Time-Dependent Changes in H1 Content, H1 Turnover, DNA Elongation, and the Survival of Cells Blocked in Early S Phase by Hydroxyurea, Aphidicolin, or 5-Fluorodeoxyuridine†

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ABSTRACT: Cells were synchronized in G_1 by isoleucine deprivation and then released into medium containing 1 mM hydroxyurea (HU), 5 $\mu g\ mL^{-1}$ aphidicolin (APC), or 1 $\mu g\ mL^{-1}$ 5-fluorodeoxyuridine (fl^{5}dU). Coulter volume, content of histone H1 per unit DNA, turnover of histone H1, the extent of DNA elongation, and the survival of cells were measured as functions of time after release into the presence of the drugs. At the concentrations used in the experiments, the drugs differ in their toxicity (fl^{5}dU > HU > APC), induction of unbalanced cell growth, and the distribution of new DNA fragment sizes allowed during block, but they all (1) allow cells to enter S phase, (2) cause similar time-dependent losses of histone H1 per unit DNA, which begin as synchronized G_1 cells begin to enter S phase, (3) retard DNA elongation beyond replicon size, and (4) retard the turnover of histone H1. The results indicate that loss of histone H1, inhibition of histone turnover, the retarded ligation of newly replicated DNA into bulk chromatin, and chromatin structural changes may be part of the cell's general response to inhibition of DNA replication. Since transient S phase block increases the frequencies of gene amplification [Mariani, B. D., & Schimke, R. T. (1984) *J. Biol. Chem. 259*, 1901–1910] and sister chromatid exchanges (SCE) [Rainaldi, G., Sessa, M. R., & Mariani, T. (1984) *Chromosoma 90*, 46–49], the observed changes in H1 content and chromatin organization may also be essential features of gene amplification and SCE.

Recently, our laboratory reported that blockage of cells in early S phase by sequential use of isoleucine deprivation and treatment with hydroxyurea (HU),[1] aphidicolin (APC), or 5-fluorodeoxyuridine (fl^{5}dU) causes (1) loss of histone H1 from the cell, (2) inhibition in the packaging of newly synthesized DNA into normal mature chromatin, and (3) changes in the structure of mature chromatin (D'Anna & Prentice, 1983a; D'Anna & Tobey, 1984). Besides causing changes in the content of H1 in chromatin and chromatin structure, drugs that inhibit DNA synthesis also affect a number of other cellular parameters. Among the effects are the following: (1) induction of a condition of unbalanced growth in which cell volume increases and excessive quantities of RNA and protein accumulate compared with DNA (Cohen & Studzinski, 1967; Ross, 1976; Frankford, 1981; Traganos et al., 1982); (2) inhibition of the ligation of newly synthesized DNA into intermediate- to bulk-sized DNA (Magnusson, 1973; Walters et al., 1976; Kurek & Taylor, 1977; Johnston, 1980; Cress & Bowden, 1981; Yagura et al., 1982; Lönn & Lönn, 1983); (3) increased cell mortality [e.g., Burchendol et al. (1959), Endinoff & Rich (1959), Mauro & Madoc-Jones (1970), and Ramseier et al. (1977)]; (4) reduction of histone mRNA levels (Heintz et al., 1983; Baumback et al., 1984; Graves & Marzluff, 1984; Helms et al., 1984; Plumb et al., 1984).

Although inhibitors of DNA synthesis can affect a number of biochemical and physiological parameters of the cell, most studies have employed unsynchronized populations of cells, different cell lines, different concentrations of drugs, and different times of treatment. Additionally, only one or a few parameters have been measured in a single system. Thus, it was not clear (1) whether drugs such as HU, APC, and fl^{5}dU would cause similar quantitative changes in cellular and biochemical parameters during S-phase block, when compared in the same system, or (2) whether there were any apparent correlations among the cellular or biochemical parameters themseleves.

Here, we report investigations of time-dependent changes in Coulter volume, cellular contents of histone H1, histone H1 turnover, the elongation of newly synthesized DNA, and the survival of cells during S-phase block induced by sequential use of isoleucine deprivation G_1 blockade and release into 1 mM HU, 5 $\mu g\ mL^{-1}$ APC, or 1 $\mu g\ mL^{-1}$ fl^{5}dU. We find that, at these concentrations [used previously (D'Anna & Prentice 1983a,b; D'Anna & Tobey, 1984)], HU, APC, and fl^{5}dU differ in their toxicity, their induction of unbalanced growth, and their effectiveness in retarding the extent of DNA elongation, but they all (1) cause similar time-dependent losses of histone H1 per unit DNA, (2) retard elongation or the ligation of newly replicated DNA beyond replicon size, and (3) retard the turnover of histone H1. The similarity in histone loss, inhibition of histone turnover, the retarded ligation of new DNA into bulk chromatin, and chromatin structural changes (D'Anna & Tobey, 1984) suggests (1) they are part of a complicated cellular response to the inhibition of DNA elongation and (2) they may play important roles in facilitating gene amplification and sister chromatid exchanges.

