G1- and S-Phase Synthesis of Histone H1 Subtypes from Mouse NIH Fibroblasts and Rat C6 Glioma Cells

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ABSTRACT: The rates of synthesis of histone H1 subtypes in synchronized mouse NIH 3T3 fibroblasts were compared with those of rat C6 glioma cells during the G0, G1, and S phases by using a combination of HPLC techniques and conventional gel electrophoresis. In the mouse cell line, all H1 subtypes, H1a–H1e including histone $H1^0$, were detectable. In the rat cell line, however, no histone H1a was found. H1c and H1e from both cell lines show in the quiescent state a relatively high specific activity comparable with that of $H1^0$. After release from the G0/G1 block, the synthesis of $H1^0$ and likewise that of H1c and H1e increase for a short period. All H1 subtypes have their maximum specific activity at the same time after stimulation. The percentage of total H1 specific activity of H1a, H1b, and H1d increases, those of H1c and H1e remain relatively constant, and that of $H1^0$ decreases while cells cycle from the G0/G1 to the S phase. These findings support our assumption that H1 subtypes could be classified into three groups with common metabolic characteristics: one consists of H1a, H1b, and H1d; another of H1c and H1e; and a third of $H1^0$ histone. Moreover, the corresponding H1 subtypes from two different species seem to have similar specific activities during the G1 and S phases.

H1 histones are a heterogeneous group of at least five different subtypes with closely related but none the less different primary structures (Kinkade & Cole, 1966; Rall & Cole, 1971). In addition to these subtypes, several mammalian species contain an H1 histone, designated $H1^0$, found both in nonreplicative tissues (Panyim & Chalkley, 1969; Yasuda et al., 1986; Helliger et al., 1992) and in rapidly proliferating cells (D'Anna et al., 1981; Gurley et al., 1984; Helliger et al., 1992). However, $H1^0$ is accumulated in excess in G1-arrested cells due to its extensive G1 synthesis compared to that of H1 (D'Anna et al., 1982, 1985). The H1 histones differ in the extent of phosphorylation in the G1, S, and late G2 phases, respectively (Bradbury et al., 1974; Gurley et al., 1974), and, moreover, these proteins exhibit a striking tissue and species specificity (Lennox et al., 1982; Lennox & Cohen, 1983).

The dominant view over a long period has been that all H1 proteins have uniform biological significance because the H1 histone subtypes possess common characteristics and only discret multiplicity. This opinion has been supported by many results obtained by insufficient analytical methods unable to resolve closely related H1 variants and their phosphorylated forms. However, during the last 10 years, it is becoming increasingly clear that H1 subtypes may have unique and specific characteristics (Cole, 1987). H1 subtypes differ from each other in their patterns of phosphorylation (Ohba et al., 1984; Matsukawa et al., 1985) and in phosphorylation sites during the cell cycle (Harrison et al., 1982; Langan, 1982). It is also known that the quantitative composition of subtypes varies with the progress of embryological development (Lennox, 1984; Lennox & Cohen, 1988) and terminal differentiation (Bustin & Cole, 1968; Kinkade, 1969). Moreover, H1 histones are synthesized at unequal rates during the cell cycle and varied in turnover (Lennox & Cohen, 1983; Sizemore & Cole, 1981; Pehrson & Cole, 1982; Higurashi et al., 1987). These last mentioned observations have been of special importance for this paper.

In our studies, we tried to characterize each H1 subtype, including $H1^0$, by comparing two different species, rat and mouse, respectively, with respect to the metabolic activity during the G0, G1, and S phases. In the past, two-dimensional gel electrophoresis or ion-exchange chromatography was most frequently applied for analyzing H1 histone synthesis. However, the application of these techniques was often complicated by the overlapping of different subtypes or phosphorylated bands from different subtypes. With our technique, we tried to avoid these complications. In order to separate all H1 subtypes including $H1^0$, we used a combination of high-performance liquid chromatography (HPLC)[1] techniques and conventional gel electrophoresis. The results described here revealed that histone H1 subtypes from both mouse and rat cell lines show common metabolic characteristics and can be classified into different groups concerning their synthesis rates.

EXPERIMENTAL PROCEDURES

Chemicals. Triton X-100, Tris base, and phenylmethanesulfonyl fluoride were obtained from Serva (Heidelberg, Germany), ethylene glycol monomethyl ether was from Aldrich-Chemie (Steinheim, Germany), and all other chemicals were from Merck (Darmstadt, Germany).

