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Recovery of DNA synthesis after ultraviolet irradiation of xeroderma pigmentosum cells depends on excision repair and is blocked by caffeine

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ABSTRACT

Normal human and xeroderma pigmentosum (XP, excision-defective group A) cells (both SV40-transformed) pulse-labeled with [^{3}H]thymidine at various times after irradiation with ultraviolet light showed a decline and recovery of both the molecular weights of newly synthesized DNA and the rates of synthesis per cell. At the same ultraviolet dose, both molecular weights and rates of synthesis were inhibited more in XP than in normal cells. This indicates that excision repair plays a role in minimizing the inhibition of chain growth, possibly by excision of dimers ahead of the growing point. The ability to synthesize normal-sized DNA recovered more rapidly than rates of synthesis in normal cells, but both parameters recovered in phase in XP cells. During recovery in normal cells there are therefore fewer actively replicating clusters of replicons because the single-strand breaks involved in the excision of dimers inhibit replicon initiation. XP cells have few excision repair events and therefore fewer breaks to interfere with initiation, but chain growth is blocked by unexcised dimers. In both cell types recovery of the ability to synthesize normal-sized DNA was prevented by growing cells in caffeine after irradiation, possibly because of competition between the DNA binding properties of caffeine and replication proteins.

Our observations imply that excision repair and semiconservative replication interact strongly in irradiated cells to produce a complex spectrum of changes in DNA replication which may be confused with parts of alternative systems such as post-replication repair.

INTRODUCTION

Previous investigations of DNA replication in normal and repair-deficient human fibroblasts led us to suggest that there may be an interaction between excision repair events and DNA replication (1-3). These investigations were based on a comparison between rates of DNA synthesis and the sizes of DNA made in cells pulse-labeled with [^{3}H]thymidine. We suggested that the single-strand breaks made during excision of pyrimidine dimers might have the same effect as direct strand breakage by ionizing radiation, which is to prevent initiation of DNA replication in clusters of replicons (4-6). If our suggestions are correct, the recovery of DNA synthesis in a cell type capable of excision repair should be different from the recovery in an excision repair-

deficient cell type. We therefore investigated DNA replication after ultraviolet irradiation of Simian Virus 40 (SV40)-transformed normal human cells and xeroderma pigmentosum (XP) group A cells. Transformed cells were chosen because of their fast rates of DNA replication, and group A XP cells were chosen because of their low rates of excision repair (7).

MATERIALS AND METHODS

Normal human (GM 637) and XP group A (XP12RO) cells were grown in Eagle's minimal essential medium with 15% fetal calf serum. Exponentially growing cultures that had been grown for several days in 0.01 μCi/ml of [^{14}C]thymidine (spec. act., 64 mCi/mmole; New England Nuclear) were drained, irradiated with 254 nm ultraviolet light at an incident dose rate of 1.3 J/m^2/sec, and grown in fresh medium. For some experiments, cultures were grown in 1-2 mM caffeine after irradiation. In our system, irradiation of human cells with 10 J/m^2 produced 0.035 ± 0.004% pyrimidine dimers, as measured by one-dimensional thin-layer chromatography (8). This corresponds to 2.0 dimers in 10^7 daltons (after correction for CC, CT, and TT dimers [9]),which is similar to the value of 2.5 *Micrococcus luteus* endonuclease-sensitive sites per 10^7 daltons reported by Paterson et al. (10) and 1.6 T4 endonuclease-sensitive sites per 10^7 daltons reported by Williams and Cleaver (9).

At various times cultures were pulse-labeled for 10-15 min with 10 μCi/ml of [^{3}H]thymidine (60 Ci/mmole); caffeine was included for those cultures grown in caffeine. Cultures were harvested immediately by trypsinization and irradiated with 1 KR of X-rays (300 kVp, G.E. Maxitron) to introduce a defined number of breaks (< 1 per 10^8 daltons) and facilitate strand unwinding (11). Cells were lysed on the top of 5-20% alkaline sucrose gradients as described previously (12) and sedimented at 20-25,000 rpm for 3-5 h. ^{3}H and ^{14}C radioactivity were determined for each fraction, and weight (M_w) and number (M_n) average molecular weights were calculated (13) with SV40 and phage λ molecular weight standards. All profiles were unimodal and had no multiple peaks (14). Because the ^{3}H pulse was short and the pool equilibration time at this concentration of [^{3}H]thymidine is about 3-10 min (Mattern, M.R. and Painter, R.B.: unpublished data), the labeling conditions produced newly synthesized molecules with ^{3}H-labeled ends. For end-labeled DNA the ^{3}H activity per fraction is approximately proportional to the number of DNA molecules per fraction, and the ^{3}H M_w corresponds approximately to the M_n. In our studies M_w was therefore used as a convenient parameter for summarizing most of our results. In studies with caffeine, however, DNA was so

