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Nucleohistone Composition in Stationary and Division Synchronized *Tetrahymena* Cultures*

Y. C. Lee and O. H. Scherbaum

ABSTRACT: The nonionic detergent Triton X-100 was used for the isolation of the macronuclei which were found by electron microscopy to be free from cytoplasmic contamination. Nuclei of stationary phase cells contain in weight per cent of substances analyzed 2.3% ribonucleic acid (RNA), 22.4% deoxyribonucleic acid (DNA), and 75.2% protein. The nuclear proteins (44%) are histones. The lysine/arginine ratio varies from 3.4 to 5.3. The amino acid composition of *Tetrahymena* histone is comparable to published results on histones of chicken 5rythrocytes. By electrophoresis on polyacrylamide gel the histones could be separated into nineteen bands. Similar electrophoresis patterns were found for log and stationary cells, as well as for cells at the end of the heat treatment (EHT) and 1 hr later (EHT + 1). Minor but distinct differences were observed between these two groups of samples. Histones extracted from log and stationary phase cells contain slow-moving components which are absent in the patterns of histones prepared at EHT and EHT + 1. The latter show fast-moving components which are absent in the patterns of histones prepared from log and stationary phase cells. The specific activity of L-[U-^{14}C]lysine was determined in 17 histone fractions. Although the nuclear histone content decreases prior to synchronized cell division (EHT + 1 hr), the rate of lysine incorporation is twice the rate found in log phase cells or in the division-blocked cells at the end of the heat treatment (EHT).

Studies of nucleohistone composition and metabolism in animal and plant tissues provide evidence for the current view that histones, among other suggested functions, are involved in regulation of gene activity (Stedman and Stedman, 1950; Huang and Bonner, 1962; Allfrey *et al.*, 1963; Irvin *et al.*, 1963; Moore, 1963; Dulbecco, 1964). Consequently, histones could serve as gene modulators in the differentiation of higher organisms and in the life cycles of single cell organisms lacking differentiation but exhibiting highly ordered development. The infraciliature of ciliates is a useful biological structure for studies of cellular development (Frankel, 1962). During the intermittent heat treatment for induction of synchronized division in *Tetrahymena*, synthesis of total protein and nucleic acids does occur, but cells are blocked at the same stage of their development in oral morphogenesis (Holz *et al.*, 1957). In preparation for synchronized division additional protein synthesis has to occur (Zeuthen, 1964).

In this investigation we have determined the amino acid composition, electrophoresis pattern, and incorporation of L-[U-^{14}C]lysine into *Tetrahymena* histones during exponential multiplication, stationary phase, and synchronized cell division. The results are compared with published data on the amino acid composition (Vande Woude, 1964, 1965; Iwai *et al.*, 1965) and electrophoresis patterns (Hardin and Lindsay, 1965) of *Tetrahymena* histone.

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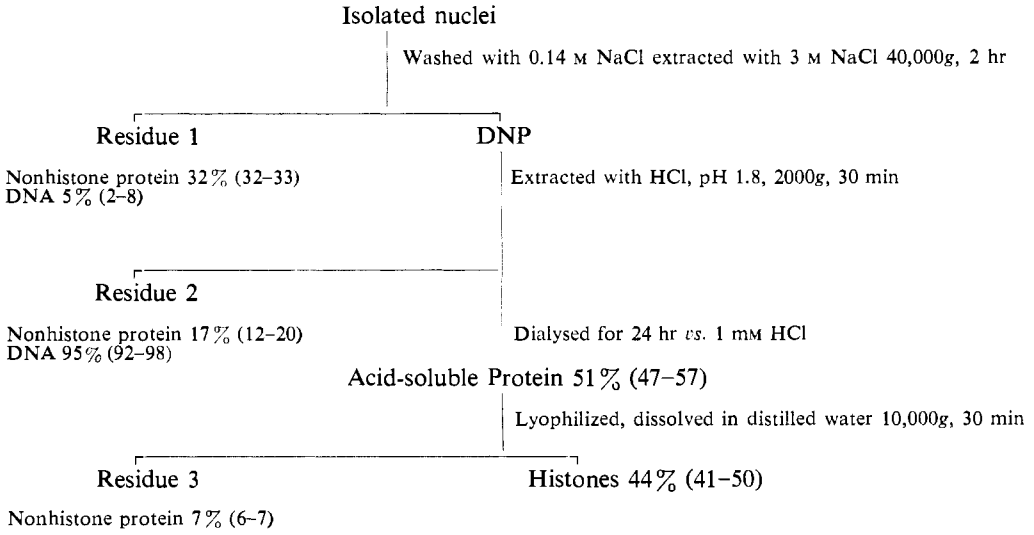

FIGURE 1: Flow sheet for the purification of deoxyribonucleoproteins (DNP), acid-soluble proteins, and histones from isolated *Tetrahymena* nuclei. All per cent values given comprise the four growth stages shown in Table II.

