De Beer, R., Duine, J. A., Frank, J., Jzn., & Large, P. J. (1980) *Biochim. Biophys. Acta 622*, 370-374.
Dekker, R. H., Duine, J. A., Frank, J., Jzn., Verwiel, P. E. J., & Westerling, J. (1982) *Eur. J. Biochem. 125*, 69-73.
Duine, J. A., & Frank, J. Jzn. (1981) *Trends Biochem. Sci. (Pers. Ed.) 6*, 278-280.
Duine, J. A., Frank, J., & Westerling, J. (1978) *Biochim. Biophys. Acta 524*, 277-287.
Duine, J. A., Frank, J., Jzn., & Verwiel, P. E. J. (1980) *Eur. J. Biochem. 108*, 187-192.
Eady, R. J., & Large, P. J. (1968) *Biochem. J. 106*, 245-255.
Eady, R. J., & Large, P. J. (1971) *Biochem. J. 123*, 757-771.
Edmondson, D. E., & Singer, T. P. (1973) *J. Biol. Chem. 248*, 8144-8149.
Edmondson, D. E., & Singer, T. P. (1976) *FEBS Lett. 64*, 255-265.
Finley, K. T. (1974) in *The Chemistry of Quinone Compounds* (Patai, S., Ed.) Part 2, pp 877-1144, Wiley, London.
Fox, T., Hinkley, D., & Larntz, K. (1980) *Technometrics 22*, 29-33.
Gainor, J. A., & Weinreb, S. M. (1982) *J. Org. Chem. 47*, 2833-2837.
Gornall, A. G., Bardawill, C. J., & David, M. M. (1949) *J. Biol. Chem. 77*, 751-766.
Kenney, W. C. (1980) in *Flavins and Flavoproteins* (Yagi, K., & Yamano, T., Eds.) pp 237-243, Japan Scientific Societies Press, Tokyo.
Matsumoto, T. (1978) *Biochim. Biophys. Acta 522*, 291-302.
Matsumoto, T., & Tobari, J. (1978) *J. Biochem. (Tokyo) 83*, 1591-1597.
Oppenheimer, L., Capizzi, T. P., & Miwa, G. F. (1981) *Biochem. J. 197*, 721-729.
Ralston, M. L., & Jennrich, R. I. (1978) *Technometrics 20*, 7-14.
Salisbury, S. A., Forrest, H. S., Cruse, W. B. T., & Kennard, O. (1979) *Nature (London) 280*, 843-844.
Segel, I. H. (1975) *Enzyme Kinetics*, pp 387-388, Wiley, New York.
Shirai, S., Matsumoto, T., & Tobari, J. (1978) *J. Biochem. (Tokyo) 83*, 1599-1607.
Singer, T. P., & Kearney, E. B. (1950) *Arch. Biochem. Biophys. 29*, 190.
Singer, T. P., & Kenney, W. C. (1974) *Vitam. Horm. (N.Y.) 32*, 1-45.
Steenkamp, D. J., & Mallinson, J. (1976) *Biochim. Biophys. Acta 429*, 705-719.
Steenkamp, D. J., McIntire, W., & Kenney, W. C. (1978) *J. Biol. Chem. 253*, 2818-2824.
Strickland, S., Palmer, G., & Massey, V. (1975) *J. Biol. Chem. 250*, 4048-4052.
Suva, R. H., & Abeles, R. H. (1978) *Biochemistry 17*, 3538-3545.
Weber, K., & Osborn, M. (1969) *J. Biol. Chem. 244*, 4406-4412.

Changes in Histone H3 Composition and Synthesis Pattern during Lymphocyte Activation†

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ABSTRACT: Freshly isolated human lymphocytes were found to synthesize histones at a significant rate even though no DNA was being synthesized. The synthesis pattern of histone variants in resting lymphocytes is similar to that found in other quiescent cells and different from that found in S-phase cells. For this reason, the histone synthesis in resting lymphocytes cannot be attributed to contamination by S-phase cells. Stimulation by the mitogen phytohemagglutinin resulted in a dramatic switch in the histone H3 variant synthesis pattern as well as a readily apparent change in the histone H3 mass pattern. Thus, the chromatin of activated lymphocytes has a different histone H3 variant composition than resting or quiescent lymphocytes. It is suggested that the proportion of H3.3 in the mass pattern of the chromatin of a cell may be related solely to how long that cell has been quiescent. Inducing resting lymphocytes to synthesize DNA by UV irradiation did not qualitatively change the histone variant synthesis pattern. No S-phase H3 variants were induced by the repair process. Furthermore, the quantity of histone synthesized neither increased nor decreased after treatment with UV light.

Recently, this laboratory (Wu & Bonner, 1981; Wu et al., 1982b) has shown that not all histone synthesis occurs during DNA replication (S phase) but that a significant amount occurs during the G_1 and G_2 phases of the cell cycle and during the quiescent or G_0 state. The variant patterns of the histone synthesis in quiescent, S-phase, and G_1/G_2-phase cells all differed from each other. Specifically, H3.3 was the only H3 variant synthesized in quiescent and in G_1/G_2 cells. All four H2A variants were synthesized in quiescent and S-phase cells, but only H2A.X and H2A.Z were synthesized in G_1/G_2 cells. No part of the G_1 or G_1/S transition could be found with a H2A synthesis pattern like that in quiescent cells (Wu et al., 1982b).

