Changes in Histone H1 Content and Chromatin Structure of Cells Blocked in Early S Phase by 5-Fluorodeoxyuridine and Aphidicolin†

Joseph A. D'Anna* and Robert A. Tobey

ABSTRACT: We have measured changes in histone H1 content and changes in chromatin structure of Chinese hamster (line CHO) cells blocked in early S phase by sequential use of isoleucine deprivation and blockade with 5-fluorodeoxyuridine or aphidicolin. Both the H1:core histone ratio in isolated nuclei and the H1 content of the cell are reduced 20–60%, depending on the duration of the block. The new deoxyribonucleic acid (DNA) synthesized during S-phase block has a shorter nucleosome repeat length than that of bulk chromatin, but it is nearly equally resistant as bulk DNA to attack by micrococcal nuclease. During the time that H1 content is decreasing, bulk chromatin also undergoes structural changes so that its nucleosome cores appear to be more closely packed along the DNA chain. The losses in H1 content and changes in chromatin structure are similar to those reported for cells blocked in early S phase by hydroxyurea [D'Anna, J. A., & Prentice, D. A. (1983) *Biochemistry 22*, 5631–5640]. The results suggest that losses of H1 and changes in chromatin structure are general events which occur when the elongation of initiated replicons or the joining of intermediate-sized DNA fragments is retarded during replication. They are consistent with the notions that (1) H1 is lost from initiated replicons and/or (2) the loss of H1 is part of an alarm response in the cell which might facilitate events leading to gene amplification.

The release of G_1 cells into hydroxyurea (HU)[1] blockade allows cells to initiate replication, but it greatly retards the elongation of initiated replicons (Walters et al., 1976a) and the ligation of small-to replicon-sized DNA fragments into bulk DNA (Walters et al., 1976b; Johnston, 1980). During the time cells are blocked in early S phase, changes occur in the composition of chromosomal proteins and chromatin structure (D'Anna & Prentice, 1983a): (1) there is a 30–70% loss of H1 from chromatin and the cell, depending on the duration of block; (2) there are increases in normally minor proteins in mononucleosome particles; (3) newly made DNA appears to have a shortened repeat length, compared to mature chromatin; (4) the old chromatin begins to undergo structural change so that its measured nucleosome repeat lengths become shorter, determined by micrococcal nuclease digestion (at 37 or 2 °C) and electrophoretic analysis of DNA.

On the basis of results in HU-blocked cells, this laboratory (1) suggested that the loss of H1 and changes in chromatin structure might be replication events which normally go unnoticed (D'Anna et al., 1982; D'Anna & Prentice, 1983a) and (2) postulated a simplified model of chromatin structural changes during replication to accommodate the results in HU-blocked cells (D'Anna & Prentice, 1983a). If those suggestions are valid, then other drugs and cell-cycle progression mutations which block cells in early S phase (and, perhaps, during other parts of S phase) also should produce losses of histone H1 and changes in chromatin structure.

Here, we report changes in H1 content and chromatin structure in cells blocked in early S phase by 5-fluorodeoxyuridine (5-FdU) and by aphidicolin (APC). These drugs were chosen because they act like HU in that they appear to allow cells to initiate replication and then retard elongation (Taylor, 1977; Pedrali-Noy, 1980; Spadari et al., 1982); however, they work by different mechanisms from HU and from one another. Aside from suspected effects (Kaplay et al., 1983; Wawra & Wintersberger, 1983), HU inhibits the enzyme ribonucleotide reductase (Reichard & Ehrenberg, 1983), but 5-FdU interferes with the methylation of deoxyuridylate in the biosynthesis of thymidylate (Taylor et al., 1973), and APC inhibits the DNA polymerase α (Huberman, 1981). Another distinction among the three drugs is that HU and 5-FdU would be expected to perturb deoxyribonucleotide triphosphate pools (Walters et al., 1973; Wawra & Wintersberger, 1983), but APC does not appear to do so (Pedrali-Noy et al., 1980). Our results indicate that the loss of H1 and changes in chromatin structure are not peculiar to HU blockade; rather, they may be general events which occur when DNA elongation is retarded. They are consistent with the notions that H1 is lost from initiated replicons or that the loss of H1 is part of an alarm response of the cell which might facilitate events leading to gene amplification.

Experimental Procedures

Cell Growth and Cell Cycle Analysis. Suspension cultures of Chinese hamster (line CHO) cells were synchronized in G_1 by maintenance in isoleucine-deprived medium (Tobey & Ley, 1971) as previously described (D'Anna & Prentice, 1983a). To block cells in early S phase, cells were released from G_1 block into (1) complete medium containing 1 mM HU, (2) complete medium containing 3 or 5 μg mL^{-1} aphidicolin (from a stock solution of 5 mg mL^{-1} in dimethyl sulfoxide), or (3) incomplete medium (F-10 lacking thymidine; 15% dialyzed newborn calf serum instead of 15% newborn calf serum) containing 1 μg mL^{-1} 5-fluorodeoxyuridine (Calbiochem-Behring Corp.). Aphidicolin (NSC 234714) was a gift from Dr. David Abraham, Investigational Drug Branch, National Cancer Institute, Bethesda, MD.

The percentage of cells entering S phase following release from G_1 block into the presence of drugs was determined by autoradiography using high concentrations of high specific activity thymidine (Walters et al., 1976a; D'Anna et al., 1982): 40 μCi mL^{-1} [^{3}H]thymidine was used with HU and aphidicolin; 5 μCi mL^{-1} was used with 5-FdU in the thymidine-deprived medium.