Methods

Culture of Organism. The growth conditions for *Tetrahymena pyriformis* GL were the same as described earlier with the exception of the composition of the culture medium which contained 2% (w/v) proteose peptone (Difco), 0.1% (w/v) bactodextrose (Difco), 0.1% (w/v) sodium acetate, and 0.1% (w/v) potassium phosphate (Scherbaum and Jahn, 1964). In pilot and labeling experiments, 1-l. cultures were grown in 2.5-l. low-form culture flasks, otherwise 5–8-l. cultures were used. The cultures were sampled at the following growth stages: (1) during exponential multiplication (*ca.* 70,000 cells/ml); (2) at the end of the intermittent heat treatment (EHT);[1] (3) 1 hr later, that is, 20 min before the synchronous division burst (EHT + 1); and (4) 24 hr after EHT (stationary phase).

The heat treatment for the induction of synchronized cell division in *T. pyriformis* GL consists of seven cyclic changes between the optimum temperature (29°) and 34° at 0.5-hr intervals. During this treatment synthesis of nucleic acids and proteins continues, but cell division is blocked. At EHT the cells are abnormally large and at EHT + 80 min more than 80% of the cells in culture divide synchronously (Scherbaum, 1964).

Isolation of Nuclei. Triton X-100 and 0.5 M sucrose in a diluted Ringer's sodium phosphate (RSP) buffer (final concentration: 0.047 M sodium chloride, 0.002 M potassium chloride, 0.001 M magnesium sulphate, and 0.0125 M sodium phosphate buffer, pH 7.3) was used for the isolation of the macronuclei. The method is essentially the same as reported by Lee and Scherbaum (1965) with the following modifications. Immediately after lysis of the cells an equal volume of 4% (w/v) polyvinylpyrrolidone in RSP buffer was added to the suspension. Polyvinylpyrrolidone was found to be more effective in protecting the nuclear integrity than the culture medium used previously. Another modification was made in the composition of the suspension medium for purification of the nuclei by centrifugation. The concentration of Triton X-100 was reduced to 1% (v/v), but polyvinylpyrrolidone (1%, w/v) was added; the concentrations of the phosphate buffer and sucrose were not changed. This modification improved the stabilization of the separated layers after centrifugation and increased the stability of the nuclei. Routine checks of the purity of the preparations and nuclei counts were made with a Leitz phase-contrast microscope. *Ca.* 30% of the nuclei present in the sample prior to cell lysis could be recovered in the final highly purified preparation.

For the examination of the ultrastructure of the isolated nuclei, samples were fixed in 6% glutaraldehyde in phosphate buffer, pH 7.3, and in 1% (w/v) osmic acid in Milonig's buffer, pH 7.4, stained with 2% (w/v) uranyl acetate and embedded in Epon. Sections, cut with a Porter-Blum ultramicrotome, were stained with lead hydroxide. The sections were examined in a Hitachi HU 11A electron microscope (Tokuyasu and Scherbaum, 1965).

Chemical Determinations. Ca. 50–60 million of purified nuclei were used in each experiment for nucleic acid and protein determinations. After three washings with ice-cold 0.25 N perchloric acid (Rozijn and Tonino, 1964), the nuclei were digested in 0.3 N KOH at 37° for 1 hr. During this treatment only negligible amounts of protein are released without impairing the quantitative recovery of RNA from rat liver (Fleck and Munro, 1962) and from isolated nuclei of *Saccharomyces cerevisiae* (Rozijn and Tonino, 1964).

The pH of the KOH digest was adjusted to 1, and

[1] Abbreviations used: EHT and EHT + 1, end of heat treatment and EHT plus 1 hr; RSP, Ringer's sodium phosphate; DNP, deoxyribonucleoprotein.

the resultant precipitate was washed twice with 0.25 N perchloric acid. The pooled supernates were used for ribonucleic acid (RNA) determinations by the spectrophotometric method of Spirin (1958). The residue was hydrolyzed twice in 0.5 N perchloric acid at 70° for 15 min. Deoxyribonucleic acid (DNA) was measured in the supernate by the method of Burton (1956). The protein content in the KOH digest was determined by a modified Lowry procedure (Chou and Goldstein, 1961), using purified *Tetrahymena* protein as a standard.

Amino Acid Analyses. For the amino acid analyses of three protein fractions, each sample (1–2 mg) was hydrolyzed in 0.2 ml 6 N HCl at 110° in sealed Pyrex tubes for 24 hr. For each experiment duplicate samples were prepared. The hydrolysates were evaporated to dryness *in vacuo* and then dissolved in 5 ml of 0.2 M sodium citrate buffer, pH 2.2, for amino acid analysis in a Beckman/Spinco automatic amino acid analyzer, Model 120 (Neelin *et al.*, 1964).