Experimental Procedures

Cell Synchrony and Radioisotropic Labeling. Suspension cultures of Chinese hamster (line CHO) cells were grown and

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[1] Abbreviations: APC, aphidicolin; EDTA, ethylenediaminetetraacetic acid; fl^{5}dU, 5-fluorodeoxyuridine; HU, hydroxyurea; kb, kilobases.

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synchronized in G_1 by maintenance in isoleucine-deficient medium (Tobey & Ley, 1971) as described (D'Anna & Prentice, 1983a). To block cells in nearly S phase, cells were released from G_1 block into (1) complete medium containing 1 mM HU, (2) complete medium containing 5 μg mL^{-1} APC (5 mg/mL^{-1} in dimethyl sulfoxide stock solution), or (3) incomplete medium (F-10 lacking thymidine and supplemented with 15% dialyzed newborn calf serum) containing 1 μg mL^{-1} fl^{5}dU. APC (NSC 234714) was a gift from Dr. David Abraham, Investigational Drug Branch, National Cancer Institute, Bethesda, MD.

Two- or four-liter suspension cultures were used to synchronize cells for measurements of H1 content per unit DNA after cells were released from G_1 block into the presence of HU, APC, or fl^{5}dU. Four liters of cells was synchronized in G_1 by isoleucine deprivation and released into 4 L or 2 × 2 L of the appropriate medium and drug. A small portion (90–120 mL) of the resuspended cells was transferred to a second suspension flask to monitor entry of cells into S phase. This secondary culture was made 40 μCi mL^{-1} in [^{3}H]thymidine when HU and APC were used or 5 μCi mL^{-1} when fl^{5}dU was used to block cells in S phase. At 2–4-h intervals after resuspension in the presence of a drug, 30 and 450 mL of cells were removed from the main cultures for flow cytometric analysis and the isolation of histone H1; 5 mL was removed from the secondary culture for autoradiography (Tobey & Ley, 1970).

For measurements of H1 turnover during S phase block, 2 L of exponentially growing cells were grown (two generations) and synchronized in G_1 in the presence of [^{3}H]lysine. They were then released into 2.0 L of the appropriate [^{3}H]-lysine-free medium and drug.

To monitor the elongation of newly replicated DNA during S-phase block, cells were grown (24 h) and synchronized in G_1 in the presence of [^{14}C]thymidine (15 μCi L^{-1}) to radiolabel old DNA. Cells were released into an appropriate medium containing one of the drugs and [^{3}H]thymidine to radiolabel new DNA. The following concentrations of [^{3}H]thymidine were used when cultures were to be harvested after 10, 17, and 24 h in the presence of drug: 10, 1.6, and 0.8 μCi mL^{-1} for HU; 18, 1.6, and 0.8 μCi mL^{-1} for APC; 0.5, 0.1, and 0.1 μCi mL^{-1} for fl^{5}dU.

Survival Studies. Cells in suspension culture were released from isoleucine-deprivation G_1 block into the presence of complete medium containing 1 mM HU, 5 μg mL^{-1} APC, or 1 μg mL^{-1} fl^{5}dU. At intervals thereafter, aliquots of cells were centrifuged and washed in fresh medium to remove the drug. The washed cells were plated in Petri dishes containing complete medium without drug. They were kept 7 days in a CO_2 incubator prior to colony (50 or more cells) assay (Tobey et al., 1982).

Flow Cytometry and Coulter Volume Measurements. The distribution of cells in the cell cycle and average DNA content per cell were determined by flow cytometry (D'Anna & Prentice, 1983a).

The average RNA content per unit DNA and simultaneous determination of DNA, RNA, and protein (Crissman et al., 1985a) were measured in a three-laser flow cytometer (Steinkamp et al., 1982). Cells were harvested by centrifugation and fixed in 70% ethanol at 4 °C for at least 18 h. They were removed from ethanol and stained with Hoechst 33342 (0.5 μg/mL), fluoresceinyl isothiocyanate (0.1 μg/mL), and pyronin Y (0.5 μg/mL) for DNA, protein, and RNA, respectively (Crissman et al., 1985a). Fluorescence was excited with UV, 457- and 530-nm laser irradiation; blue, red, and green fluorescence provided quantitation of DNA, RNA, and protein for each cell. Data are presented as contour density profiles. At least 3 × 10^4 cells were analyzed per sample.

Mean Coulter volumes were measured with an instrument assembled and programmed by James Freyer and Mark Wilder of the Toxicology and Experimental Pathology Groups. A Coulter Model ZF particle counter was serially connected to a Quantum 8 multichannel pulse-height analyzer (The Nucleus, Inc.) and a Hewlett-Packard HP-86 computer.

Isolation and Analysis of Histone H1. Histone H1, histone H1^0, and HMG proteins were extracted by blending whole cells directly in 0.83 M $HClO_4$ and recovered by precipitation with Cl_3CCO_2H (D'Anna & Tobey, 1984). H1 was then separated from the other proteins by electrophoresis in 0.5 × 25 cm cylindrical acid–urea–polyacrylamide gels (Panyim & Chalkley, 1969; D'Anna et al., 1982), and its content per unit DNA was quantified as described (D'Anna & Tobey, 1984). Specific activities were calculated from the absorbance of amido black bound to H1 and the radioactivity of H1 in the acid–urea gels (D'Anna et al., 1982).

