HISTONE F1 OF *TETRAHYMENA* MACRONUCLEI

Unique Electrophoretic Properties and Phosphorylation of F1 in an Amitotic Nucleus

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ABSTRACT

Histone fraction F1 has been isolated and purified from macronuclei of the ciliated protozoan, *Tetrahymena pyriformis*. In many respects, *Tetrahymena* F1 is similar to that of other organisms. It is the only *Tetrahymena* histone soluble in 5% perchloric acid or 5% trichloroacetic acid, has a higher molecular weight than any other *Tetrahymena* histone, is the histone most easily dissociated from *Tetrahymena* chromatin, and is susceptible to specific proteolytic cleavage. However, unlike F1 in all other organisms, *Tetrahymena* F1 is not the slowest-migrating histone fraction when analyzed by polyacrylamide gel electrophoresis at low pH. *Tetrahymena* F1 also exhibits unusual behavior in sodium dodecyl sulfate-containing polyacrylamide gels, migrating faster than calf thymus F1 at pH 10, and slower than calf thymus F1 at pH 7.6. *Tetrahymena* F1 was found to be highly phosphorylated in rapidly growing cells, suggesting that the relationship between cell replication and F1 phosphorylation previously observed in mammalian cells may extend to all eukaryotes. The observation that extensive F1 phosphorylation occurs in macronuclei, which divide amitotically, argues against a unique role for F1 phosphorylation in the process of chromosome condensation at mitosis.

INTRODUCTION

In many ways, F1 is the most interesting of the five major histone fractions found in eukaryotes. When the histones of different organisms are compared, F1 shows much greater variability in electrophoretic mobility than other histone fractions (Nelson and Yunis, 1969; Fambrough and Bonner, 1969; Mohberg and Rusch, 1969; Cohen and Gotchel, 1971; Panyim et al., 1971; Phelan et al., 1972; Oliver and Chalkley, 1972). It has also been suggested that F1 isolated from a single mammalian tissue shows a greater degree of sequence diversity than the other histones (Bustin and Cole, 1968; Kinkade and Cole 1966 *a*, *b*; Rall and Cole, 1971), and both qualitative and quantitative differences have been reported when F1 histone fractions isolated from different tissues of the same organism have been compared (Sheriden and Stern, 1967; Bustin and Cole, 1968; Kinkade, 1969; Panyim and Chalkley, 1969 *a*; Bustin and Stollar, 1972). Recently, considerable interest has centered around the fact that histone F1 can be phosphorylated in vivo, and it has been suggested that phosphorylation of F1 may play an important role either in chromo-

some replication (Oliver et al., 1972 *a*) in the control of genetic activity (Kleinsmith et al., 1966; Guttierrez-Cernosek and Hnilica, 1971; Meisler and Langan, 1969), or in mitosis (Lake et al., 1972; Bradbury et al., 1973).

We have been studying the histones of the ciliated protozoan, *Tetrahymena pyriformis*, in an effort to understand the underlying molecular basis for the differences in structure and function of the ciliate macro- and micronucleus (see Gorovsky, 1973), and to compare the histones of this lower eukaryote to those of mammalian cells. In particular, we wished to determine whether the correlation between cell replication and phosphorylation of histone F1 which had been observed in mammalian cells (Balhorn et al., 1972 *b*; Stevely and Stocken, 1968) could be extended to lower eukaryotes as well. Moreover, since macronuclei divide amitotically, with no signs of chromosome condensation (Flickinger, 1965; Nilsson, 1970), analysis of macronuclei should provide insight into whether phosphorylation of histone F1 plays a unique role in the condensation of chromosomes during mitosis (Bradbury et al., 1973).

MATERIALS AND METHODS

Culture Methods

T. pyriformis (syngen I, mating-type I, strain WH-6) were cultured in enriched proteose-peptone as described previously (Gorovsky et al., 1973). Cell counts were performed by measuring the optical density (OD) at 550 nm on a Bausch and Lomb 340 spectrophotometer, (Bausch and Lomb Inc., Scientific Instrument Div., Rochester, N. Y.). Under the conditions used, 1.0 OD = approximately 1×10^6 cells/ml.

Isolation of Macronuclei

Macronuclei were isolated as previously described (Gorovsky, 1970) except that 0.1% spermidine trihydrochloride was added to all media to preserve nuclei and to inhibit proteolysis.

Extraction of Total Histone

Isolated macronuclei were washed with 0.1% spermidine trihydrochloride, adjusted to pH 3.6 with 1.0 N acetic acid. Histones were extracted from the washed nuclei by repeated extraction with either 2.4 M urea-0.4 N H_2SO_4, or with 0.4 N H_2SO_4 alone. Histones were precipitated from the pooled extracts by treatment overnight at −20°C with 4 vol of 95% or 100% ethanol, washed in 95% ethanol, and dried under vacuum at room temperature. Whole calf thymus histone was isolated from calf thymus chromatin as described by Panyim et al. (1971).

Isolation of F1

FROM ISOLATED NUCLEI: Histone fraction F1 was removed from isolated nuclei be repeated extraction with 0.5 M or 0.74 M perchloric acid (PCA) (Johns, 1964), precipitated by addition of 50% trichloroacetic acid (TCA) to a final concentration of 20%, washed with acetone, and air-dried under vacuum.

FROM WHOLE HISTONES: F1 was isolated from whole histone either by repeated resuspension of the dried histone in 0.001 N HCl followed by addition of PCA to a concentration of 0.50 M (Oliver et al., 1972 *b*); or by direct extraction of the histone powder with 0.50 M or 0.74 M PCA. F1 was obtained from the PCA supernate either by addition of H_2SO_4 to a final concentration of 0.4 N followed by precipitation in 6 vol of acetone (Oliver et al., 1972 *b*), or by addition of 50% TCA to a final concentration of 20% followed by centrifugation and washing of the TCA precipitate in acetone.