Extraction of Histones. The method of Neelin and Butler (1959) was used for the extraction of histones. Isolated nuclei were washed with 0.14 M NaCl, collected by centrifugation at 1700*g*, and extracted for 18–24 hr at 3° with 3 M NaCl to a final concentration of 50–100 μg of DNA/ml. Insoluble material was removed by centrifugation at 40,000*g* for 2 hr. The pH of the supernate, containing the purified deoxyribonucleoprotein (DNP) was carefully adjusted to pH 1.8 by addition of a few drops of 1 N HCl. The precipitate was removed by centrifugation (2000*g*, 30 min) and analyzed for its DNA and protein content. The supernatant containing the acid soluble nuclear protein was dialyzed *vs.* 1 mM HCl (ratio 1:150) at 5° for 24 hr. After lyophilization, 3–6 mg of material was dissolved in 5 ml of glass-distilled water and centrifuged at 10,000*g* for 30 min. The supernate will be referred to as the histone fraction. The relative distribution of proteins in various fractions is shown in Figure 1.

Electrophoresis. Purified histones were fractionated by electrophoresis in polyacrylamide gels, using a Canalco Model 12 electrophoresis apparatus (McAllister *et al.*, 1963). The cleaned gel tubes (soft glass, 6 × 90 mm) were rinsed in 0.5% photoflo solution prior to casting of the gels (Davis, 1964). The length of the gel columns was 75 mm. The gels were gently layered with distilled water and photopolymerized for 3 hr at room temperature. The samples were prepared by dissolving 200–400 μg of histones in 100 μl of electrophoresis buffer containing 1 M sucrose. The samples were transferred to the gel tubes and overlaid gently with the electrophoresis buffer (glycine–glacial acetic acid, pH 4.0). Vertical columns (10) were run simultaneously with the cathode in the bottom vessel (3 ma/column, 3–4 hr, room temperature). The gels were removed from the glass tubes by rimming with a hypodermic needle (no. 22, 4.5 in.) in 7% acetic acid. Amido Black (0.5%) in 7% acetic acid was used for staining (2 hr). The gels were then washed for 12 hr in excess volume of 7% acetic acid, placed in tapered destaining tubes. Destaining (5 ma/tube, cathode in upper vessel) was continued until the background was clear.

Fractionation of L-[*U*-^{14}*C*]*Lysine-Labeled Histones.* After electrophoresis and destaining the gels were gently inserted into a glass tube with an inner diameter slightly larger than the thickness of the gels. Each gel was then sectioned with a razor blade by pushing it from one end with a glass rod of gel diameter and severing the other end at the rim of the glass tube. The thickness of each cut disk was measured with a transparent ruler attached to the fluorescent back illuminator (Canalco). Hydrolysis of the gels and determination of radioactivity was carried out by the method of Young and Fulhorst (1965). The samples were counted at infinite thinness on nickel plated planchets in a Nuclear-Chicago thin-window, gas-flow counter. Samples were plated at least in duplicate and a minimum of 640 counts was recorded for each. Counts were corrected for background which ranged from 17 to 20 cpm.

Results

Effect of RNAase on Structure of Isolated Nuclei. To a suspension of nuclei isolated from stationary phase cells RNAase (bovine pancreas, salt free, A grade, Cal Biochem) was added to make a final concentration of 0.2% and the mixture was incubated at 35° for 45 min. Samples were removed at 5-min intervals and examined in a phase-contrast microscope. Most of the finely granulated, rather homogeneous material had already disappeared after 5 min of incubation. No significant changes could be observed in the number, size, and appearance of the larger granules during the 45-min incubation period with RNAase. These granules seem to be identical with the nucleoli in *T. pyriformis* WH 6 (variety 1, mating type I) described by Elliott *et al.* (1962). These larger granules appear clustered or fused in heat-treated cells (Figure 2a and b). The electron micrograph in Figure 2c shows well preserved nuclear membranes, chromatin bodies, and prominent nucleoli. Nonnuclear cell contamination could not be detected in the preparation.

Nucleic Acid and Protein Content of Isolated Nuclei. In three experiments with a total of 6 l. of culture, macronuclei were isolated from stationary phase cells. Nuclei were counted in the final purified suspensions, which were used for nucleic acid and protein determinations. The results are shown in Table I. The average protein content was found to be nearly three times to that of both nucleic acids combined. The DNA content shown in Table I is less than previously reported for whole cells (Scherbaum, 1957). Factors contributing to this discrepancy are considered in the "Discussion."

