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Histone Synthesis by Lymphocytes in G_0 and G_1†

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ABSTRACT: Peripheral blood lymphocytes are a naturally occurring population of G_0 cells which can be activated in vitro to grow and divide. Upon activation with phytohemagglutinin (PHA), they enter G_1 and, after a 24-h lag, begin DNA replication (S phase). Using radioisotope labeling and gel electrophoresis of acid-soluble chromatin proteins, we investigated histone synthesis in G_0, G_1, and S phase cultures of human and pig lymphocytes. In G_0 and G_1 cultures, which have less than 0.1% S phase cells, all five histones are synthesized and are incorporated into chromatin in equimolar amounts. In G_0 lymphocytes histone synthesis accounts for at least 6% of nuclear protein radioactivity, and the rate of synthesis is about 2-3% of that of S phase lymphocytes. In contrast to histone synthesis by S phase cultures, G_0 and G_1 histone synthesis was completely resistant to treatment with hydroxyurea.

Although it is now well established that most histone synthesis is coupled to DNA replication [reviewed in Hohmann (1981)], evidence is accumulating which shows that some histone synthesis, especially of H1, occurs outside of the S phase of the cell cycle. This evidence has most often come from studies which involved detection of newly synthesized histones or their mRNA in established mammalian cell lines in G_1, obtained by synchronization or G_1 arrest (Curley et al., 1972; Melli et al., 1977; Tarnowka et al., 1978; Pehrson & Cole, 1982). However, there have been conflicting results, often with the same cell lines (Rickles et al., 1982; Marashi et al., 1982), and the G_1 synthesis observed in these cell lines has often been ascribed to the relatively large numbers (from 2 to 25%) of S phase cells which usually persist in such preparations (Rickles et al., 1982; Delegeane & Lee, 1982).

Circulating peripheral blood lymphocytes are a naturally occurring population of G_0 cells which can be activated in vitro by plant lectins to grow and divide [reviewed in Ling & Kay (1975); Hume & Weidemann, 1980]. During the course of studies on the synthesis of nuclear proteins by unstimulated and phytohemagglutinin- (PHA-) activated human and pig lymphocytes, we observed significant incorporation of labeled amino acids into histones of G_0 and G_1 phase lymphocyte cultures. We present here evidence that there is incorporation of all five newly synthesized histones into chromatin of G_0 and G_1 lymphocytes which is not due to contaminating S phase cells and which is resistant to hydroxyurea.

Materials and Methods

Lymphocyte Cultures. Human and pig lymphocytes were isolated from EDTA[1] anticoagulated or defibrinated blood by density gradient centrifugation on Ficoll-Isopaque as described by Boyum (1976) and washed and suspended in RPMI 1640 medium as previously described (Dauphinais & Waithe, 1977). The cells were incubated at 37 °C for 16-22 h before the start of the experiment to allow them to recover from the isolation procedure. Of the nucleated cells in the cultures, 80-90% were lymphocytes, and the rest were approximately equal numbers of granulocytes and monocytes (neither of which survive or divide in the cultures). Pure (over 99%) human T lymphocytes, prepared by rosette formation with neuraminidase-treated sheep red blood cells (Weiner et al., 1973), were used in some experiments and gave the same results. The cells were cultured at a concentration of 2×10^6 mononuclear cells/mL in 50 mL or conical-tip disposable culture tubes or in flat-

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[1] Abbreviations: EDTA, ethylenediaminetetraacetic acid; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; PMSF, phenylmethanesulfonyl fluoride; Tris, tris(hydroxymethyl)aminomethane; NaDodSO$_4$, sodium dodecyl sulfate.

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bottomed vessels [(3–5) × 10^6 cells/cm^2]. Unless otherwise specified, unstimulated and G_1-PHA-stimulated cultures were incubated for 10 h before labeling (the incubation time referred to is the time *after* the recovery period). PHA (Wellcome HA 16/17) was used at a final concentration of 2 μg/mL. When used, hydroxyurea, at a final concentration of 2 mM, was added to the cultures 30 min before labeling.

