

# STUDIES ON HISTONE FRACTION F2A1 IN MACRO- AND MICRONUCLEI OF *TETRAHYMENA PYRIFORMIS*

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## ABSTRACT

Histone fraction F2A1 has been isolated and purified from macronuclei of the ciliate *Tetrahymena pyriformis*. It migrates as a single species on sodium dodecyl sulphate-acrylamide gel electrophoresis, with a molecular weight indistinguishable from that of calf thymus F2A1. The solubility properties of *Tetrahymena* F2A1 are also similar to those of calf thymus F2A1. Electrophoretic analyses on urea-acrylamide gels indicate that *Tetrahymena* F2A1 consists of four or five subspecies, the two fastest having electrophoretic mobilities identical with those of the two major electrophoretically separable forms of calf thymus F2A1. High resolution (long gel) electrophoresis coupled with incorporation of radioactive acetate both in vivo and in vitro suggest that, as in the case of calf thymus F2A1, differential acetylation of a parent molecule can explain the observed electrophoretic heterogeneity of *Tetrahymena* F2A1. Electrophoretic analysis of histones isolated from the micronucleus, which is genetically less active than the macronucleus, indicates that it contains largely the relatively unacetylated (parent) form of histone F2A1.

## INTRODUCTION

Although macro- and micronuclei of *Tetrahymena* develop from daughter nuclei formed by the second postzygotic division during conjugation and reside in the same cytoplasm during vegetative growth, they differ markedly in their structure and function. Macronuclei contain considerably more DNA than micronuclei (Cleffmann, 1968; Gibson and Martin, 1971; Woodard et al., 1972), and the bulk of chromatin is organized into distinct bodies 0.1–0.3  $\mu\text{m}$  in diameter (Swift et al., 1964). The micronuclear chromatin, on the other hand, is found in a dense, chromosome-like thread (Gorovsky, 1968).

Macro- and micronuclei synthesize DNA at different times in the cell cycle (McDonald, 1962; Prescott and Stone, 1967; Woodard et al., 1972) and also divide at different times by different mechanisms (Elliott, 1963; Flickinger, 1965). Per-

haps of greatest interest is that the two nuclei appear to differ in their genetic activity. Macronuclei contain large amounts of cytochemically detectable RNA which is largely localized in typical nucleoli and interchromatin granules (Swift et al., 1964; Gorovsky, 1968). By contrast, micronuclei contain little, if any, cytochemically detectable RNA and do not contain any detectable nucleoli or interchromatin granules (Gorovsky, 1968). While there is some question as to whether there is any RNA synthesis in micronuclei, it is clear that micronuclei synthesize very little RNA when compared to macronuclei (Gorovsky and Woodard, 1969; Murti and Prescott, 1970). Thus, the macro- and micronucleus of *Tetrahymena* can serve as a model system in which to study the mechanisms by which the same (or related) genetic information in a eukaryotic cell is main-

tained in very different structural and functional states.

We have previously reported that histones extracted from isolated macro- and micronuclei differ from each other when examined by quantitative polyacrylamide gel electrophoresis (Gorovsky, 1970 *b*). In this report we describe the purification and characterization of histone fraction F2A1 from macronuclei of *Tetrahymena*. The solubility properties, relative electrophoretic mobility, and molecular weight of macronuclear F2A1 were found to be indistinguishable from those of purified calf thymus F2A1. Like F2A1 in other organisms (see Allfrey, 1971 for review), macronuclear F2A1 consists of multiple acetylated subspecies, and acetylation was shown to occur within macronuclei, after the protein was synthesized. Evidence is also presented that the molecular basis for one of the differences between macro- and micronuclear histones resides in the degree of acetylation of this fraction: F2A1 isolated from macronuclei consists of a parent molecule plus three or four acetylated subspecies, while micronuclear F2A1 consists largely, if not entirely, of the unacetylated parent<sup>1</sup> molecule. These observations are discussed in relation to the suggestions (see Allfrey, 1971) that histone acetylation may play a role in the control of gene activity.