These results have implications in two quite different areas. The first is that histone synthesis is not necessarily linked to DNA synthesis. The second is that the histone variant synthesis pattern may usefully serve as an indicator of the physiological state of the cell. Since these observations and conclusions were drawn from studies performed on continuous

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cell lines adapted for in vitro culture, density-inhibited cells, and cells arrested by deprivation of essential nutrients, it is essential to know whether these observations and conclusions can be applied to normal cells that are *physiologically* in a nonproliferating state. Lymphocytes provide an ideal opportunity to investigate this question.

Resting lymphocytes, like the great bulk of mammalian cells, are thought to be in a G_0 nonproliferating state, rarely if ever replicating their DNA or undergoing mitosis (Cooper, 1973). With the appropriate stimuli, however, they can be induced to initiate either of two types of DNA synthesis. Upon activation with mitogens such as phytohemagglutinin (PHA) (Hartog et al., 1967) or T-cell growth factor (Morgan et al., 1976), they initiate DNA replication (Cooper, 1973). On the other hand, if subjected to DNA damaging agents such as UV light, carcinogens, or drugs, they initiate repair DNA synthesis (Evans & Norman, 1968; Clarkson & Evans, 1972).

In this paper, we report that resting lymphocytes isolated directly from blood were found to synthesize histones at a reduced but significant rate. Because of its characteristic pattern, this synthesis cannot be attributed to the possible presence of S-phase cells in the culture. The histone variant synthesis pattern of resting lymphocytes is similar to that found in confluent or quiescent Chinese hamster ovary (CHO) cells and is also insensitive to hydroxyurea treatment.

Stimulation by PHA resulted in a dramatic switch in the histone variant synthesis pattern, as well as a readily apparent change in the histone mass pattern. Thus, chromatin of activated lymphocytes has a different histone variant composition than resting or quiescent lymphocytes. Inducing DNA repair synthesis in resting lymphocytes by UV irradiation did not qualitatively or quantitatively alter the histone variant synthesis pattern.

Materials and Methods

Purification and Culture of Lymphocytes. The buffy coat fraction of human whole blood from normal donors was obtained from the platelet phoresis center of NIH and subjected to Ficoll–Hypaque gradient centrifugation (Bøyum, 1968; Wu et al., 1981). The band containing lymphocytes was isolated and washed 3 times with serum-free RPMI 1640. An aliquot of the unfractionated lymphocytes was removed and cultured in RPMI 1640 supplemented with 10% fetal calf serum. To obtain T lymphocytes, the remainder of the sample was incubated for 2 h in the presence of sheep red blood cells, followed by centrifugation through a Ficoll–Hypaque gradient (Jondal et al., 1972). The T-cell rosettes were collected from the bottom of the gradient, washed 3 times in serum-free RPMI 1640, and resuspended in 0.83% NH_4Cl to lyse the sheep red blood cells. The T cells were centrifuged, washed 3 times with serum-free RPMI 1640, and cultured in RPMI 1640 supplemented with 10% fetal calf serum. Unfractionated lymphocytes and T cells were activated with phytohemagglutinin [Hartog et al., 1967; for a review, see Rieke (1969)].

UV Irradiation. Ultraviolet irradiation was delivered to cells by a General Electric 245-nm germicidal lamp calibrated to deliver 1 W/m^2. The tops of the plastic dishes were removed during irradiation.

Histone Extraction. Whole cell pellets were extracted with 0.5 N HCl containing 1% mercaptoethanol and 1 mM phenylmethanesulfonyl fluoride (PMSF). The acid extracts were frozen and lyophilized. The lyophilization of the acid extracts improved the morphology of the H3 spots in polyacrylamide gels.

Polyacrylamide Gel Electrophoresis. First-dimension acetic acid–urea–Triton X-100 (AUT) gels and second-dimension acetic acid–urea–hexadecyltrimethylammonium bromide (AUC) gels were prepared according to the methods described in Bonner et al. (1980). First-dimension gels contained 15% acrylamide–6 M urea. Second-dimension gels contained 17.5% acrylamide–6 M urea in the resolving gel. The second-dimension stacking gel contained 5.5 M urea to minimize hexadecyltrimethylammonium bromide precipitation. The running conditions were those described in Wu et al. (1982b).