Composition of Nuclear Proteins in Growing and Stationary Cells. Nuclei were isolated from cultures in four different growth stages and further treated as shown in Figure 1. Protein determinations were made in two nonhistone protein fractions and one acid-soluble nuclear protein fraction. The results in Table II show

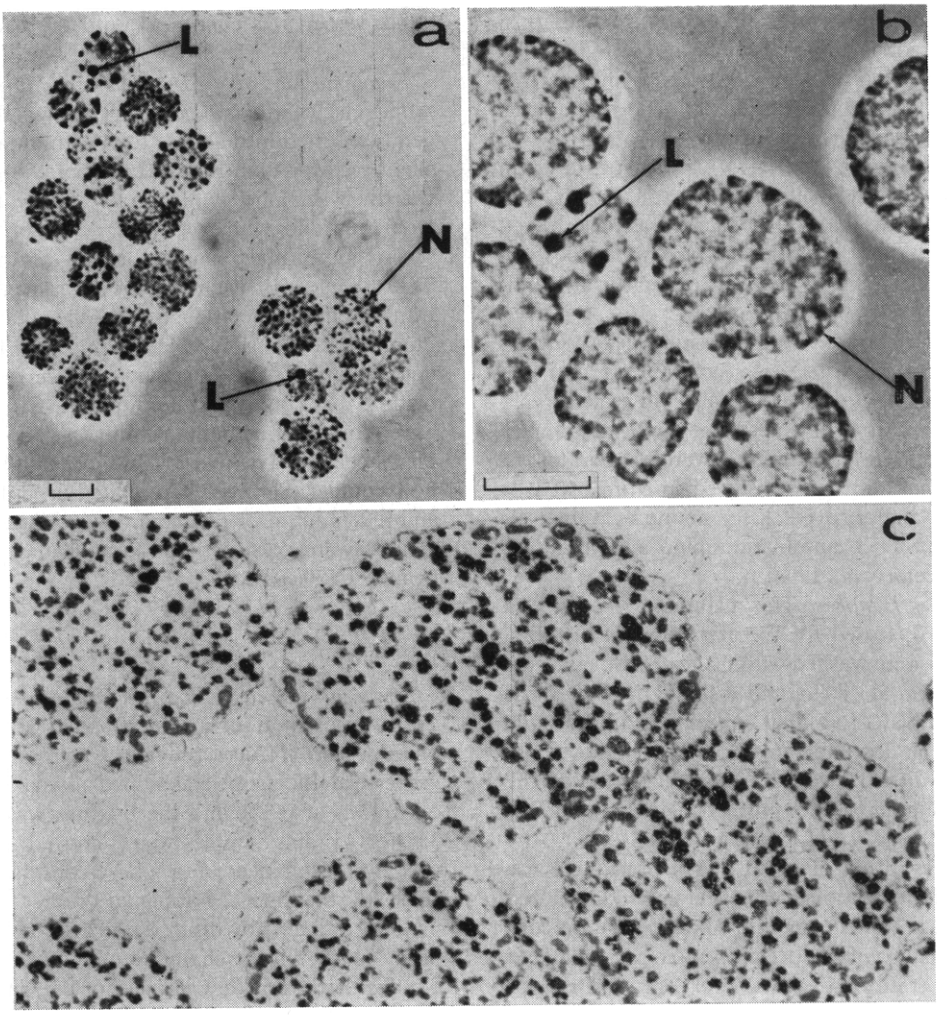

FIGURE 2: Photographs of isolated nuclei from *T. pyriformis* GL. (a, b) Phase-contrast photomicrographs of nuclei isolated from cells after the second heat shock of the standard temperature cycle treatment for induction of synchronized cell division. (N) individual nucleoli; (L) aggregated nucleoli. The scales indicate 10 μ. (c) Electron-micrograph showing nuclei from stationary phase culture (magnification 12,000×).

TABLE I: Chemical Composition of Macronuclei Isolated from Stationary Phase Cultures.

	Compn (in pg/nucleus)			Ratio (%)[b]
Expt[a]	RNA	DNA	Protein	RNA/DNA/Protein
1	0.97	8.94	29.81	2.4:22.5:75.0
2	0.73	7.86	25.94	2.1:22.7:75.1
3	0.93	8.25	28.52	2.5:21.9:75.5
Av	0.88	8.35	28.09	2.3:22.4:75.2

[a] Nucleic acids and protein were separated and assayed as outlined under "Chemical Determinations." No difference in the protein content could be found in preparations washed with cold 0.25 N perchloric acid (Table I) or 0.14 M NaCl (Table II) prior to analysis. [b] Expressed in per cent of total nucleic acid and protein content.

that *ca.* 50% of the proteins are nonhistone proteins (residues 1 and 2), and range from 43.5 (stationary phase) to 52.5% (EHT). The acid-soluble nuclear proteins (containing the histones) contribute 47.2 at EHT + 1 hr, but they account for 56.5% of the nuclear proteins in stationary phase cells.

Ratio of Acid-Soluble Proteins/DNA in Growing and Stationary Cells. The results in Table II show an increase of 1.7 times in macronuclear DNA during the synchrony-inducing heat treatment, while the DNA content of stationary phase cells is comparable to log cells. Cells at EHT and stationary phase contain twice the amount of acid-soluble nuclear proteins found in log phase cells. As a result the acid-soluble protein/DNA ratio of log cells increased 20% during the heat treatment, but 90% in stationary cells. During the preparatory phase for cytokinesis (EHT + 1 hr) the acid-soluble protein/DNA ratio is only 80% of log phase cells.