Labeling of Proteins. Cultures were concentrated to 10^7 cell/mL and labeled for 2 h (or as specified) with either 50 μCi/mL [^{3}H]leucine (50–65 Ci/mmol), 5 Ci/mL [^{14}C]leucine (312 mCi/mmol), or 10 μCi/mL [^{3}H]lysine (80 Ci/mmol). Two methods of labeling were used: In the short term experiments (10-h G_0 and G_1 cultures) when labeled leucine was used, the cells were cultured in conical-tip tubes in medium containing one-fourth the normal leucine concentration. [Reduction of the leucine concentration by this amount did not affect lymphocyte stimulation during the first 18 h of culture (results not shown)]. At the time of labeling, four-fifths of the medium overlying the cells was removed, and the labeled leucine was added as a concentrated solution. In the other experiments the cells were cultured in medium containing the normal concentration of leucine (4 mM). Before labeling, the cells were centrifuged (150*g*, 10 min), the medium was removed, ant the cells were suspended at 10^7/mL in fresh 37 °C medium containing 25 mM Hepes buffer and one-tenth the normal concentration of leucine (or without lysine). The suspension was incubated for 15 min before addition of the labeled amino acid. Incorporation of label into protein was linear for over 2 h with both methods. Labeling was terminated by washing the cells twice (800*g*, 3 min) with 4 °C phosphate-buffered isotonic saline and rapid freezing of the pellet with dry ice–acetone. The cell pellets were stored at −70 °C until used.

Cell Fractionation. Nuclei were isolated by the detergent method of Levy et al. (1973). PMSF (1 mM) was included in all buffers. The nuclear preparations were free of intact cells and cytoplasmic tags (examined by phase and electron microscopy). Some ribosomes, apparently attached to strands of cytoskeletal framework, remained attached to the nuclei. Chromatin was prepared by homogenizing the nuclei with a motor-driven Potter–Elvehjem tissue grinder in buffer 1 (75 mM NaCl, 24 mM EDTA, and 1 mM PMSF, pH 8.0) followed by centrifugation (16000*g*, 10 min) and homogenization of the pellet in buffer 2 (50 mM Tris-HCl and 1 mM PMSF, pH 8.0). The supernatants of buffers 1 and 2 constituted the nucleoplasmic fraction. In some experiments, the chromatin was subsequently extracted with a high salt buffer (0.35 M NaCl, 10 mM Tris-HCl, and 1 mM PMSF, pH 8.0), and H1 was extracted with 5% perchloric acid by the method of Sanders & Johns (1974). The acid-soluble proteins were extracted from the chromatin with 0.4 N H_2SO_4, and the histones of the acid-soluble fraction (AS) were precipitated with ethanol. Non-histone chromatin proteins (NHCP) were extracted with 1% $NaDodSO_4$ by the method of Elgin & Bonner (1970). Incorporation of label into whole cells, nuclei, and the subcellular fractions was determined by liquid scintillation counting of trichloroacetic acid (Cl_3CCOOH) precipitated protein. Protein content was determined by the Lowry method as modified by Hartree (1972) with bovine serum albumin as standard. DNA was determined by the method of Burton (1956), using calf thymus DNA as standard. Ninety percent of the nuclear protein radioactivity and 95% of the nuclear protein were recovered in the fractions.

Electrophoresis. Acetic acid–urea–polyacrylamide gel electrophoresis was performed according to Panyim & Chalkley (1969) by using 10% acrylamide and 6.25 M urea in the 10-cm gels and 15% acrylamide with 1.0 M urea in the 25-cm gels. $NaDodSO_4$ gel electrophoresis was performed as described by Thomas & Kornberg (1975). After electrophoresis, the gels were stained with Coomassie Blue R-250, photographed, and then sliced (1 mm). The slices were extracted overnight at 40 °C in 1 mL of NCS solubilizer containing 10% water, and the radioactivity was determined by scintillation counting. Over 90% of ^{3}H and ^{14}C counts were released into the scintillation fluid. Quench correction and calculation of disintegrations per minute (dpm) were by external standardization. Correlation of the stained bands and the radioactive peaks was verified by measurements of relative mobility, using the gel photographs.