## MATERIALS AND METHODS

### *Culture Methods*

*Tetrahymena pyriformis* (syngen I, mating type I) was cultured axenically in enriched proteose peptone as previously described, (Gorovsky, 1970 *a*) except that 2 drops/liter of Antifoam SAG 471 (Union Carbide Corporation, New York) was used instead of Antifoam B (Dow Corning Corp., Midland, Mich.).

Macronuclei were isolated by a slight modification of the method described previously (Gorovsky, 1970 *a*). 0.01%–0.10% spermidine trihydrochloride (Nutritional Biochemicals Corporation, Cleveland, Ohio) was routinely used in all media to preserve nuclei and to inhibit proteolytic degradation of histones (Gorovsky, unpublished observations). Micronuclear fractions were isolated from postmacronuclear supernatants by centrifugation at 16,000 *g* for

<sup>1</sup>The acetylation referred to throughout this paper is that occurring after histone synthesis, probably on  $\epsilon$ -amino groups of lysine (see Allfrey, 1971). It is entirely possible that molecules referred to here as unacetylated do, in fact, contain acetyl groups (probably at the amino terminus; Allfrey, 1971), which are incorporated at the time of histone synthesis.

10 min frequently followed by sedimentation at unit gravity (McBride and Peterson, 1970) or by filtration through Nucleopore filters (3.0  $\mu$ m or 5.0  $\mu$ m) to remove intact macronuclei or large macronuclear fragments. Micronuclear histones isolated by these techniques probably contain about 20% macronuclear histones as contamination, as estimated by the amount of macronuclear-specific, methylated DNA which is found in micronuclear preparations (Gorovsky et al., 1973).

### *Extraction of Histone*

Histones were prepared from isolated *Tetrahymena* nuclei by repeated extraction either with 2.4 M urea – 0.4 N H<sub>2</sub>SO<sub>4</sub> or with 0.4 N H<sub>2</sub>SO<sub>4</sub> alone. The pooled extracts were precipitated with 4 vol of 95% or 100% ethanol at –20°C for at least 12 h washed in 95% ethanol, and air dried under vacuum. In most experiments, either the isolated nuclei or the isolated histones were extracted with 0.50 N or 0.74 N perchloric acid (Johns, 1964) to remove histone fraction F1 and the products of proteolytic digestion of F1 which were incompletely resolved from histone fraction F2A1 during electrophoresis. The nature of histone fraction F1 and its digestion by endogenous protease will be the subject of another communication. Similar F1-specific proteolysis has been described in calf thymus chromatin (Panyim et al., 1968; Bartley and Chalkley, 1970). Histones were extracted from calf thymus chromatin by the method of Panyim et al. (1971).

### *Histone Fractionation*

Calf thymus and *Tetrahymena* histones were fractionated by method 2 of Johns (1964), and by the method of Oliver and Chalkley (personal communication). *Tetrahymena* histone fraction F2A1 was isolated by a modification of the method of Oliver and Chalkley for separating histone F2A1 and F2A2 of calf thymus. *Tetrahymena* histones were dissolved (10 mg/ml) in 2 M urea–0.01 N HCl. Samples (10–20 mg) were layered on a 1.5 cm  $\times$  60 cm column of Bio-gel P-100 and eluted with 2 M urea–0.01 N HCl. Fractions were collected, the absorbance was read at 230 nm, and the appropriate samples were pooled (see Results) and then dialyzed for 8–12 h against 5 vol of 0.4 N H<sub>2</sub>SO<sub>4</sub> (two changes, 5°C) followed by dialysis for 4–8 h against 5 vol of 95% ethanol (two changes, 5°C). The resulting precipitate was collected by centrifugation (16,000 *g*  $\times$  10 minutes), washed twice with acetone, and air dried under vacuum at room temperature.

### *Acrylamide Gel Electrophoresis*

Electrophoresis in long (25 cm) 2.5 M urea–15% polyacrylamide gels was performed by the method of

Panyim and Chalkley (1969). Gels were stained in 1.0% fast green (Gorovsky et al., 1970) and were scanned at 630 nm, using a Gilford 2400 spectrophotometer equipped with a 2410 linear transport. Areas under peaks were measured by cutting and weighing appropriate portions of the gel tracings (Gorovsky et al., 1970).