Alkaline Sucrose Gradient Centrifugation. The size of single-stranded DNA replicated during S-phase block was compared with that of bulk DNA by alkaline sucrose gradient centrifugation (Walters & Hildebrand, 1975). Cells radiolabeled with [^{14}C]thymidine and [^{3}H]thymidine, described above, were lysed by treatment with heparin, sodium *N*-laurylsarcosine, and 0.5 N NaOH on top of linear 5–20% sucrose gradients containing 0.4 N NaOH and 10 mM EDTA. The gradients were then loaded onto a Beckman SW-27 rotor and subjected to centrifugation at 2.2 × 10^4 rpm for 6 h.

Gradients were fractionated from the top. Aliquots of 1.0 mL were adsorbed to Whatman GF/B glass filters, and DNA was precipitated by adding 5% trichloroacetic acid–1% sodium pyrophosphate (Walters et al., 1974). The filters were batch washed 3 times with the trichloroacetic acid–pyrophosphate solution and twice with 100% ethanol. They were then air-dried before being counted in glass vials containing Altex EP scintillation cocktail.

The size of newly replicated DNA was estimated from its position in the gradient relative to the position of radiolabeled bacteriophage λ (Abelson & Thomas, 1966; Fritsch, 1973) and the size–sedimentation relationship developed by Studier (1965).

Results

Heterogeneity and the Survival of Cells Blocked in Early S Phase. Measurements of relative Coulter volume and FCM-derived DNA content (Figure 1) confirm the general condition of unbalanced growth that might be expected of cells blocked in early S phase (Cohen & Studzinski, 1967; Traganos et al., 1982). When cells are released from isoleucine deprivation G_1 block into the presence of HU, APC, or fl^{5}dU, they enter S phase [D'Anna & Tobey (1984) and below], and their Coulter volumes increase compared with those of control G_1 cells exposed to the drugs. After 25 h in the presence of the drugs, the mean Coulter volume increases 1.6–2.1 times compared with those of control-treated G_1 cells and 1.5–2.0 times compared with those of exponentially growing cells. At the same time, the cell distributions in the drug-blocked cells become broader, indicated by the standard deviation from the mean cell volume, which doubles compared with those of G_1 or exponentially growing cells.

Cells released from G_1 block in the absence of drugs also exhibit time-dependent increases in their mean Coulter volume (Anderson et al., 1969; Steen & Lindmo, 1978), but the increases are smaller than those of cells blocked in early S phase.

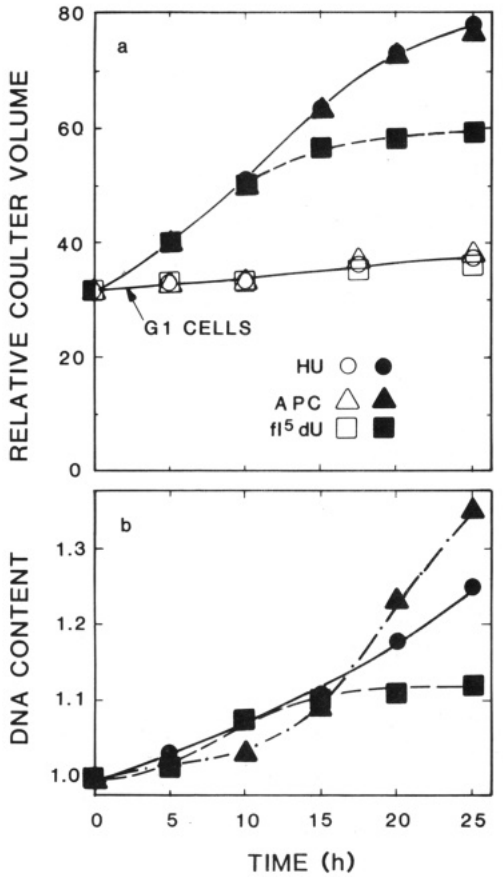

FIGURE 1: Relative mean Coulter volume (a) and DNA content (b) as functions of time for G_1-blocked cells (34 h in isoleucine-deprived medium) exposed to 1 mM HU (○), 5 μg mL^{-1} APC (△), or 1 μg mL^{-1} fl^5dU (□) and cells released from G_1 block into the presence of the same drugs (respective closed symbols). The Coulter volumes of control G_1 cells not treated with drugs were the same as those of cells exposed to drugs in (a) and are not shown. The DNA content of G_1 cells remains constant during exposure to drugs in (b); these data are also not shown.