† From the Toxicology Group, Life Sciences Division, MS M880, Los Alamos National Laboratory, Los Alamos, New Mexico 87545. *Received February 14, 1984.* This work was supported by National Institutes of Health Grant GM-24564, the U.S. Department of Energy, and the Los Alamos Flow Cytometry and Sorting Research Resource funded by NIH Grant P41-RR01315-02.

[1] Abbreviations: APC, aphidicolin; bp, base pairs of DNA; DNA, deoxyribonucleic acid; FCM, flow cytometry; 5-FdU, 5-fluorodeoxyuridine; HU, hydroxyurea; Me_2SO, dimethyl sulfoxide.

0006-2960/84/0423-5024$01.50/0

The distribution of cells in the growth cycle was determined from the distribution of cellular DNA by flow cytometry (FCM) as described (D'Anna et al., 1980).

Other Procedures. To distinguish new DNA from old DNA in drug-blocked S-phase cells, exponentially growing cultures were first prelabeled for 24 h and then synchronized in G_1 in the presence of 5 μCi L^{-1} [^{14}C]thymidine (53 Ci mM^{-1} in H_2O) to radiolabel "old DNA". The cells were then released into medium without radioactive thymidine; after 1.5 h, [^{3}H]thymidine (83 Ci mM^{-1} in H_2O) was added to radiolabel "new DNA". Concentrations of 9.4 mCi L^{-1} [^{3}H]thymidine were used with APC, and concentrations of 50–100 μCi L^{-1} were used with 5-FdU when cells were to be harvested 10 h after release into the drug. Concentrations of 1.8 mCi L^{-1} [^{3}H]-thymidine were used with APC, and concentrations of 50–100 μCi L^{-1} (18-h exposure period) were used with 5-FdU when cells were to be harvested after 24 h in the presence of drug.

With one exception, other procedures, including isolation of nuclei and histones, digestion of nuclei with micrococcal nuclease, electrophoresis, and determination of nucleosome repeat length, were as described (D'Anna & Prentice, 1983a). Whole histones were extracted with 0.2 M H_2SO_4 from homogenates of nuclei isolated with Nonidet P-40 detergent, total histone H1 was extracted from whole cells by blending the cells directly in $HClO_4$, histones were separated by electrophoresis in 0.5 × 25 cm acid–urea–polyacrylamide gels (Panyim & Chalkley, 1969), and DNA fragments were separated by electrophoresis in composite 0.5% agarose–2.5% polyacrylamide gels. The one change was employed for quantification of histone H1 per unit DNA: cells were harvested and transferred to a graduated tube in a total of 10.0 mL of complete medium in place of isotonic saline.

Results

Flow Cytometry and Autoradiography of Cells Blocked in Early S Phase by HU, 5-FdU, and APC. Previously, Kurek & Taylor (1977) showed that the release of mitotically selected cells into thymidine-deprived medium containing 5-FdU allows cells to initiate DNA synthesis, but it allows only very limited progression through S phase. Since we required larger quantities of cells than we would get from mitotic selection, we synchronized cells in G_1 by the isoleucine deprivation method (Tobey & Ley, 1971) and released them into thymidine-deprived medium containing 5-FdU. For the same reason, we released cells from isoleucine deprivation G_1 block into complete medium containing 5 μg/mL aphidicolin and, as previously described (D'Anna et al., 1982), into complete medium containing 1 mM HU to block cells in early S phase.

Figure 1 shows FCM histograms of cellular DNA content from cultures blocked with HU, 5-FdU, or APC for the times used for examination of H1 contents and chromatin structure. Analysis of the histograms indicates that there is an average increase in DNA content of 0–5% in cultures after 10 h. In general, the increase in DNA content during the first 10 h is least in the APC-blocked cells.

After 18–24 h of block, there is increased slippage into S phase in the presence of each of the drugs. Cultures blocked with HU and 5-FdU exhibit average movement of 8–15% into S phase after 24 h (Figure 1), while cells "blocked" with APC for 24 h exhibit as much as 30% movement into S phase (not shown). After only 18 h in APC, the slippage into S phase is comparable to that observed with HU and 5-FdU after 24 h (Figure 1). Even then, there is greater asymmetry of the major peak in the histograms of APC-blocked cells; this suggests that between 10 and 18 h some cells slip into S phase at a faster rate than others.

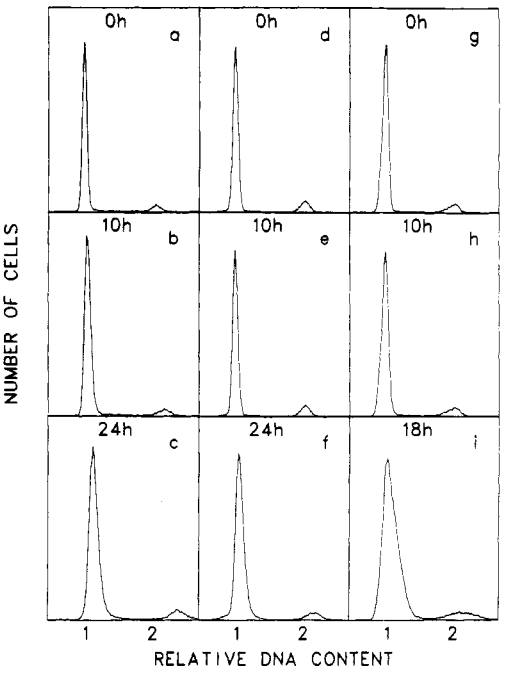

FIGURE 1: FCM DNA histograms of cells before and after release from G_1 block into the presence of 1 mM HU for 0 (a), 10 (b), and 24 h (c), into the presence of 1 μg mL^{-1} 5-FdU for 0 (d), 20 (e), and 24 h (f), and into the presence of 5 μg mL^{-1} APC for 0 (g), 10 (h), and 18 h (i). The small bands in the G_2 regions of the histograms appear to be doublets, because they contain a DNA content that is twice that of the major band in the histogram.

