CHANGE IN HISTONE/DNA RATIO DURING POPULATION GROWTH IN *TETRAHYMENA*

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INTRODUCTION

Lee and Scherbaum (6) have shown that stationary phase populations of *Tetrahymena* maintain a histone/DNA ratio of 2:1 while logarithmically growing cells exhibit a ratio of 1. In their studies on strain GL, they found that stationary phase cultures and logarithmically growing cultures had the same amount of DNA per cell, but that stationary phase cells contained twice as much histone as logarithmically growing cells (accounting for the 2:1 ratio during stationary phase).

The correlation of increased histone with the lack of cell division may be a reflection of the suggested function of histones as possible genetic regulators (1, 2, 5, 9, 12). For example, the high histone/DNA ratio in nondividing cell populations may be directly responsible for cessation of cell division, and conversely the equalization of histone and DNA may be a prerequisite for cell division. It should be possible to determine whether the changes in the histone/DNA ratio precede changes in the division rate of a cell population or whether they are only a consequence of changes in the division rate, thus indicating the presence of or lack of possible causal relationships between the amount of histone and the growth conditions of a population of cells.

The experiments to be reported here determine, in a population of *Tetrahymena* cells, the time course of change in histone/DNA ratio during the transition from a nonproliferating culture to a dividing cell population, defined here as lag phase, throughout the period of logarithmic cell growth and continuing into the period of stationary phase. These ratios are correlated with the initiation of DNA synthesis, incorporation of amino-^{3}H acids into histone and nonhistone fractions, and the beginning of cell division.

MATERIALS AND METHODS

Tetrahymena pyriformis (strain HSM) was grown in 1000 ml of Bacto-tryptone medium (Difco Laboratories, Inc., Detroit, Mich.) (4), and adjusted to pH 7.4, in 1-liter culture flasks at room temperature for 6 days to a final cell concentration of 2–3 $\times$ 10^5 cells/ml.

For producing the transition from stationary phase to a logarithmically dividing culture, cells were diluted with fresh medium to between 2 and 4 $\times$ 10^4 cells/ml. Total histone and DNA determinations were performed on samples of 10–20 $\times$ 10^6 cells at known times after dilution.

The percentage of the cell population in DNA synthesis following refeeding was determined from radioautographs of cells incubated with 2.5 μc/ml of thymidine-^{3}H (sp. act. 18.6 c/mmole) added at the time of dilution. A liquid emulsion procedure (10) utilizing Kodak NTB3 emulsion was employed for radioautography.

Incorporation of DL-leucine, -^{3}H 0.25 μc/ml (sp. act. 5 c/mmole), plus DL-lysine-^{3}H, 0.25 μc/ml (sp. act. 4c/mmole), into whole cell homogenate protein and into the histone fraction was determined from refed 1-liter cultures. The incorporated radioactivity was assayed by plating 10 or 25 μl of protein solution on planchets and counting in a windowless Nuclear-Chicago low-background gas flow detector. The results are presented as counts per minute per mg protein.

Samples of 10–20 $\times$ 10^6 cells were harvested from culture and maintained at 0-5°C during nuclear isolation and extraction procedures. 6–7 ml of cold 0.1% Triton X-100, 0.02% spermine tetrahydro-

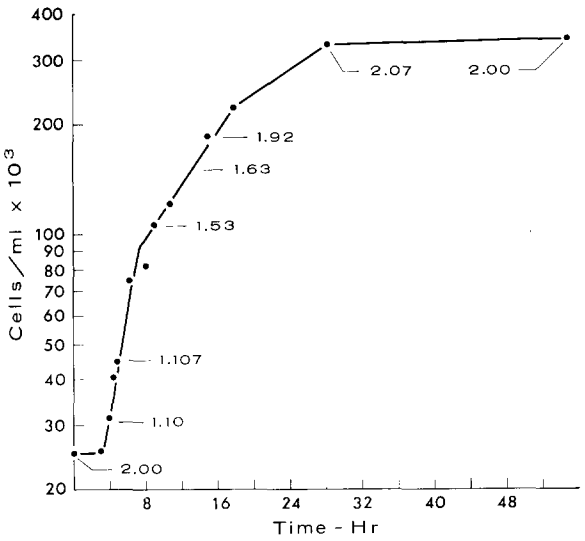

FIGURE 1 Complete growth curve for *Tetrahymena* culture correlated with histone/DNA ratios. One value, 1.107, has been presented for log phase; however, additional experimental data indicate that the values range between 0.90 and 1.2.

chloride in 0.25 M sucrose pH 6.5 (11) was added to a pellet and mixed. This procedure resulted in 100% lysis in pellets containing under 20 × 10^6 cells.

