G_1- and S-Phase Syntheses of Histones H1 and H1° in Mitotically Selected CHO Cells: Utilization of High-Performance Liquid Chromatography†

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ABSTRACT: We have employed high-performance liquid chromatography (HPLC) to investigate the syntheses of histones H1 and H1° as synchronized cells traverse from mitosis to S phase. Chinese hamster (line CHO) cells were synchronized by mitotic selection, and, at appropriate times, they were pulse labeled for 1 h with [^{3}H]lysine. Histones H1 and H1° were extracted by blending radiolabeled and carrier cells directly in 0.83 M $HClO_4$; the total $HClO_4$-soluble, Cl_3CCO_2H-precipitable proteins were then separated by a modification of an HPLC system employing three μBondapak reversed-phase columns [Gurley, L. R., D'Anna, J. A., Blumenfeld, M., Valdez, J. G., Sebring, R. J., Donahue, D. K., Prentice, D. A., & Spall, W. D. (1984) *J. Chromatogr. 297*, 147–165]. These procedures (1) produce minimally perturbed populations of synchronized proliferating cells and (2) maximize the recovery of radiolabeled histones during isolation and analysis. Measurements of rates of synthesis indicate that the rate of H1 synthesis increases (3.6 ± 0.5)-fold as cells traverse from early to mid G_1; as cells enter S phase, the rate of H1 synthesis increases an additional ≃22-fold and is proportional to the number of S-phase cells. In contrast to H1, the rate of H1° synthesis is nearly constant throughout G_1. As cells progress into S phase, the rate of H1° synthesis increases (3.1 ± 0.2)-fold so that it also appears to be proportional to the number of S-phase cells. Except for the first 1–2 h after mitotic selection, these results are similar to those obtained when cells are synchronized in G_1 with the isoleucine deprivation procedure.

Histone H1 and histone H1° appear to be diverse variants of the same lysine-rich family of histones (Smith et al., 1980; Pehrson & Cole, 1981; Mura & Stollar, 1981). Both H1 and H1° (1) are phosphorylated in nearly identical cell-cycle-dependent fashions (Gurley et al., 1975, 1978; D'Anna et al., 1980, 1981), (2) have similar "nose–head–tail" structures in solution (Cary et al., 1981), (3) are associated with DNA on the exterior of the 164–166 base pair platysome particle (McGhee & Felsenfeld, 1980; Keppel et al., 1979; Smith & Johns, 1980), and (4) can fold oligonucleosomes into more compact structures (Finch & Klug, 1976; Biard-Roche et al., 1982). In contrast to these similarities, H1 and H1° exhibit substantial differences in (1) the sequence in their central region which is highly conserved among H1 subspecies (Pehrson & Cole, 1981), (2) their content in chromatin [e.g., see Panyim & Chalkley (1969a); Smith & Johns (1980), and D'Anna et al. (1982) and references cited within], and (3) their rates of synthesis (D'Anna et al., 1982; Pehrson & Cole, 1982) and turnover (Pehrson & Cole, 1982).

With regard to the syntheses of histones H1 and H1°, it is becoming increasingly clear that both proteins can be synthesized in proliferating as well as nonproliferating cells [e.g., see Gurley et al. (1974), Tarnowka et al. (1978), Zlatanova (1981), Pehrson & Cole (1982), and D'Anna et al. (1982)]. Nevertheless, (1) there remains considerable controversy pertaining to the range of H1 synthesis rates and the extent of coupling between H1 and DNA syntheses during cell-cycle progression, and (2) it is not known whether H1 and H1° are synthesized throughout G_1 or whether there are large variations in their individual rates of synthesis during traverse of G_1 (see the following).

Investigation of H1 synthesis employing short 15–20-min pulses in CHO cells synchronized by mitotic selection or in S49 cells synchronized by centrifugal elutriation and flow cytometric cell sorting suggests that the rate of H1 synthesis during G_1 is nearly the same as during S phase (Groppi & Coffino, 1980). On the other hand, measurements of H1 rates of synthesis (Tarnowka et al., 1978) and H1 specific activities (Gurley et al., 1974; D'Anna et al., 1982) from cells synchronized by isoleucine-deprivation G_1 arrest (Tobey & Ley, 1971) suggest that the G_1 rates of H1 synthesis are between 10% and 30% of the values that might be obtained if all cells were in S phase. Although the data from all three reports employing isoleucine deprivation are in reasonable agreement, the results of Chiu & Marzluff (1982) suggest that the synchrony procedure itself produces G_1 rates of H1 synthesis that differ from those of unperturbed proliferating cells.