Culture and Synchronization of Cells. NIH 3T3 fibroblast were grown in monolayer culture and cultivated in DMEM (Biochrom, Berlin, Germany) supplemented with 10% FCS,[1] penicillin (60 μg/mL), and streptomycin (100 μg/mL) in the presence of 5% CO_2. The rat C6 glioma cell line was grown in monolayer culture in MEM with Earl's salt (Biochrom) supplemented with 10% FCS, vitamins, and essential and nonessential amino acids in the presence of 5% CO_2. For synchronization, cells were seeded at a density of 4×10^5 cells/dish (48 cm^2), grown for 12 h in normal supplemented DMEM or MEM, respectively. After this time, cells were

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[1] Abbreviations: FCS, fetal calf serum; PBS, phosphate-buffered saline; HPLC, high-performance liquid chromatography; TFA, trifluoroacetic acid; SDS/PAGE, sodium dodecyl sulfate/polyacrylamide gel electrophoresis; AU/PAGE, acid urea/polyacrylamide gel electrophoresis.

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washed once with prewarmed PBS and then incubated in starvation medium (a 1:1 mixture of HAM's F12 with DMEM or MEM) supplemented with 0.1% FCS (NIH 3T3) or without FCS (C6 glioma) for 72 h to accumulate the cells in the G0/G1 phase. To release the cells from G0/G1-phase arrest, fresh medium supplemented with 10% FCS was added. Several hours after the beginning of restimulation, [^{3}H]-thymidine or [^{3}H]lysine incorporation was measured.

Thymidine Incorporation. Cells were grown on round coverslips in multiwell dishes. Starvation and stimulation were performed as described above. Cells were pulsed with [6-^{3}H]-thymidine (29 Ci/mmol, Amersham, England) at 37 °C for 30 min at a concentration of 2 μCi/mL. Fixation of cells and autoradiography have been described in detail elsewhere (Knosp et al., 1991). The developed and fixed slides were evaluated by phase-contrast microscopy.

Labeling Conditions and Isolation of H1 Histones. For labeling H1 histones with [^{3}H]lysine, cells were incubated for 30 min in lysine-deficient medium (Sigma Chemie, Munich, Germany) containing 25 μCi/mL L-[4,5-^{3}H]lysine (85 Ci/mmol, Amersham). The reaction was stopped by washing the cells 2 times with ice-cold PBS. For isolation of H1 histones, the labeled cells were incubated in ice-cold lysis buffer [50 mM Tris, pH 7.5, 25 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 0.25 M sucrose, 10 mM 2-mercaptoethanol, 0.1 mM phenylmethanesulfonyl fluoride, and 0.1% (w/v) Triton X-100] for 5 min and were removed from the dish by a rubber policeman. Nuclei were pelleted at 2500*g* for 15 min at 4 °C and washed with lysis buffer without Triton. Whole histones were isolated from the resulting nuclear preparation by extraction with 0.2 M H_2SO_4 at 4 °C for 1 h. The mixture was centrifugated at 10000*g* in a microfuge for 20 min. The supernatant was mixed with 5 volumes of chilled acidified acetone and allowed to stand for 12 h at −20 °C. The precipitate was washed once with acetone, resuspended in water containing 10 mM 2-mercaptoethanol, and freeze-dried.

HPLC. The equipment used consisted of two Beckman Model 114M pumps, a 421A system controller, and a Model 165 variable-wavelength UV/VIS detector. The effluent was monitored at 210 nm, and the peaks were recorded using Beckman System Gold software. The protein separations were performed on self-packed columns filled with Nucleosil 300-5 C4 obtained from Macherey-Nagel, Düren, Germany (12.5 cm × 0.8 cm, 5-μm beads, 300 Å). The freeze-dried proteins were dissolved in water containing 0.1% TFA, and samples of 100 μg of histone were injected onto the column. At a constant flow rate of 1 mL/min, the H1 histones were eluted within 35 min using a linear gradient from 41 to 61% B [solvent A is water containing 10% ethylene glycol monomethyl ether and 0.1% TFA; solvent B is ethylene glycol monomethyl ether (10%)/70% acetonitrile (90%) with 0.1% TFA].

Polyacrylamide Gel Electrophoresis. H1 histone fractions from HPLC runs were collected, freeze-dried, and stored at −20 °C. Rat H1 histone fractions were analyzed by SDS/PAGE (15% polyacrylamide/0.1% SDS) as described by Laemmli (1970) and mouse H1 histones by AU/PAGE (15% polyacrylamide/0.9 M acetic acid/2.5 M urea) according to Lennox et al. (1982). The gels were stained with 0.1% Coomassie blue and destained by diffusion. Processing for fluorography was as described (Knosp et al., 1991). Bands of stained protein and the blackening of the X-ray films were quantitated in the linear range by densitometry.