FROM WHOLE CELLS: F1 was extracted directly from whole cells with 5% TCA by the method of DeNooij and Westenbrink (1962).

Polyacrylamide Gel Electrophoresis

Electrophoresis at low pH in 2.5 M urea–15% polyacrylamide gels was performed by the method of Panyim and Chalkley (1969 *b*). Gel lengths were either 25 cm (long) or 8.5 cm (short). Gels were stained in fast green as described previously (Gorovsky et al., 1970).

Electrophoresis in sodium dodecyl sulfate (SDS)-containing polyacrylamide gels at both pH 7.6 and pH 10.0 was performed according to the method of Panyim and Chalkley (1971). Whole calf thymus histones or isolated fractions of calf thymus histone were used as standards. SDS-containing gels were stained overnight in 0.1% fast green, 20% acetic acid, and 50% methanol and were destained by repeated changes of 7.5% acetic acid–5.0% methanol.

Fast green-stained gels were scanned at 630 nm using a Gilford 2400 spectrophotometer (Gilford Instrument Laboratories, Inc., Oberlin, Ohio), equipped with a 2410 linear transport. Electrophoretic mobilities of the various histone fractions were calculated either from measurements made on the densitometer tracings or from measurements made directly on the gels. Mobilities are expressed relative to the fastest-migrating subspecies of fraction F2A1 of either calf thymus or *Tetrahymena*, which have been

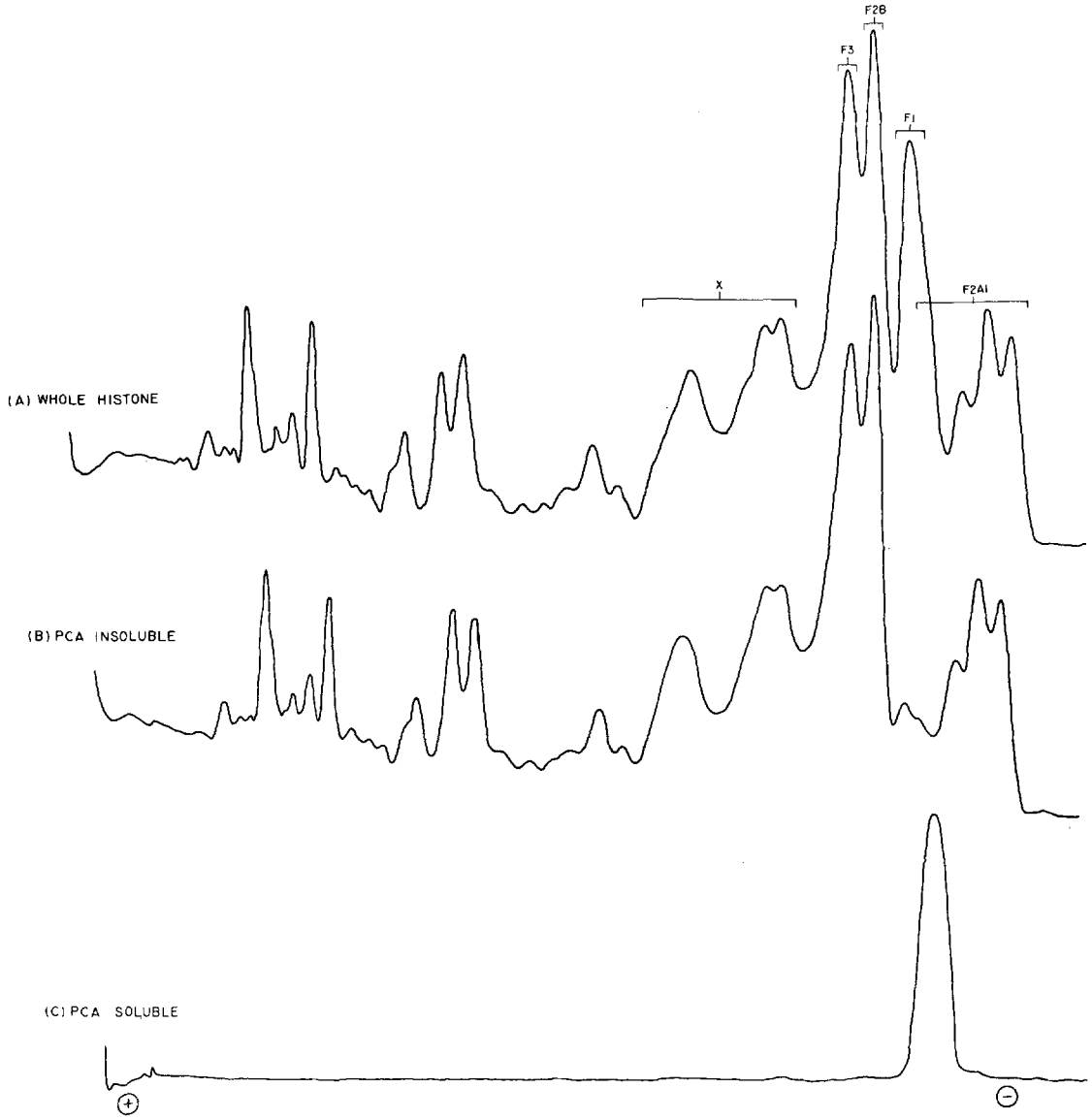

FIGURE 1 Densitometer tracings of short polyacrylamide gels containing (A) whole histone, (B) PCA-insoluble histones, and (C) PCA-soluble histones extracted from macronuclei of *T. pyriformis*. Electrophoretically separable components have been identified as previously described (Gorovsky et al., 1973). The fraction(s) labeled *X* have not yet been identified. The peaks to the left of fraction(s) *X* represent nonhistone contamination (probably nucleolar or ribosomal contaminants) which can be largely removed by isolating histones from chromatin instead of directly from macronuclei. It should be noted that while a considerable number of acid-soluble slow-migrating proteins can be extracted from isolated macronuclei, none of them is soluble in PCA. Electrophoresis at 130 *V* for 240 min.

shown to have identical mobilities in both types of gel (Gorovsky et al., 1973).