DNA Synthesis and Autoradiography. Replicate samples of 10^6 cells were labeled for 30 min with 2 μCi/mL [^{3}H]-thymidine (Tdr) (6.7 Ci/mmol). Measurement of the incorporation of [^{3}H]Tdr into lymphocyte DNA by liquid scintillation counting and preparation of stained slides of lymphocytes for autoradiography were as described by Waithe & Hirschhorn (1978). The slides were dipped in Kodak NTB-2 emulsion and exposed for 5 days. Background grains were 0–2 grains per cell; cells with 5 or more grains were scored as labeled. Most (94%) of the labeled cells in the S phase cultures had well over 30 grains per cell. Most (98%) of the labeled cells in the hydroxyurea-treated S phase cultures had less than 30 grains per cell (80% had from 5 to 20 grains).

Results

Histone Synthesis by Unstimulated Lymphocytes. Peripheral blood lymphocytes are nondividing quiescent cells with a low rate of protein and RNA synthesis. Within 1 h after the addition of PHA to lymphocyte cultures, the rate of protein and RNA synthesis increases, and after a 24–30-h lag, cells begin to enter the S phase. Under optimal culture conditions, about 50% of the cells are in S by 40–50 h after the addition of PHA. Lymphocyte cultures can thus be operationally defined as G_0 (unstimulated), G_1 (0–24-h PHA-activated cultures), or S (PHA-activated cultures after 30 h).

Incorporation of newly synthesized histones into chromatin of lymphocytes in G_0 was evaluated in 10-h [^{3}H]- or [^{14}C]-leucine-labeled, unstimulated lymphocyte cultures. The acid-soluble chromatin protein fraction (AS), which had a protein:chromatin DNA ratio of 1.0 ± 0.02 (n = 18; ±SEM), represented 47 ± 3% (n = 7) of the recovered nuclear protein and 15 ± 0.9% of the total nuclear radioactivity. The specific activity of the AS fraction (145–595 dpm/μg of protein in [^{3}H]leucine-labeled cultures) was one-fourth that of the other nuclear fractions. The ethanol-precipitated proteins of the AS fraction were electrophoresed on 10-cm acetic acid–urea gels. A representative pattern of the radioactive distribution is shown in Figure 1. In seven cultures the major histone bands accounted for 57 ± 3% of the label on the gel and ubiquinated H2A (uH2A) for 20 ± 2%. The radioactivity recovered in the five histones (excluding uH2A) was 5.8 ± 0.4% of the total nuclear label. This is a minimum value since it does not take into account chromatin losses to the nucleoplasmic fraction. From the portion of the AS fraction radioactivity contributed by the histones, it can be estimated that their specific activity is about one-tenth that of the other nuclear proteins.

To ensure that the radioactivity in the histone bands was not due to comigration of non-histone proteins and that the label was, in fact, incorporated into histones, the following experiments were performed:

(1) The histone fraction of [^{3}H]leucine-labeled, unstimulated lymphocytes was electrophoresed in high-resolution (25 cm)

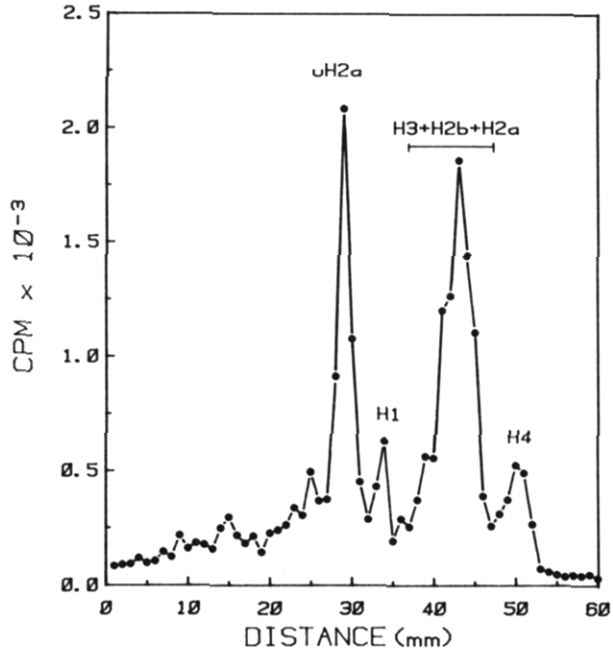

FIGURE 1: Radioactive distribution of the histone fraction of [^{3}H]-leucine-labeled, unstimulated lymphocytes (10-cm acetic acid–urea gel). The position of the histone bands was determined from the Coomassie blue stained gel before slicing and measurement of the radioactivity. uH2A, ubiquinated H2A.