Incubation of Histone H1 with Alkaline Phosphatase. To prevent the appearance of phosphorylated bands in AU/PAGE, the mouse samples were incubated with alkaline phosphatase. About 100 μg of whole histones in 0.25 mL of 10 mM Tris-HCl, pH 8.0, and 1 mM phenylmethanesulfonyl fluoride was mixed with 210 μg of *Escherichia coli* alkaline phosphatase (60 units/mg; Sigma Chemie) for 12 h at 37 °C (Sherod et al., 1970; Harisanova & Ralchev, 1986).

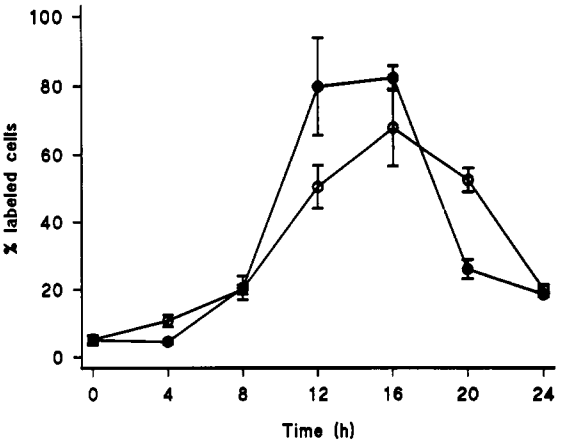

FIGURE 1: DNA synthesis during serum stimulation. Cells were serum-starved for 72 h and afterward stimulated with DMEM (NIH cells) or MEM (C6 cells) containing 10% FCS. At the indicated times, cells were labeled for 30 min with [^{3}H]thymidine (2 μCi/mL) and processed for autoradiography as described under Experimental Procedures. 0 h means 72-h serum starvation. Mouse NIH fibroblasts (●); rat C6 glioma cells (○). Each point is the mean ● SD of four independent experiments.

RESULTS

Cultures of NIH mouse fibroblasts or C6 rat glioma cells were synthronized by serum starvation for 72 h. During this period, the fraction of nuclei that incorporated thymidine dropped to levels lower 5% and stayed at this level. These findings are similar to those previously regarded (Knosp et al., 1991). After addition of serum, both cell lines started DNA synthesis after a lag period of 4–6 h with a maximum of [^{3}H]tymidine incorporation at 12–16 h, where about 70–80% of the nuclei were labeled (Figure 1). However, 24 h after the beginning of serum stimulation, both cell lines reached incorporation values of about 20%.

We used this cell synchronization system to compare the course of the synthesis of different H1 isoprotein species in a mouse and a rat cell line, respectively. Cells were labeled with [^{3}H]lysine for 30 min at the end of serum deprivation and at different time points after serum stimulation. In this work, whole histones were extracted from isolated nuclei at each time point; histone H1 was resolved by reversed-phase HPLC and assigned to the Lennox nomenclature using two types of gel electrophoreses and amino acid analyses of each H1 subtype as previously described (Lindner et al., 1990; Lindner & Helliger, 1990). Figure 2 illustrates the chromatography of the H1 subtypes from quiescent cells on a reversed-phase column as outlined under Experimental Procedures. Within 35 min, five H1 subtypes and $H1^0$ were isolated. Following the Lennox nomenclature (Lennox et al., 1982) in the NIH fibroblast cell line, we found H1a, H1b, H1c, and $H1^0$ as pure H1 subtypes. One subfraction, however, was a mixture of the variants H1d and H1e (Figure 2A). Interestingly, the rat glioma cell line lacks H1a. Consequently, we found only H1b, H1e, and $H1^0$ as pure subtypes, whereas H1c and H1d were not resolved from each other (Figure 2B). Therefore, we had to separate the unfractionated H1 subtypes with gel electrophoretic methods. For the analysis of the mouse subtypes H1d and H1e, we used the AU/PAGE system as

