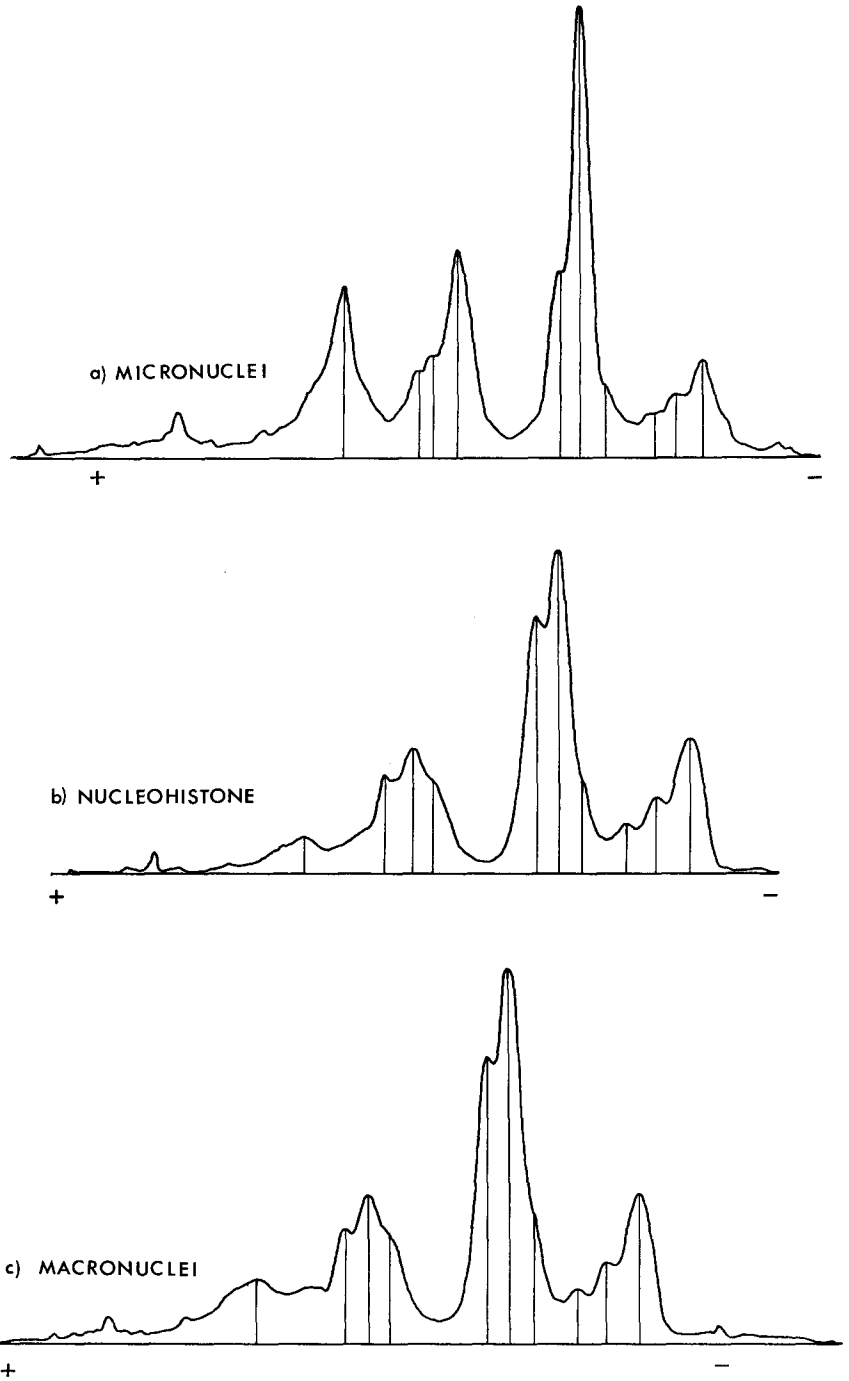


FIGURE 2 Quantitative microdensitometer tracings of the polyacrylamide gels in Fig. 1. Fig. 2 *a*, Histones extracted from the micronuclear fraction. Fig. 2 *b*, Histones extracted from nucleohistone fibers prepared from the macronuclear fraction. Fig. 2 *c*, Histones extracted from the macronuclear 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 mμ. The *Tetrahymena* histone fractions showed slightly more absorption in the region of 260 mμ 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 micronuclei were not artifacts, a number of experiments were performed to determine the effects of many of the variables in the isolation and electrophoretic analysis of histones by the methods employed in this study. It could be shown that histones were not lost in the saline washes, and that the differences which were observed between 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 micronuclear 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, 1965; Huang et al., 1964; Huang and Bonner, 1965; Pogo et al., 1968). In a number of these mechanisms, it might be expected that the histones isolated from condensed, inactive chromatin would have different electrophoretic properties than histones from more extended, active chromatin. Although there is presently little information as to the underlying molecular basis for the differences 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 marco- and micronuclei.

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