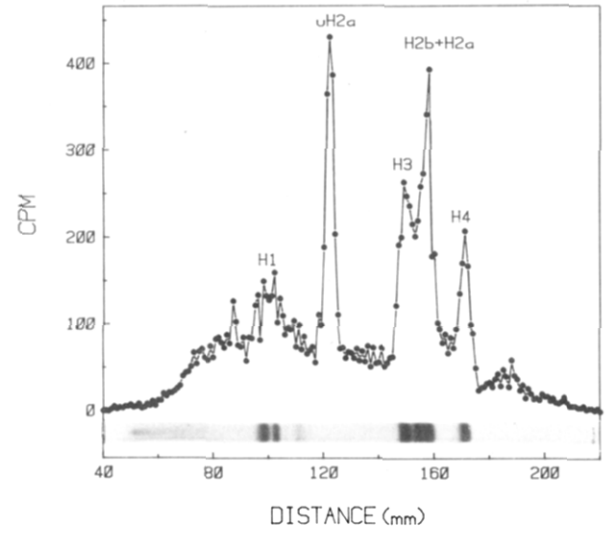

FIGURE 2: High-resolution (25-cm) $NaDodSO_4$–polyacrylamide gel electrophoresis of the histone fraction of [^{3}H]leucine-labeled, unstimulated human lymphocytes (other details as in Figure 1). A photograph of the corresponding portion of the stained gel is shown beneath the radioactive profile.

acetic acid–urea and $NaDodSO_4$–polyacrylamide gels. The $NaDodSO_4$ gel is shown in Figure 2. In both gel systems, the radioactive peaks coincided with the histone bands. When the radioactivity in the histone bands is divided by their respective number of leucine residues per molecule, a molar ratio of synthesis of approximately one is obtained (Table I). In five experiments with leucine- or lysine-labeled G_0 and G_1 cultures the H1:core histone ratio was 1.1 ± 0.1.

(2) Histones isolated from 0.35 M NaCl washed chromatin of [^{3}H] lysine-labeled, unstimulated lymphocytes were separated on a two-dimensional (acid–urea, $NaDodSO_4$) gel (Figure 3), and all stained spots (as well as random unstained areas of the gel) were cut out and counted. The five histones and uH2A accounted for 97% of the recovered label.

Table I: Histone Synthesis by Unstimulated (G_0) Lymphocytes[a]

gel	relative molar synthesis H1	H3,2A,2B	H4	*molar ratio,* H1:H3,2A,2B:H4
$NaDodSO_4$	154	120	136	1.1:0.9:1.0
acid–urea	137	170	165	0.8:1.0:1.0

[a] The histone fraction of [^{3}H]leucine-labeled, unstimulated lymphocytes was electrophoresed on 25-cm $NaDodSO_4$ and acetic acid–urea gels and the radioactive distribution determined as described under Materials and Methods. The relative molar synthesis was obtained by dividing the total dpm in the gel slices comprising the histone bands by their respective molar leucine contents. The values (H1, 9; H3 + 2A + 2B, 31; H4, 8) are those of calf thymus histones. For the calculation, the total dpm values for H3, 2A, and 2B were divided by their total number of leucyl residues per molecule.

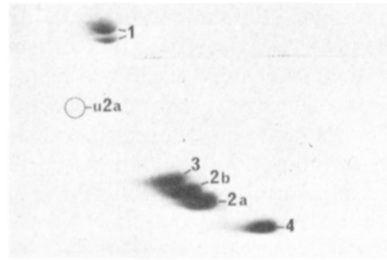

FIGURE 3: Two-dimensional gel electrophoresis of unstimulated lymphocyte histones. Histones were isolated from 0.35 M NaCl washed chromatin of unstimulated, [^{3}H]lysine-labeled pig lymphocytes. A portion of the fraction equivalent to 10^7 cells (6000 dpm) was applied to the gel. First dimension, acetic acid–urea (0.3 × 16 cm). Second dimension, $NaDodSO_4$ disc electrophoresis (14% acrylamide). Migration is from left to right and top to bottom of figure. The position of uH2A (not visible on the photograph) is indicated. The histone spots of the Coomassie blue stained gel, as well as uH2A, several faint spots (not visible on the photograph), and random, unstained areas of the gel were cut out, and their radioactivity was measured by liquid scintillation. No counts above background were detected in the unstained areas. Of the recovered label (2000 dpm), 87% was in the five histones, 10% in uH2A, and 3% in the faintly staining spots.