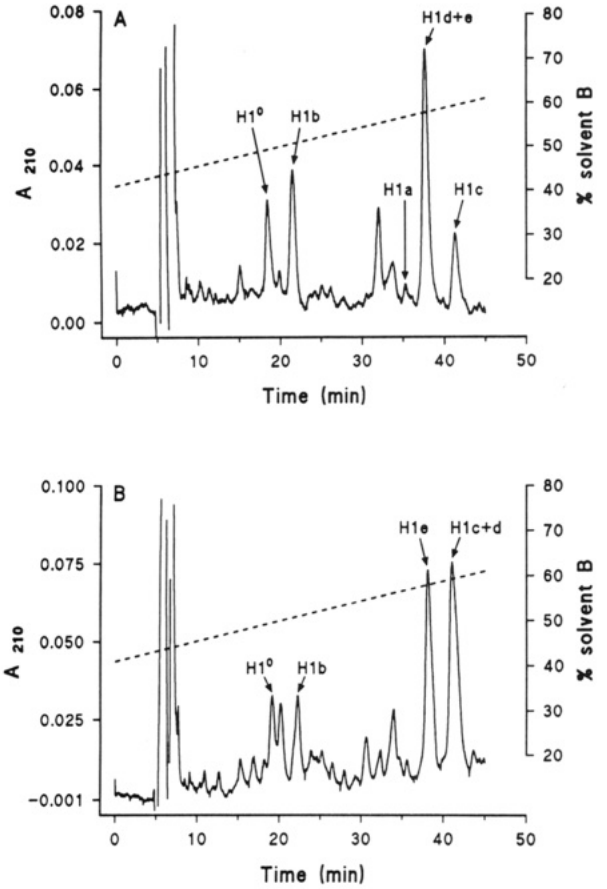

FIGURE 2: HPLC analysis of histone H1⁰ and H1 subtypes in 72-h serum-starved NIH fibroblasts and C6 glioma cells. Whole histones were isolated from cell nuclei and separated by reversed-phase HPLC as described under Experimental Procedures. The column (12.5 cm × 0.8 cm) contained Nucleosil 300-5 C4, and the flow rate was 1 mL/min with a linear acetonitrile gradient (- - -). Within 35 min, all H1 subtypes including H1⁰ were isolated as single peaks with the exception of H1d and H1e (NIH cells) and H1c and H1d (C6 cells) which were not resolved. All unmarked peaks are non-histone proteins. (A) NIH fibroblasts; (B) C6 glioma cells.

described by Lennox et al. (1982), because these variants cannot be separated by SDS/PAGE. The rat subtypes H1d and H1c, however, were analyzed by SDS gel electrophoresis as described by Laemmli (1970), because the resolution of these histone variants is not sufficient on AU gels. In addition, all unassigned peaks in Figure 2 were analyzed with gel electrophoretic methods and identified as non-H1 histone proteins (data not shown). After evaluation of HPLC and electrophoretograms, the percentages of the H1 subtypes from quiescent cells were calculated. For the NIH cells, the percentages of the H1 subtypes H1a, -b, -c, -d, and -e and H1⁰ were 3%, 20%, 16%, 26%, 22%, and 13%, respectively. The C6 cells showed for subtypes H1b, -c, -d, and -e and H1⁰ the following values: 11%, 23%, 22%, 32%, and 11%.

Figure 3 shows H1 mass and synthesis patterns in quiescent C6 glioma cells (Figure 3a,b) and from those in the S phase 12 h after restimulation with fetal calf serum (Figure 3c,d). As previously observed by D'Incalci et al. (1986), H1 subtype H1c dominates the synthesis pattern in G0, but also in G1 (Figure 3b, lane 3, lower band). In our cell system, H1⁰ (Figure 3b, lane 1) and H1e (Figure 3b, lane 4) reveal a high lysine incorporation rate as well. The incorporation rate of subtype H1b (Figure 3b, lane 2) and H1d (Figure 3b, lane 3, upper band), however, is very low. During the S phase, all subtypes seem to have different lysine incorporation rates graded from H1c to H1⁰ with the following order of extent: H1c > H1d > H1e > H1b > H1⁰ (Figures 3d and 6A).