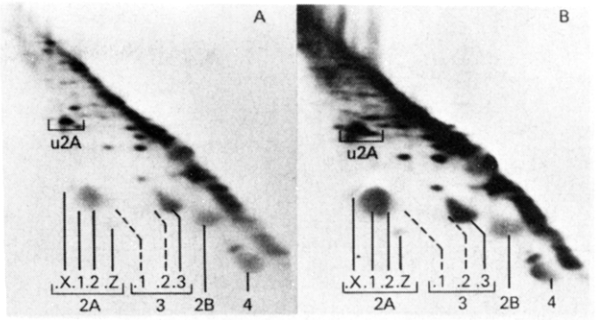

FIGURE 1: Histone synthesis in resting lymphocytes. Unfractionated human lymphocytes of the buffy coat fraction were purified and cultured for 24 h as described under Materials and Methods. A total of 2×10^7 cells were separated into two aliquots; one aliquot was treated with hydroxyurea (1 mM) for 1 h. Then the respective aliquots were labeled in the presence or in the absence of 1 mM hydroxyurea for 2 h with 10 μCi/mL [^{14}C]-R in arginine-free RPMI 1640 medium supplemented with 10% fetal calf serum. The histones were extracted from whole cells and separated on two-dimensional gels. (A) Control; (B) 1 mM hydroxyurea treated. Both fluorograms were exposed for 1 month.

Determination of Radioactivity. Fluorograms were prepared by the methods of Bonner & Laskey (1974) and Laskey & Mills (1975). Gel slices were oxidized overnight at 37 °C in 1 mL of H_2O_2–NH_4OH (95 parts/5 parts) in a tightly capped scintillation vial. Fifteen milliliters of Aquassure scintillation liquid (NEN) was added to each vial for counting. A modification of the method of Schmidt & Thannhauser (1945) as described by Wu & Wilt (1974) was used to determine the amount of [^{3}H]-T incorporation into acid-precipitable material after UV irradiation. The specific radioactivity (cpm/OD_{260}) of the DNA was obtained. Autoradiograms of lymphocytes after activation with PHA were obtained as follows. Labeled cells were fixed and washed in a mixture of methanol and acetic acid (3:1). Aliquots of the fixed-cell suspension were dropped on slides and allowed to air-dry. The slides were dipped in Kodak NTB-2 emulsion, exposed for 1 day, developed in Kodak D-19 solution, and fixed in Kodak fixative. Slides were then washed for 1 h in H_2O and stained with Giemsa.

Radioactive Compounds. [^{14}C]Arginine (R) (300 mCi/mmol) was obtained from ICN, Irving, CA. [*methyl*-^{3}H]-Thymidine ([^{3}H]-T) (84 Ci/mmol) was purchased from NEN, Boston, MA.

Results

Histone Synthesis in Quiescent Cells. Recently, we have shown that quiescent CHO cells exhibited a reduced but significant amount of histone synthesis with a distinctive variant pattern (Wu et al., 1982b). Freshly isolated human T lymphocytes are physiologically in a quiescent nongrowing state and incorporate little if any radioactive thymidine into DNA (Cooper, 1973). However, when incubated with [^{14}C]arginine, resting lymphocytes did show significant incorporation into histones (Figure 1A). The pattern of histone synthesis lacked H3.1 and H3.2 but contained all four H2A variants. This pattern is the same as that found in the