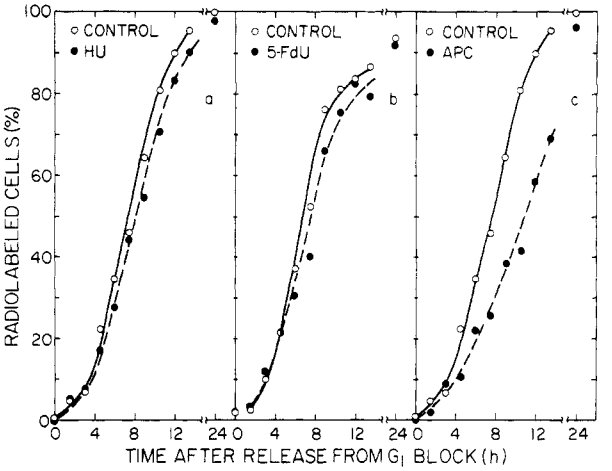

FIGURE 2: Percentage of radiolabeled cells as a function of time following release from isoleucine deprivation G_1 block into medium containing (●) or lacking (○) one of the following drugs: HU (a); 5-FdU (b); or APC (c).

To compare the rates of entry into S phase of drug-treated cells with those of untreated controls, cells were released from G_1 block into the presence of drug and high concentrations of high specific activity [^{3}H]thymidine for autoradiography (Figure 2). The increase in the percentage of labeled cells in cultures treated with HU or 5-FdU is somewhat slower than that of untreated controls, but it is substantially less in cultures treated with APC. After 24 h of block, nearly all of the cells move into S phase in the presence of each of the drugs. Thus, all of the cells eventually enter into S phase in the presence of any of the drugs; however, with APC, entry is slower during the first 10 h. Since there is a more rapid increase in DNA content between 10 and 18 h, determined by FCM (Figure 1), APC appears to slow the entrance of G_1 cells into S phase (or to retard elongation to such an extent that grains cannot be seen by autoradiography) for ~10 h, but it then becomes

Table I: Changes in the H1:H4 Ratio in Isolated Nuclei of Cells Blocked in Early S Phase by Sequential Use of Isoleucine Deprivation and 5-FdU, APC, or HU Blockade

cell culture	DNA content[a]	H1:H4 absorbance ratio	H1:H4[b]
exponential	1.27 ± 0.02	0.50 ± 0.03	1.00 ± 0.06
$Ile^- \rightarrow$ 5-FdU (10 h)	1.04	0.38 ± 0.01	0.76 ± 0.02
$Ile^- \rightarrow$ 5-FdU (24 h)	1.06 ± 0.02	0.18 ± 0.03	0.36 ± 0.06
$Ile^- \rightarrow$ APC (3 $\mu g/mL^{-1}$, 10 h)	1.00	0.41 ± 0.01	0.82 ± 0.02
$Ile^- \rightarrow$ APC (3 $\mu g\ mL^{-1}$, 18 h)	1.13	0.23 ± 0.01	0.46 ± 0.02
$Ile^- \rightarrow$ APC (5 $\mu g\ mL^{-1}$, 18 h)	1.10	0.21 ± 0.01	0.42 ± 0.02
$Ile^- \rightarrow$ HU (10 h)	1.04[c]	0.36 ± 0.02[c]	0.72 ± 0.04[c]
$Ile^- \rightarrow$ HU (24 h)	1.15[d]	0.18 ± 0.02[d]	0.37 ± 0.02[d]

[a] Relative to a DNA content of 1.0 in G_1 cells. [b] Fraction of value in exponentially growing cells. [c] Taken from D'Anna et al. (1982). [d] Taken from D'Anna & Prentice (1983a).

Table II: Changes in H1 Content per Unit DNA in Cells Blocked in Early S Phase by Sequential Use of Isoleucine Deprivation (Ile^-) and 5-FdU, APC, or HU Blockade

cell culture	DNA content[a]	H1:DNA content[b]
exponential	1.26 ± 0.04	1.00 ± 0.03
$Ile^- \rightarrow$ 5-FdU (10 h)	1.04	0.77 ± 0.03
$Ile^- \rightarrow$ 5-FdU (24 h)	1.09 ± 0.05	0.39 ± 0.04
$Ile^- \rightarrow$ APC (5 $\mu g\ mL^{-1}$, 10 h)	1.07	0.69 ± 0.01
$Ile^- \rightarrow$ APC (5 $\mu g\ mL^{-1}$, 18 h)	1.16 ± 0.05	0.44 ± 0.05
$Ile^- \rightarrow$ APC (5 $\mu g\ mL^{-1}$, 24 h)	1.21	0.41 ± 0.01
$Ile^- \rightarrow$ HU (24 h)	1.15[c]	0.35 ± 0.02[c]
exponential + 5 $\mu g\ mL^{-1}$ APC (18 h)	1.40	0.48 ± 0.02

[a] Relative to a DNA content of 1.0 in G_1 cells. [b] Relative to exponentially growing cells. [c] From D'Anna & Prentice (1983a).

less effective and allows the cells to slip into S phase. Whether the slippage arises from continued production of polymerases (Pedrali-Noy et al., 1980) or from metabolic destruction of APC (Sala et al., 1983) is not known.