Immediately following lysis, the mixture was diluted up to 50 ml with 0.25 M sucrose and centrifuged at 750 *g* for 15 min, the supernatant was aspirated, and the nuclei were washed again in 50 ml of 0.25 M sucrose. Following the second centrifugation, the pellet was suspended in 50 ml of 0.15 M KCl pH 7.0, twice for 30 min. These preparations of nuclei were only partially purified, in that some oral areas containing kinetosomes were usually present with the nuclei. The nuclei were not completely free of adhering material, and some nuclei tended to clump.

The nuclear pellet was extracted three times with 0.5 N H_2SO_4, and the extracts were pooled, brought to 20% TCA to precipitate nuclear basic proteins, and permitted to stand overnight in the cold. The proteins were measured by the Lowry method (7) standardized against commercially prepared calf thymus histone (Sigma Chemical Co., St. Louis Type II) and were subjected to the divalent mercury test described by Mirsky and Pollister (8) for detection of possible contamination by nonhistone proteins. The nuclear pellet was washed with acetone following acid extraction, and DNA was determined by the diphenylamine reaction (3).

RESULTS

Fig. 1 illustrates the histone:DNA ratios correlated with the different stages of the complete growth curve of a population of *Tetrahymena*. The values given at the different points represent the mean values of triplicate samples for this experiment.

TABLE I

Values for μg Histone and DNA per Million Cells Sampled at Various Times after Refeeding

Time after dilution	Histone/10^6 cells	DNA/10^6 cells	Histone/DNA
min	*μg*	*μg*	
0	22.5	11.00	2.04
30	22.00	11.04	1.99
60	20.40	10.69	1.90
90	22.70	16.78	1.35
180	24.00	18.68	1.28
240	19.60	17.30	1.13

The range of values for any given point did not exceed 0.2 of a unit. The ratio dropped from 2:1 to 1.1:1 by early lag phase and remained at approximately 1.1:1 during logarithmic growth, with a generation time of 2–3 hr. At a concentration of about 100,000 cells/ml, the generation time shifted to 6–8 hr, and the ratio began to increase and reached a value of approximately 2:1 prior to complete cessation of cell division.

The time at which the decrease in the ratio from 2:1 to near 1:1 occurred during lag phase was examined by determining the histone and DNA content at six intervals during the 4 hr following refeeding. Cell division was not detected until 4 hr after refeeding. The results for one such experiment are given in Table I.

The total amount of acid-soluble proteins remained relatively constant at 20–25 μg per 10^6 cells during stationary and lag phases of culture

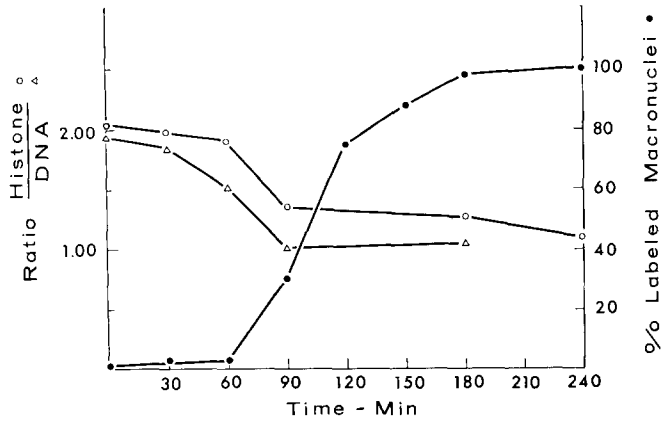

FIGURE 2 Two experiments measuring the histone/DNA ratio (○, △) at intervals following refeeding. The two experiments represent the largest variation in results for complete experiments of this type. ●, Per cent of population incorporating thymidine-^{3}H during continuous labeling. Cell division was not detected until 240 min.