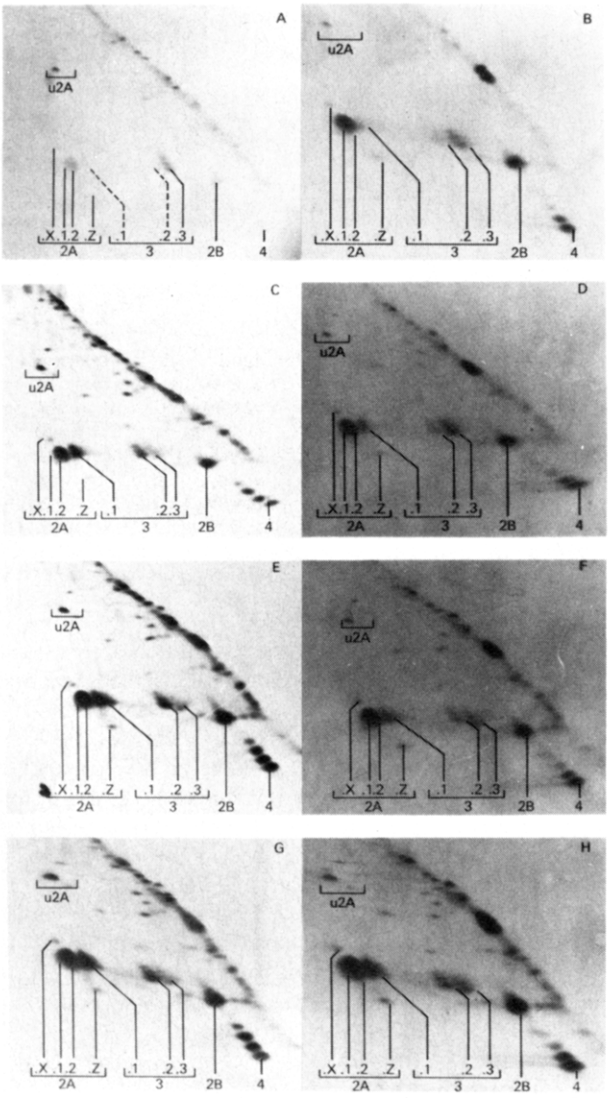

FIGURE 2: Time course of histone variant synthesis in activated human T lymphocytes. Human T lymphocytes were purified as described under Materials and Methods. A total of 5×10^7 cells were separated into two aliquots; 4×10^7 cells were activated with PHA, and the remainder (1×10^7 cells) served as controls. At various times (t = 12, 36, 60, and 84 h) after activation, the T cells were labeled for 2 h with 10 μCi/mL [^{14}C]-R or 1 μCi/mL [^{3}H]-T in arginine-free medium supplemented with 10% fetal calf serum. The histones were extracted from whole cells and separated on two-dimensional gels. Gels shown in panels A, C, E, and G are fluorograms of the respective mass patterns shown in panels B, D, F, and H. (A and B) Control, t = 0; (C and D) t = 36 h; (E and F) t = 60 h; (G and H) t = 84 h. The fluorogram of the gel shown in panel A was exposed for 1 month; all other fluorograms were exposed for 2 weeks. The fluorogram and mass pattern of the gel for t = 12 h are similar to those for the control sample and thus not shown. The gels for unactivated controls at t = 12, 36, 60, and 84 h were identical with the t = 0 sample shown in panels A and B. See Table I for quantitation from these gels.

quiescent CHO cells (Wu et al., 1982b). Since DNA replication is supposedly absent in quiescent cells, these findings suggested that histone synthesis in resting lymphocytes is not coupled to it. In CHO cells, quiescent histone synthesis was shown to be unaffected by hydroxyurea, a DNA synthesis inhibitor. Figure 1B shows that the histone synthesis in resting lymphocytes was also not inhibited by hydroxyurea treatment, and even seemed to be somewhat stimulated by it.

Figure 1 also shows that the ubiquitin adducts of the H2A's in quiescent lymphocytes are much more heavily labeled than the H2A's themselves; this is due to the rapid turnover of

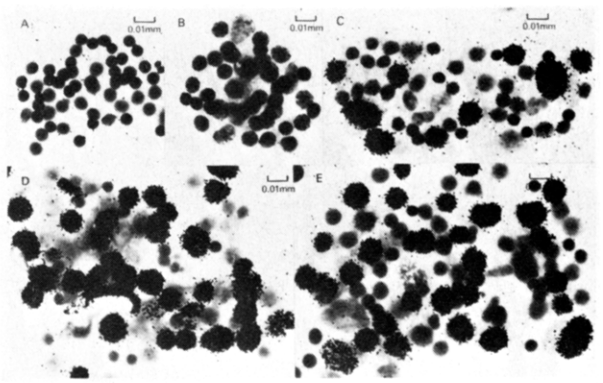

FIGURE 3: DNA synthesis in resting and activated human T lymphocytes. Human T lymphocytes were purified, cultured, and labeled as described in the legend for Figure 2. Autoradiograms were prepared as described under Materials and Methods. (A) Control, t = 0 h; (B) t = 12, (C) t = 36, (D) t = 60, and (E) t = 84 h after activation.

Table I: T Lymphocytes[a]

time (h)	relative DNA synthesis	relative histone synthesis	(H3.1 + H3.2)/ H3.3	(H2A.1 + H2A.2)/ (H2A.X + H2A.Z)
0	0.3	1.8	0.3	7.8
12	0.7	3.9	0.3	9.4
36	99	36	2.0	11.6
60	100	68	5.3	10.4
84	94	100	4.7	11.0

[a] Data were obtained from the appropriate gels as described under Materials and Methods. DNA synthesis was calculated as cpm per culture.

ubiquitin as shown by Wu et al. (1981). In that earlier study, however, the quiescent histone synthesis was not apparent due to the lower specific activity of the labeling medium.

Although the synthesis patterns of quiescent lymphocytes and CHO cells are similar, there is a notable difference between the mass patterns of these cells. In quiescent CHO cells, H3.1 and H3.2 dominate the H3 *mass* pattern, although H3.3 is the only H3 variant being synthesized. However, in quiescent lymphocytes, the H3 mass pattern very closely resembles the H3 synthesis pattern; H3.3 dominates both (Figure 2A,B).

Histone Synthesis and DNA Replication. Very little is known about the underlying mechanisms which result in particular variant patterns. The presence of H3.3 as the predominant H3 variant in quiescent lymphocytes suggests that H3.3 might also be the predominant variant in activated lymphocytes. An alternative possibility is that H3.1 and H3.2 synthesis will resume during S phase in lymphocytes, as occurs in tissue culture cells. If this should occur, then there should be a noticeable shift in the H3 variant mass composition between the quiescent and the activated cells.