Molecular weight determinations were performed on sodium dodecyl sulphate (SDS)-containing acrylamide gels at both pH 7.6 and pH 10 as described by Panyim and Chalkley (1971). Whole calf thymus histones and isolated fractions of calf thymus histones were used as standards. SDS-containing gels were stained overnight in 0.1% (wt/vol) fast green, 20% (vol/vol) acetic acid, and 50% (vol/vol) methanol. Destaining was achieved by shaking in repeated changes of 7.5% acetic acid-5.0% methanol. Gels were traced as described above and the relative electrophoretic mobilities (using calf thymus fractions as standards) were calculated either from measurements made on the tracings or from measurements made directly on the gels.

#### Isotopic Labeling and Counting Procedures

Cells growing logarithmically at 36°C were collected by centrifugation at 2,250 *g* for 3 min, washed once in Dryl's solution (Dryl, 1959), and resuspended in Dryl's solution containing a 1:100 dilution of antibiotic-antimycotic mixture (Grand Island Biological Co., Grand Island, N.Y.), 20 µg/ml cycloheximide (actidione, Mann Research Labs., Inc., New York), and 10 µCi/ml <sup>14</sup>C-labeled sodium acetate (New England Nuclear Corp., Boston, Mass.). After 15 min at 36°C, macronuclei were isolated and histones were extracted in the usual manner.

Isolated nuclei (1 × 10<sup>8</sup> nuclei/ml) were incubated at 28°C in a medium containing 0.006 M MgCl<sub>2</sub>, 0.01 M Tris-HCl, pH 7.2, 0.10 M KCl, 0.001 M dithiothreitol, 0.25 M sucrose, 0.01% spermidine trihydrochloride, 0.001 M ATP, 0.00025 M GTP, 0.01 M creatine phosphate, 1% creatine phosphokinase, and 1 µCi/ml tritium-labeled acetyl coenzyme A (New England Nuclear Corp.). After 30 min, histones were isolated in the usual manner.

Specific activities of fractions separated on polyacrylamide gels were determined by cutting and weighing appropriate portions of tracings of the gels containing radioactive proteins, subsequently cutting out the bands and slicing them into 1 mm segments. The radioactive protein was then solubilized in NCS (Amersham/Searle Corp., Arlington Heights, Ill.). Counting was performed in a Packard #3380 liquid scintillation counter, using Spectrafluor (Amersham/Searle Corp.) as a scintillant.

## RESULTS

### Urea-Acrylamide Gels of *Tetrahymena* Histones

Fig. 1 *a* shows a densitometer tracing of histones isolated from macronuclei. Since *Tetrahymena* contains a histone protease with activity similar to that described in calf thymus (Bartley and Chalkley, 1970), we have routinely removed fraction F1 and its digestion products with perchloric acid so that they do not interfere with the visualization of fraction F2A1. The other electrophoretically separable fractions of *Tetrahymena* histones have been identified by the electrophoretic criteria of Panyim et al. (1971) and by differential solubilization using the fractionation criteria described by Johns (1964, 1967). A more detailed description of *Tetrahymena* histones will be published elsewhere (Gorovsky, Pleger, and Keevert, in preparation). We might