Other studies also indicate that H1 is synthesized at noticeable rates during G_1 (Wu & Bonner, 1981; Delegeane & Lee, 1982; Waithe et al., 1983), but the values were not quantified during traverse of cells through G_1 into S.

Investigations of H1° synthesis during the cell cycle are not so numerous as those of H1. During 1982, this laboratory (D'Anna et al., 1982) reported measurements of H1° specific activities following the release of CHO cells from isoleucine-deprivation G_1 block. We found that the H1° specific activities during G_1 were 50–70% of the maximum values when 60–70% of the cells were in S phase. On the other hand, Chabanas et al. (1983) have concluded from indirect evidence that histone H1° is synthesized at a nearly constant rate throughout the cell cycle. In addition to this discrepancy, there is the concern that, as with H1 synthesis, isoleucine deprivation synchronization produces metabolic perturbations that do not reflect the G_1 biochemical processes in proliferating cells.

In this report, we have combined mitotic selection with $HClO_4$ extraction of histones and high-performance liquid chromatography (HPLC)[1] to quantify the rates of H1 and H1°

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synthesis as cells progress from mitosis through G_1 into S phase. The combined use of these procedures in a single experiment provides a number of technical advantages compared with previous studies of H1 and H1° synthesis: (1) mitotically selected cells provide a minimally perturbed population of proliferating cells (Tobey et al., 1967); (2) isolation of proteins by blending cells directly in $HClO_4$ leads to reproducible quantitation of H1 histones (D'Anna & Tobey, 1984); (3) large quantities of histones may be loaded on the HPLC (Gurley et al., 1984); thus, a suitably large number of carrier cells can be combined with a small number of radiolabeled cells to maximize protein recovery and quantification of isotope incorporation. These procedures also avoid the introduction of a gel matrix in protein separation which might cause quenching in scintillation spectrometry or problems of quantifying large ranges of isotope incorporation by fluorography. The general method should be applicable to other proteins, as well as to histones.

Experimental Procedures

Cell Synchrony and Isotopic Labeling. Monolayer and suspension cultures of Chinese hamster (line CHO) cells were grown in F-10 medium supplemented with 15% neonatal calf serum, streptomycin, and penicillin (Tobey et al., 1966).

To examine protein synthsis as cells traversed from mitosis into S phase, cells were synchronized by mitotic selection *in the absence of Colcemid* (Tobey et al., 1967; Gurley et al., 1978). These procedures yielded a series of preparations that were 94%, 98%, 97%, and 96% mitotic cells as determined by light microscopy. After mitotic selection, the cells ($\simeq 1 \times 10^8$) were maintained on ice to keep them in mitosis. They were then concentrated by centrifugation at 4 °C and resuspended in $\simeq$600 mL of 37 °C F-10 ($t = 0$ in the protocol) to allow cells to resume cell-cycle traverse. [We note that these procedures do not affect the rate of cell-cycle progression from mitosis (Tobey et al., 1967).] Measured aliquots of 120–130 mL were dispensed in suspension flasks for measurements of [^{3}H]lysine incorporation and autoradiography.

Starting at $t = 0$ and at appropriate times thereafter, the cell suspensions (120 mL at the time of addition of isotope) were radiolabeled for 1 h with [^{3}H]lysine (80–110 Ci mmol^{-1}; New England Nuclear) at 4.2 μCi mL^{-1}. Isotope incorporation was stopped by pouring the cells onto 60 mL of crushed frozen culture medium in an open-faced centrifuge cone. Cells were harvested by centrifugation at 4 °C. Fifteen minutes before the harvest of each culture and at hourly intervals, 5 mL of cells was withdrawn for autoradiography from a succeeding culture in the series for measurements limited to G_1 or from a parallel culture for measurements spanning G_1 and S. Each aliquot was radiolabeled for 15 min with [^{3}H]thymidine at 2 μCi mL^{-1} in preparation for autoradiography (Tobey & Ley, 1970).