(3) When H1 was extracted with 5% perchloric acid, its corresponding peak of radioactivity was selectively extracted, while the label in the other histone bands was unaffected. When the remaining core histones were separated by Biol-Gel P-100 chromatography and the fractions subjected to $NaDodSO_4$ electrophoresis, the label remained with the histones (results not shown).

Histone Synthesis by PHA-Stimulated Lymphocytes. When lymphocytes are activated by PHA, there is a continual increase in the rate of protein synthesis. Studies of double-labeled proteins (isolated from [^{3}H]leucine-labeled, PHA-stimulated cultures combined with [^{14}C]leucine-labeled unstimulated lymphocytes prior to fractionation and gel electrophoresis) show that by 10 h, PHA has induced a 2-fold increase in the rate of synthesis of proteins of all subcellular fractions. Gel electrophoresis of the histone fraction from these double-label experiments shows that there is no accompanying increase in histone synthesis (W. I. Waithe et al., unpublished results). Table II shows the results of an experiment in which the PHA-induced increase of label incorporation into whole cell and that of nuclear proteins of G_1 and S phase cultures were compared. PHA had no effect on the rate of histone synthesis in the G_1 culture, whereas by 39 h, the rate of histone synthesis was over 20 times that of unstimulated and G_1 lymphocyte cultures. In parallel experiments under the same conditions, 50% of the cells were in S at 39 h after PHA. Thus, on the basis of the data in this table, the rate of histone synthesis in G_1 (and G_0) is about 2–3% of that in S.

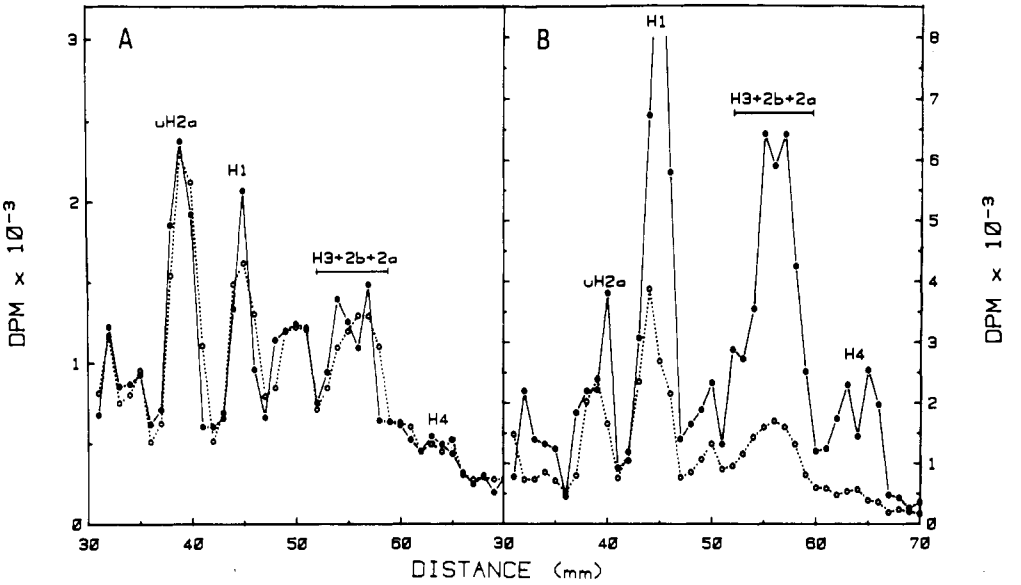

FIGURE 4: Effect of hydroxyurea on histone synthesis in G_1 and S phase cultures. The histone fractions from the 18- and 66-h PHA-activated, [^{3}H]lysine-labeled cultures shown in Table III were electrophoresed on 10-cm acetic acid–urea gels. (●) Without hydroxyurea; (○) with hydroxyurea (see footnotes to Table III for details). (A) G_1 culture (18 h with PHA); (B) S phase culture (66 h with PHA).