Changes in H1 Content in Cells Blocked with 5-FdU or APC. To determine whether there were decreases in the content of histone H1 in isolated nuclei and in whole early S-phase cells blocked with 5-FdU or APC, we measured (1) H1:H4 molar ratios from isolated nuclei and (2) the relative H1:DNA content from cells blended directly in $HClO_4$. Those measurements from independent cultures (Tables I and II) clearly show that there are losses of H1 from both the nucleus and the cell and that the losses for a given set of conditions are the same. Furthermore, they are similar to the losses observed in HU-blocked cells (Tables I and II). Thus, as observed for HU (D'Anna & Prentice, 1983a), there are losses of H1 from both nuclei and whole early S-phase cells blocked with 5-FdU or APC, and there is no need to postulate the existence of large cytoplasmic pools of H1 or the accumulation of extraordinary quantities (an excess greater than ~10%) of core histones during the block.

Besides examining the loss of H1 from cells blocked in early S phase, we also examined the H1 content of (1) exponentially growing cells treated with APC for 18 h and (2) cells released from isoleucine deprivation G_1 block into nearly complete medium containing regular serum and 1 $\mu g\ mL^{-1}$ 5-FdU. With these conditions, some cells become blocked in S phase and/or some cells proceed well into S phase, compared with untreated controls.

Flow cytometric histograms of exponentially growing cells treated with APC for 18 h (Figure 3) indicate that a large portion of cells is located in the first 50% of S phase, a lesser

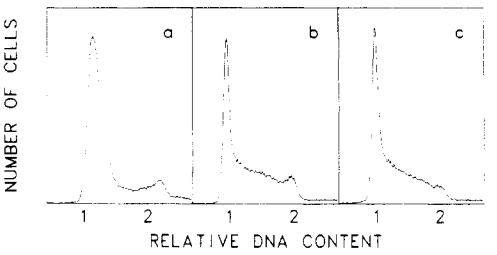

FIGURE 3: FCM DNA histograms of exponentially growing cells treated with APC for 18 h (a) or cells released from G_1 block into the presence of nearly complete thymidine-deprived medium containing regular serum and 1 $\mu g\ mL^{-1}$ 5-FdU for 10 (b) or 24 h (c).

Table III: H1:DNA Contents in Isoleucine-Deprived G_1-Blocked Cells and G_1-Blocked Cells Treated with 5-FdU or APC

culture	H1:DNA content[a]	culture	H1:DNA content[a]
exponential	1.00 ± 0.03	Ile^- (60 h)	1.14 ± 0.02
Ile^- (36 h)	1.15 ± 0.06	Ile^- (60 h) + 5-FdU (24 h)	1.12 ± 0.03
Ile^- (46 h)	1.07 ± 0.01		
Ile^- (46 h) + 5-FdU (10 h)	1.02 ± 0.05	Ile^- (54 h) + 0.1% Me_2SO (18 h)	1.10 ± 0.05
Ile^- (46 h) + 5 $\mu g\ mL^{-1}$ APC (10 h)	1.05 ± 0.03	Ile^- (54 h) + 5 $\mu g\ mL^{-1}$ APC (18 h)	0.97 ± 0.03

[a] Compared with exponentially growing cells.

portion is distributed in the rest of S phase, and there is evidence for cell dimers. From the histograms, we calculate an average DNA content of 1.40 relative to G_1 cells and a 50% loss of H1 per unit DNA compared with exponentially growing cells (also see Table II). Hence, omission of G_1 block prior to treatment with APC does not stop the loss of H1 (i.e., the loss of H1 is not an artifact associated with the earlier maintenance of the cells in isoleucine-deprived medium).

Losses of H1 are observed when isoleucine-deprived G_1 cells are released into nearly complete medium (thymidine-deprived medium plus regular, *as opposed to dialyzed*, serum) containing 5-FdU for 10 or 24 h. Flow cytometry histograms (Figure 3) indicate that, under those conditions, portions of the cells accumulate in early S phase but a large portion of the cells progresses further into S. At 10 h after release, there is no loss of H1 per unit DNA, but after 24 h, the H1:DNA content is 0.61 ± 0.03 that of exponentially growing cells. Thus, it is conceivable that when cells progress well into S phase, the loss of H1 from the culture may be somewhat less than that observed during efficient early S-phase block.

In contrast to the clear losses of H1 from S-phase-blocked cells, treatment of isoleucine-deprived G_1-blocked cells with 5-FdU or APC causes, at best, moderate losses of histone H1 (Table III). Treatment of G_1-blocked cells for 10 h causes reductions in the measured H1 contents of 2–5%, and treatment for 18–24 h causes losses of 2–15%. Since the random error in the measurements of H1:DNA content is on the order of 5%, the changes occurring in G_1 cells are of questionable significance and, clearly, are much less than the 26–31% and 56–59% losses of H1 measured from cells blocked in early S phase. Thus, while there may be a small drug-induced loss of H1 from G_1 cells (especially with APC), the losses are severalfold less than those observed during S-phase block.