Phosphatase Treatment

Histone fraction F1 isolated from *Tetrahymena* macronuclei was treated with *Escherichia coli* alkaline phosphatase as described by Sherod et al. (1970).

RESULTS

Low pH-Acrylamide Gel Electrophoresis

Extraction with 5% PCA routinely solubilized 20–25% of the total fast green staining-material from whole histones or from isolated nuclei (Fig. 1).

The bulk of the extracted material had a relative electrophoretic mobility (on low pH acylamide gels) greater than that of any other *Tetrahymena* histone fraction except three of the four fastest-migrating subspecies of fraction F2A1 (Fig. 1), and migrated approximately 30% faster than purified calf thymus F1 (Fig. 2). Since PCA solubility is a diagnostic characteristic of fraction F1, and since this PCA-soluble fraction from *Tetrahymena* showed an amino acid composition not unlike that of calf thymus F1 (Table I), we entatively concluded that, in spite of its unusual electrophoretic mobility, this fraction corresponded to F1 from other organisms.

However, while it is clear that the precise relative electrophoretic mobilities of F1s isolated from different organisms vary considerably (Panyim et al., 1971), F1 is the slowest-migrating histone fraction in all organisms examined to date. Since histone F1 is particularly susceptible to specific proteolytic degradation which can result in the appearance of new molecular species on polacrylamide gels (Bartley and Chalkley, 1970), we examined the possibility that the unusual

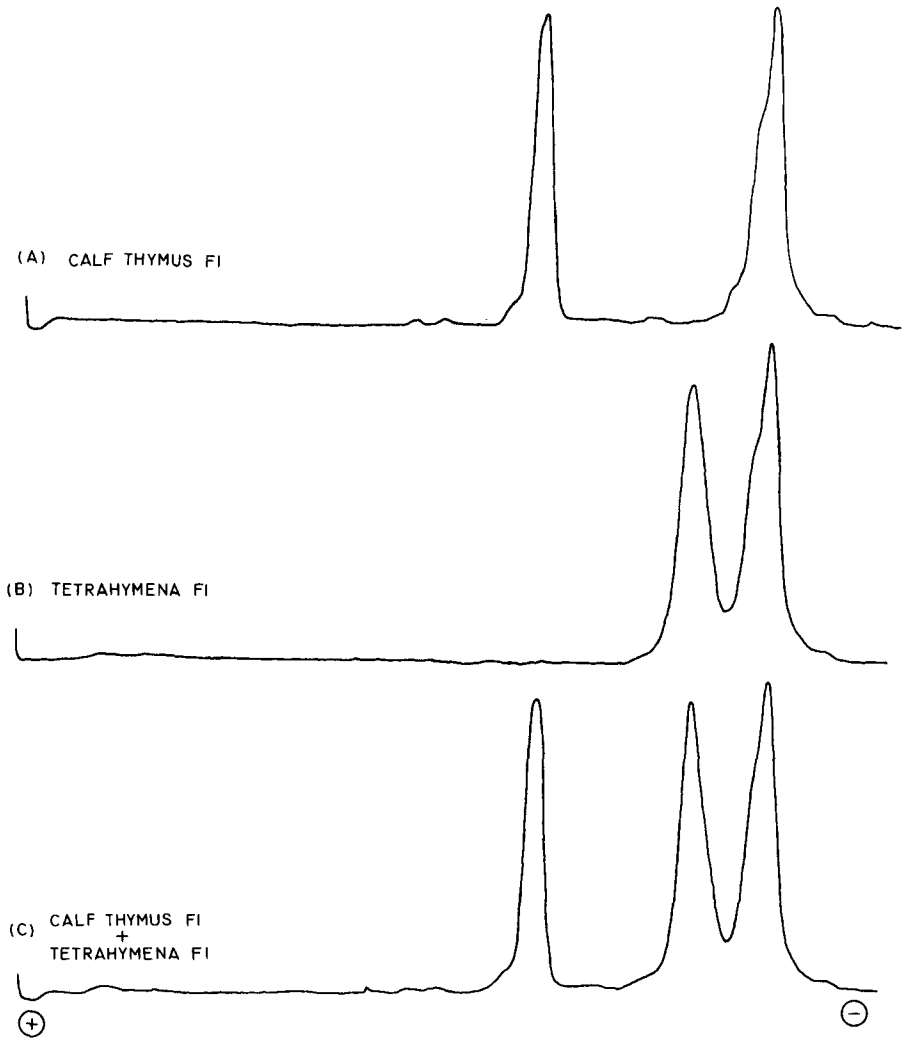

FIGURE 2 Densitometer tracings of short polyacrylamide gels containing (A) calf thymus F1, (B) *Tetrahymena* F1, and (C) *Tetrahymena* F1 plus calf thymus F1. The fastest-migrating peak in each tracing is calf thymus F2A1 which was added to each gel as a mobility marker. Electrophoresis at 120 V for 265 min.