Table II: Effects of PHA on Leucine Incorporation into Proteins of G_1 and S Phase Cultures[a]

fraction	radioactivity (dpm × 10^{-3})			relative incorporation	
	G_0	G_1	S	G_1/G_0	S/G_0
whole cells	3927 ± 109	8947	25430	2.3	6.5
NHCP	652 ± 18	1352	4735	2.1	7.3
acid soluble	290 ± 14	555	3382	1.9	11.7
uH2A	17.0 ± 0.4	38.3	117	2.2	6.9
histones	31.8 ± 0.4	32.5	722	1.0	22.7

[a] Unstimulated pig lymphocyte cultures were labeled at 14 h with [^{14}C]leucine and PHA-stimulated cultures at 14 (G_1) and 39 h (S phase culture) with [^{3}H]leucine. The values for unstimulated (G_0) lymphocytes (mean ± SEM of three cultures) were converted to equivalent ^{3}H dpm by multiplying the ^{14}C dpm by the ^{3}H:^{14}C ratio (=22.0) of double-labeled protein from a control consisting of [^{3}H]- and [^{14}C]leucine-labeled, PHA-stimulated (14 h) cultures which were combined after labeling. The values for uH2A and histones were determined from 10-cm acetic acid–urea gels of the acid-soluble fraction.

Effects of Hydroxyurea on DNA and Histone Synthesis of G_0, G_1, and S Phase Lymphocyte Cultures. For determination of whether the basal level of histone synthesis observed in G_0 and G_1 cultures could be due to the presence of DNA-replicating cells, [^{3}H]thymidine incorporation into DNA and the proportion of cells in S were determined in freshly isolated lymphocytes, 18-h unstimulated cultures, and G_1 and S phase PHA-activated cultures, in the presence and absence of hydroxyurea. In some cases, portions of the same cultures were simultaneously labeled with [^{3}H]leucine or [^{3}H]lysine for measurement of histone synthesis (Table III). There was a low rate of [^{3}H]Tdr incorporation (less than 0.5% of maximum) and very few labeled cells (1–3/5000) in 18-h G_0 and G_1 cultures. The freshly isolated lymphocyte suspension had a slightly higher thymidine incorporation and number of labeled cells. Hydroxyurea effectively inhibited both the low level of DNA replication in G_0 and G_1 cultures and that of the S phase cultures as well as 64% of [^{3}H]lysine incorporation into S phase culture histones but had no effect on histone synthesis in G_0 and G_1 cultures (Table III and Figure 4).

Discussion

Our results show that there is significant synthesis, and incorporation into chromatin, of all five histones in G_0 and G_1 lymphocytes. The rate of incorporation of labeled amino acid into chromatin histones in G_0 and G_1 is about 2–3% of that of S phase lymphocytes. We have estimated the rate of histone synthesis from the rate of incorporation of labeled leucine and lysine into histones, and it is possible that the changes observed during growth activation are partly due to changes in amino acid transport and the size of the intracellular pool. However, extensive studies on protein synthesis in activated pig and human lymphocytes render this unlikely. In contrast to the Na^+-dependent A system for amino acid transport, the Na^+-independent L and Ly^+ systems, responsible for the transport of leucine and lysine, show little or no increase during activation (Segel & Lichtman, 1981; Borghetti et al., 1979), and the intracellular leucine pool does not change significantly (Kay et al., 1971; Wettenhall & London, 1974). Although some depletion of the external (medium) amino acid pool does occur after 48 h of activation, this was circumvented by suspension of the cells in fresh medium for 30 min before labeling for 2 h. Under these conditions, the intracellular pool of leucine and lysine equilibrate rapidly with the external pool (our unpublished results; Kay et al., 1971). Finally, the rate of amino acid incorporation into histones of G_1 and S phase cultures changes differently from that of other cytoplasmic and nuclear proteins, and one would thus have to assume a separate amino acid pool for these proteins to ascribe the effect to transport or pool changes.

In these experiments, the labeled histone was measured in chromatin extracts rather than whole cell extracts. The data thus refer to the incorporation of newly synthesized histones into chromatin. It is possible that the changes observed are due to changes in partitioning between cell compartments (Groppi & Coffino, 1980). Examination of the other nuclear fractions of G_0, G_1, and S phase cultures revealed that, although there were minor amounts of histones in the nucleoplasmic fraction, their radioactivity was insufficient to account for the increased incorporation into chromatin of S phase cultures (unpublished observations).