reduced in size that normal chain growth rates would have produced small, uniformly ^{3}H-labeled DNA; therefore, M_n was used to summarize these experiments. Data were expressed as molecular weights relative to control values of M_w of 5.2 x 10^7 daltons and 4.7 x 10^7 daltons for GM 637 and XP12RO, respectively.

Samples of each ^{3}H and ^{14}C cell suspension analyzed on sucrose gradients were fixed in 4% $HClO_4$ at 4°C overnight and collected on Whatman GF/C glass fiber filters. $^{3}H/^{14}C$ ratios were determined by liquid scintillation counting. During the first few hours after irradiation the $^{3}H/^{14}C$ ratio (the measure of specific activity of the culture) is an accurate reflection of the rate of synthesis per cell, but at later times the entry of G_1 cells into S will cause it to become an increasingly inaccurate measure. We therefore confined our attention to the hours immediately after irradiation to minimize errors arising from population changes.

RESULTS

Ultraviolet irradiation of replicating human cells caused decline and recovery of the average size of DNA synthesized and the rate of DNA synthesis per cell (Figs. 1,2). The kinetics of decline and recovery were dose and cell-type dependent, and minimum molecular weights were observed in the first

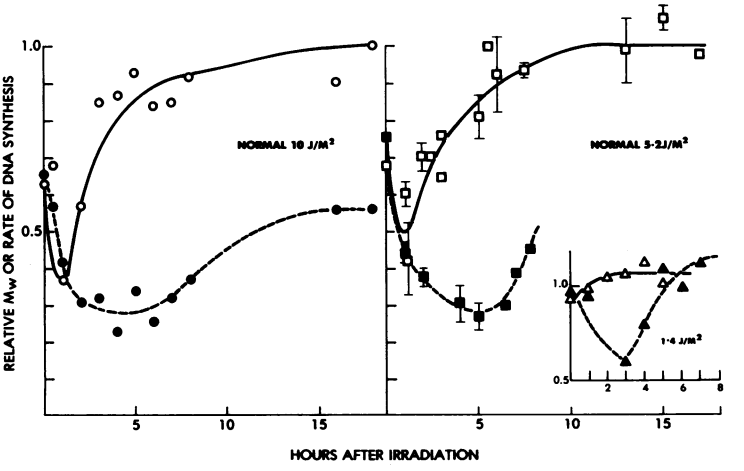

Figure 1. Relative molecular weights (△ ,□ , ○) and rates of DNA synthesis (▲ ,■ , ●) in normal (GM637) cells labeled for 10 min with [^{3}H]dThd (10 µCi/ml, 60 Ci/mmole) at various times after irradiation with 1.4, 5.2, or 10 J/m^2 UV light. Standard errors are indicated when three or more measurements were made at one time.

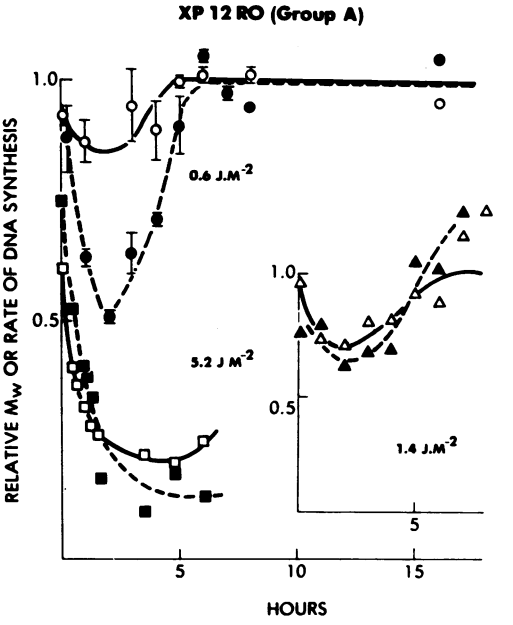

Figure 2. Relative molecular weights (△ ,□ , ○) and rates of DNA synthesis (▲ ,■ ,●) in XP12RO cells labeled for 10 min with [^{3}H]dThd (10 μCi/ml, 60 Ci/mmole) at various times after irradiation with 0.6, 1.4, or 5.2 J/m^2 UV light. Standard errors are indicated when three or more measurements were made at one time.