T lymphocytes were activated by PHA (Hartog et al., 1967; Stjernholm & Falor, 1970). Histone synthesis still showed the quiescent pattern at 12 h (data not presented) before DNA synthesis began (Figure 3B). However, when DNA synthesis commenced (Figure 3C), the synthesis of H3 variants .1 and .2 also commenced (Figure 2C). As lymphocyte proliferation continued (Figure 3D,E), H3.1 and H3.2 synthesis increased relative to H3.2 synthesis (Figure 2E,G). Table I shows that the H3 variant synthesis ratio changed dramatically as DNA synthesis commenced and histone synthesis increased; the (H3.1 + H3.2)/H3.3 ratio increased from 0.3 to 5.3. At the same time, the H2A variant ratio remained relatively constant

Table II: Buffy Coat Fraction[a]

time (h)	relative DNA synthesis	relative histone synthesis	(H3.1 + H3.2)/ H3.3	(H2A.1 + H2A.2)/ (H2A.X + H2A.Z)
0	0.3	2.4	0.16	16.3
12	0.2	nd[b]	nd	nd
36	65	40	1.7	20.8
60	100	98	3.7	23.0
84	73	100	2.2	16.7

[a] Data were obtained as described in the legend to Table I.
[b] nd, not determined.

(Table I). The initiation of H3.1 and H3.2 synthesis affected the variant content in that there was clearly an increase in the mass of H3.1 and H3.2 relative to H3.3 in the chromatin of activated lymphocytes (Figure 2B, D, F, H). It is notable that the histone variant mass pattern of the human T lymphocytes at 84 h after activation is almost identical with the mass pattern of human cell lines maintained in exponential culture. These include a normal human fibroblast line (IMR-90) as well as HeLa cells (data not presented). Thus, T lymphocytes are similar to other types of mammalian cells tested in that H3.1 and/or H3.2 synthesis predominates during S phase.

The data in Figure 2 and Table I were obtained with a purified T lymphocyte preparation. Table II shows that a similar experiment with whole buffy coat fraction yielded data with the same overall pattern. Gels of whole buffy coat fraction also showed the same changes in mass of the H3 variants after activation.

Histone Synthesis and DNA Repair Synthesis. DNA synthesis occurs not only during replication but also when the chromatin is damaged by chemical (Roberts et al., 1968; Clarkson & Evans, 1972) or physical agents (Rasmussen & Painter, 1964; Evans & Norman, 1968). In contrast to DNA replicative synthesis, DNA repair synthesis does not lead to a net increase in the amount of DNA and can occur in the presence of inhibitors of DNA replication such as hydroxyurea (Cleaver, 1969).

The fate of existing histones during DNA repair and the effect of DNA repair synthesis on histone variant synthesis are not known. When DNA repair synthesis was induced in resting lymphocytes with UV light, it rapidly increased with increasing UV dose to a maximum plateau at about 4–5 J/m^2 (Figure 4). Incorporation of radioactive arginine into the acid-insoluble fraction of cells remained constant up to 4–5 J/m^2 and then decreased with higher UV flux. Incorporation into the acid-soluble fraction, which contains the histones, also remained constant until 4–5 J/m^2 and then slightly decreased. When the respective acid-soluble fractions were analyzed on acid–urea–Triton X-100 gels to resolve all the histone variants, no major differences in the quantity of synthesis or the qualitative pattern of synthesis were observed between UV-irradiated cells and the nonirradiated controls (Figure 5). Thus, no specific histone variants were induced by DNA repair synthesis as were by DNA replicative synthesis. Furthermore, the overall level of histone synthesis neither increased nor decreased after treatment with UV light between 0.3 and 35 J/m^2.

Discussion

This study showed that quiescent T lymphocytes, like quiescent CHO cells, maintain a reduced but significant level of histone synthesis, and this histone synthesis differs qualitatively from both S-phase histone synthesis and basal histone