TABLE II: Composition of Nuclear Protein and DNA Fractionated from Normal and Synchronized *Tetrahymena*.

Cell Stage[a]	Compn (pg/nucleus)							Acid-Soluble Protein/DNA
	Nuclear Protein				DNA			
	Residue 1	Residue 2	Acid-Soluble Protein	Total	Residue 1	Residue 2	Total	
Log phase	5.0 (32.0)[b]	2.6 (16.8)	8.1 (51.2)	15.7 (100)	0.2 (2.1)	7.9 (97.9)	8.1 (100)	1.0
EHT	11.7 (32.8)	7.0 (19.7)	16.9 (47.5)	35.6 (100)	1.1 (8.2)	12.8 (91.8)	13.9 (100)	1.2
EHT + 1 hr	7.7 (33.0)	4.6 (19.8)	11.1 (47.2)	23.4 (100)	0.6 (4.7)	13.1 (95.3)	13.7 (100)	0.8
Stationary phase	9.0 (32.0)	3.3 (11.5)	16.0 (56.5)	28.3 (100)	0.4 (5.0)	7.9 (95.0)	8.3 (100)	1.9

[a] The isolated nuclei were fractionated as shown in the flow sheet of Figure 1. [b] Percentages are listed in parentheses

Amino Acid Composition of Tetrahymena Histones. The acid-soluble nuclear protein was prepared as outlined in Figure 1, dissolved in distilled water, and centrifuged. The supernatant was used for a determination of the amounts of histones. The yield of histones was *ca.* 87% of the acid-soluble, or 44% of the total nuclear protein. These values vary but little at the four growth stages.

Histones were prepared from *Tetrahymena* cultures in maximum stationary phase and heat-induced stationary phase (EHT). Acid-soluble proteins were obtained from stationary phase cultures to permit a comparison with published data. Lyophilized, dry powder (2 mg) of each fraction was hydrolyzed for a determination of the amino acid composition. The data in Table III are expressed in mole per cent of the total amino acids recovered in each analysis. The acid-soluble protein has a high content in lysine, aspartic acid, glutamic acid, glycine, and alanine. These five amino acids account for 53% of the 16 amino acids present.

The histone fractions are rich in lysine and alanine. These two amino acids constitute 41.7% of the total amino acid content in stationary phase cells and 34.1% in cells at EHT. The ratio basic/acidic amino acids is highest in stationary phase cells where it is 3.2, as compared to 2.2 in cells at EHT. A difference in the composition of these two histone fractions is also observed in the lysine/arginine ratio; it is 5.3 in stationary phase cells, but only 3.4 in cells at EHT. Differences in the amino acid composition of histones isolated from cultures in stationary phase and at the end of the synchrony-inducing heat treatment (EHT) have been tabulated in the column at right of Table III.

A comparison of the data on acid-soluble proteins of *Tetrahymena* in stationary phase (Table III) with published information on a comparable fraction of *Tetrahymena* in late log phase of growth (Iwai *et al.*, 1965) shows a slightly higher amount in aspartic and glutamic acids with a lower arginine content in stationary cells (Table III). All other amino acids differ only by <1 mole %. The high lysine/arginine ratio in *Tetrahymena* histone has also been observed by Vande Woude (1964, 1965).

TABLE III: Amino Acid Compositions of the Acid-Soluble Nuclear Protein and Histones.

Amino Acid	Cell Stage			
	Stationary Phase			Difference (mole %)[a]
	Acid-Soluble Protein	Histone	EHT Histone	
Lysine	15.8	28.0	23.0	+5.0
Histidine	1.8	1.9	2.2	−0.3
Arginine	4.6	5.3	6.7	−1.4
Aspartic acid	9.4	5.4	6.8	−1.5
Threonine	6.3	6.9	6.0	+0.9
Serine	8.0	5.5	8.1	−2.6
Glutamic acid	10.6	5.5	7.8	−2.3
Proline	3.9	5.0	5.6	−0.6
Glycine	8.0	6.0	6.3	−0.3
Alanine	9.5	13.7	11.1	+2.6
Cystine	0	0	0	0
Valine	5.1	5.2	5.0	+0.2
Methionine	0.1	0.6	0.6	0
Isoleucine	4.9	4.0	3.6	+0.4
Leucine	7.2	4.4	4.5	−0.1
Tyrosine	2.0	0.9	1.0	−0.1
Phenylalanine	2.9	1.7	1.7	0
Basic/Acidic	1.1	3.2	2.2	
Lysine/Arginine	3.4	5.3	3.4	

[a] Stationary phase − EHT. The sum of the differences in the right column is ±18.2.

The relative concentration of the amino acids in the two histone fractions shown in Table III are similar to those found by Neelin *et al.* (1964) in unfractionated histones of chicken erythrocytes with the following exceptions: *Tetrahymena* histone contains relatively more aspartic acid and threonine, but less alanine.