Protein Extraction. Histones H1 and H1° and other $HClO_4$-soluble proteins were extracted essentially as described (D'Anna & Prentice, 1983; D'Anna & Tobey, 1984); however, nonlabeled carrier cells were mixed with the radiolabeled cells in the open-faced centrifuge cone. In turn, total cells and successive multiple washings were transferred from the cone to a 12-mL Corex centrifuge tube where they were pelleted by centrifugation (5 min at 1000g). The supernate was removed from the pelleted cells with a Pasteur pipet, cells were transferred and blended in 0.83 M $HClO_4$, and proteins were extracted and stored as described (D'Anna & Prentice, 1983).

High-Performance Liquid Chromatography and Data Analysis. Histones H1 and H1° and other $HClO_4$-soluble fractions were separated by multicolumn high-performance liquid chromatography (HPLC) employing minor modifications of the reversed-phase columns and linear H_2O/CH_3CN gradients containing 0.2% F_3CCO_2H (Gurley et al., 1984). This procedure resolves histones H1 and H1° but not their individual subfractions (Gurley et al., 1984).

Three end-capped reversed-phase HPLC columns (Waters Associates) were used in the separations: (1) a 30 cm × 3.9 mm i.d. steel column containing μBondapak CN packing; (2) a 30 × 3.9 mm i.d. steel column containing μBondapak phenyl packing; (3) a radial-PAK cartridge containing μBondapak C_{18} packing and pressurized in a Waters radial compression Z-module. The use of these columns and the basic procedures outlined below have been described in detail by Gurley et al. (1984).

The total $HClO_4$-soluble, Cl_3CCO_2H-precipitable proteins containing H1, H1°, and HMG proteins were dissolved in 150 μL of 0.2% F_3CCO_2H in water in preparation for HPLC. The solution and 2 × 150 μL washings of the sample tube were injected into the HPLC containing a μBondapak CN column equilibrated with 0.2% F_3CCO_2H in H_2O. The proteins were then eluted with a linear gradient of CH_3CN/0.2% F_3CCO_2H increasing from 0% to 24% CH_3CN in 144 min at a flow rate of 1.0 mL min^{-1}. At the end of the elution, the C_{18} radial-PAK column was connected to the outlet of the CN column, and the linear gradient was resumed for 27 min so that the final concentation of CH_3CN was 29.5% instead of the 29% previously used by Gurley et al. (1984). This minor change allows nearly complete elution of H1 and H1° from the CN column and their adsorption to the C_{18} Radial-PAK column, but HMG1, HMG2, and other proteins which might contaminate H1 and H1° are not eluted from the CN column.

Next, the CN column which retained HMG1, HMG2, and other minor proteins was removed from the system, and the linear gradient was continued at 1 mL min^{-1} from 29.5% to 40% CH_3CN for 126 min employing the C_{18} column alone. The rate of change in the percentage of CH_3CN in the gradient in this column was half that previously employed (Gurley et al., 1984), because it facilitates the collection of H1° that is free of proteins in neighboring bands.

Finally, the C_{18} column was removed, and the original CN column (containing HMG1 and HMG2 proteins in 29.5% CH_3CN/0.2% F_3CCO_2H) was attached followed by a phenyl column equilibrated with the same eluting solution. This tandem set of columns was eluted at 1 mL min^{-1} for 123 min with a linear CH_3CN/0.2% F_3CCO_2H gradient that went from 29.5% to 50% CH_3CN. The HMG1 and HMG2 proteins eluted from the CN columns, readsorbed on the phenyl column, and then eluted with a profile characteristic of the phenyl column. This profile was examined for indications of H1 which might not have been eluted onto the C_{18} column.

Proteins eluted from the column were detected by their absorbance at 206 nm and by scintillation spectrometry. Fractions of 1.0 mL were collected directly in Beckman Poly-Q vials for determination of isotope incorporation profiles. Twelve milliliters of Altex Ready-Solv EP scintillation fluid was added to the vials, and the samples were counted for 10 min each in a Packard Model 3320 liquid scintillation spectrometer.

In order to span the cell cycle from mitosis to mid-S phase, three separate mitotic selection experiments were performed. The rates of synthesis among the three experiments were then

[1] Abbreviations: HPLC, high-performance liquid chromatography; $NaDodSO_4$, sodium dodecyl sulfate; HMG, high mobility group.

compared by (1) normalizing the rates of incorporation during the third hour after mitotic selection (measured in all experiments) and (2) proportionately scaling the other rates relative to the normalized rate durng the third hour. Since total $HClO_4$-soluble, Cl_3CCO_2H-precipitable proteins were loaded on the HPLC columns, the measured rates of incorporation are directly proportional to the number of cells in the cultures (i.e., synthesis of H1 per hour per cell).