We note that the H1:DNA content of isoleucine-deprived G_1 cells is ~15% greater than that of exponentially growing cells. This is in general agreemewnt with the previous observation that the H1:core histone ratio of isoleucine-deprived G_1 cells is ~10% higher than that of exponentially growing cells (D'Anna et al., 1982).

Chromatin Structural Changes in S-Phase-Blocked Cells. To determine whether cells blocked in early S phase with

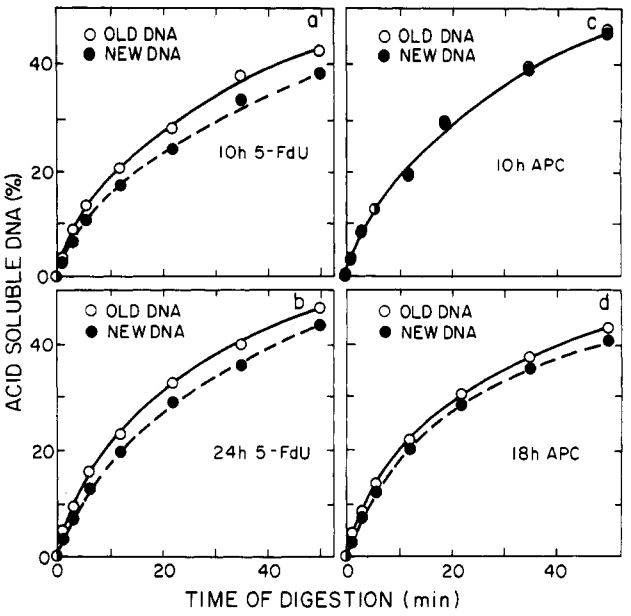

FIGURE 4: Percentages of acid-soluble new DNA and old DNA released by micrococcal nuclease digestion of nuclei as functions of time. Cells were released from G_1 block into thymidine-deprived medium containing 1 $\mu g\ mL^{-1}$ 5-FdU for 10 (a) and 24 h (b), or they were released into complete medium containing 5 $\mu g\ mL^{-1}$ APC for 10 (c) and 18 h (d).

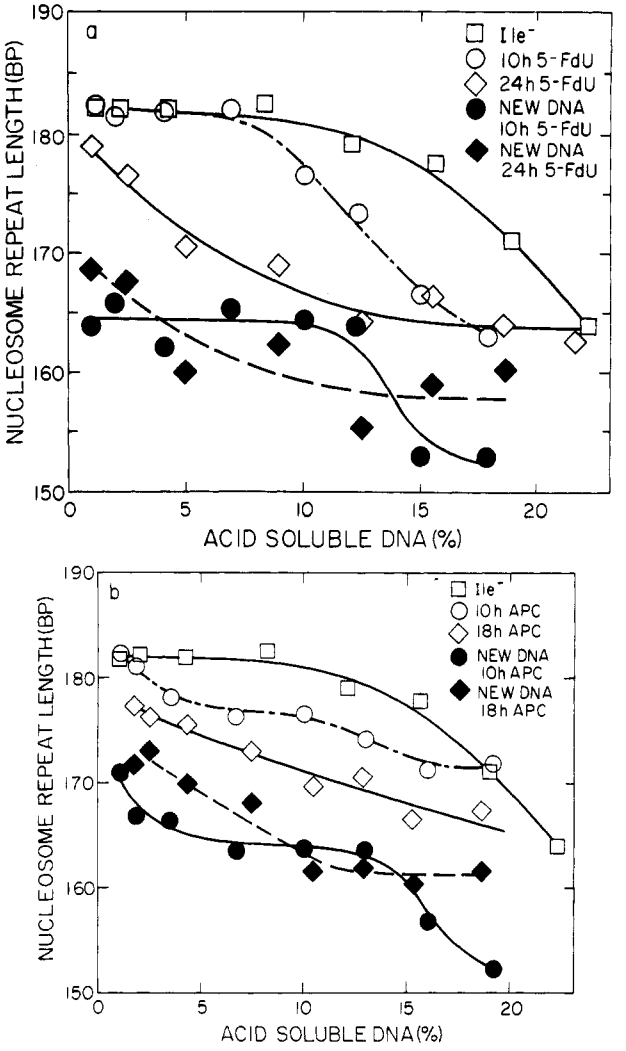

FIGURE 5: Nucleosome repeat length as a function of percent acid-soluble DNA. Cells were released from G_1 block into the presence of 5-FdU (a) or APC (b) to block cells in early S phase: total DNA from G_1-blocked cells (□); cells blocked in S phase 10 (○) or 18–24 h (◇) after release from G_1 block; new DNA from cultures blocked in S phase 10 (●) or 18–24 h (◆) after release from G_1 block.

5-FdU or APC undergo chromatin structural changes which resemble those of HU-blocked cells (D'Anna & Prentice, 1983a), we monitored the following properties at 10 and 18 (or 24) h after release from G_1 block into the presence of the drugs: (1) the relative rates of digestion of new and old DNA (in isolated nuclei) by micrococcal nuclease; (2) the measured nucleosome repeat lengths of new and bulk (old) chromatin as functions of the percentage of DNA rendered acid soluble by micrococcal nuclease. As previously described (D'Anna & Prentice, 1983a), new DNA is that DNA synthesized after cells are released from G_1 block into the presence of the drug.