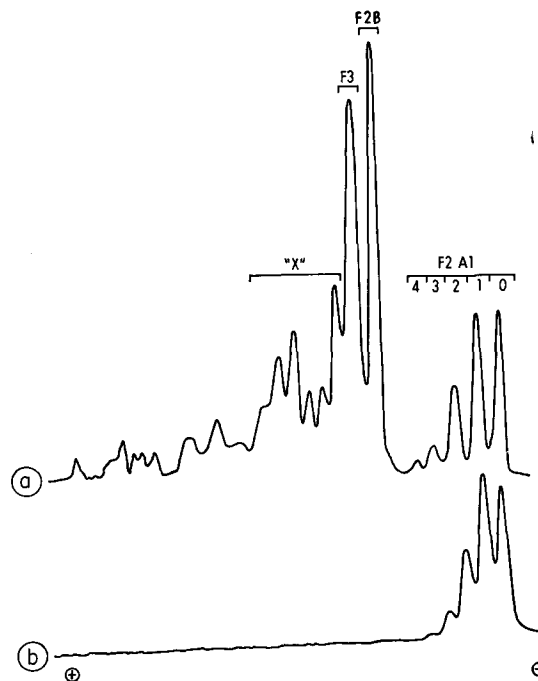


FIGURE 1 Densitometer tracings of polyacrylamide gels. (a) Macronuclear histones from which fraction F1 has been removed by extraction with perchloric acid. The peaks to the left of fraction(s) X represent non-histone (probably nucleolar or ribosomal) contaminants which can be largely removed by isolating histones from chromatin instead of directly from macronuclei. (b) *Tetrahymena* histone fraction F2A1 isolated by column chromatography on Bio-gel P-100.

note here, however, that fractions F3 and F2B of *Tetrahymena* have solubility and electrophoretic properties similar to those of comparable fractions from calf thymus, while histone fraction F1 has solubility properties and molecular weight similar to those of calf thymus F1 but migrates considerably faster in urea-acrylamide gels, presumably owing to its higher positive charge. We have not yet been able to identify a fraction corresponding to fraction F2A2 in higher organisms, nor have we yet been able to characterize the fraction(s) labeled X on Fig. 1 a.

#### Identification of *Tetrahymena* Fraction F2A1

We have identified the four fastest migrating bands in Fig. 1 a as F2A1 by the following criteria:

(a) The ratio of the relative electrophoretic mobility of band F2A1<sup>0</sup> to that of histone F3 is identical with the ratio of mobilities of histone fractions F3 and F2A1 in all other organisms examined (Panyim et al., 1970).

(b) The electrophoretic mobilities of bands F2A1<sup>0</sup> and F2A1<sup>1</sup> are identical with those of the two major electrophoretically separable forms of purified calf thymus fraction F2A1 when these are run on the same gel as *Tetrahymena* histones (Fig. 2).

(c) The solubility properties of all four bands are the same as those of calf thymus F2A1 when *Tetrahymena* histones are fractionated by Johns's Method 2 (1964) or by selective extraction with GuCl (Johns, 1967).

(d) The electrophoretic mobility of the five bands on SDS-acrylamide gels is identical with that of calf thymus F2A1 (see below).

We conclude from these results that the four fastest migrating bands in urea-acrylamide gels of *Tetrahymena* histone correspond to histone fraction F2A1 of calf thymus. The fifth band, seen in Fig. 1 a, is also probably a subspecies of F2A1 (and is so labeled in Fig. 1), but is present in so low a concentration that it is not always detected.<sup>2</sup>

<sup>2</sup> It should be noted that Wangh et al. (1972) have reported that an acetylated fragment of fraction F3 may be found in the region of the gel containing the fifth subfraction of F2A1 (F2A1<sup>4</sup> in Fig. 1). However, the fifth subfraction has the precise electrophoretic mobility expected of a subspecies of F2A1 containing four acetylated amino groups, and we have been unable to detect any material migrating in this region of the gel which reacts with [<sup>14</sup>C]N-ethylmaleimide (Wangh et al., 1972) so that subfraction F2A1<sup>4</sup>, if it is produced by proteolytic digestion of F3, does not contain the (single) sulfhydryl group found in ungraded *Tetrahymena* F3.