Since incorporation was measured for a 1-h period, the counts incorporated into H1 or H1° represent average rates of synthesis per hour rather than "instantaneous" rates of synthesis. Thus, rates of synthesis and ratios of rates of synthesis were plotted at the midpoint of the labeling period. Because CHO H1 and H1° contain approximately the same numbers of lysines per molecule (D'Anna et al., 1981), the ratios of rates of synthesis are essentially the same as the ratios of rates of [^{3}H]lysine incorporation.

Electrophoresis. HPLC fractions were lyophilized and subjected to electrophoresis in acid–urea–polyacrylamide gels (Panyim & Chalkley, 1969b) or in the sodium dodecyl sulfate ($NaDodSO_4$) system of Laemmli (1970). The details of the procedures have been described (D'Anna et al., 1982).

Results

HPLC of $HClO_4$-Soluble, Cl_3CCO_2H-Precipitable Cellular Proteins. Recently, this laboratory has shown that (1) histones H1 and H1° could be purified by multicolumn HPLC from the $HClO_4$-soluble, Cl_3CCO_2H-precipitable proteins isolated by the first method of Johns (1964) from cells blended in isotonic saline (Gurley et al., 1984) and (2) H1 histones could be quantified from separate cultures by electrophoretic analysis of the total Cl_3CCO_2H-precipitable proteins obtained by blending cells directly in $HClO_4$ (D'Anna & Tobey, 1984). Although both methods are based on the procedures of Johns (1964), blending cells directly in $HClO_4$ leads to the extraction of additional proteins not obtained when cells are first blended in isotonic saline [e.g., see D'Anna et al. (1983)]. We, therefore, investigated whether histones H1 and H1° from cells blended directly in $HClO_4$ could be suitably resolved by multicolumn HPLC.

HPLC of the Cl_3CCO_2H-precipitable proteins from cells blended in $HClO_4$ (Figure 1) yields several bands, including some not observed from cells blended in isotonic saline (Gurley et al., 1984). Identification of the eluted proteins in acid–urea–polyacrylamide gels (Figure 2b) and further analysis of H1 and H1° in $NaDodSO_4$ gels (Figure 2a) indicate that H1 is eluted as a homogeneous band from the C_{18} Radial-PAK column and in trace amounts with HMG1 and HMG2 in the effluent from the tandem μBondapak CN–μBondapak phenyl columns. On the basis of the proportion of the total sample applied to gels and the absorbance of the stained proteins in three different gel experiments, we estimate that >98% of the H1 is eluted electrophoretically pure in the major band from the μBondapak C_{18} column. Furthermore, when isotope incorporation profiles (1-h pulse) from exponentially growing cells are examined on gels, we find that 97% of the H1 is located in the major H1 band from the C_{18} column. Since the fractionation appears to be reproducible and there is no apparent preferential incorporation of isotope into H1 in either region, the small amount of H1 in the HMG-1/2 region has been ignored in the following synthesis experiments.

Histone H1° is also eluted from the μBondapak CN column (Figure 1). Although H1° from the HPLC column migrates as a single band in $NaDodSO_4$ gels (Fiure 2a), a trace impurity of slower migrating material (not visible in the photographs but observable by absorbance measurements) is detectable on