Measurement of the percent acid-soluble DNA as a function of the time of micrococcal nuclease digestion in isolated nuclei shows that new DNA and old DNA are digested at nearly the same rates (Figure 4). Since (1) the rates of digestion of new DNA and old DNA are similar to one another (Figure 4) and to those of exponentially growing cells (data not shown) and (2) there is no evidence for the rapidly degraded new DNA observed with pulse-labeling experiments in the absence of drugs (Seale, 1975; Hildebrand & Walters, 1975; Levy & Jacob, 1978), we conclude that, like HU, 5-FdU and APC do not prevent new chromatin from maturing to a structure that is equally or more resistant than that of old chromatin to attack by micrococcal nuclease.

In contrast to maturation with respect to digestibility by micrococcal nuclease, new chromatin from cells blocked with 5-FdU or APC does not mature with respect to those features which determine its measured nucleosome repeat length. Following digestion with micrococcal nuclease, (1) the new DNA migrates faster than old DNA in agarose–acrylamide gels [results not shown; similar data are shown in D'Anna & Prentice (1983a)], and (2) the measured nucleosome repeat lengths of new chromatin are less than those of old chromatin and the mature chromatin of G_1-blocked cells at any extent of digestion (Figure 5). Additionally, the measured nucleosome repeat lengths of bulk chromatin become shorter during the period of S-phase block (Figure 5). Thus, early S-phase block induced by 5-FdU, APC, or HU (D'Anna & Prentice, 1983a) (1) prevents new chromatin from achieving a mature repeat length and (2) causes old chromatin to undergo structural changes so that the nucleosome cores appear to be more closely packed along the DNA chain.

Although the overall changes in chromatin structure are similar whether 5-FdU, APC, or HU is used, there are subtle differences. First, when cells are blocked with 5-FdU or HU for 10 or 24 h, the new DNA is always less sensitive than bulk DNA to attack by micrococcal nuclease [Figure 4 and Figure 2 of D'Anna & Prentice (1983a)]; however, when cells are blocked with APC for 10 h (but not at 18 h), the new DNA differs somewhat from culture to culture from slightly less sensitive to slightly more sensitive than bulk DNA. Second, the repeat length of bulk DNA at 10 h of APC block (Figure 5b) follows a different functional dependence than those observed when 5-FdU (Figure 5a) or HU [Figure 4 of D'Anna & Prentice (1983a)] is used. With 5-FdU or HU, there is a plateau in the repeat length plots between 0 and 7% acid-soluble DNA; then, between 7% and 18–20% acid-soluble DNA, there is a decrease to ~160–164 bp of DNA. With APC, there is a gradual change in the repeat length throughout the extent of digestion, but the repeat length is still ~172 bp after digestion to ~18% acid solubility. Although we do not know why this is so, we suspect it is related to the percentage of cells that enters S phase during block and the extent of

DNA elongation from the point of initiation.

Discussion

These results demonstrate that cells blocked in early S phase undergo compositional and chromatin structural changes regardless of whether the blocking drug is HU, 5-FdU, or APC. There is a loss of histone H1, new chromatin does not reach a stable mature nucleosome repeat length, and old chromatin undergoes structural changes which are reflected in a shortened nucleosome repeat length. As stated previously (D'Anna & Prentice, 1983a), we do not know whether the changes in nucleosome repeat length occur prior to or during digestion; nevertheless, the changes clearly illustrate that new chromatin does not truly mature and that old chromatin undergoes structural change during S-phase block. Furthermore, since chromatin undergoes structural changes as H1 is lost, it appears that the changes in chromatin structure and the loss of histone H1 are directly related (D'Anna & Prentice, 1983a), or they may arise from related processes.

While this work was in progress, Leffak (1983) reported investigations with results that resemble those reported here and previously (D'Anna & Prentice, 1983a). Leffak investigated histone synthesis, histone deposition onto chromatin, and the structure of chromatin in exponentially growing cells treated for 2–4 h with arabinocytidine, another DNA-inhibitory drug known to slow cell progression through S phase (Tobey & Crissman, 1972). Among his observations, Leffak found that (1) the DNA synthesized in the presence of the drug had a shortened repeat length and (2) the new and old DNA exhibited similar sensitivites to attack by micrococcal nuclease. Although a shortened nucleosome repeat length for old chromatin was not detected and changes in histone stoichiometry were not examined, the short time of the block (on the basis of our results) may have precluded such observations. Thus, we suspect that arabinocytidine will also produce a loss of H1 and changes in the structure of bulk chromatin.

Several lines of evidence support the contention that the observed losses of H1 and, perhaps, changes in chromatin structure are normal replication events which are enhanced by S-phase block with HU, 5-FdU, and APC: (1) in the absence of drugs, the H1:core histone ratio decreases when cells enter S phase following release from G_1 block (D'Anna et al., 1983a); (2) up to 70% of histone H1 can be lost from cells blocked in early S phase by HU, 5-FdU, or APC (drugs which inhibit DNA replication by different mechanisms); and (3) minimal loss of H1 is observed when G_1-blocked cells are treated with 5-FdU, APC, or HU (D'Anna & Prentice, 1983a).