TABLE I

Amino Acid Composition of Histone Fraction F1 Isolated from T. pyriformis and from Calf Thymus

Amino acid	Moles/100 moles of amino acids		
	Tetrahymena* strain WH-6	Calf thymus‡	Tetrahymena§ strain GL
Lysine	29.9	27.3	33.4
Histidine	2.3	0.0	2.1
Arginine	2.4	2.1	2.9
Aspartic acid	9.9	2.6	7.6
Threonine	8.6	6.0	10.6
Serine	7.5	6.9	7.8
Glutamic acid	7.3	4.7	4.8
Proline	6.1	8.4	6.1
Glycine	3.5	6.7	2.2
Alanine	14.0	23.4	12.7
Cysteine	0.0	0.0	0.0
Valine	3.9	6.0	6.9
Methionine	Trace	Trace	0.5
Isoleucine	2.3	0.8	2.3
Leucine	1.7	3.9	1.1
Tyrosine	0.5	0.5	0.2
Phenylalanine	0.4	0.4	0.0
Lysine/arginine	12.5	13.0	11.5
Basic/acidic	2.0	4.0	3.1

* We are grateful to Dr. Roger Chalkley, Department of Biochemistry, School of Medicine, University of Iowa, for providing the amino acid analysis of *Tetrahymena* F1.

‡ From Panyim et al., 1971.

§ Average values for fractions III and C7 computed from data in Hamana and Iwai, 1971.

mobility of *Tetrahymena* F1 was due to proteolytic degradation of a larger, slower-migrating molecule. Conditions which are known to inhibit proteolysis in other systems, or which might reasonably be expected to reduce proteolysis, consistently resulted in F1 with the electrophoretic shown in Figs. 1 and 2. Thus, isolating nuclei rapidly, at pH 4.0 or 5.0, in the presence of 0.05 M $NaHSO_3$, of 0.5 M mercaptoethanol, or of 0.01 M dithiothreitol did not significantly alter the electrophoretic pattern of *Tetrahymena* histones. Similarly, isolating F1 directly from whole cells with 5% TCA containing either 0.05 M bisulfite, 0.001 M phenyl methyl sulfonyl fluoride, or 0.1 M phenylhydrazine hydrochloride yielded F1 prep-a rations consisting almost entirely of a fraction with an electrophoretic mobility like that in Figs. 1 and 2. Evidence of proteolytic degradation of *Tetrahymena* F1 was observed under some conditions, particularly when isolations were done slowly or in the absence of inhibitors. Such proteolysis involved a loss of material migrating as in Fig. 2 and the appearance of material with a relative electrophoretic mobility somewhat greater than F2A1. This material, like the slower-migrating species from which it is probably derived, is soluble in 5% PCA. We conclude, therefore, that the unusual electrophoretic mobility of *Tetrahymena* F1 is probably not due to proteolysis (see also results on SDS acrylamide gel electrophoresis below).

In the course of these studies on F1 proteolysis we also observed that spermidine trihydrochloride at a concentration of 0.1% was an effective inhibitor of F1 degradation in *Tetrahymena* (Grrovsky and Keevert, unpublished observations). Since spermidine also stabilized nuclear strueture during isolation of nuclei, it was routinely added to all isolation media.

SDS Acrylamide Gel Electrophoresis

In a further attempt to determine if limited proteolysis might be responsible for the unusual electrophoretic mobility of *Tetrahymena* F1, we have examined the properties of isolated F1 by electrophoresis in SDS-containing polyacrylamide gels. We reasoned that if *Tetrahymena* F1 migrated more rapidly than calf thymus F1 because it was partially degraded, it should have a significantly lower molecular weight than calf thymus F1. Fig. 3 shows that *Tetrahymena* F1 migrates more slowly than calf thymus F1 on SDS-containing gels at pH 7.6, suggesting that *Tetrahymena* F1 has a greater molecular weight. However, when the two F1s are compared by electrophoresis on SDS-containing gels at pH 10, (Fig. 4), *Tetrahymena* F1 migrates slightly faster than calf thymus F1. The reason for this discrepancy is unclear (see Discussion). It is clear, however, that in SDS-containing gels at either pH, *Tetrahymena* F1 migrates more slowly (has a higher molecular weight) than any other *Tetrahymena* histone fraction. These results suggest that the unusually high mobility of *Tetrahymena* F1 in low pH acrylamide gels is due, at least in part, to its having a higher positive charge (at low pH) than either calf thymus F1 or than any of the other *Tetrahymena* histones (see Discussion).