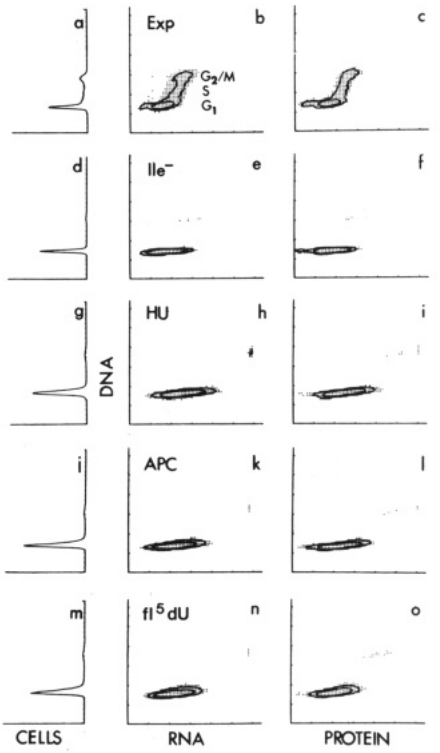

FIGURE 2: Single-parameter distributions of cells as functions of DNA content (a, d, g, j, and m) and two-parameter contour distributions of cells as functions of DNA (the ordinate) and RNA contents (b, e, h, k, and n) or DNA (the ordinate) and protein contents (c, f, i, l, and o) from exponentially growing cells (a–c), cells blocked in G_1 by isoleucine deprivation (d–f), and cells released from G_1 block into the presence of HU (g–i), APC (j–l), or fl^5dU (m–o) for 15 h. Dotted areas in the two-parameter contour distributions represent at least 10 cells, and sequential contours represent at least 50 and 200 cells [see Crissman et al. (1985a)].

Synchronized CHO cells released from G_1 arrest in the absence of drugs exhibit a maximum mean Coulter volume of 45 units [compared with 50–60 units at the same times in the presence of drugs (Figure 1)] between 10 and 14 h after release from G_1 block; then, the mean Coulter volume decreases as cells divide and reenter G_1 (results not shown).

Simultaneous measurements of DNA, RNA, and protein contents per cell by three-laser flow cytometry indicate that cells blocked in G_1 or in early S phase also became heterogeneous with respect to their RNA and protein contents per cell. This is illustrated by the examples in Figure 2. When exponentially growing cells (Figure 2a–c) are blocked in G_1 (Figure 2d–f), the distributions of RNA and protein become broader (standard deviation 50% greater) than those seen for the G_1 population in the exponentially growing culture (Figure 2b–c). Then, as cells are released from G_1 block into HU, APC, or fl^5dU, the heterogeneity increases further (Figure 2g–o). Interestingly, those cells that exhibit increased DNA content (ordinate in Figure 2) also have the more elevated levels of protein and RNA contents per cell (abscissas in Figure 2). Thus, whereas >85% of the cells enter S phase after 15 h [D'Anna & Tobey (1984) and Figure 5), those cells with the highest RNA and protein contents exhibit the largest increase in DNA content. This same relationship is observed in the absence of drugs in exponentially growing cells (Figure 2b,c; Traganos et al., 1982; Darzynkiewicz et al., 1982) and in cells released from G_1 block (Crissman et al., 1985b).

Although three-color flow cytometry appears best suited for examination of populations in a single sample, measurements from multiple samples indicate that the average RNA/DNA content per cell increases 90 ± 40% with HU, 30 ± 20% with APC, and 15 ± 2% with fl^5dU after 24 h of block.

During S phase block, the survival of the cells decreases in a time-dependent fashion, dependent on the drug (Figure 3). At the concentrations used in this and previous studies (D'Anna & Prentice, 1983b; D'Anna & Tobey, 1984), the toxicity of the drugs increases in the order APC < HU < fl^5dU (Figure 3).

Time-Dependent Loss of Histone H1. Previously, we examined the content of histone H1 per unit DNA in cells that had been blocked in early S phase for fixed periods of 0, 10, 18, or 24 h (D'Anna & Prentice, 1983a; D'Anna & Tobey, 1984). Although those measurements and controls clearly showed that H1 becomes depleted in S phase blocked cells, they did not indicate (1) the time that cells begin to lose H1 following release from G_1 block into the presence of drugs nor (2) whether the loss of H1 follows the same functional dependence from drug to drug. To address these questions, we measured the H1 content per unit DNA and the percentage of S-phase cells as functions of time after the release of G_1 cells into complete medium or complete medium containing HU, APC, or fl^5dU.

Initial measurements in the presence or absence of HU, APC, or fl^5dU (Figure 4) indicated that (1) loss of histone H1 begins within the first 2–4 h after release from G_1 block and is similar for the first 5–6 h, regardless of whether or not drug is present, and (2) the loss of H1 appears to follow a similar functional dependence with all of the drugs. In contrast