Besides blocking cells in early S phase and causing changes in H1 content and chromatin structure, APC, 5-FdU, HU, or other inhibitors of DNA synthesis produce similar effects, from drug to drug, on other parameters of S-phase cells: (1) HU and arabinocytidine reduce, but do not totally inhibit, the synthesis of histones (Gurley et al., 1974; Chiu & Marzluff, 1982; Leffek, (1983); (2) HU, APC, 5-FdU, and arabinocytidine cause histone messenger ribonucleic acid levels to decrease (Chiu & Marzluff, 1982; Baumback et al., 1983; Graves & Marzluff, 1983); and (3) HU and arabinocytidine enhance the frequency of gene amplification [e.g., see Brown et al. (1983)] or double replication (Woodcock & Copper, 1981; Mariani & Schimke, 1984). Even though all of these parameters of S-phase cells are perturbed, release of cells from early S-phase block (produced by sequential use of isoleucine deprivation and blockade with HU, APC, or arabinocytidine) leads to the immediate preferential synthesis of specific parts of highly amplified dihydrofolate reductase genes (Heintz & Hamlin, 1981, 1983; Heintz et al., 1983). Thus, it is possible that changes in all of the affected parameters, including histone H1 content, chromatin structure, initiation of replication, modulation of histone synthesis and histone messenger pools, and even events leading to gene amplification are interdependently regulated.

Previously, this laboratory suggested a simplified model of chromatin structural changes during replication to account for the loss of histone H1 and changes in chromatin structure in HU-blocked cells (D'Anna & Prentice, 1983a). In that model, it was assumed that histone H1 is dissociated from initiated replicons (in the presence or absence of drugs) and it does not reassociate with the initiated replicon in a concerted fashion until a considerable part of the replicon (i.e., 40–80 nucleosome lengths of DNA) or the whole replicon (D'Anna & Prentice, 1983b) has been replicated. This loss of H1 from initiated replicons would facilitate nucleosome "sliding" along chromatin so that the new chromatin and old chromatin could rearrange on the initiated replicons (as opposed to dissociation of H1 and rearrangement of old chromatin at random). While our results are not inconsistent with such a model and with the observations of Chambers et al. (1983) that the nucleosome repeat length may be shorter in rapidly dividing cells, such a relationship is only speculative. It seems equally plausible that the loss of H1 in drug-blocked cells is part of a general alarm response in the cell [see Varshavsky (1983)] which might (1) activate the H1 proteolytic system reported by Surowy & Berger (1983) and (2) facilitate events leading to gene amplification and survival [which are enhanced when cells blocked in mid-S phase are released into the presence of toxic concentrations of methotrexate (Brown et al., 1983; Mariani & Schimke, 1984)].

In conclusion, it is clear that histone H1 is lost from cells blocked during early S phase by drugs, and there is good reason to suspect that H1 will also be lost (1) in cells with mutations which interfere with the pathways involved in DNA synthesis or (2) in S-phase cells whose progression is perturbed by other chemical or physical agents. These possibilities and the mechanisms by which H1 is lost remain to be clarified.

Acknowledgments

We thank Mary Luedemann, Joe Valdez, Judy Tesmer, Evelyn Campbell, Robert Sebring, Marieke Thayer, and Elizabeth Saunders for their technical assistance in the performance of this research. We also thank Marla Griffith and Noyola Scott for typing and handling the manuscript, Mark Wilder for his help in preparing the computer-drawn figures, and Lawrence Gurley for his helpful comments during the preparation of the manuscript.

Registry No. 5-FdU, 50-91-9; APC, 38966-21-1; HU, 127-07-1.

References

Baumbach, L. L., Plumb, M., Stein, G., & Stein, J. (1983) *Fed. Proc., Fed. Am. Soc. Exp. Biol. 42*, 1959.

Brown, P. C., Tlsty, T. D., & Schimke, R. T. (1983) *Mol. Cell. Biol. 3*, 1097–1107.

Chambers, S. A. M., Vaughn, J. P., & Shaw, B. R. (1983) *Biochemistry 22*, 5626–5631.

Chiu, I. M., & Marzluff, W. F. (1982) *Biochim. Biophys. Acta 699*, 173–182.

D'Anna, J. A., & Prentice, D. A. (1983a) *Biochemistry 22*, 5631–5640.

D'Anna, J. A., & Prentice, D. A. (1983b) *Fed. Proc., Fed. Am. Soc. Exp. Biol. 42*, 2118.

D'Anna, J. A., Tobey, R. A., & Gurley, L. R. (1980) *Biochemistry 19*, 2656–2671.