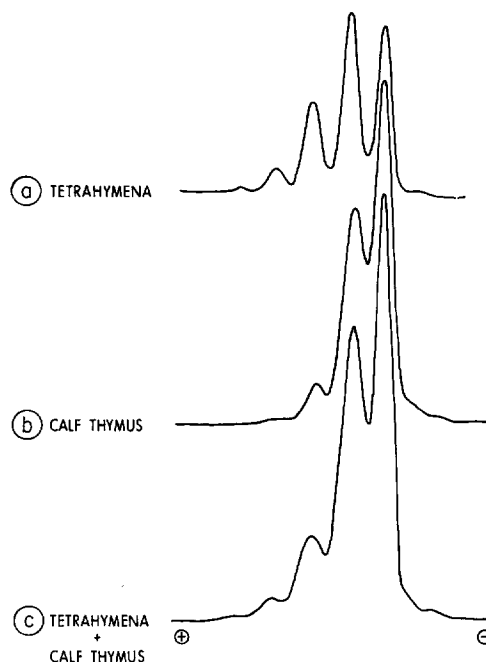


FIGURE 2 Densitometer tracings of polyacrylamide gels containing (a) fraction F2A1 isolated from *Tetrahymena*, (b) fraction F2A1 isolated from calf thymus, and (c) a mixture of *Tetrahymena* and calf thymus F2A1.

#### Purification of *Tetrahymena* Fraction F2A1

Although methods commonly used to isolate and purify calf thymus F2A1 (Johns, 1964; 1967) also resulted in marked enrichment for fraction F2A1 when used with *Tetrahymena* histones, we were unable to obtain completely purified F2A1 from *Tetrahymena* by these techniques. We have therefore purified F2A1 by gel exclusion chromatography, taking advantage of the fact that F2A1 has a lower molecular weight than any other histone fraction. Fig. 3 shows a typical separation of *Tetrahymena* histones by column chromatography on Bio-gel P-100. When examined by high resolution acrylamide gel electrophoresis, the fraction obtained by this technique is seen to consist almost entirely of the fast migrating components identified as histone fraction F2A1 (Fig. 1 b).

#### SDS-Acrylamide Gel Electrophoresis

When purified *Tetrahymena* F2A1 was analyzed by SDS-acrylamide gel electrophoresis, it migrated as a single band which could not be distinguished from purified calf thymus F2A1 which was run in the same gel. These results indicate that it has a molecular weight of approximately 11,000 daltons

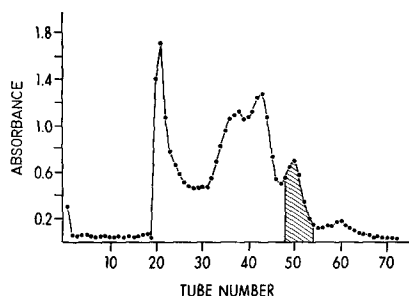


FIGURE 3 Elution profile of macronuclear histones separated on Bio-gel P-100. 13 mg of macronuclear histone from which fraction F1 had been removed were dissolved in 1.3 ml of 2 M urea-0.01 N HCl and applied to a 1.5 cm  $\times$  60 cm column. Fractions of 1.2 ml were collected at a flow rate of 3.5 ml/h. The shaded area indicates the fractions which were pooled to obtain the F2A1 fraction.

(DeLange et al., 1969 a), and that the five species observed on urea-acrylamide gels all have similar molecular weights when examined on SDS-acrylamide gels.

#### *In Vivo* Acetylation of *Tetrahymena* F2A1

It has been suggested that the electrophoretic heterogeneity of calf thymus and pea F2A1 could be explained by differential acetylation of a (relatively unacetylated) parent molecule (DeLange et al., 1969 a; Wangh et al., 1972). This suggested to us that different degrees of acetylation might explain the observation that *Tetrahymena* F2A1 could be separated into four or five subspecies by urea-acrylamide gel electrophoresis (which separates proteins on the basis of both charge and molecular weight), but migrated as a single species on SDS-acrylamide gels. Since the available evidence suggests that such differential acetylation occurs after the histone molecules are synthesized (see Allfrey, 1971 for review), we have measured the incorporation of radioactive acetate into *Tetrahymena* histones under conditions where protein synthesis has been completely inhibited by cycloheximide. Under these conditions radioactive acetate is incorporated preferentially into histone fraction F2A1 and into histones in the region of F3 and the bands labeled  $X^3$  in Fig. 1 a. Little incorporation is detectable in fractions F1 and F2B. When the specific activities (counts per minute of

<sup>3</sup> It should be noted that the region of the gel labeled  $X$  on Fig. 1 a might be expected to contain the multiply acetylated forms of histone F3 (Wangh et al., 1972).

acetate incorporated per unit protein staining) of the electrophoretically separable subspecies of F2A1 are compared (Tables I and III), it is seen that the fastest migrating species, F2A1<sup>0</sup>, shows relatively little incorporation of [<sup>14</sup>C]acetate, while the slower moving forms contain significant amounts of radioactivity. These results are consistent with the hypothesis that the subspecies represent an unacetylated (parent) molecule and three or four more highly acetylated forms of that molecule.