1-2 h (Figs. 1,2). In normal cells the rate of DNA synthesis per cell reached a minimum 3-4 h later than the relative molecular weights after doses of 1.4 - 10 J/m^2, and recovery was slower. At late times after irradiation (8 h and beyond) cells were synthesizing normal-sized DNA even though the rate of DNA synthesis per cell was about half that of controls (Fig. 1). At the lowest dose, 1.4 J/m^2, the rate of DNA synthesis decreased to about 60% of control, 3 h after irradiation, whereas the molecular weights of pulse-labeled DNA changed little, or perhaps even increased slightly. This observation is very similar to observations made after ionizing radiation (4-6). An increase in molecular weights and a decrease in rates of synthesis occur because of specific inhibition of replicon initiation by X-ray damage and consequent loss of the lower molecular weight components in the distributions of pulse-labeled DNA in sucrose gradients (4-6). In XP cells the rate of DNA synthesis per cell and the size of DNA synthesized reached their minimum at approximately the same times, and recovery was in phase (Fig. 2). At a dose

of 5.2 J/m^2 the relative reductions of DNA synthesis and molecular weights were greater in excision-defective XP12RO cells than in excision-competent GM 637 cells (Figs. 1,2).

Growth of both cell types in 1-2 mM caffeine immediately after irradiation and during pulse-labeling completely prevented the recovery in the size of DNA synthesized, but caffeine did not affect the rate of DNA synthesis per cell in normal cells (Fig. 3). This contrasts with our previous observations in untransformed normal cells, in which the effect of caffeine was much less (3). We have also observed that if the pulse-labeled DNA is allowed to extend and be linked to higher molecular weight DNA by subsequent growth in unlabeled medium, the rate of linkage is independent of the repair capacity of the cells and relatively insensitive to caffeine (14).

DISCUSSION

Our results indicate that the rates of DNA synthesis and the sizes of DNA strands in normal and XP fibroblasts undergo rapid changes after irradiation that represent complex disturbances of DNA replication caused by damage and

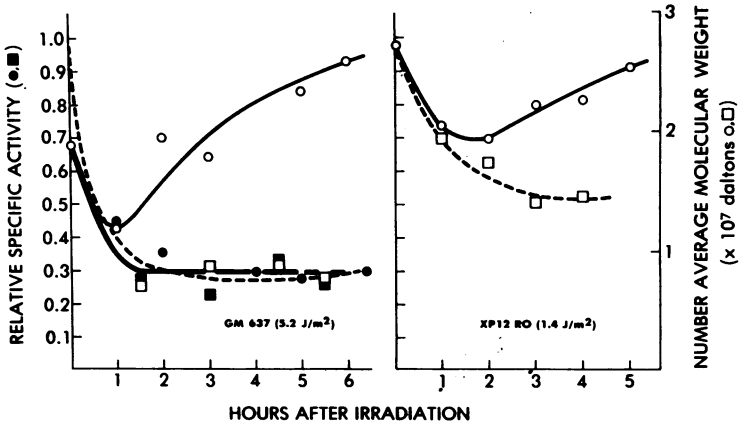

Figure 3. Relative molecular weights and specific activities in normal (GM 637) and XP12RO cells labeled for 10 min with [^{3}H]dThd (10 μCi/ml, 60 Ci/mmole) at various times after irradiation with 5.2 or 1.4 J/m^2 UV light, respectively. Cells were grown in 10^{-3} M caffeine after irradiation and during the labeling period. Data for the molecular weights were calculated from weight averages for the cells grown in the absence of caffeine and from number averages for the cells grown in caffeine (see Materials and Methods for explanation). ○, Molecular weights of cells grown without caffeine; □, molecular weights of cells grown in caffeine; ●, specific activities of cells grown without caffeine; ■, specific activities of cells grown in caffeine.

excision repair events. The main features of our results that need interpretation are (1) the greater relative inhibition of DNA synthesis and molecular weights in XP than in normal cells, (2) the lag between the recovery of DNA synthesis and molecular weights observed in normal but not XP cells, and (3) the inhibition of recovery by caffeine.