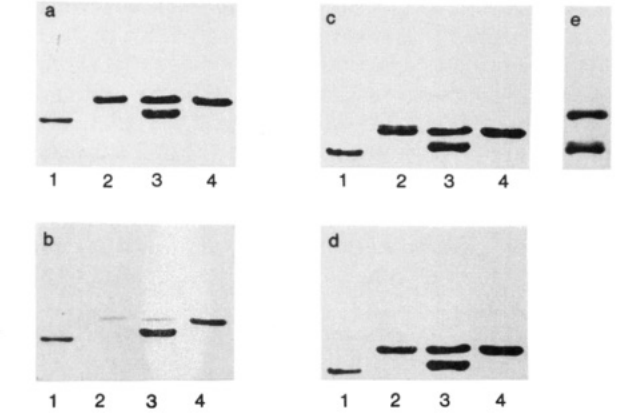

FIGURE 3: H1 mass and synthesis patterns in quiescent and S-phase rat C6 glioma cells. Synchronized C6 cells were pulse-labeled 30 min with [³H]lysine (25 μCi/mL) in lysine-deficient medium. Whole histones were extracted as described under Experimental Procedures and separated with reversed-phase HPLC. The HPLC-fractionated H1 histones were loaded on a 16-cm SDS/PAGE gel (15% polyacrylamide/0.1% SDS). Fluorographies of gels were exposed to X-ray film (Kodak X-Omat) for 7 days. The mass patterns (Coomassie blue stained gel) of quiescent (a) and S-phase (c) cells and the corresponding fluorographies during quiescence (b) and S phase (d) are shown. Lane 1, H1⁰; lane 2, H1b; lane 3, H1c (lower band) and H1d (upper band); lane 4, H1e. The same proteins as in (c) lane 3 were loaded on a 32-cm SDS/PAGE gel (e) (lower two bands H1c, upper band H1d).

H1 subtypes undergo cell cycle related specific phosphorylations, principally during the S phase and mitosis (Ajiro et al., 1981a,b). In SDS gels, generally the phosphorylated subtypes cannot be seen. However, the phosphoryation alters the electrophoretic mobility of H1, and, therefore, it is possible to see the appearance of phosphorylated H1 subtype bands caused by changes in the conformation of the H1 molecule (Blumenfeld, 1979; Billings et al., 1979). We found that not only the subtype H1b as previously described (Lennox et al., 1982) but also H1c produced an altered electrophoretic mobility if these H1 variants were in higher states of phosphorylation. This altered mobility is characterized in the appearance of an additional band migrating just above the main band of the corresponding H1 variant. In the 16-cm SDS gel electrophoresis, the additional band is well visible at the H1b variant (Figure 3c, lane 2) but hardly detectable at the H1c subtype (Figure 3c, lane 3). However, using a long SDS gel (32 cm), we observed a distinct resolution of these two bands of H1c (Figure 3e, lower bands).

As mentioned above, for separating the mouse cell histone subtypes H1d and H1e, we used an AU/PAGE system. In this gel system, however, the phosphorylated forms of the H1 subtypes appearing in the S phase are detectable as well. This leads to an overlapping of phosphorylated bands of H1e with both unphosphorylated and phosphorylated bands of H1d. Figure 4 demonstrates this shift of phosphorylated bands of H1e in the H1d area (lane 1). In order to avoid ambiguities concerning the calculation of specific activities of both subtypes which result from the overlapping gel mobilities of the two subcomponents phosphorylated to different levels (Figure 4, lane 2), we digested the phosphorylated histone preparations with *E. coli* alkaline phosphatase.

The mass and synthesis patterns of quiescent and S-phase mouse NIH fibroblasts are shown in Figure 5. In principal, the mass pattern and the results of [³H]lysine incorporation of mouse fibroblasts are comparable with those obtained from the rat cell line. However, in the mass pattern, two differences

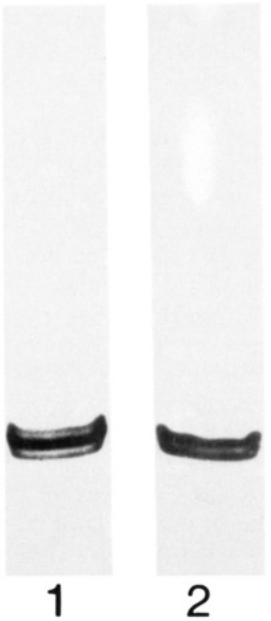

FIGURE 4: AU gel electrophoresis of H1d and H1e after alkaline phosphatase digestion. Whole histones of NIH 3T3 fibroblasts passing the S phase (16 h after 10% FCS stimulation) were extracted and afterward digested with alkaline phosphatase as described, fractionated with reversed-phase HPLC, and electrophoresed in AU gels. Lane 1 shows untreated H1d and H1e; lane 2 shows phosphatase-treated H1d (upper band) and H1e (lower band) on a Coomassie blue stained AU gel.