Our finding that there are very few DNA replicating cells in unstimulated lymphocyte cultures is supported by many

Table III: DNA and Histone Synthesis in G_0, G_1, and S Phase Cultures with and without Hydroxyurea[a]

expt	PHA	incubation time (h)	[^{3}H]Tdr incorporation[b] (dpm × 10^{-3})		% labeled cells[c]		^{3}H-labeled histones (dpm/μg of DNA)[e]	
			C	HU	C	HU	C	HU
1	−	0	1.5	0.1	0.2	0.02	190	195
2	−	18	0.1		0.05			
2	+	18	0.4	0.1	0.06	0	197	195
3	+	50	71.6	2.6	17	12[d]		
3	+	54	99.6		21			
2	+	66	20.0	1.2	5	4[d]	752	268

[a] Human lymphocyte cultures were incubated without PHA (G_0), with PHA for 18 h (G_1), or with PHA for 50–66 h (S phase cultures). The 0-h cultures are freshly isolated lymphocytes, labeled 30 min after isolation. At the end of the indicated incubation time, replicate samples were removed and labeled with [^{3}H]thymidine (Tdr) and the rest of the culture labeled with [^{3}H]leucine or -lysine for 60 min. HU, hydroxyurea; C, controls without HU. [b] Means of triplicate samples (the SEMs were in all cases less than 10% of the means). [c] Means of duplicate samples. Five thousand cells were counted for the 0–18-h cultures and 1000 for the 50–66-h cultures. [d] Lightly labeled (see Materials and Methods). [e] Incorporation of [^{3}H]leucine (experiment 1) or [^{3}H]lysine (experiment 2) into the histone bands (excluding uH2A) of 10-cm acetic acid–urea gels of the acid-soluble fraction was measured as described under Materials and Methods. The results are expressed as dpm/μg of chromatin DNA. The DNA content ranged from 210 to 270 μg/4 × 10^7 cells and did not differ significantly in control and HU-treated cultures.

other studies (Akifjev & Aingorn, 1972; Dau, 1975; Darzynkiewicz et al., 1976). It is unlikely that the histone synthesis that we detect in G_0 and G_1 cultures is due to the few DNA synthesizing cells present. From the data in Tables II and III, we can calculate that these cells would have to synthesize histones at a rate at least 40 times greater than that of the DNA-replicating cells in the S phase cultures. Furthermore, under conditions in which hydroxyurea abolished over 90% of [^{3}H]thymidine incorporation into DNA and completely eliminated the DNA-replicating cells in the G_1 cultures, histone synthesis was unaffected.

There have been several studies of the synthesis of nonhistone chromatin proteins during growth activation of lymphocytes, but no detailed studies of their histone synthesis have been reported. Hemminki (1975), in a study of T and B lymphocyte chromatin proteins, reported that the PHA-induced increase in protein synthesis of the acid-soluble fraction was due to contaminating non-histone proteins and concluded that H1 was labeled in unstimulated and stimulated lymphotytes. Wu et al. (1981) reported that unstimulated lymphocytes do not synthesize histones but that there was labeling of uH2A. Their failure to detect histone synthesis in G_0 lymphocytes may be due to the low specific activity of the G_0 histones and the rapid turnover of uH2A. They labeled unstimulated lymphocytes for 3 days with [^{14}C]lysine and detected labeled proteins by fluorography. Under these conditions, the label incorporated into uH2A would be much greater than that in the histones, and the latter might not be detected in fluorographs exposed long enough to reveal labeled uH2A. We also find that the label in uH2A turns over rapidly (W. I. Waithe et al., unpublished results) and, as shown in the data presented here, the increased labeling of the histone fraction of PHA-activated lymphocytes in G_1 is to a large extent due to increased labeling of uH2A.