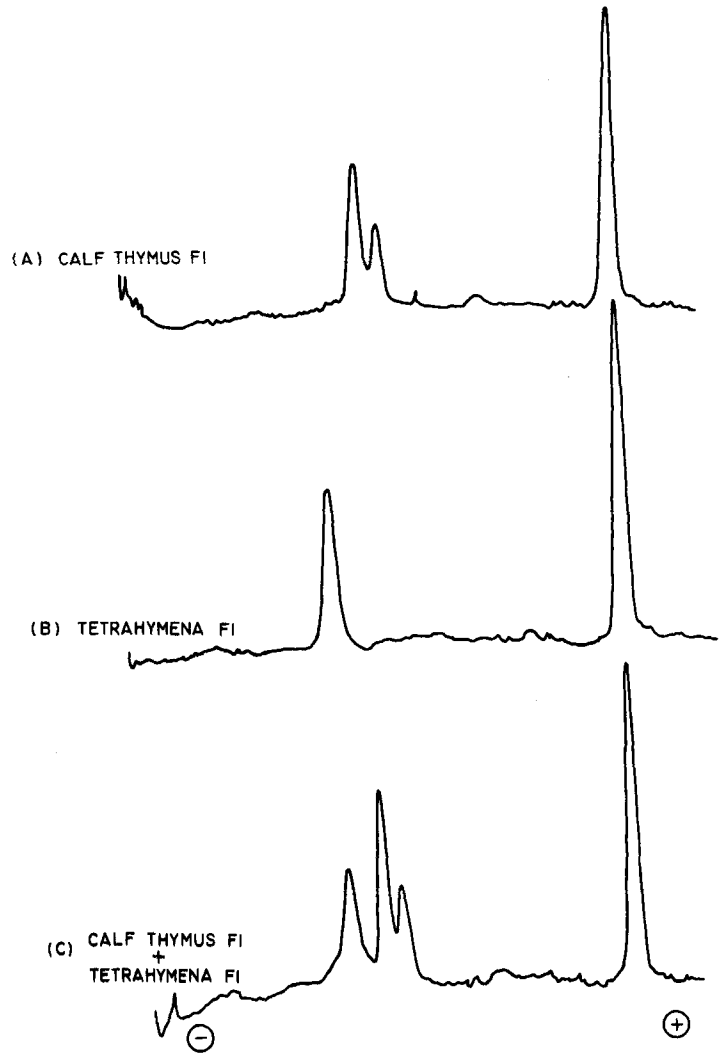

FIGURE 3 Densitometer tracings of SDS-polyacrylamide gels, pH 7.6, containing (A) calf thymus F1, (B) *Tetrahymena* F1, and (C) *Tetrahymena* F1 plus calf thymus F1. The fastest-migrating peak in each tracing is calf thymus F2A1 which was added to each gel as a mobility marker. Electrophoresis at 80 V for 390 min.

Phosphorylation of Tetrahymena F1

When histones isolated from rapidly growing cells (log phase; cell density = 250,000 cells/ml) were compared to those isolated from slowly growing cells (decelerator growth phase; 850,000 cells/ml), a striking difference was observed in the electrophoretic mobility of histone fraction F1. In short gels, F1 from slowly growing cells migrated as a sharper band with a significantly greater mobility than that of F1 from log phase cells (Fig. 5). Since a correlation has recently been demonstrated between phosphorylation of histone fraction F1 and the rate of cell division in a number of mammalian cell types (Stevely and Stocken, 1968; Balhorn et al., 1971; 1972 *a*; 1972 *b*), we tested the possibility that phosphorylation might explain the differences in mobility of F1 isolated from the different stages of culture growth. When F1 isolated from log phase cells is analyzed on long polyacrylamide gels, the broad, slow-migrating peak is better resolved into multiple components (Fig. 6). After treatment with alkaline phosphatase, these

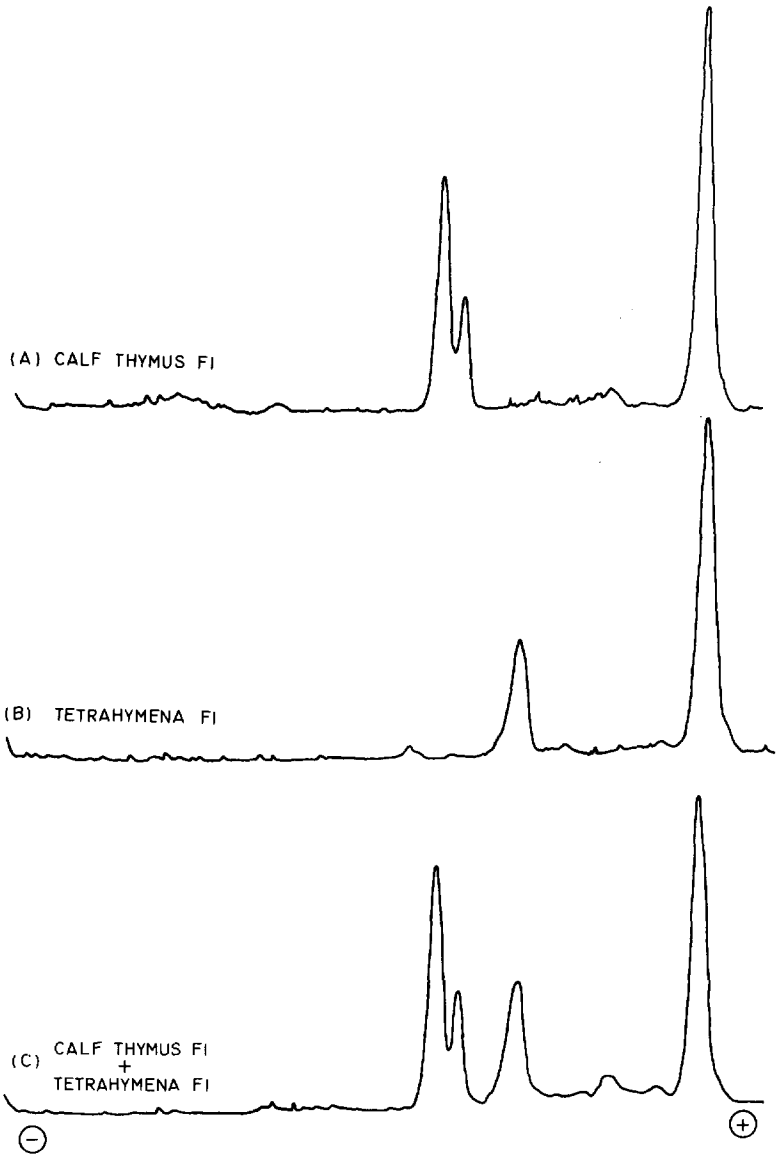

FIGURE 4 Densitometer tracings of SDS-polyacrylamide gels, pH 10.0, containing (A) calf thymus F1, (B) *Tetrahymena* F1, and (C) *Tetrahymena* F1 plus calf thymus F1. The fastest-migrating peak in each tracing is calf thymus F2A1 which was added to each gel as a mobility marker. Electrophoresis at 100 V for 490 min.