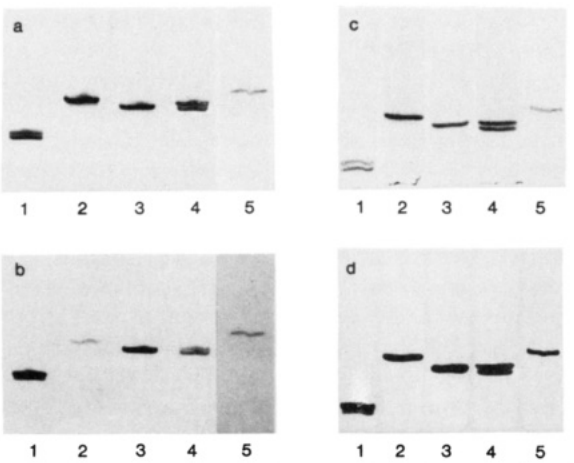

FIGURE 5: H1 mass and synthesis patterns in quiescent and S-phase mouse NIH 3T3 fibroblasts. Synchronization and lysine labeling of cells and extraction of histones were as in Figure 3. The histones were fractionated with reversed-phase HPLC and loaded on a 16-cm AU/PAGE gel (15% polyacrylamide/0.9 M acetic acid/2.5 M urea). Fluorographies of all lanes were exposed to X-ray films for 7 days with the exception of (b) lane 5 which was exposed for 14 days. The mass patterns (Coomassie blue stained gel) of quiescent (a) and S-phase (c) cells and the corresponding fluorographies during quiescence (b) and the S phase (d) are shown. Lane 1, $H1^0$; lane 2, H1b; lane 3, H1c; lane 4, H1d (upper band) and H1e (lower band); lane 5, H1a.

could be observed. First, in NIH fibroblasts, H1a exists, and second, because an AU/PAGE was performed, there are two protein bands of $H1^0$ visible (Figure 5a,c, lane 1). The distribution of label among H1 components when quiescent NIH cells were pulsed with radioactive lysine was similar to the distribution in C6 cells. All subtypes were slightly labeled, but the most label was in H1c (Figure 5b, lane 3) and the upper band of $H1^0$ (Figure 5b, lane 1) followed by H1e (Figure 5b, lane 4, lower band). During the S phase (12 and 16 h after serum stimulation), the mouse H1 subtypes, like the rat subtypes, showed different synthesis patterns with a gradation

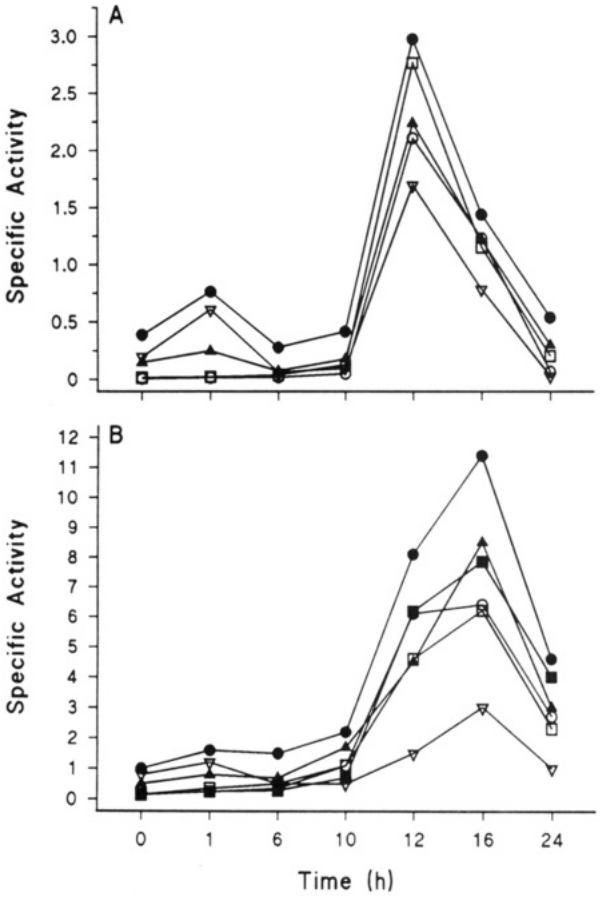

FIGURE 6: Time course of the H1 subtype synthesis rate during serum stimulation in C6 glioma cells (A) and NIH fibroblasts (B). After 72 h of serum starvation, cells were stimulated with 10% FCS. At the indicated time points, cells were labeled and histones were fractionated as in Figures 3 and 5. The specific activities were calculated as follows: histone bands on SDS gels (C6 cells) or AU gels (NIH cells) and corresponding fluorographies were quantitated by densitometry and the values divided by each other. $H1^0$ (▽); H1a (■); H1b (○); H1c (●); H1d (□); H1e (▲). Each point represents the mean value of three (C6 cells) or four (NIH cells) independent experiments.

from H1c to $H1^0$. The order of the extent of the lysine incorporation rate was H1c > H1e > H1a > H1b > H1d > $H1^0$ (Figures 5d and 6B).