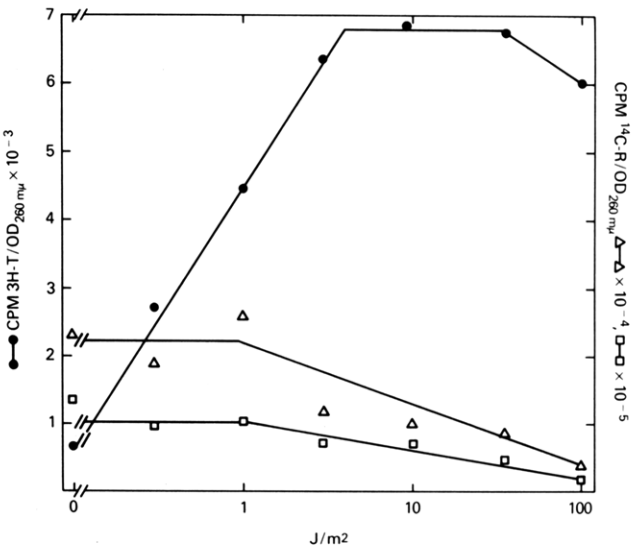

FIGURE 4: Effect of various doses of UV irradiation on DNA and protein synthesis. Unfractionated human lymphocytes of the buffy coat fraction were purified and cultured for 24 h as described under Materials and Methods. All cultures were treated with 1 mM hydroxyurea for 1 h before UV irradiation. A total of 2 × 10^7 cells pretreated with 1 mM hydroxyurea served as unirradiated controls. These control cells were divided into two equal aliquots by centrifugation and resuspension in arginine-free RPMI 1640 medium supplemented with 10% fetal calf serum and 1 mM hydroxyurea. The remainder of the culture (6 × 10^7 cells) was also centrifuged, resuspended in arginine-free RPMI 1640 medium containing 10% fetal calf serum and 1 mM hydroxyurea, and divided into 12 dishes. The plated cultures were then UV irradiated (0.3, 1, 3, 10, 35, and 100 J/m^2). The respective UV-irradiated and control cultures were labeled for 2 h with [^{3}H]-T (10 μCi/mL) or [^{14}C]-R (10 μCi/mL) by direct addition of the respective radioactive compounds into the cultures. Specific activity of [^{3}H]-T (●) in DNA was measured by the Schmidt–Thannhauser procedure as described under Materials and Methods. Incorporation of [^{14}C]-R into proteins was determined by trichloroacetic acid precipitation of the respective HCl-soluble and HCl-insoluble fractions of whole cells. The cpm in trichloroacetic acid precipitable material was normalized by the amount of DNA in the cultures [(Δ) HCl-insoluble fraction; (□) HCl-soluble fraction].

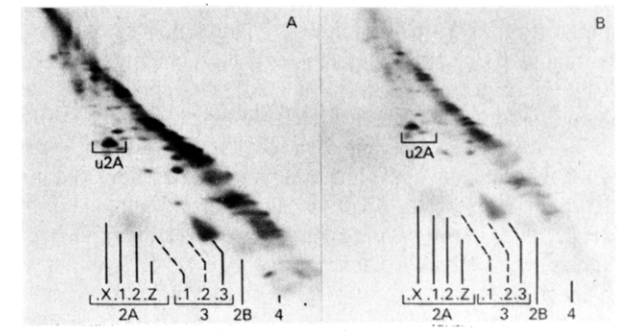

FIGURE 5: Effect of various doses of UV irradiation on histone variant synthesis. The HCl-soluble fraction of whole cells (Figure 4) was analyzed on two-dimensional gels. (A) 1 J/m^2; (B) 35 J/m^2. The unirradiated control of this experiment is shown in Figure 1B. Fluorograms were exposed for 1 month.

synthesis found in the G_2/G_1 phases of cycling cells. These results with lymphocytes extend previous findings in three ways. First, since T lymphocytes are a normal bodily constituent, quiescent histone synthesis is not an aberration of cells cultured in vitro (Wu et al., 1982b) but a characteristic of normal quiescent cells. Second, since T lymphocytes are in suspension and not attached to a surface, quiescent histone synthesis is not the result solely of contact inhibition (Todaro et al., 1965) but more generally of a quiescent nondividing state. Third, since T lymphocytes are in fresh complete me-

dium, quiescent histone synthesis need not be only the result of depletion of nutrients from the growth medium.

In quiescent T lymphocytes, H3.3 is the only H3 variant synthesized and is also the major variant by mass. When T lymphocytes are induced to proliferate, the histone synthesis pattern quickly changes to the S-phase pattern also found in other cultured cells. H3.1 or H3.2 synthesis dominates the pattern. Gradually the H3 mass pattern found in resting lymphocytes also changes to resemble that found in cultured cells. Thus, the chromatins of resting and activated lymphocytes differ from each other in H3 variant content.

The primary sequences of the H3 variants differ from each other in only a couple of positions. H3.1 differs from H3.2 and H3.3 at position 96, a change of Cys in H3.1 to Ser in H3.2 and H3.3 (Marzluff et al., 1972). H3.3 differs from H3.1 and H3.2 at positions 89–90, Ile-Gly in H3.3 instead of the Val-Met found in H3.1 and H3.2 (Franklin & Zweidler, 1977). In fact, the H3 primary sequences are so well conserved that only five amino acid differences occur between H3's of pea and man (Isenberg, 1979).

A relevant question to address here is whether these differences among the nonallelic variants could lead to functional consequences. Some evidence suggests that in many cases the existence of histone variants may not lead to functional diversity, because certain variants seem to be expendable. For example, the relative contents of H3 variants .1 and .2 differ greatly in different mammals (West & Bonner, 1980a,b; Wu et al., 1981). In yeast (Rykowski et al., 1981; Kolodrubetz et al., 1982), mutants lacking one of the H2B variants are viable. In mouse (Franklin & Zweidler, 1977), there are two H2B variants, one of which is not found in any substantial quantity in other mammals. On the other hand, in some cases there is suggestive evidence for functional consequences of changes in variant composition (Simpson, 1981; Blankstein & Levy, 1976; Wu et al., 1982a).

The predominance of the .3 variant in the H3 mass pattern of quiescent lymphocytes could be the result of any of several different mechanisms. One possibility is that H3.3 is the particular H3 variant synthesized as lymphoblasts multiplied and differentiated into lymphocytes. The alternative is that as lymphoblasts multiplied H3.1 and H3.2 dominated the mass and synthesis pattern, and the H3 shift took place after cell division ceased, during either differentiation or quiescence. The shift would be due to histone turnover and would be apparent only for those histones with different variant synthesis patterns in different cell states. Grove & Zweidler (1982) have some evidence for shifts in variant ratios during terminal differentiation of murine erythroleukemia cells in vitro. Indirect evidence for shifts in histone variant ratios during quiescence can be found by analyzing quiescent tissues of whole animals. In mouse liver (Bonner et al., 1980), brain, and kidney (Zweidler, 1976, 1980), H3.3 dominates the mass pattern, while in proliferating cells in culture, H3.3 is usually a minor component. Therefore, it may be that the fraction of H3.3 in the H3 mass pattern of a resting lymphocyte or of any other quiescent cell can be attributed solely to the length of its quiescence. In the case of the extranucleosomal histone H1, there is direct evidence that certain H1 variants turn over rather rapidly as cells become quiescent (Pehrson & Cole, 1982; Lennox et al., 1982; D'Anna et al., 1982).