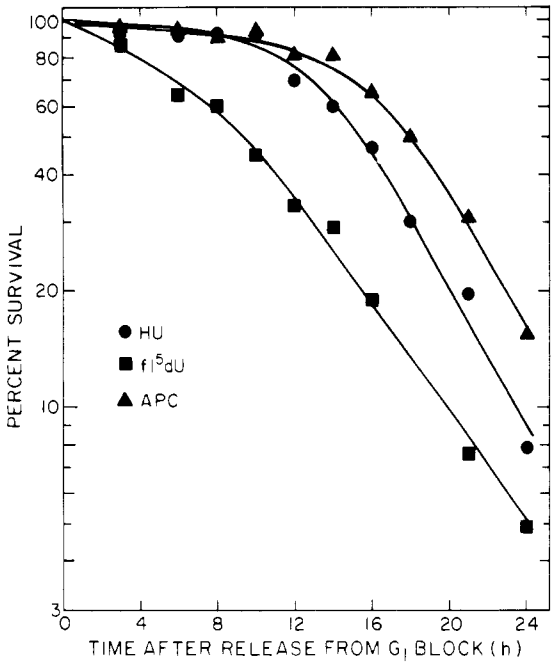

FIGURE 3: Survival of cells as a function of the duration of block in early S phase. Synchronized G_1 cells were released into the presence of 1 mM HU (●), 5 μg mL^{-1} APC (▲), or 1 μg mL^{-1} fl^{5}dU (■). After the indicated times in the presence of drug, the cells were washed and plated in medium without drug as described under Experimental Procedures.

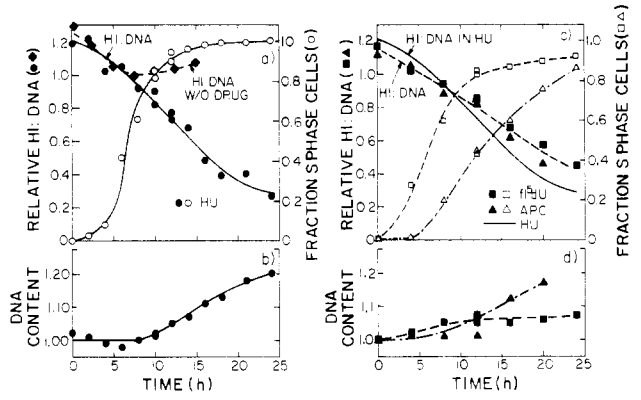

FIGURE 4: Relative H1:DNA contents [closed symbols in (a) and (c)], fraction of S-phase cells [open symbols in (a) and (c)], and DNA contents (b and d) as functions of time after the release of cells from G_1 block into the presence of medium without drug (♦) or with 1 mM HU (●, ○), 5 μg mL^{-1} APC (▲, △), or 1 μg mL^{-1} fl^{5}dU (■, □).

to the similarities, (1) the percentage of S-phase cells monitored by autoradiography increased at a slower rate with APC than with HU and fl^{5}dU [which enter S phase at nearly the same rate as drug-free controls (D'Anna & Tobey, 1984)], and (2) the ultimate loss of H1 per unit DNA with APC and fl^{5}dU may not have been so great as with HU.

To investigate whether APC truly retarded the entry of cells into S phase or our autoradiographic procedures were not sensitive enough to detect the initiation of DNA synthesis in the presence of APC, we employed thymidine-deprived medium and dialyzed serum, instead of regular F-10 medium which contains thymidine, for autoradiography. At those conditions (Figure 5), the percentage of apparent S-phase cells increases much more rapidly than that in Figure 4, and the curve is more like those obtained with HU and fl^{5}dU (D'Anna & Tobey, 1984). There is, nevertheless, a somewhat greater difference in the rate of incorporation between the APC-treated culture and its controls than observed for HU and

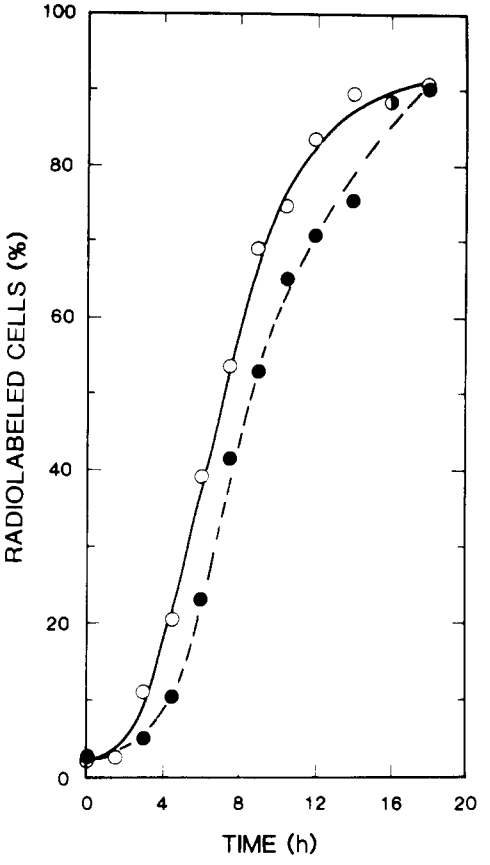

FIGURE 5: Percentage of radiolabeled cells (S-phase cells) following release of G_1-blocked cells into the presence (●) or absence (○) of 5 μg mL^{-1} APC in thymidine-deprived medium supplemented with 15% dialyzed newborn calf serum.

fl^{5}dU. Thus, it appears that APC may retard, to some extent, the entry of cells into S phase (see Discussion).