The specific radioactivities of the labeled H1 histones were calculated from stained gel and fluorography scans for both cell lines. The time course of the histone H1 subtype synthesis rate during serum stimulation is shown in panels A and B of Figure 6 for rat and mouse cells, respectively. Previous studies showed that immediately after release from G0/G1 block, the synthesis of core histones (Knosp et al., 1991), and after release from G1 block the synthesis of $H1^0$ histone and total H1 histones (D'Anna et al., 1982), increased for a short period. Recently, D'Anna et al. (1985) have reported that even mitotically selected cells as they traverse through G1 show nearly the same results.

Our measurements of $H1^0$ and H1 subtype syntheses agree with D'Anna et al., who showed that $H1^0$ synthesis increases 1 h after restimulation of blocked cells. However, we could show that the synthesis of total H1 histone subtypes 1 h after serum stimulation can be attributed mainly to the synthesis of histone $H1^0$ and subtypes H1c and H1e whereas the remaining H1 subtypes showed clearly lesser portions of synthesis. The inequality of synthesis rates among the subtypes and the fact that during this period no increase in the

Table I: Distribution of Histone H1 Subtype Synthesis in C6 Glioma Cells and NIH Fibroblasts Expressed as Percent of Total H1 Specific Activity[a]

	Histone H1 Subtypes from C6 Cells				
time (h)[b]	H1b	H1c	H1d	H1e	$H1^0$
0	4 ± 1[c,d]	53 ± 7[d]	3 ± 2[d]	15 ± 6	25 ± 4[d]
1	3 ± 1[d]	41 ± 4[d]	4 ± 1[d]	15 ± 2	36 ± 3[d]
6	4 ± 2	49 ± 11	12 ± 3	18 ± 3	16 ± 2
10	7 ± 3	48 ± 9	15 ± 2	18 ± 4	12 ± 2
12	18 ± 5[d]	25 ± 3[d]	23 ± 3[d]	19 ± 2	14 ± 5[d]
16	21 ± 4[d]	25 ± 3[d]	20 ± 4[d]	21 ± 2	13 ± 2[d]
24	11 ± 5	42 ± 7	16 ± 3	25 ± 4	6 ± 2

	Histone H1 Subtypes from NIH Fibroblasts					
time (h)	H1a	H1b	H1c	H1d	H1e	$H1^0$
0	5 ± 2[d]	6 ± 2[d]	36 ± 6[d]	5 ± 1[d]	18 ± 3	30 ± 7[d]
1	6 ± 4[d]	6 ± 3[d]	37 ± 4[d]	5 ± 2[d]	17 ± 6	29 ± 9[d]
6	8 ± 5	9 ± 4	41 ± 6	13 ± 3	20 ± 3	9 ± 5
10	9 ± 3	15 ± 4	30 ± 4	15 ± 4	23 ± 3	7 ± 2
12	20 ± 3[d]	19 ± 3[d]	26 ± 5[d]	15 ± 2[d]	15 ± 2	5 ± 4[d]
16	18 ± 5[d]	15 ± 2[d]	26 ± 3[d]	14 ± 1[d]	20 ± 7	7 ± 2[d]
24	20 ± 6	14 ± 5	23 ± 5	12 ± 3	21 ± 4	10 ± 4

[a] The total specific activity is the sum of the H1 subtype and $H1^0$ activities (at the different time points) and was set to 100%. [b] Time means hours of serum stimulation after 72 h of serum deprivation. [c] The values, given as the percent of the total H1 specific activity at the corresponding time points, are expressed as means ± SD for three or four independent experiments in the case of C6 cells or NIH fibroblasts, respectively. [d] Significant differences were calculated with the *t* test between G0/G1 (0 h, 1 h) and the S phase (12 h, 16 h). *p* values are given under Results.

incorporation of [^{3}H]thymidine into DNA occurs eliminate the possibility that synthesis is due to a low level of DNA replication.