Histone turnover might function to maintain chromatin integrity in quiescent cells but might also be involved with transcription or DNA repair. Simpson et al. (1980) have proposed a model for transcription of nucleosome-associated genes in which nucleosomes dissociate from the DNA as the RNA polymerase reads through and then reassociate with the DNA. Quiescent and basal histone synthesis opens the possibility that the dissociated histones are simply degraded with newly synthesized histones replacing them in the nucleosome. During such a process, it is possible that the chromatin could be put into various active or inactive configurations.

During UV-induced DNA repair, histone synthesis is not increased. Our results agree with those DNA repair studies of Stein et al. (1976) on this point. In addition, our results indicate that the syntheses of no specific histone variants are induced or accumulated in the core particles by the DNA repair process.

Wu et al. (1982b) suggested that patterns of histone variant synthesis may be useful in distinguishing different cellular growth states irrespective of any functional consequences of changes in variant composition. They reported that during G_0 in CHO cells the (H3.1 + H3.2)/H3.3 synthesis ratio is very low and the (H2A.1 + H2A.2)/(H2A.X + H2A.Z) synthesis ratio is high whereas during G_1 both the H3 and H2A variant synthesis ratios are very low. The results presented here show that resting lymphocytes exhibit a G_0 pattern of histone variant synthesis and thus do not seem to be arrested in some portion of G_1.

References

Blankstein, L. A., & Levy, S. B. (1976) *Nature (London) 260*, 638–640.

Bonner, W. M., & Laskey, R. A. (1974) *Eur. J. Biochem. 46*, 83–88.

Bonner, W. M., West, M. H. P., & Stedman, J. D. (1980) *Eur. J. Biochem. 109*, 17–23.

Bøyum, A. (1968) *Scand. J. Clin. Lab. Invest. 21 (Suppl. 97)*, 1–108.

Clarkson, J. M., & Evans, H. J. (1972) *Mutat. Res. 14*, 413–430.

Cleaver, J. E. (1969) *Radiat. Res. 37*, 334–348.

Cooper, H. L. (1973) in *Drugs and the Cell Cycle* (Zimmerman, A. M., Padilla, G. M., & Cameron, I. L., Eds.) pp 137–194, Academic Press, New York.

D'Anna, J. A., Gurley, L. R., & Tobey, R. A. (1982) *Biochemistry 21*, 3991–4001.

Evans, R. G., & Norman, A. (1968) *Radiat. Res. 36*, 287–298.

Franklin, S. G., & Zweidler, A. (1977) *Nature (London) 266*, 273–275.

Grove, G. W., & Zweidler, A. (1982) *J. Cell Biol. 95*, 81a.

Hartog, M., Cline, M. J., & Grodsky, G. M. (1967) *Clin. Exp. Immunol. 2*, 217–228.

Isenberg, I. (1979) *Annu. Rev. Biochem. 48*, 159–191.

Jondal, M., Holm, G., & Wigzell, H. (1972) *J. Exp. Med. 136*, 207–215.

Kolodrubetz, P., Rykowski, M. C., & Grunstein, M. (1982) *Proc. Natl. Acad. Sci. U.S.A. 79*, 7814–7818.

Laskey, R. A., & Mills, A. D. (1975) *Eur. J. Biochem. 56*, 335–341.

Lennox, R. W., Oshima, R. G., & Cohen, L. H. (1982) *J. Biol. Chem. 257*, 5183–5189.

Marzluff, W. F., Jr., Sanders, L. A., Miller, D. M., & McCarty, K. S. (1972) *J. Biol. Chem. 247*, 2026–2033.

Morgan, D. A., Ruscetti, F. W., & Gallo, R. (1976) *Science (Washington, D.C.) 193*, 1007–1008.

Pehrson, J. R., & Cole, R. D. (1982) *Biochemistry 21*, 456–460.

Rasmussen, R. E., & Painter, R. B. (1964) *Nature (London) 203*, 1360–1362.

Rieke, W. O., Ed. (1969) *Third Annual Leukocyte Culture Conference*, Appleton-Century-Crofts, New York.