#### *Acetylation of Histone F2A1 by Isolated Nuclei*

It has been reported (see Allfrey, 1971) that postsynthetic acetylation of histones occurs in the cell nucleus. We have found that the transfer of radioactive acetate from acetyl Co A to histones can occur in isolated *Tetrahymena* nuclei. Identical results were obtained in the presence or absence of cycloheximide. Acetylation of histones in isolated nuclei is remarkably similar to postsynthetic acetylation of histones in intact cells: the same histone fractions (F2A1, F3,  $X$ ) are acetylated, and the subspecies of histone fraction F2A1 are differentially acetylated just as they are in vivo (Tables II and III). We conclude from these results that acetylation of histones in *Tetrahymena*, as in other organisms, probably occurs within the nucleus, and that the acetylation reaction measured in isolated nuclei retains specificity similar to that observed in vivo.

#### *Comparison of Macro- and Micronuclear F2A1*

Fig. 4 shows densitometer tracings of urea-acrylamide gels of histones extracted from purified

TABLE I  
*A Typical Experiment Measuring the Incorporation of [<sup>14</sup>C]Acetate into Subfractions of Histone F2A1 of Tetrahymena Labeled In Vivo*

Subfraction	% of total F2A1 staining	cpm	Relative* specific activity
F2A1 <sup>0</sup>	42	61	0.05
F2A1 <sup>1</sup>	33	1,049	1.00
F2A1 <sup>2</sup>	17	1,163	2.15
F2A1 <sup>3+4</sup>	07	856	3.84

\* Calculated by setting the specific activity of subfraction F2A1<sup>1</sup> equal to 1.00.

TABLE II  
A Typical Experiment Measuring the Incorporation of [<sup>3</sup>H]Acetate from Acetyl Co A into Subfractions of Histone F2A1 in Isolated Nuclei

Subfraction	% of total F2A1 staining	cpm	Relative* specific activity
F2A1 <sup>0</sup>	36	82	0.04
F2A1 <sup>1</sup>	37	2,396	1.00
F2A1 <sup>2</sup>	19	2,226	1.81
F2A1 <sup>3+4</sup>	07	976	2.15

\* Calculated by setting the specific activity of subfraction F2A1<sup>1</sup> equal to 1.00.

TABLE III  
Summary of Relative Specific Activities of F2A1 Subfractions

Subfraction	Relative specific activity	
	in vivo* †	in vitro* †
F2A1 <sup>0</sup>	0.06	0.09
F2A1 <sup>1</sup>	1.00	1.00
F2A1 <sup>2</sup>	1.90	1.76
F2A1 <sup>3+4</sup>	2.20	2.14

\* Specific activity of F2A1<sup>1</sup> arbitrarily set at 1.00.  
† Average values of three experiments.

macro- and micronuclei. As noted earlier, macronuclear F2A1 consists of at least four subspecies. By contrast, histone F2A1 from micronuclei consists mostly of the fastest migrating species. If the heterogeneity of macronuclear F2A1 is, in fact, due to differential acetylation of a parent molecule (see Discussion), then histone F2A1 exists in micronuclei largely, if not entirely (see Discussion), in the relatively unacetylated, parent form of the molecule. The other differences between macro- and micronuclear histones which are apparent in Fig. 4 will be discussed in a later communication (Gorovsky, Pleger, and Keevert, in preparation).