components are converted into a sharp, rapidly migrating species (Fig. 6), with an electrophoretic mobility indistinguishable from that of the major component of F1 isolated from slowly growing cells (Fig. 7). When F1 isolated from slowly growing cells is treated with alkaline phosphatase, the small amounts of the slower-migrating components are rapidly converted to a faster-migrating form (Fig. 7). These studies suggest that the difference in electrophoretic mobility between F1 of rapidly growing and of slowly growing cells is due to the presence of large amounts of covalently bound phosphate groups on F1 of log phase cells. In addition, fully dephosphorylated F1 from both rapidly and slowly growing cells seems to consist almost

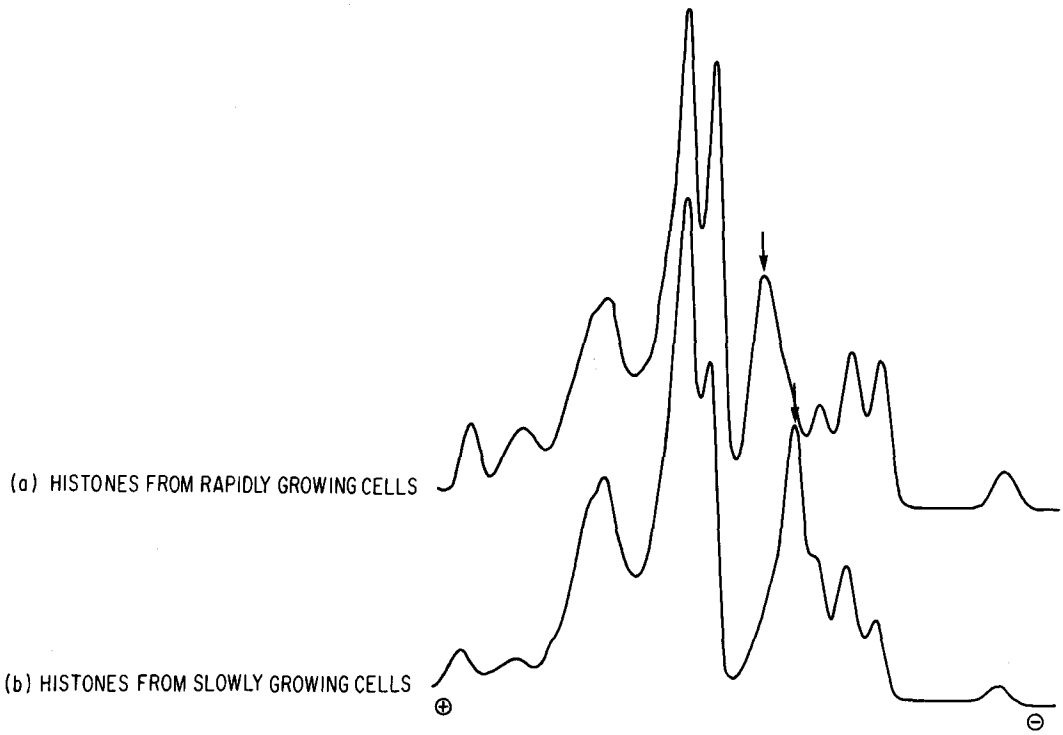

FIGURE 5 Densitometer tracings of short polyacrylamide gels containing histones isolated from (A) rapidly growing cells and (B) slowly growing cells. The arrows indicate the position of fraction F1. Electrophoresis at 150 V for 270 min.

entirely of a single electrophoretic species having an electrophoretic mobility identical to that of the major F1 subspecies isolated from slowly growing cells (Fig. 6).

DISCUSSION

Electrophoretic Properties of Tetrahymena

We have observed that *Tetrahymena* F1 has a higher apparent molecular weight than any other *Tetrahymena* histone when examined on SDS-containing acrylamide gels either at pH 7.6 or at pH 10.0 and that F1 is eluted before the other *Tetrahymena* histone fractions when examined by molecular exclusion chromatography (unpublished observations). Clearly then, on the basis of molecular weight alone, F1 should migrate more slowly than any other *Tetrahymena* histone.

One possible explanation of the unusual electrophoretic mobility in urea-containing acrylamide gels at low pH is that *Tetrahymena* F1 has a significantly higher number of basic amino acids than calf thymus F1 and than the other *Tetrahymena* histones. This is borne out by an examination of the amino acid composition of *Tetrahymena* F1 which indicates that it contains approximately 18% (34.6% basic residues in *Tetrahymena* F1, compared to 29.4% in calf thymus; see Table I) more basic residues than does calf thymus F1. Nonetheless, it is unlikely that the difference in the number of basic groups between *Tetrahymena* and calf thymus F1 is sufficient to account for the large (30%) difference in relative electrophoretic mobilities of these two proteins in low pH gels. Our studies on SDS-containing gels (see below) at pH 10.0 indicate that the molecular weight of *Tetrahymena* F1 is somewhat lower than that of calf thymus F1. It is likely, therefore, that these two properties of *Tetrahymena* F1, an increase in total basic residues coupled with a lower molecular weight (compared to calf thymus F1), together account for its unique electrophoretic mobility on low pH, urea-containing polyacrylamide gels.

It should be noted that Smith et al. (1970) have suggested that the histone of slowest mobility in a low pH urea-acrylamide gel of *Tetrahymena* histones is fraction I (=F1). However,

they have presented no evidence (other than the low electrophoretic mobility) for identifying this fraction as F1 and, on the basis of our results, it is likely that this fraction is not F1, but is rather a fraction which is as yet uncharacterized (fraction X, Fig. 1).