Electrophoresis of Histones. Purified histones were fractionated by electrophoresis on acrylamide gel into

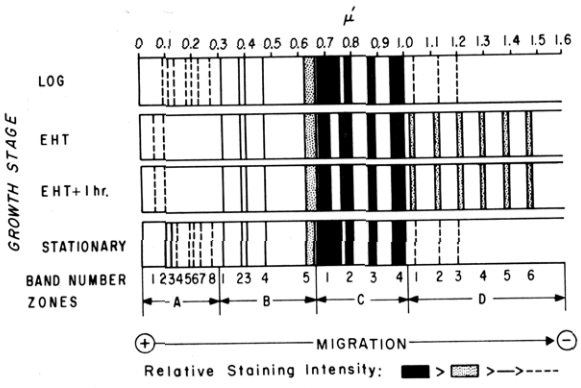

FIGURE 3: Schematic electrophoresis patterns of *Tetrahymena* histones representing four growth stages. Further explanation in text.

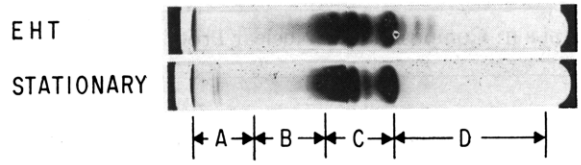

FIGURE 4: Photographs of electrophoretic patterns of *Tetrahymena* histones in polyacrylamide gel. Direction of migration from left to right. Samples from cells at the end of the synchrony-inducing heat treatment (EHT) and from stationary phase cultures are shown. A–D denote four zones described in Figure 3.

17–19 bands. A schematic diagram of the well reproducible patterns are shown in Figure 3. Each pattern was arbitrarily divided into four zones (A–D) depending on the relative mobility (μ') of the bands. The front of the reference band moving 45 mm from the origin was assigned a μ' value of 1.00 (McAllister *et al.*, 1963). A visual analysis of histone patterns representing four growth stages show striking similarities between log and stationary phases as well as between EHT and EHT + 1 hr. However, the patterns of log and stationary phases differ markedly from the patterns of synchronized growth (EHT and EHT + 1 hr). This difference is illustrated in the photograph of Figure 4 and in the diagram of Figure 3. In zone A seven bands (A_{2-8}) are found in log and stationary phase, but only two (A_{1-2}) in EHT and EHT + 1 hr. In zone D three faint bands (D_{1-3}) are found in log and stationary phases, but six weak bands (D_{1-6}) are present in EHT and EHT + 1 hr. The patterns in zone C showing four distinct bands seem to be identical in the four growth stages.

Among the numerous histone bands found in the electrophoretic patterns, the four bands in zone C were always by far the heaviest. In an attempt to quantitate the material in this zone, gels were subjected to densitometric scanning. The area below each recorded peak was measured with a planimeter and the relative proportions of the four areas were tabulated. The results (Table IV) show considerable quantitative differences between the four bands of stationary phase and those of EHT and EHT + 1 hr. In the stationary phase the concentration of C_1 has increased 2–3 times, but C_2 and C_3 has each decreased to $\lesssim 10\%$, while C_4 showed no appreciable change.

Incorporation of L-[*U*-^{14}C]*Lysine into Acid-Soluble Nuclear Proteins and Histones.* At each growth stage 2000 ml of *Tetrahymena* cultures were collected by centrifugation, washed, and resuspended in 600 ml of phosphate buffer containing 20 μc of L-[U-^{14}C]lysine. After 1 hr of incubation the nuclei were isolated and the acid soluble proteins were extracted according to the scheme in Figure 1 and counted. The data shown

TABLE IV: Relative Amounts of Histones in Zone C.[a]

Band	EHT (%)	EHT + 1 hr (%)	Stationary Phase (%)
1	23.5	18.5	48.2
2	29.0	30.7	12.1
3	13.2	13.7	6.0
4	34.3	37.1	33.7
Total	100.0	100.0	100.0

[a] The histones were separated by disk electrophoresis in 15% acrylamide gel, stained, and destained as described under "Methods." The gels were scanned in the Canalco Model F microdensitometer. The data give the relative proportions of four recorded peaks of the bands shown in the first column at the left. The location of zone C in the whole histone pattern is given in Figure 3.

TABLE V: Incorporation of L-[U-^{14}C]Lysine into the Histones of Normal and Synchronized *Tetrahymena* Cultures.

Growth Stage[a]	Total cpm Recovered from Acid-Sol. Nucleoprotein	Total Nuclei ($\times 10^6$)	Incorp/Nucleus cpm ($\times 10^{-6}$)	Incorp/Nucleus Rel. Ratio (%)[b]
Log phase	5,140	41.6	124	100
EHT	4,990	44.0	113	91
EHT + 1 hr.	10,870	43.2	252	203

[a] Cultures (600 ml) were incubated for 1 hr in RSP buffer containing 20 μc L-[U-^{14}C]lysine. At the three growth stages shown in the left column, the cells were harvested and nuclei were isolated. [b] Using the value for log phase as 100%.

in Table V indicated that the normal rate of lysine incorporation (log phase) is reduced to 91% at EHT, but increased double the normal rate prior to synchronized division (EHT + 1 hr).