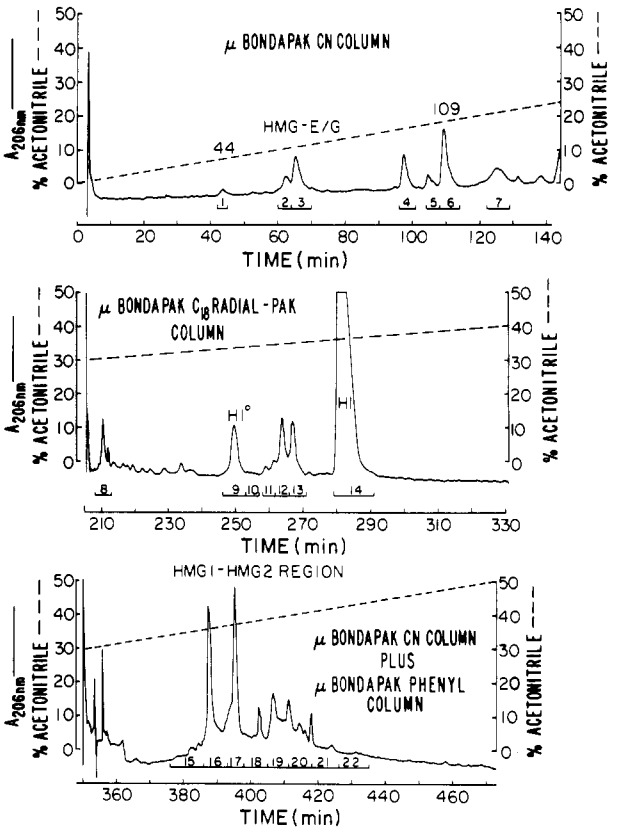

FIGURE 1: Multicolumn HPLC of the $HClO_4$-soluble, Cl_3CCO_2H-precipitable cellular proteins frm exponentially growing cells. Sample (0.9 mg) was injected, and HPLC was performed as described under Experimental Procedures. Pooled fractions (indicated by the bracketed numbers below the absorbance tracing) were used for the electrophoretic analyses in Figure 2. The numbers of the pooled fractions correspond to the numbers of the gels.

Table I: Percentage of Total [^{3}H]Lysine in the H1° Peak Eluted from the C_{18} Column That Is Incorporated into H1° during a 1.0-h Pulse[a]

sample	% G_1 cells	% S cells	% ^{3}H in H1°
exponentially growing culture	54[b]	37[b]	97
1 h after mitotic selection	100[c]	0	95
3 h after mitotic selection	100[c]	0	94
9 h after mitotic selection	29[c]	71	97

[a] The effluent in the H1° peak from the C_{18} column was subjected to electrophoresis in acid–urea–polyacrylamide gels, as described under Experimental Procedures. [b] Determined by flow cytometry. [c] Determined by autoradiography, assuming that there were no G_2/M cells in the culture.

the acid–urea gels (Figure 2b, gel 9). To determine whether this trace impurity might interfere with investigations of isotope incorporation into H1°, (1) exponentially growing and mitotically selected cells were pulse labeled for 1 h with [^{3}H]-lysine, (2) the $HClO_4$-soluble, Cl_3CCO_2H-precipitable proteins were subjected to HPLC, and (3) the H1° fraction was further separated on acid–urea–polyacrylamide gels. Isotope incorporation profiles of the acid–urea gels indicate that during G_1, ≥94% of the ^{3}H cpm are incorporated into H1° and during S phase, ≥97% of the ^{3}H cpm are incorporated into H1° (Table I). Thus, as we shall see below, the non-H1° component has, practically, no effect on the H1° rates of synthesis during the G_1 and S phases.

Besides H1 and H1°, we have labeled other fractions in the HPLC chromatogram of Figure 1. Of these, the regions containing the high mobility group proteins are not homogeneous. The HMG-E/G region [a fraction whose composition