It is tempting to suggest that the unusual behavior of *Tetrahymena* F1 on SDS-containing polyacrylamide gels is also due to its unusually large number of positively charged groups. It is known, for example, that histones fall on a different standard curve than do other proteins when their relative electrophoretic mobilities are plotted against the logarithms of their molecular weights (Panyim and Chalkley, 1971). Therefore it is likely that the net positive charge on a histone molecule affects its mobility in SDS-containing

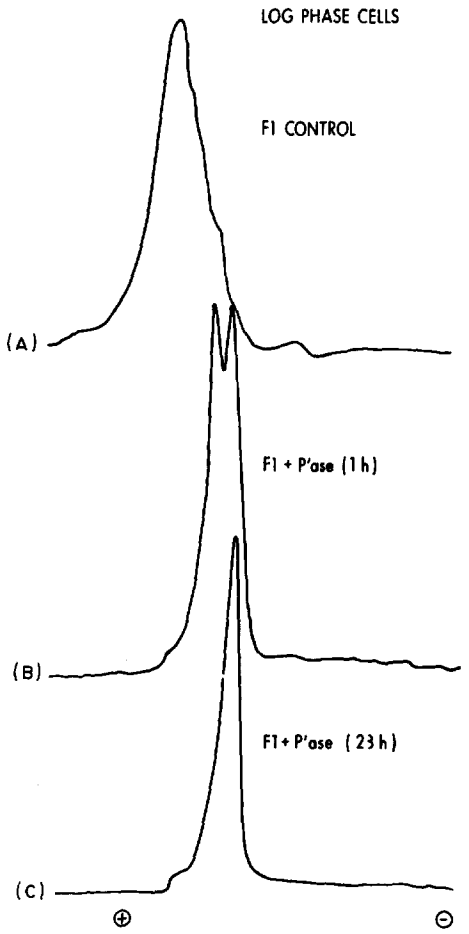

FIGURE 6 Densitometer tracings of long polyacrylamide gels containing F1 isolated from rapidly growing cells before and after treatment with alkaline phosphatase. (A) Control, (B) treatment for 1 h, (C) treatment for 23 h. Tracings were lined up by including calf thymus F2A1 (not shown) as a marker in each gel. Electrophoresis at 250 V for 25 h.

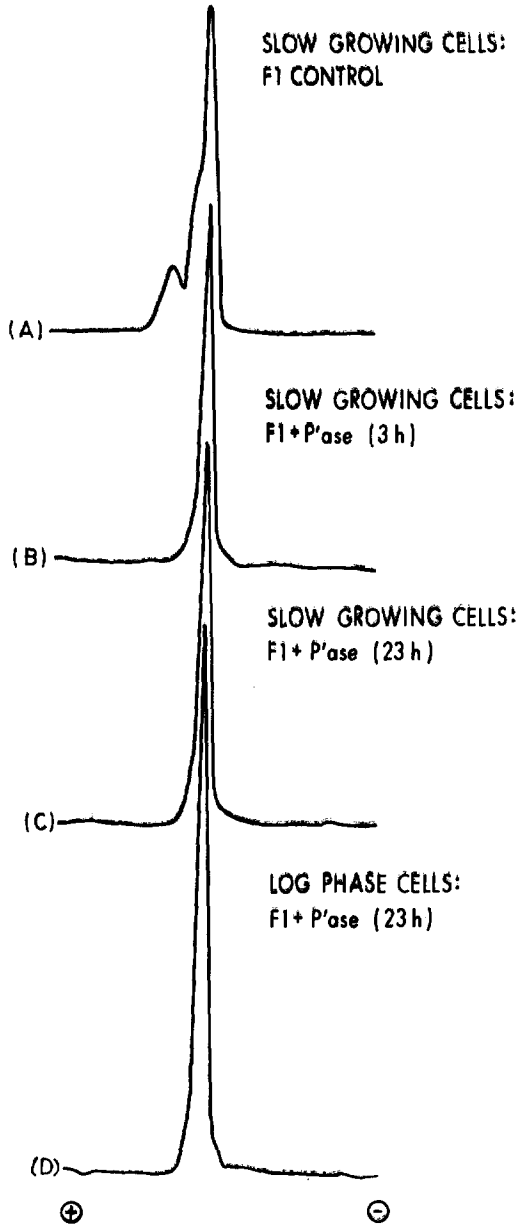

FIGURE 7 Densitometer tracings of long polyacrylamide gel containing F1 isolated from slowly growing cells (A–C) before and after treatment with alkaline phosphatase. (D) shows a sample of F1 from rapidly growing cells which has been extensively (23 h) treated with phosphatase. Tracings were lined up by including calf thymus F2A1 (not shown) as a marker in each gel. Electrophoresis at 250 V for 25 h.

gels, and the marked pH dependence of the mobility of *Tetrahymena* F1 in SDS-containing gels may be related, in part, to the large number of basic groups which it contains. Unfortunately, the correlations between molecular weights and relative electrophoretic mobilities in SDS gels, even for the calf thymus histones which are used as standards, are not precise. For example, calf thymus fractions F2B and F3 have identical electrophoretic mobilities on SDS gels, although their molecular weights determined by primary sequence analysis differ considerably (Iwai et al., 1972; DeLange et al., 1972). Moreover, the amino acid analyses (Table I) indicate that while *Tetrahymena* F1 contains a greater number of basic amino acids than does calf thymus F1, it also contains a significantly greater number of acidic amino acids, so that the net positive charge of *Tetrahymena* F1 is probably smaller than that of calf thymus at both pH 7.6 and at pH 10.0. At the present time, therefore, we cannot determine whether it is the large number of basic residues or other unknown factors (such as peculiarities of amino acid composition or charge distribution which affect SDS binding, a pH-dependent conformational change, etc.) which account for the behavior of *Tetrahymena* F1 in SDS-containing gels.