The distribution of H1 subtype synthesis expressed as the percent of total H1 specific activity at the different time points is shown in Table I. During the G0/G1 phase of the cell cycle, the portion of total H1 histone specific activity differed among the various H1 subtypes. For the H1b and H1d subtypes of both cell lines and the H1a subtype in the mouse cell line, this portion is about 3–6%. On the other hand, the portion of H1c was about 53 or 36%, that of $H1^0$ about 25% or 30%, and that of H1e about 15% or 18% for C6 or NIH cells, respectively. If cells enter the S-phase, the percentage of total H1 histone specific activity of H1a, H1b, and H1d increases significantly (for both cell lines, $p < 0.025$) compared to G0/G1. The percentage of H1e remains constant (no significant difference between G0/G1- and S-phase values) whereas that of H1c significantly decreases (C6, $p = 0.025$; NIH, $p = 0.018$). The behavior of subtype $H1^0$ is as expected because the $H1^0$ percentage of total H1 activity decreased from G0/G1 to the end of the S phase from about 30% to 10% (C6; $p = 0.023$; NIH; $p = 0.005$). The *p* values were calculated with the *t* test.

DISCUSSION

In order to measure the specific activities of H1 subtypes, we used in this study a combination of HPLC techniques and gel electrophoretic methods. Up to now (Lindner et al., 1986, 1990; Karhu et al., 1988), it was hardly possible to resolve the H1 subtypes with HPLC and reversed-phase columns (Gurley et al., 1975; D'Anna et al., 1981). D'Anna et al. (1985) have used HPLC to measure $H1^0$ synthesis during the cell cycle without resolving all other H1 subtypes. However, our method enables us to examine the synthesis of all different histone H1 subtypes including histone $H1^0$. Comparing the different forms of H1 from two species, namely, rat and mouse, we could classify the H1 subtypes in both cell lines into groups with common metabolic characteristics. One group is represented by H1c and H1e, because in the quiescent state of the cells both subtypes showed a relatively high specific activity comparable with that of $H1^0$ (Figure 6). Furthermore, these two subtypes have a relatively constant distribution of synthesis in the G1 and S phases (Table I). The second group is represented by H1a, H1b, and H1d, because these subtypes have a greatly increased distribution of synthesis in the S phase. On the contrary, $H1^0$ appears to be a third class because it was synthesized to a considerable extent during G0 and G1 and decreased continuously in distribution of total specific activity during the S phase, reaching a minimum in the late S phase. The present study demonstrates that unequal turnover of H1 subfractions is a fact. This conclusion was implied by earlier reports; however, the former studies were carried out with one- or two-dimensional gel electrophoretic methods and were unable to resolve all H1 subtypes or their phosphorylated forms (Pehrson & Cole 1982; D'Incalci et al., 1986).

We could show that the synthesis of the various groups differed in respect to its linking to DNA synthesis: at the time point where no DNA synthesis could measured (0 and 1 h after serum stimulation), the specific activities of $H1^0$, H1c, and H1e are higher than those of H1a, H1b, and H1d. On the other hand, in general we could not observe that one or more H1 subtypes are synthesized early while other H1 subtypes are preferentially made late in the S phase (Sizemore & Cole, 1981; Higurashi et al., 1987). We found that all subtypes have their maximum activity at the same time after stimulation. One difficulty to measuring the synthesis of H1 subtypes during the S phase is the specific phosphorylation of the different H1 subtypes at this cell cycle state, leading to the overlapping of additional appearing bands in gel electrophoresis or peaks in ion-exchange chromatography. In contrast to others, we tried to avoid the shift and overlapping of bands caused by phosphorylation of H1 subtypes. Another explanation for the differences between our results and others could be the different way to obtain cell synchronization because we used serum deprivation to block cells at the G0/G1 phase. However, the above described metabolic properties of H1c and H1e are partially in agree with Higurashi et al. (1987), who reported a common metabolic behavior of their subtypes III and V, and it is likely, though not proven, that these subtypes III and V correspond to our subtypes H1c and H1e. The fact that some H1 subtypes, namely, $H1^0$, H1c, and H1e, are synthesized in the absence of DNA replication and some are not (H1a, H1b, and H1d) might represent a design for the regulation of function in particular parts of the chromatin rather than merely a repair mechanism (Pehrson & Cole, 1982). Our results support the proposal of Urban and Zweidler (1983) and Wu and Bonner (1981) that histone subtypes could be classified into two general classes: the so-called replication subtypes which are synthesized only in the S phase during DNA replication, and the so-called replacement subtypes which are also synthesized independently of DNA replication. This proposal refers only to the core histones and $H1^0$; however, our results indicate that this classification applies to the H1 subtypes as well.

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