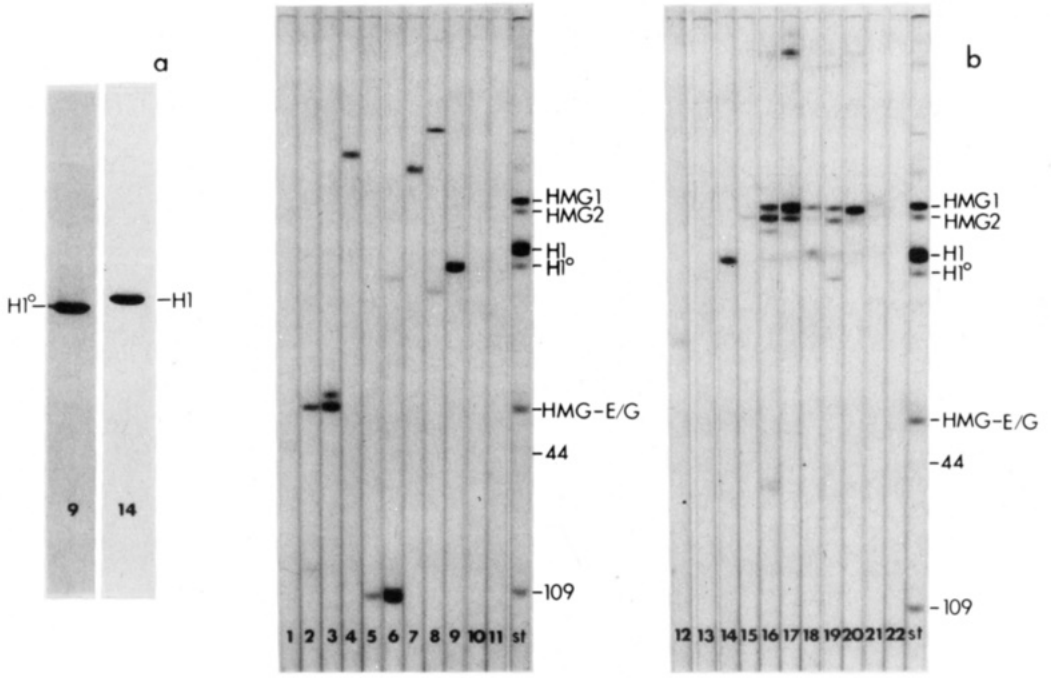

FIGURE 2: $NaDodSO_4$ gel electrophoresis of histone H1 and H1° fractions separated with the HPLC three-column system used in Figure 1 (a) and acid–urea gel electrophoresis of the HPLC fractions eluted from the μBondapak CN, C_{18}, and phenyl columns in Figure 1 (b). The numbers of the gels correspond to the pooled fractions from the HPLC bands designated in Figure 1.

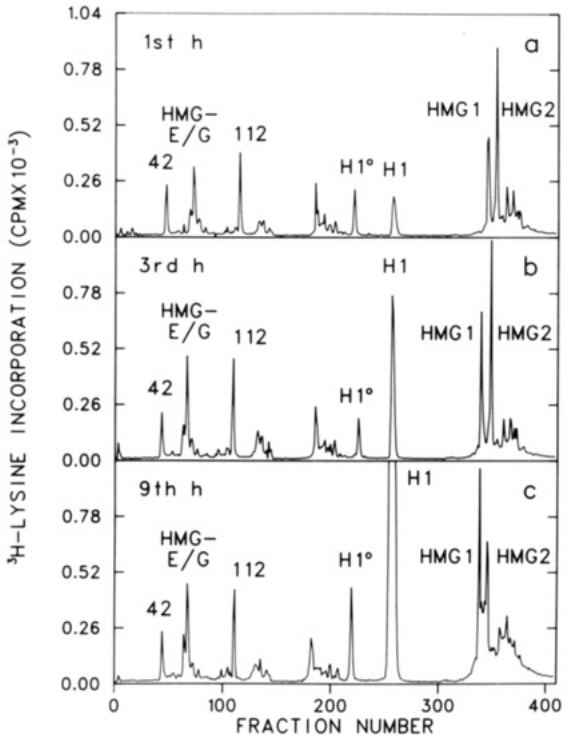

FIGURE 3: Examples of [^{3}H]lysine incorporation profiles of the $HClO_4$-soluble, Cl_3CCO_2H-precipitable proteins from mitotically selected cells that were subjected to multicolumn HPLC. The panels illustrate [^{3}H]lysine incorporated into the proteins (1-h pulses) during the first hour (a), third hour (b), and ninth hour (c) after the release of mitotically selected cells into warm medium. The numbers on the abscissa correspond to the numbers of the vials that were collected and counted.

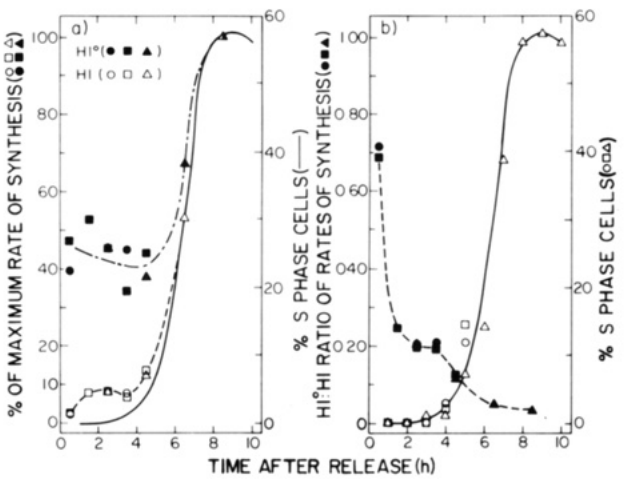

FIGURE 4: Percentages of H1 and H1° maximum rates of synthesis (a), H1°:H1 ratio of rates of synthesis (b), and percentages of S-phase cells (a and b) plotted as functions of time after the release of mitotically selected cells. The opened or closed forms of each of the symbols denote measurements from a single mitotic selection experiment.