We had also hoped to determine the apparent molecular weight of *Tetrahymena* F1 at a pH which is sufficiently high so that the positive charges of both the ϵ-amino groups of lysine and the guanido groups of arginine are neutralized. However, technical difficulties have prevented us from performing analyses on SDS gels above pH 10 (Panyim and Chalkley, 1971; S. Mancuso, unpublished observations).

Heterogeneity of Tetrahymena F1

Hamana and Iwai (1971) have reported that the amino acid compositions of a number of histone fractions (their fractions I, I-C6, C6-1, C6, C6-1, III, and C7) extracted from *Tetrahymena* macronuclei (strain GL) and purified by column chromatography had amino acid compositions similar to that of calf thymus F1. However, five o these fractions had lower molecular weights and were less basic than either fraction III (purified by chromatography on amberlite CG-50) or fraction C7 (purified by chromatography on CM-cellulose). Thus, they may have been products of proteolytic degradation of fraction III or C7 whose electrophoretic properties and amino acid compositions were indistinguishable. We have found that when care is taken to avoid proteolytic degradation and to completely dephosphorylate F1 with alkaline phosphatase, F1 isolated from strain GL (unpublished observations) as well as from the strain (WH-6) studied here migrates as a single, homogeneous band during electrophoresis on both urea-acrylamide gels at low pH, and on SDS-containing acrylamide gels at either pH 7.6 or pH 10.0.

It should also be noted that a comparison of the amino acid composition of either fraction III or C7 of strain GL (Hamana and Iwai, 1971), and that reported here for F1 from strain WH-6 indicates that they are remarkably similar. Both the GL and WH-6 fractions contain more basic residues than calf thymus F1, but also contain considerably more acidic amino acids and less alanine than calf thymus F1 (Table I). *Tetrahymena* F1 also appears to contain histidine which is absent in calf thymus F1.

Evolution of F1

Histone fraction F1 is unusual in that the electrophoretic mobilities of F1s isolated from different organisms vary considerably, while the mobilities of the other fractions remain relatively constant. In low pH polyacrylamide gels, mobilities ranging from approximately 20% slower (*Drosophila* F1, Cohen and Gotchel, 1971; Oliver and Chalkley, 1972) to approximately 30% faster (this report) than calf thymus F1 have been reported. In both of these extreme cases, a major change in the number of basic residues of the molecule has probably played a role in the altered electrophoretic mobility (arbitrarily assuming calf thymus F1 is "normal"). It should be noted, however, that while the variability of histone fraction F1 from species to species is often emphasized, a surprising number of the physical, chemical, and physiological properties of this fraction appear to be common to all eukaryotic organisms. Most, if not all, eukaryotic organisms examined to date contain a histone fraction (F1) which: (*a*) is uniquely soluble in PCA; (*b*) is the most lysine-rich fraction, and (*c*) is the histone fraction of greatest molecular weight. In organisms as diverse as mammals and ciliates, this fraction is the histone most easily dissociated from chromatin by salts and acid, is susceptible to specific proteolysis, and is highly phosphorylated in

rapidly dividing cells. Thus, in spite of the considerable variation in electrophoretic mobility, the common properties of F1s from widely different organisms make it likely that the function of F1 in all organisms is similar.

Kinkade and Cole (1966 *b*) have suggested that different F1s may have quite similar amino acid sequences in part of the molecule, while the sequences of other parts of the molecule differ in varying degrees. If different F1s do, in fact, contain such evolutionarily constant as well as evolutionarily variable sequences, the constancy of many of the physical and chemical properties of F1 from different organisms as well as the variability in electrophoretic properties, molecular weight, and charge density may be attributable to different portions of the molecule. Such a correlation awaits the complete sequence of at least two different F1 molecules.

Phosphorylation of Histone F1

With the exception of recent studies on the histones of sea urchin testes (Subirana and Unzeta, 1972) and of *Physarum* (Bradbury et al., 1973), phosphorylation has been extensively studied only in higher organisms, and considerable controversy exists regarding its function. It has been variously suggested that phosphorylation plays a role in gene activation (Kleinsmith et al., 1966; Guttierrez-Cernosek and Hnilica, 1971), in chromosome replication (Oliver et al, 1972 *a*), and in mitosis (Lake et al., 1972; Bradbury et al., 1973). As is the case in mammalian cells (Balhorn, et al., 1971; 1972 *a*; 1972 *b*), we have observed that phosphorylation of histone F1 in *Tetrahymena* is correlated with the stage of growth of the cells. While these observations seem to suggest a relationship between F1 phosphorylation and cell replication, it is still possible that the level of genetic activity in slowly growing cells is lower than that in rapidly growing cells. Our finding of extensive phosphorylation of F1 in macronuclei of rapidly dividing cells does, however, rule out a unique function of F1 phosphorylation in chromosome condensation (Bradbury et al., 1973) or in the process of mitosis itself (Lake et al., 1972), since macronuclei divide amitotically without any marked changes in chromatin structure (Flickinger, 1965; Nilsson, 1970).

Finally, *Tetrahymena* offers some particular advantages for the study of F1 phorphorylation in greater detail. Since unphosphorylated *Tetrahymena* F1 exists as a single electrophoretic species, it is particularly easy to distinguish between phosphorylated and unphosphorylated forms by electrophoretic criteria (Balhorn et al., 1971). Finally, the micronucleus of *Tetrahymena* undergoes DNA replication, but is genetically inactive (see Gorovsky, 1973 for review) so that a demonstration that phosphorylation of F1 occurs in micronuclei might implicate phosphorylation in the process of chromosome replication, while the absence of F1 phosphorylation in micronuclei may suggest that it played a role in gene activation. These studies are now in progress.

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