As we noted above, there are quantitative differences in the H1 contents per unit DNA between cells blocked with HU and cells blocked with APC or fl^{5}dU in Figure 4. Since the percentages of cells that ultimately enter S phase are different in the experiments in Figure 4a,c it seemed likely that the differences might arise from variations in the cell populations. Indeed, measurements comparing H1 content between cells released from a single G_1 culture into HU or APC or from another G_1 culture into HU or fl^{5}dU produce essentially the same values between 10 and 24 h, the times when the differences were greatest in Figure 4c (Figure 6). Thus, it appears that (1) the time-dependent loss of H1 per unit DNA is essentially the same with each of the drugs and (2) depletion of H1 exceeding that of the control begins at about the time cells enter S phase (see Discussion).

Extent of New DNA Elongation. Previously, Walters et al. (1976) showed that following release of mitotically selected CHO cells into 1 mM HU the DNA replicated during the first 10 h of block does not reach replicon size. In related experiments, (1) Johnston (1981) found that treatment of exponentially growing yeast cells with HU concentrations of 25–125 mM for 20–80 min inhibited the ligation of pulse-labeled DNA beyond replicon size, and (2) Yagura et al. (1982) found similar effects when exponentially growing mouse cells were treated with 1.4 μg mL^{-1} APC for 2 h. Because of these results, we investigated whether the new DNA from the cells blocked in early S phase was elongated to subreplicon, replicon, or bulk-sized DNA during a block period of 24 h.

Analyses of isotope incorporation profiles from alkaline sucrose gradients (Figure 7) indicate that, at the employed

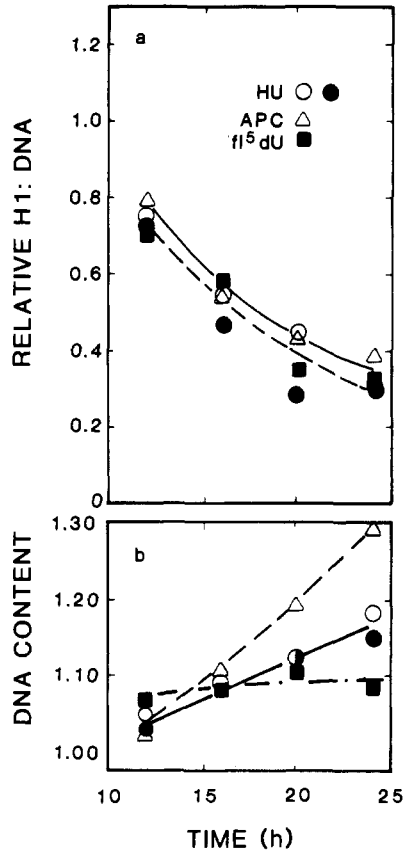

FIGURE 6: Relative H1:DNA contents (a) and DNA contents (b) of cells released from G_1 block into the presence of 1 mM HU (●, ○), 5 μg mL^{-1} APC (△), or 1 μg mL^{-1} fl^{5}dU (■). Closed or open symbols designate parallel experiments in which cells were released from the same G_1 culture.

concentrations of HU, APC, and fl^{5}dU, the drugs differ qualitatively in their inhibition of DNA ligation in S-phase cells. After 10 h of block in 1 mM HU, the new DNA is centered about ~60 kb; by 17 h, there is an accumulation of smaller fragments with a maximum near 15 kb; and by 24 h, there is a distribution of sizes ranging from ~15 kb to bulk DNA. At 24 h, about one-third of the new DNA (≃6% of total DNA) appears elongated to sizes greater than 200 kb.

Block with 5 μg mL^{-1} APC leads to relatively homogeneous distributions of new DNA that appear to increase in size throughout the block period. The size at the band maximum increases from ≃25 kb at 10 h, to ~70 kb at 17 h, and to ~95 kb at 24 h, and there is some incorporation into bulk DNA.

Block with 1 μg mL^{-1} fl^{5}dU leads to a rather broad distribution at 10 h, but as the block is prolonged, there is an accumulation of small fragments of ~10 kb and very limited incorporation of new DNA into bulk DNA.

Regardless of the drug used, the band maximum in new DNA is less than ~100 kb in length at up to 17 h. Even at 24 h, there is negligible incorporation of new DNA into bulk DNA with fl^{5}dU, but with HU and APC, some incorporation of new DNA into bulk DNA does occur. In contrast to the results with drugs, when cells are released from G_1 block in their absence, the isotope incorporation profile of the new DNA synthesized by 10 h is the same as that of bulk DNA, as one might expect (results not shown).

Turnover of H1 during S-Phase Block. Measurements of H1 turnover are of interest, because (1) the measurements of others have shown that HU treatment does not totally inhibit histone synthesis (Gurley et al., 1972; Nadeau et al., 1978) and (2) it is possible that reassociation of H1 may be linked