## DISCUSSION

### Evolution of Histone F2A1

*Tetrahymena* histone fraction F2A1 has been found to be remarkably similar to calf thymus F2A1 in solubility properties, electrophoretic mobility on urea-acrylamide gels, molecular weight, and in the fact that it is subject to post-synthetic acetylation in the nucleus. Moreover, the amino acid composition of a histone fraction isolated and purified from *Tetrahymena* by techniques similar to the column chromatographic method described here is like that of calf thymus F2A1 (Hamana and Iwai, 1971). Thus, while primary

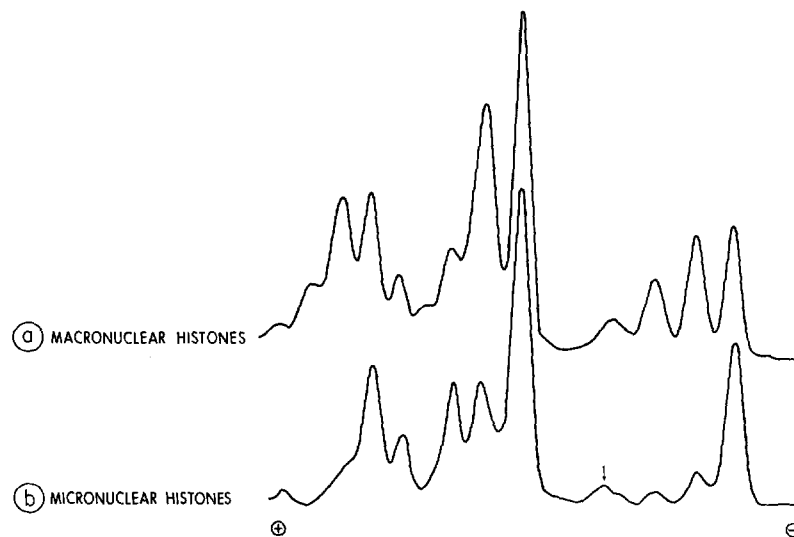


FIGURE 4 Densitometer tracing of polyacrylamide gels containing (a) macronuclear histones minus fraction F1 and (b) micronuclear histones minus F1. The band at the arrow is a variable contaminant which was frequently observed in micronuclear preparations. Its electrophoretic mobility is similar to, but not identical with, that of the slowest migrating subspecies of F2A1 observed in Fig. 1.

sequence data are not yet available for *Tetrahymena* F2A1, it is likely that the remarkable evolutionary conservatism of F2A1 which has been noted for plants and animals (DeLange et al., 1969 *b*) will extend to lower eukaryotes as well.

### *Acetylation of Histone F2A1*

We have found that postsynthetic acetylation of histone fraction F2A1 occurs in the *Tetrahymena* nucleus and that the acetate donor is acetyl Co A (free acetate is incorporated poorly by *Tetrahymena* nuclei). Similar results have been reported in a variety of other cell types (Allfrey, 1971). Therefore, with only a few exceptions (see below), the ability to acetylate histone fraction F2A1 seems to be a general property of eukaryotic nuclei, and, along with the remarkable evolutionary stability of this histone, must be accounted for by any theory seeking to explain the function of the protein.

We have demonstrated the presence of four or five subspecies of F2A1 in *Tetrahymena* macronuclei. While final proof of the hypothesis that these electrophoretic subspecies represent different degrees of acetylation of the same (relatively) unacetylated parent molecule will require chromatographic separation and chemical analysis of the subfractions as recently described by Wangh et al. (1972), the following considerations strongly support this hypothesis: (*a*) The molecular weights of the five subspecies are indistinguishable on SDS-acrylamide gel electrophoresis, suggesting that the difference in mobilities on urea-acrylamide gels is due to difference in charge, not to a significant difference in molecular size. (*b*) The two fastest migrating subspecies co-electrophorese precisely in long gels with the two major electrophoretic subspecies of calf thymus F2A1 which have been shown to differ in their degree of acetylation (Wangh et al., 1972) and to consist of a single polypeptide chain in which approximately one-half of the F2A1 (= histone IV) molecules contain an acetylated lysine residue at position 16 (DeLange et al., 1969 *a*). (*c*) Each one of the subspecies differs in relative electrophoretic mobility from the next fastest (or slowest) subspecies by 2%-3%, the same mobility difference which we have measured between the two major calf thymus subspecies which are known to differ in the acetylation of a single lysine residue. Therefore, it seems reasonable to assume that each of the subspecies differs from its