is characteristic of the HMG14 and HMG17 class of proteins (D'Anna et al., 1983)] contains at least one contaminant (gel 3, Figure 2b) not seen when the proteins are extracted from the chromatin of cells blended in isotonic saline (Gurley et al., 1984). The region containing HMG1 and HMG2 proteins is broad, the HMG1 and HMG2 proteins are not resolved from one another, and the region contains traces of H1 and other unidentified contaminants. Two other fractions (44 and 109), designated by the tube numbers at which they elute, have been singled out, because they appear suitable for future identification and analysis. Protein 44 (barely discernible in this set of gels) appears to be homogeneous, and protein 109 exhibits a minor contaminant which is not seen when the protein is extracted from chromatin of cells blended in isotonic saline (Gurley et al., 1984).

Isotope Incorporation during the G_1 and S Phases. To determine relative rates of synthesis for H1 and for H1° during the traverse of cells from mitosis to S phase, cells were mitotically selected and pulse labeled for 1.0 h with [^{3}H]lysine as described under Experimental Procedures. The total $HC10_4$-soluble, Cl_3CCO_2H-precipitable proteins from the homogenized (labeled plus carrier) cells were then subjected to multicolumn HPLC. Figure 3 shows examples of isotope

incorporation profiles obtained by HPLC, and Figure 4 shows (1) measured H1 and H1° rates of synthesis expressed as percentages of their maximum values, (2) H1°:H1 ratios of rates of synthesis, and (3) percentages of S-phase cells plotted as functions of time after mitotic selection. In general, these data verify a number of qualitative aspects of H1 and H1° syntheses that were ascertained from previous studies employing synchronization by isoleucine deprivation and other procedures: (1) H1 is synthesized at a measurable rate during traverse of G_1 (Gurley et al., 1974; Tarnowka et al., 1978; Groppi & Coffino, 1980; Wu & Bonner, 1981; D'Anna et al., 1982); (2) as cells enter S phase, the rate of H1 synthesis increases enormously and appears to be proportional to the increased fraction of S-phase cells (Gurley et al., 1974; Tarnowka et al., 1978; D'Anna, 1982); (3) H1° is extensively synthesized (relative to its S-phase values) during traverse of G_1 (D'Anna et al., 1984); (4) there are large variations in the H1°:H1 ratio of rates of synthesis as cells traverse G_1 into S (D'Anna et al., 1982).

Besides confirming these general aspects, the data reveal a number of previously undetermined quantitative features about the syntheses of H1 and H1°. The rate of H1° synthesis is nearly constant throughout the G_1 period—even during the first hour after mitotic selection. We calculate an average uncorrected G_1 rate that is 43 ± 5% of the maximum rate when ≃57% of the cells are in S phase. This is somewhat lower than the 50–60% of maximum (when 60–70% of the cells are in S phase) determined after release from isoleucine-deprivation G_1 block (D'Anna et al., 1982). If we now assume that the rates of H1° synthesis are proportional to the numbers of G_1- and S-phase cells, we can estimate the relative rates of H1° synthesis during the G_1 and S phases. Using the measured rates of synthesis and the percentages of G_1- and S-phase cells during the third, sixth, and ninth hours, we calculate that the rate of H1° synthesis during G_1 is ~32 ± 3% of its value when all of the cells have entered S phase. The small variation in the results is consistent with the assumptions, and the result is the same if a correction is made for isotope incorporation into the minor impurity that elutes with H1° in HPLC.

These data also show that the rate of H1 synthesis is variable during G_1 (Figure 4). There is a (3.6 ± 0.5)-fold increase in the rate of H1 synthesis between the first and third hours after mitotic selection (the increase exceeds that observed for H1° throughout the experiment; see below). This result is different from those obtained when cells are synchronized by isoleucine deprivation. Under those conditions, the rate of H1 synthesis (Gurley et al., 1974; Tarnowka, 1978; D'Anna et al., 1982) decreases after release from G_1 block and goes through a minimum as cells traverse G_1; thus, the initially higher rate of H1 synthesis in cells synchronized by isoleucine deprivation most likely results from metabolic differences between G_1-arrested and proliferating cells (Chiu & Marzluff, 1982). These differences in the rates of synthesis between the two methods are reflected in the ratio of H1°:H1 rates of synthesis during G_1. In cells synchronized by isoleucine deprivation, the H1°:H1 ratio of rates of synthesis goes through a maximum during G_1 (D'Anna et al., 1982); here (Figure 4b), the ratio of H1°:H1 rates of synthesis declines from the earliest time point in G_1.