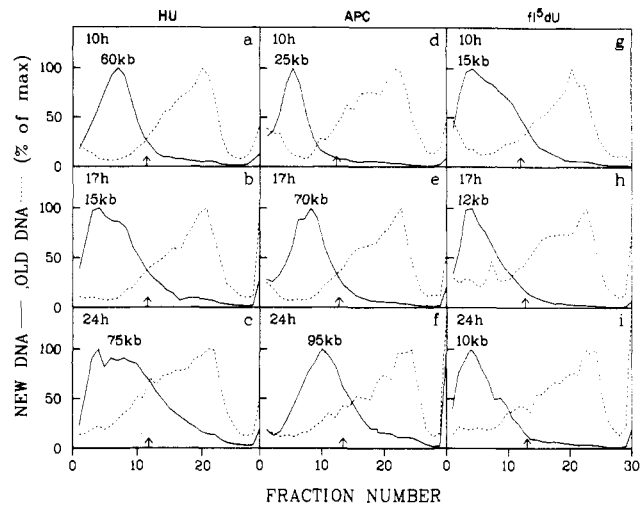

FIGURE 7: Alkaline sucrose gradients of DNA from cells at 10, 17, and 24 h after release from G_1 block into the presence of 1 mM HU (a–c), 5 μg mL^{-1} APC (d–f), or 1 μg mL^{-1} fl^{5}dU (g–i). Cells were prelabeled with [^{14}C]thymidine to distinguish old DNA (---) and labeled with [^{3}H]thymidine to distinguish the new DNA (—) synthesized during S-phase block. Sedimentation is from left to right. The arrow on the abscissa indicates 200 kb.

to the ligation of new DNA into bulk DNA (D'Anna & Prentice, 1983b). Previously, we measured the specific activities of histones H1 and H4 (prelabeled with [^{3}H]lysine prior to and during G_1 block) isolated from the total histones of cells blocked in S phase by HU (D'Anna & Prentice, 1983a). Those measurements indicated that <10% of H1 remaining at 24 h had been synthesized during the block. Since small quantities of histone H1 were employed (which caused considerable fluctuation from point to point), we have repeated measurement of H1 specific activities from cells blocked with HU and have measured the H1 specific activities of cells blocked with APC or fl^{5}dU. The results (Figure 8) indicate that with HU and APC there is some turnover of H1 by 17 h and more turnover between 17 and 24 h, but there is no measured turnover of H1 with fl^{5}dU. Since the H1 content per unit DNA is only 20–30% of the value in S-phase cells, only a small part (4–6%) of the normal histone H1 complement is synthesized and persists between 17 and 24 h in the APC- and HU-blocked cells.

Discussion

We have investigated several parameters of cells blocked in early S phase by HU, APC, or fl^{5}dU: Coulter volume, RNA and protein contents per cell, survival, loss and turnover of histone H1, and the ligation of newly replicated DNA into bulk DNA. At the concentrations employed in these investigations, the drugs differ quantitatively in their toxicity, perturbation of cell-size RNA contents, protein contents, and the size of new DNA intermediate fragments allowed during block; however, they all produce heterogeneous populations of cells, similar time-dependent changes in H1:DNA ratios, and, for the most part, inhibition of the ligation of new DNA into bulk DNA.

Within the first 4 h after release from G_1 block, cells begin to enter S phase, their Coulter volumes increase, and their content of H1 per unit DNA declines. Previously, we reported (D'Anna & Tobey, 1984) that (1) cells released from isoleucine deprivation G_1 block into the presence of 1 mM HU or 1 μg mL^{-1} fl^{5}dU enter S phase at nearly the same rate as controls but cells released into complete medium containing APC did not and (2) cells blocked with APC exhibit the

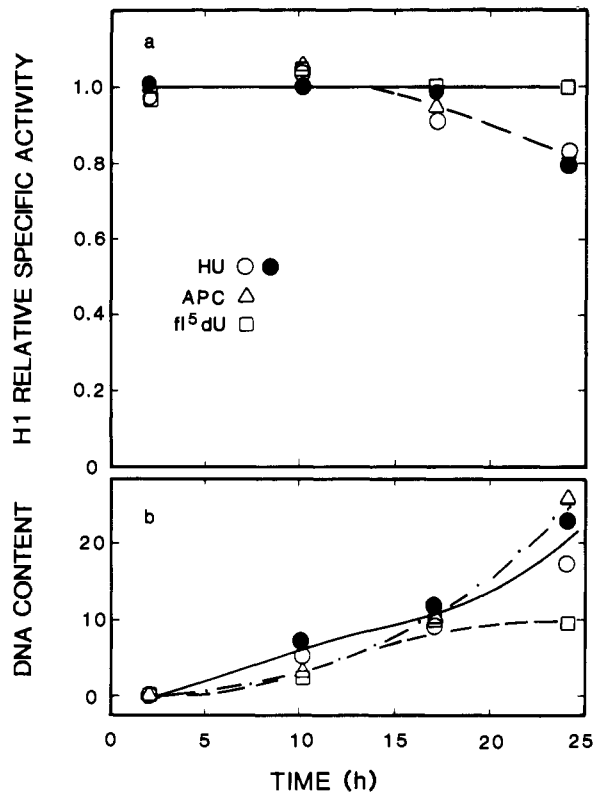

FIGURE 8: Turnover of histone H1 during S-phase block. H1 relative specific activities (a) and DNA contents relative to G_1 cells (b) are plotted as functions of time after the release of G_1-blocked cells into the presence of 1 mM HU (●, ○), 5 μg mL^{-1} APC (△), or 1 μg mL^{-1} fl^5dU (□). Cells were prelabeled with [^{3}H]lysine prior to release from G_1 block into medium containing the drugs (no [^{3}H]lysine present). The two symbols for HU represent the results from two separate experiments.