In another series of experiments *Tetrahymena* cells were harvested at two growth stages and resuspended in phosphate buffer containing L-[U-^{14}C]lysine (20 μc/600 ml). Conditions for growth and harvest of cells were the same as given in Table V. Histones were isolated, subjected to acrylamide gel electrophoresis, and the radioactivity in 17 bands was determined. Although most of the material is found in zone C (*cf.* Figure 3), only 60–65% of the radioactivity is associated with this zone (Table VI). Despite the identical appearance of the electrophoresis patterns (Figure 3) distinct differences in the incorporation patterns do exist such as noted in fractions A_2, B_5, C_1, and D_6.

TABLE VI: Electrophoretic Fractionation of *Tetrahymena* Histones Labeled with L-[U-^{14}C]Lysine.

		Growth Stage[a]			
		EHT Radioactivity		EHT + 1 hr. Radioactivity	
Zone	Band	cpm	%	cpm	%
A	1	21	1.8	32	1.3
	2	72	6.1	46	1.9
B	1	14	1.2	70	2.9
	2	30	2.5	96	3.9
	3	48	4.1	80	3.3
	4	69	5.9	97	4.0
	5	85	7.2	93	3.8
C	1	153	13.0	490	20.1
	2	209	17.8	388	15.9
	3	120	10.2	221	9.1
	4	217	18.4	476	19.5
D	1	49	4.2	76	3.1
	2	32	2.7	75	3.1
	3	32	2.7	29	1.2
	4	7	0.6	19	0.8
	5	10	0.8	19	0.8
	6	9	0.8	130	5.3
Total		1177	100.0	2437	100.0

[a] Cultures (2000 ml) were harvested 1 hr prior to the end of the heat treatment (EHT) or at EHT, resuspended in 600 ml of RSP buffer containing 20 μc L-[U-^{14}C] lysine, and incubated for 1 hr. The cultures were harvested, and nuclei and histones were isolated as described under "Methods." Each acrylamide gel cylinder was cut into 17 disks representing the bands in zones A–D shown in the first two columns at left and in Figure 3. The disks were hydrolyzed and the radioactivity determined.

Discussion

Contamination and Recovery of Histones. Since clean nuclei preparation are essential for the extraction of authentic histones (Butler, 1964), microscopic checks were made routinely to insure that the nuclei were free from visual contamination. Furthermore, the purified nuclei were thoroughly washed with 0.14 M NaCl to remove contaminating nonhistone proteins (Laurence *et al.*, 1963). To reduce the loss of histones during the extraction of deoxyribonucleoprotein (DNP), sodium chloride was added to the saline-washed nuclei (final concentration, 3 M), and the resulting suspension was gently agitated with a stirring rod. Vigorous stirring has to be avoided since it causes foaming and results in a low yield of histones (Sporn and Dingman, 1963). The loss of DNA during the extraction was used for estimating the loss of histones. After separation of the DNP by centrifugation (40,000*g*, 2 hr), 2–8% of the total DNA was found in the residue. It appears, therefore, that 2–8% of the nuclear histones were lost during extraction. Steele and Busch (1963) reported that histones made approximately one-third of the nuclear proteins in rat liver and Walker tumor. The higher value for *Tetrahymena* (44%, Figure 1) may be in part due to a loss of some nonhistone proteins during the initial washing of the nuclei with 0.14 M NaCl (Busch, 1965).

The possible loss of nucleoproteins after cell lysis and during the purification process prior to extraction of the histones is more difficult to assess. The ratio of RNA/DNA is only 1:10 in isolated nuclei (Table I). For rat liver nuclei a ratio of 3:10 has been reported (Steele and Busch, 1963).

The DNA content in the isolated nuclei of stationary phase cells reported here (Table I) is less than the values obtained on whole cells from the exponential growth phase; *i.e.*, 8.4 *vs.* 13.6 pg in strain GL (Scherbaum, 1957) or 9–14 pg in strain HSM (Cameron and Guile, 1965). Although cytoplasmic organelles such as kinetosomes (Randall and Disbrey, 1965) and mitochondria (Luck and Reich, 1964; Parsons, 1965) contain DNA, the total amount in these structures may probably not exceed 2% of the DNA content in a *Tetrahymena* cell. In addition *ca.* 1.5% of cellular DNA is found in the subnuclear aggregates in stationary phase cells (Scherbaum *et al.*, 1958). These aggregates are formed during the amitotic division of the macronucleus and comprise 0.7–2.8% of the macronuclear volume, depending on the growth stage.