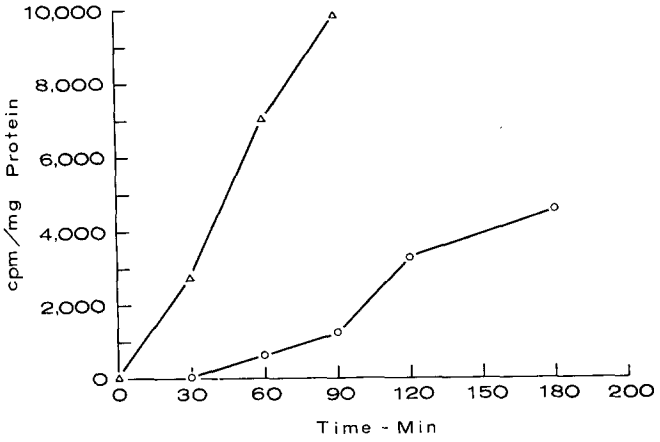

FIGURE 3 Incorporation of lysine-^{3}H, during continuous labeling, into whole homogenate protein (△) and histone (○) following refeeding of a stationary culture.

growth. An increase in the total DNA per 10^6 cells was detectable at 90 min. The effect was to produce a drop in histone/DNA ratio during lag phase of culture growth by a net increase in DNA. DNA synthesis was first detected radioautographically between 60 and 90 min, as seen in Fig. 2. The percentage of the population entering DNA synthesis by 180 min was between 75 and 100%. 75% represents the lowest value obtained in four separate experiments. The ratios obtained from two different experiments during the first 4 hr are also plotted in Fig. 2 for illustrating the close relationship between the ratio change and the initiation of DNA synthesis.

Although the total histone per 10^6 cells did not appear to change significantly during lag phase, labeling experiments were performed for determining whether radioactive amino acids were incorporated into the histone fraction during lag phase. The results of this type of experiment are given in Fig. 3. Incorporation into total protein was detectable at 30 min after dilution of the culture, while incorporation into acid-soluble protein fraction was detected at 60 min after dilution of the culture and continued to rise until the experiment was terminated at 180 min.

DISCUSSION

The results reported in this paper indicate that the changes in the histone:DNA ratio are closely associated with changes in the division rate of a cell population. The critical questions are now related to the mechanism of ratio change, taking into account absolute values of DNA and histone content. In view of the inhibitory action of histones on RNA and DNA syntheses (2, 5), a simple hypothesis might state that the initiation of DNA synthesis and subsequent cell division during lag phase is mediated through a decrease in histone content prior to the initation of DNA synthesis.

On preliminary examination, the data presented in this paper do not agree with this hypothesis since the ratio appears to decrease because of an increase in DNA content.

Another possibility is that a loss of histones begins immediately prior to the beginning of DNA synthesis and that concomitant with DNA replication there is synthesis of new histones. This possibility is primarily supported by the observations that, although there is little, if any, increase in total histone content, incorporation of radioactive amino acids into the histone fraction begins at or near the time of initiation of DNA synthesis. Accordingly, one would not necessarily expect to see a rapid change in total histone if some histone was being lost at only a slightly faster rate than new histones were being synthesized.

The possibility that the amino acid incorporation observed during the DNA synthesis period represents a very small amount of new histone synthesis and that actually there is no loss of histone cannot be entirely ruled out yet. However, if equivalent amounts of DNA and histone are synthesized during the first cell cycle following refeeding, it must mean that one-half of the histones present in stationary phase are lost during this first cell cycle. More precise information on the extent and time of histone turnover as related to the initiation of DNA synthesis is needed to determine whether the temporal sequence of events is compatible with the idea that initiation of DNA synthesis and subsequent cell division is mediated through histones. Experiments are now in progress for determining whether histone turnover is extensive enough during the transition from a ratio of 2:1 to 1:1 to account for a loss of 10–12 μg histone per 10^6 cells, and secondly, whether all the histone fractions turn over at the same rate.

The interpretation that histones can turn over rapidly comes from the work of Lee and Scherbaum (6) with populations of *Tetrahymena* synchronized for cell division. They demonstrated that from the end of heat treatment to the first synchronous division the total histone decreased by 30%, but that labeled lysine was being incorporated into the histone fraction.

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