Roberts, J. J., Crathorn, A. R., & Brent, T. P. (1968) *Nature (London) 218*, 970-972.
Rykowski, M., Wallis, J., Choe, J., & Grunstein, M. (1981) *Cell (Cambridge, Mass.) 25*, 477-487.
Schmidt, G., & Thannhauser, S. J. (1945) *Biochem. J. 167*, 83-89.
Simpson, R. T. (1981) *Proc. Natl. Acad. Sci. U.S.A. 78*, 6803-6807.
Simpson, R. T., Stein, A., Bitter, G. A., & Kunzler, P. (1980) in *Novel ADP-Ribosylation of Regulatory Enzymes and Proteins* (Smulson, M. E., & Sugimura, T., Eds.) pp 133-142, Elsevier/North-Holland, New York.
Stein, G. S., Park, W. D., Stein, J. L., & Lieberman, M. W. (1976) *Proc. Natl. Acad. Sci. U.S.A. 73*, 1466-1470.
Stjernholm, R. L., & Falor, W. H. (1970) *Reticuloendothel. Soc. 7*, 471-483.
Todaro, G. J., Lazar, G. K., & Green, H. (1965) *J. Cell. Comp. Physiol. 66*, 325-334.
West, M. H. P., & Bonner, W. M. (1980a) *Biochemistry 19*, 3238-3245.
West, M. H. P., & Bonner, W. M. (1980b) *Nucleic Acids Res. 8*, 4671-4680.
Wu, R. S., & Wilt, F. H. (1974) *Dev. Biol. 41*, 352-370.
Wu, R. S., & Bonner, W. M. (1981) *Cell (Cambridge, Mass.) 27*, 321-330.
Wu, R. S., Kohn, K. W., & Bonner, W. M. (1981) *J. Biol. Chem. 256*, 5916-5920.
Wu, R. S., Nishioka, D., & Bonner, W. M. (1982a) *J. Cell Biol. 93*, 426-431.
Wu, R. S., Tsai, S., & Bonner, W. M. (1982b) *Cell (Cambridge, Mass.) 31*, 367-374.
Zweidler, A. (1976) *Life Sci. Res. Rep. 4*, 187-196.
Zweidler, A. (1980) in *Gene Families of Collagen and other Proteins* (Prockop, D. J., & Champe, P. C., Eds.) pp 47-56, Elsevier/North-Holland, New York.

Structure of Chromatin at Deoxyribonucleic Acid Replication Forks: Nuclease Hypersensitivity Results from both Prenucleosomal Deoxyribonucleic Acid and an Immature Chromatin Structure†

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ABSTRACT: Relative to nonreplicating DNA in mature simian virus 40 (SV40) chromosomes, newly synthesized DNA in replicating SV40 chromosomes was found to be hypersensitive to the nonspecific endonucleases, micrococcal nuclease (MNase), DNase I, and DNase II. Nascent DNA, pulse labeled in either intact cells or nuclear extracts supplemented with cytosol, was digested about 5-fold faster and about 25% more extensively than uniformly labeled DNA in mature viral chromosomes. Pulse–chase experiments in vitro revealed a time-dependent chromatin maturation process that involved two distinct steps: (i) conversion of prenucleosomal DNA (PN-DNA) into immature nucleosomal oligomers and (ii) maturation of newly assembled chromatin into a structure with increased nuclease resistance. PN-DNA was hypersensitive to MNase, releasing short DNA fragments which were subsequently solubilized by the nuclease. However, when the nascent PN-DNA was specifically removed by digestion of replicating viral chromosomes with *Escherichia coli* exonuclease III (3′–5′) and phage T7 exonuclease (5′–3′), subsequent digestion of the remaining chromatin with MNase revealed the same degree of hypersensitivity observed prior to exonuclease treatment. Furthermore, newly assembled nucleosomal oligomers, isolated after a brief MNase digestion of replicating viral chromosomes, were also hypersensitive to MNase relative to oligomers isolated from mature chromosomes. Hybridization analysis of the DNA in these immature oligomers revealed that it originated from both sides of replication forks. Inhibition of DNA polymerase α by aphidicolin inhibited conversion of PN-DNA into nucleosomes but did not inhibit loss of nucleosomal hypersensitivity to MNase. In contrast, components in the soluble fraction of the subcellular system ("cytosol") were required for both DNA replication and chromatin maturation. Analysis of the nucleoprotein products from a MNase digestion of replicating and mature SV40 chromosomes failed to detect a change in nucleosome structure that corresponded to the loss of nuclease hypersensitivity. However, the results presented demonstrate that *both* PN-DNA and newly assembled immature chromatin, present on both arms of SV40 replication forks, contribute to the commonly observed hypersensitivity of newly replicated chromatin to endonucleases.

Since eukaryotic chromatin structure varies as a function of gene activity (Weisbrod, 1982) and DNA sequence (Wu et al., 1979; Wigmore et al., 1980; Bryan et al., 1981; Cremisi, 1981; Herbomel et al., 1981), it seems likely that chromatin structure greatly influences where and when transcription, replication, and, perhaps, recombination occur. Therefore, to preserve these structural landmarks, chromosome replication presumably requires accurate duplication of protein organization, as well as DNA sequence (Weintraub, 1979). To understand the mechanisms involved in this duplication process requires detailed knowledge of the assembly pathway at DNA replication forks.

Chromatin assembly primarily involves the organization of nucleosomes containing a minimum of 145 base pairs (bp)[1]

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