That we find a H1:core histone molar synthesis ratio of 1 in G_0 lymphocytes, rather than the expected ratio of 0.5, agrees with other reports of excess synthesis of H1 histones in G_1-arrested cell lines (Gurley et al., 1972; Tarnowka et al., 1978; Pehrson & Cole, 1982). Pehrson & Cole (1982) have demonstrated differences in the rate of turnover of H1 subfractions and $H1^0$ in G_1-arrested mouse neuroblastoma cells. $H1^0$ is known to increase when cell division is inhibited (Pehrson & Cole, 1980), but the mechanism and physiological significance of these changes are not yet resolved.

Recently, Wu & Bonner (1981) demonstrated that in several cultured mammalian cell lines, G_1 histone synthesis ("basal" synthesis) could be distinguished from S phase synthesis on the basis of the histone variants synthesized and the relative resistance of basal synthesis to inhibition by hydroxyurea. Our finding that histone synthesis in G_0 and G_1 lymphocytes is resistant to hydroxyurea confirms their results and extends the observation to include diploid, unpassaged cells.

In view of the apparent differences in the degree of coupling of histone and DNA synthesis in different eukaryotic cell types, it has been suggested (Nadeau et al., 1978; Marashi et al., 1982) that transcriptional, posttranscriptional, and, perhaps, even posttranslational control (Groppi & Coffino, 1980) of histone gene expression may function to different degrees in different cell types. In this context, peripheral blood lymphocytes, being a population of naturally occurring G_0, diploid cells which have not undergone in vitro passage, represent an interesting system for further investigations on the mode of control of histone gene expression during the cell cycle.

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Histone H5 Can Increase the Internucleosome Spacing in Dinucleosomes to Nativelike Values†

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ABSTRACT: Chicken erythrocyte chromatin was assembled with inner histones at about 60% of the ratio found in vivo and subsequently incubated with histone H5 (or H1 + H5) in a solution containing 0.1 M NaCl and poly(glutamic acid). Micrococcal nuclease digestion produced dinucleosomes of 360–390 base pair (bp) DNA content, similar to those from native chromatin and contrasting with the 270–280 bp species found in material incubated without H5. On sucrose gradients a dinucleosome sedimenting at 16 S containing 360 bp DNA was isolated. Removal of H1 + H5 after reconstitution did not change these results; H5 thus can induce rearrangements of nucleosome cores with respect to their neighbors. The results are interpreted as an H5-induced "sliding apart" of histone octamers, complementary to the "sliding together" found in native chromatin after removal of H1 + H5.

Histone H1 has long been implicated in the condensation of chromatin (Bradbury et al., 1973; Billett & Barry, 1974). More recently, detailed studies of the ionic strength dependence of the condensation of chromatin have shown that H1 is required for chromatin to condense to a high degree and with structural regularity (Renz et al., 1977; Thoma et al., 1979; Strätling, 1979; Ruiz-Carrillo et al., 1980). It has also been shown that when chromatin is stripped of H1 by procedures that do not disturb the nucleosome spacing, many of the properties of native chromatin can be reestablished by adding H1 back (P. P. Nelson et al., 1979; Allan et al., 1980; Thoma & Koller, 1981).

Whereas the role of H1 in chromatin condensation is fairly well established, the relationship between H1 and the nucleosome spacing is not. The native nucleosome spacing has been achieved in vitro only for unfractionated cell homogenates from *Xenopus* eggs (Laskey et al., 1977; Laskey & Earnshaw, 1980) or *Drosophila* embryos (T. Nelson et al., 1979). These extracts contain endogenous histones, many other proteins, and a variety of enzymatic activities. The "active ingredients" for nucleosome spacing have not been identified. Because of the tissue and species specific variability of H1 and the apparent interaction of H1 with linker DNA, it has been suggested that H1 may specify the nucleosome repeat length in chromatin (Morris, 1976a; Noll, 1976; Compton et al., 1976). However, for chromatin reconstituted in vitro from purified histones and DNA, it has been found that nucleosomes pack closely together with repeat lengths much shorter than those generated in vivo (Yaneva et al., 1976; Steinmetz et al., 1978; Thomas & Butler, 1978; Spadafora et al., 1978; Fulmer & Fasman, 1979; Ruiz-Carrillo et al., 1979; Noll et al., 1980). Similarly, when H1 is removed from chromatin, nucleosomes tend to slide together under conditions where the nucleosome spacing is stable in native chromatin (Spadafora et al., 1979; Weischet,

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