adjacent member(s) in the acetylation of one charged residue. We had hoped to support this hypothesis with evidence showing an arithmetic progression in the specific activities of the various subspecies, (i.e.,  $F2A1^0 = 0$ ;  $F2A1^1 = 1$ ,  $F2A1^2 = 2$ , etc.) Although there is a suggestion that such a stepwise progression in specific activities does occur for the three fastest migrating subspecies (Table III), it does not seem to continue to the slowest forms. However, the actual demonstration of such a progression requires ideal conditions in which the number of acetate molecules incorporated is proportional to the number of acetates already present, and in which there is complete purity of the subfractions separated on acrylamide gels. Since there is little information available on the rates of acetylation and deacetylation of the subspecies of F2A1, we have no reason to believe that these ideal conditions were met in our experiments. Further work on this question is now in progress.

### *F2A1 in Macro- and Micronuclei*

While our results strongly suggest that macronuclei contain multiple, differentially acetylated forms of F2A1, we have not yet proven that such forms are completely absent from micronuclei. However, as we have developed better techniques for purifying micronuclei, the levels of all but the fastest migrating subspecies of F2A1 in micronuclear histones have greatly decreased. We are therefore inclined to the view that only the unacetylated (parent) form of F2A1 exists in micronuclei, since the low levels of acetylated forms which we have observed in micronuclear histones can be accounted for by low levels of contamination with macronuclear histones and by the products of proteolytic degradation of other histone fractions which occasionally appear in small amounts in the region of the gel between fraction F2B and F2A1.<sup>2</sup>

Although the small amounts of the slower migrating acetylated subspecies in micronuclear histones may reflect the absence of acetylating enzymes from micronuclei, it should be noted that other factors, such as rapid deacetylation, or the masking of appropriate sites on F2A1 in micronuclear chromatin might also explain these observations. Further work on this question is now in progress.

### F2A1 Acetylation and RNA Synthesis

Allfrey and his collaborators (see Allfrey, 1964, 1970, 1971) have shown that there is a correlation between the degree of histone acetylation and RNA synthesis in different chromatin subfractions isolated from lymphocyte nuclei, in phytohemagglutinin-stimulated human peripheral lymphocytes, and in regenerating rat livers. More recently, it has been reported that acetylated subspecies of fraction F2A1 are not present in *Arbacia* sperm, while several acetylated forms are present in the *Arbacia* embryo (Wangh et al., 1972; Easton and Chalkley, 1972), and Berlowitz and Pallotta (1972) have presented evidence that the condensed, genetically inactive heterochromatic chromosome set of male mealy bugs incorporated much less acetate from acetyl Co A than the extended, genetically active euchromatic set. These results support the hypothesis (see Allfrey, 1971) that histone acetylation is correlated with genetic activity (RNA synthesis). Our findings can be interpreted in similar fashion since the genetically inactive micronucleus probably contains only the unacetylated parent F2A1 while the genetically active macronucleus contains a multiplicity of acetylated forms. It should be noted, however, that the correlation between histone acetylation or the amount of acetylated histone subspecies and RNA synthesis is not a simple one. Thus, while there appear to be differences between the rates of histone acetylation and the amounts of acetylated subspecies of F2A1 in immature (genetically active) and mature (genetically inactive) red blood cells (Allfrey, 1970; Wangh et al., 1972), the genetically inactive nucleus of mature erythrocytes still appears to contain significant amounts of the acetylated forms of F2A1 (Panyim et al., 1971; Wangh et al., 1972). Plant nuclei, on the other hand, which might reasonably be assumed to be synthesizing RNA, contain only small amounts of acetylated F2A1 (DeLange et al., 1969 *b*). Also, we have found only small differences in the ratios of acetylated to nonacetylated forms of F2A1 when comparing the histones of inactive, condensed chromatin with that of the active, extended chromatin of mouse liver nuclei (Johmann, Eckhardt and Gorovsky, unpublished observations). Clearly, the precise role played by histone acetylation in the structure and function of eukaryotic chromatin remains to be elucidated.

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