Problems of enzymic degradation of histones during isolation of nuclei and purification of the histones have been discussed by several authors (Butler *et al.*, 1954; Moore, 1959; Crampton *et al.*, 1957; Rasmussen *et al.*, 1962). The histone patterns shown in Figure 3 were reproducible in duplicate experiments using fresh material or lyophilized powder stored at −10° for several weeks. If the fast moving components in zone D are degradation products (Busch, 1963), we feel that such degradation could occur *in vivo* as a result of the heat treatment. In samples of log and stationary phase cells this material is virtually absent (Figure 3). A

definite proof whether degradation occurs during the isolation process will have to await further experimentation using diisopropylfluorophosphate as an inhibitor of protease action and end group analyses.

Histone/DNA Ratio. The ratio of acid-soluble nuclear proteins/DNA of one in log phase *Tetrahymena* (Table II) is comparable to data reported for rat liver (Umana *et al.*, 1964). The highest content of acid-soluble nuclear protein was found in *Tetrahymena* cells with low synthetic activity such as during the maximum stationary phase and at EHT. In cells at EHT + 1 hr the nuclei contain only 66% of the value at EHT, while the DNA content does not change appreciably during this period. These results will be discussed later in connection with the evaluation of the turnover studies.

From other studies it appears that the ratio of acid-soluble nuclear protein/DNA does not change between EHT + 80 min and EHT + 180 min, *i.e.*, during the interphase between the first two synchronized division peaks. In this period histones and DNA are synthesized at the same time and rate (Hardin and Lindsay, 1965).

Composition of Tetrahymena Histones. Polyacrylamide gels have been used successfully in the separation of histones (McAllister *et al.*, 1963). Driedger *et al.* (1963) resolved calf thymus histone into 18 components. About 13 bands were recently described for *Tetrahymena* histone (Hardin and Lindsay, 1965).

The degree of heterogeneity of *Tetrahymena* histone (Figure 3) is comparable to the results on calf thymus reported by Driedger *et al.* (1963). The patterns of histones extracted from log and stationary phase *Tetrahymena* show seven components in zone A (Figure 3) similar to the pattern of β-histone fraction of ox thymus (Cruft, 1964). The patterns of *Tetrahymena* at EHT and EHT + 1 hr show six components in zone D (Figure 3) similar to the pattern of the α-histone fraction of ox thymus. The α-histone is a lysine-rich fraction and the β fraction is an arginine-rich fraction (Cruft, 1964). Since the comparable components in the *Tetrahymena* histone patterns are only a small fraction of the total histones in each fraction (Figure 3), possible changes in the lysine/arginine ratio due to changes in the slow- and fast-moving components can not be detected in the amino acid analyses (Table III). The fast-moving components in zone D (Figure 3) may not only have a high positive charge, but may also have a low molecular weight, since the separation of histones in the acrylamide gels is based to a considerable degree on the molecular sieving effect of the gels (Umana *et al.*, 1964).

Turnover of Histones. The problem of nuclear protein synthesis in *Amoeba proteus* has been studied extensively by radioautography (Prescott and Bender, 1963; Prescott, 1964). The results show a continuous turnover of all nuclear proteins during the cell cycle. Although the present study does not pertain to the normal cell cycle directly, the results indicate a rapid turnover of histones prior to synchronized cell division in *Tetrahymena*. During 1 hr of the "recovery period" (*i.e.*, between EHT and EHT + 1 hr, which is 20 min prior to the first synchronized division) the nuclear protein content decreases by 30%, but at the same time the rate of L-[U-^{14}C]lysine incorporation is 2.23 times the rate found during the last hour of the heat treatment. The data suggest an accelerated loss of histones from the nuclei in *Tetrahymena* cells preparing for synchronized division. These data support earlier cytological observations. With the method of Alfert and Geschwind (1953) using fast green as a specific stain for basic proteins, cytoplasmic basic proteins were found in rapidly growing cultures of *Tetrahymena*, but not in stationary cells, where the stain was restricted to the nuclei only (Alfert and Goldstein, 1955). This observation was later extended to synchronized *Tetrahymena* cells (Scherbaum *et al.*, 1959). Cells at EHT were deeply stained in both the nucleus and cytoplasm, while 5% of exponentially growing cells were stained only in the nucleus and the remaining 95% were stained in both the nucleus and the cytoplasm. Considering the possible role the histones may have in heat-induced synchronous division in *Tetrahymena*, it is interesting to note that the electrophoresis patterns of histones representing rapid growth (log phase) and no appreciable growth (stationary phase), are remarkably similar; the same can be said for the composition of patterns representing growth prior to synchronized division (EHT + 1 hr) and heat-induced stationary phase (EHT). However, a comparison of the histone/DNA ratios at these stages and the rate of lysine incorporation at EHT and EHT + 1 hr leaves no doubt that metabolic processes do occur which are not reflected in the histone patterns separated on acrylamide gels.

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