If we again assume that the rates of protein synthesis are directly proportional to the numbers of cells in G_1 and in S phase, we calculate that the rate of synthesis for H1 is 1.3 ± 0.2% of the S-phase rate during the first hour of G_1, and it increases to 4.5 ± 0.6% of the S-phase rate at mid G_1.

We note that during the first hour after mitotic selection, H1° is synthesized at ≃30% of its S-phase rate compared with a value of only ≃1% for H1; nevertheless, only 0.7 molecule of H1° is synthesized for every molecule of H1. As cells enter S phase, there is a (≃3.1 ± 0.2)-fold increase in the rate of H1° synthesis (assuming pure populations of G_1- and S-phase cells); even then, however, the increase in H1° synthesis is less than the 3.6-fold increase in the rate of H1 synthesis in going from the first to third hours after mitotic selection. Thus, the maximum rate of H1° synthesis (i.e., new molecules per hour) throughout G_1 and early S phase is always less than 60% of the rate of H1 synthesis during mid G_1 (2–3 h after mitotic selection).

Discussion

These results illustrate that HPLC and quantitative isolation procedures may be combined to measure rates of protein synthesis from mitotically selected cells. Measurements of rates of synthesis indicate the rate of H1 synthesis is variable during traverse of G_1: there is a nearly 4-fold increase in the rate of H1 synthesis as cells progress from the first hour (≃1.2% of the rate in S phase) to the third hour (≃4.5% of the rate in S phase) after mitotic selection. As cells progress into S phase, the rate of H1 synthesis increases ~22-fold and appears to be proportional to the number of S-phase cells.

In contrast to the variable rate of H1 synthesis during G_1, the rates of H1° synthesis are essentially constant during that period. This constancy is observed even during the first hour of G_1; thus, there is no appreciable lag between cell division and the onset of H1° synthesis. As cells progress into S phase, the rate of H1° synthesis increases ~3.1-fold, so that it appears to be proportional to the increase in the number of S-phase cells.

Comparison of these results with those obtained from cells released from isoleucine-deprivation block (Gurley et al., 1974; Tarnowka et al., 1978; D'Anna et al., 1982) indicates that, except for the first hour of G_1, the results are nearly the same. When cells are synchronized by the isoleucine-deprivation procedure, the rate of H1 synthesis (compared with S phase) during the first hour after release appears to be 6–8 times greater than the values measured here (≃1.3% of S phase) from mitotically selected cells; however, by the second hour, the rate (4–6% of the S-phase value) is only 1.2–1.5 times as great. During S phase, the rates of synthesis appear to be proportional to the numbers of S-phase cells in both procedures. Thus, whereas isoleucine deprivation produces a metabolic state that, most likely, differs from G_1 in proliferating cells (Chiu & Marzluff, 1982), it would appear that by 1–2 h after release the procedure yields cells whose capacity for H1 synthesis is similar to that of mitotically selected cells.

Recently, Plumb et al. (1984) have reported the relative abundance of H1 messenger RNA (probed with a HeLa H1 subfraction DNA clone) during cell-cycle progression. Beginning 2 h after mitotic selection, they obtained measurable quantities of H1 mRNA which increased as cells entered S phase. Although their results and ours are not quantitatively comparable, they appear to be qualitatively consistent.

So far as we know, this is the first application of HPLC in the examination of protein synthesis during the cell cycle. Since the reversed-phase columns do not resolve the H1 species H1(I) and H1(II) (Gurley et al., 1975; D'Anna et al., 1981) nor the H1° species H1°a and H1°b (D'Anna et al., 1981), we have not been able to look for variable rates of synthesis among H1 or H1° subfractions as reported for H1 during S phase by Sizemore & Cole (1981). On the other hand, these results clarify when the syntheses of CHO H1 and H1° class proteins

begin after cell division, the limits of variation in the rates of H1 and H1° synthesis during the G_1 and S phases, and the degree of coupling between the synthesis of H1 or H1° class proteins with DNA synthesis. We suspect that this combination of mitotic selection, quantitative protein recovery, and HPLC should be useful to study the synthesis of other proteins besides H1 histones.

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