smallest average increase in DNA content after 10 h. At that time, we raised the possibility that APC might allow cells to enter S phase at the same rate as controls, but DNA elongation might be retarded to such an extent that grains could not be seen in autoradiograms. The results in this paper support that suggestion: (1) when thymidine-deprived medium is used (Figure 5) instead of complete F-10 (Figure 4c) for autoradiography, the percentage of cells incorporating [^{3}H]thymidine (S-phase cells) increases more rapidly; (2) after 10 h of exposure to APC, cells blocked with APC exhibit shorter DNA fragments than cells blocked with HU or fl^5dU (Figure 7). Thus, while the rate of increase in the percentage of cells incorporating isotope is somewhat slower with APC compared with controls, we suspect that the actual rate of entry into S phase is approximately the same.

Several lines of evidence support the notion that the enormous loss of histone H1 in S phase blocked cells is directly related to the initiation of DNA replication or a step that directly precedes or follows it: (1) loss of histone H1 per unit DNA exceeding that of controls begins at approximately the time G_1 cells enter S phase (Figures 4 and 5); (2) treatment of G_1 cells with drug does not induce the explicit loss in H1 content per unit DNA (D'Anna & Tobey, 1984); (3) similar time-dependent losses in H1 content are observed regardless of whether cells are block with HU, APC, or fl^5dU [which differ in their extents of DNA elongation during the first 10 h of block (Figure 7)]. Regarding the time-dependent loss of histone H1 that is observed when cells are released from G_1 block, it appears that a potentially inordinately high H1 content in isoleucine-deprived G_1 cells may contribute to the initial loss of H1 observed in Figure 4. When G_1-blocked cells are released from isoleucine deprivation G_1 block in the absence of drugs (Figure 4), there is a 20–30% decrease in H1 content during the first 5–6 h and a 15–20% decrease in the H1:core histone ratio by 8 h (D'Anna et al., 1983). We note, however, the H1 content per unit DNA in the isoleucine-deprived G_1 cells is 15–20% greater than in exponentially growing cells (D'Anna & Tobey, 1984). Thus, (1) an increased H1 content in G_1-blocked cells and the resumption of cell-cycle traverse cause the initial loss of H1, or (2) there is a transient loss of H1 as cells enter S phase [see D'Anna & Prentice (1983a)]. Measurement of H1 contents in G_1 cells not subjected to G_1 block will be required to address this question.

Treatment of cells released from G_1 block with either 1 mM HU, 5 μg mL^{-1} APC, or 1 μg mL^{-1} fl^5dU also inhibits or retards DNA elongation; however, the size of the accumulated new DNA varies considerably, depending on the drug. fl^5dU allows a broad distribution after short times (perhaps some residual thymidine in the medium) but ultimately leads to an accumulation of very small fragments. In the other extreme, HU blockade produces a sharp distribution of fragments of intermediate size at 10 h, but then it allows a greater distribution of both small and large DNA fragments during prolonged block. In contrast to results with HU and fl^5dU, the size of the new DNA in APC-blocked cells increases in a more uniform continuous manner to ~95 kb after 24 h. The 95-kb size is similar to the 137-kb size observed by Ogawa et al. (1982) with shorter treatment times. Although the size of replicon units appears to vary considerably (Edenberg & Huberman, 1975), the average size has been estimated to be near 137 kb (Ogawa et al., 1982). Thus, drug-induced block for as long as 24 h retards the elongation of most of the DNA to replicon size.

Measurements of histone H1 turnover (Figure 8), DNA content (Figure 8), and DNA elongation (Figure 7) provide some support for the notion that DNA elongation or ligation into bulk chromatin facilitates the reassociation of H1 with chromatin in the drug-treated cultures (D'Anna & Prentice 1983a,b). Whereas the H1:DNA ratios are similar for all of the drugs (Figures 4 and 6), new DNA gets longer, and there may be slightly greater increases in the DNA contents per cell in the cells blocked with HU or APC, compared with cells blocked with fl^5dU. Correspondingly, cells blocked with HU and APC exhibit H1 turnover between 17 and 24 h, but cells blocked with fl^5dU do not.

In summary, these experiments indicate that drugs that inhibit DNA elongation appear to differ in their efficiency to retard DNA elongation and in their ability to sustain inhibition of histone synthesis *at the tested concentrations*; yet, they all induce similar losses of histone H1 per unit DNA and changes in chromatin structure. (1) Since drugs that retard DNA elongation also enhance the frequencies of gene amplification (Brown et al., 1983; Mariani & Schimke, 1984), and, perhaps, sister chromatid exchanges (Rainaldi et al., 1984), and (2) there appear to be optimum conditions that enhance gene amplification and sister chromatid exchanges (same references), we suspect that changes in chromatin structure, the extent of DNA elongation, and the dissociation–reassociation of histone H1 are critical factors in the processes of gene amplification and sister chromatid exchanges.

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