D'Anna, J. A., Gurley, L. R., & Tobey, R. A. (1982) *Biochemistry 21*, 3991–4001.
D'Anna, J. A., Becker, R. R., Tobey, R. A., & Gurley, L. R. (1983) *Biochim. Biophys. Acta 739*, 197–206.
Graves, R. A., & Marzluff, W. F. (1983) *Fed. Proc., Fed. Am. Soc. Exp. Biol. 42*, 1893.
Gurley, L. R., Walters, R. A., & Tobey, R. A. (1974) *Arch. Biochem. Biophys. 164*, 469–477.
Heintz, N. H., & Hamlin, J. L. (1982) *Proc. Natl. Acad. Sci. U.S.A. 79*, 4083–4087.
Heintz, N. H., & Hamlin, J. L. (1983) *Biochemistry 22*, 3557–3562.
Heintz, N. H., Milbrandt, J. D., Greisen, K. S., & Hamlin, J. L. (1983) *Nature (London) 302*, 439–441.
Hildebrand, C. E., & Walters, R. A. (1976) *Biochem. Biophys. Res. Commun. 73*, 157–163.
Huberman, J. A. (1981) *Cell (Cambridge, Mass.) 23*, 647–648.
Johnston, L. H. (1980) *Curr. Genet. 2*, 175–180.
Kaplay, M., Prabhakar, V., & Rao, K. S. (1983) *Biochem. Int. 6*, 283–289.
Kurek, M. P., & Taylor, J. H. (1977) *Exp. Cell Res. 104*, 7–14.
Leffak, I. M. (1983) *Nucleic Acids Res. 11*, 5451–5466.
Levy, A., & Jakob, K. M. (1978) *Cell (Cambridge, Mass.) 14*, 259–267.
Mariani, B. D., & Schimke, R. T. (1984) *J. Biol. Chem. 259*, 1901–1910.
Panyim, S., & Chalkley, R. (1969) *Biochemistry 8*, 3972–3979.
Pedrali-Noy, G., Spadari, S., Miller-Faures, A., Miller, A. O. A., Kruppa, J., & Koch, G. (1980) *Nucleic Acids Res. 8*, 377–387.
Reichard, P., & Ehrenberg, A. (1983) *Science (Washington, D.C.) 221*, 514–519.
Sala, F., Galli, M. G., Nielson, E., Magnien, E., Devreux, M., Pedrali-Noy, G., & Spadari, S. (1983) *FEBS Lett. 153*, 204–208.
Seale, R. (1975) *Nature (London) 255*, 247–249.
Spadari, S., Sala, F., & Pedrali-Noy, G. (1982) *Trends Biochem. Sci. (Pers. Ed.) 7*, 29–32.
Surowy, C. S., & Berger, N. A. (1983) *Proc. Natl. Acad. Sci. U.S.A. 80*, 5510–5514.
Taylor, J. H. (1977) *Chromosoma 62*, 291–300.
Taylor, J. H., Adams, A. G., & Kurek, M. P. (1973) *Chromosoma 41*, 361–384.
Tobey, R. A., & Ley, K. D. (1971) *Cancer Res. 31*, 46–51.
Tobey, R. A., & Crissman, H. A. (1972) *Cancer Res. 32*, 2726–2732.
Varshavsky, A. (1983) *Cell (Cambridge, Mass.) 34*, 711–712.
Walters, R. A., Tobey, R. A., & Ratliff, R. (1973) *Biochim. Biophys. Acta 319*, 336–347.
Walters, R. A., Tobey, R. A., & Hildebrand, C. E. (1976a) *Biochem. Biophys. Res. Commun. 69*, 212–217.
Walters, R. A., Tobey, R. A., & Hildebrand, C. E. (1976b) *Biochim. Biophys. Acta 447*, 36–44.
Wawra, E., & Wintersberger, E. (1983) *Mol. Cell. Biol. 3*, 297–304.
Woodcock, D. M., & Cooper, I. A. (1981) *Cancer Res. 41*, 2483–2490.

Carbon-13 Nuclear Magnetic Resonance Studies of Myocardial Glycogen Metabolism in Live Guinea Pigs†

Klaus J. Neurohr,* Gerald Gollin, Janet M. Neurohr, Douglas L. Rothman, and Robert G. Shulman

ABSTRACT: Myocardial glycogen metabolism was studied in live guinea pigs by ^{13}C NMR at 20.19 MHz. Open-chest surgery was used to expose the heart, which was then positioned within a solenoidal radio frequency coil for NMR measurements. The time course of myocardial glycogen synthesis during 1-h infusions of 0.5 g of D-[1-^{13}C]glucose (and insulin) into the jugular vein was investigated. The possible turnover of the ^{13}C-labeled glycogen was also studied in vivo by following the labeled glucose infusion with a similar infusion of unlabeled glucose. The degree of ^{13}C enrichment of the C-1 glycogen carbons during these infusions was measured in heart extracts by ^{1}H NMR at 360 MHz. High-quality proton-decoupled ^{13}C NMR spectra of the labeled C-1 carbons of myocardial glycogen in vivo were obtained in 1 min of data accumulation. This time resolution allowed measurement of the time course of glycogenolysis of the ^{13}C-labeled glycogen during anoxia by ^{13}C NMR in vivo. With the solenoidal coil used for ^{13}C NMR, the spin–lattice relaxation time of the labeled C-1 carbons of myocardial glycogen could be measured in vivo. For a comparison, spin–lattice relaxation times of heart glycogen were measured in vitro at 90.55 MHz. Natural abundance ^{13}C NMR studies of the quantitative hydrolysis of extracted heart glycogen in vitro at 90.55 MHz showed that virtually all the carbons in heart glycogen contribute to the ^{13}C NMR signals. The same result was obtained in ^{13}C NMR studies of glycogen hydrolysis in excised guinea pig heart.

Mobilization of endogenous glycogen stores is of vital importance in maintaining adequate myocardial performance during episodes of limited oxygen supply to the heart (Opie, 1976; Randle & Tubbs, 1979; Capasso et al., 1981; Liedtke, 1981). Studies on isolated, perfused hearts have shown that myocardial glycogen stores are rapidly depleted during anoxia and, on a somewhat slower time scale, during ischemia (Rovetto et al., 1973). Glucose and insulin have been shown to increase glycogen in normal heart tissue (Villar-Palasi & Larner, 1968), and glucose–insulin–potassium (GIK) infusions increased glycogen levels in experimental baboon and dog infarcts (Opie et al., 1975; Opie & Owen, 1976). A protective role of increased myocardial glycogen stores in cardiac anoxia has been demonstrated (Scheuer & Stezoski, 1970; Hewitt et al., 1974). Rose et al. (1976) found a close association between

† From the Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut 06511. *Received February 14, 1984.* This work was supported by National Institutes of Health Grant GM 32087.

0006-2960/84/0423-5029$01.50/0