Extent of inhibition

UV irradiation initially produces the same number of photoproducts in the DNA of normal and XP cells (Thomas, G.H. and Cleaver, J.E., unpublished measurements). The greater effect of irradiation on DNA synthesis in XP cells suggests that the excision of some photoproducts between irradiation and labeling may account for the difference between these two cell types. A similar observation, based solely on rates of DNA synthesis measured by autoradiography, was made by Rudé and Friedberg (15). They noted that the extent of inhibition of DNA synthesis was correlated with the extent of cell killing by UV irradiation. In our experiments the inhibition of DNA synthesis was significantly different in the two cell types within 2-4 h after irradiation with 5.2 J/m^2 UV light. Enzymatic measurements of the excision of pyrimidine dimers suggest that within this time about 30% of the pyrimidine dimers may be lost from the DNA of excision-competent cells, but none from group A cells (10). This difference in excision may be enough to account for the difference between DNA synthesis in the two cell types, especially if some of the excision occurs preferentially near the growing points. Some evidence has been reported for preferential repair of UV and alkylated DNA damage near the growing point in normal cells (16,17).

Recovery of DNA synthesis and molecular weights

In normal and XP cells the relative molecular weights were always larger than the relative rate of DNA synthesis (Figs. 1,2). After UV irradiation, the rates of DNA synthesis recovered in phase with molecular weights (Fig. 2) in XP cells but lagged behind in normal cells (Fig. 1). Molecular weights were always above relative rates of synthesis because some newly synthesized DNA would be attached to higher molecular weight DNA synthesized before the pulse-labeling period, but the lag in recovery in normal cells compared to XP must have another cause. The major difference between these two cell types is that XP group A cells make very few incisions in their DNA after irradiation because of the reduced activity of the endonucleolytic step of excision repair (7,18). A mechanism linking the known repair capacity of these cell types and our observations on DNA synthesis after UV irradiation can be proposed if we make use of observations in cells damaged by ionizing radia-

tion (4-6). These studies demonstrated that DNA replication occurs from multiple replication origins and proceeds bidirectionally and that the origins are arranged in tandem arrays of replicating units (replicons) to form clusters that initiate together (19). Single-strand breaks at very low frequency (1 or 2 per cluster) produced by ionizing radiation (4-6), bromouracil photolysis (20), or alkylating agents (21) effectively prevent replicon initiation throughout the cluster carrying the breaks, presumably because of a relaxation in the coiled configuration of the chromatin (22,23). We propose that breaks introduced into DNA during excision repair may also prevent initiation of replication in the clusters during repair; only those clusters in which no repair occurs at the time of initiation may begin DNA replication. This mechanism explains the difference between normal and excision repair-deficient cells and points to a homeostatic mechanism that minimizes the interference between replication and excision repair that could be lethal to the cell.

Inhibition of recovery by caffeine

The recovery of DNA replication in normal and excision-defective cells (Figs. 1,2) and in untransformed normal, XP heterozygous, and XP variant cells (3) represents a steady increase in the ability of cells to replicate their DNA despite damage ahead of the replicating forks. In normal cells the recovery occurred more rapidly than the rate of dimer excision and therefore must represent a mechanism of DNA replication that allows cells to bypass dimers and other DNA damage without interruption and with increasing efficiency. In transformed cells caffeine blocked this mechanism (Fig. 3) and cells continued to synthesize DNA in fragments about the length of interdimer intervals.

We have argued (3) that the recovery of DNA replication may occur either by induction of *de novo* replication proteins, as previously suggested by Buhl et al. (24) and d'Ambrosio and Setlow (25), or by a constitutive resynthesis of replication proteins displaced by the initial irradiation. We have also demonstrated that recovery of DNA chain growth in the XP variant is abnormally slow and caffeine sensitive (3). Although definitive discrimination between the induced versus constitutive mechanisms for explaining recovery has not yet been made, several arguments, including the absence of a long lag period before recovery begins, lead us to prefer the constitutive explanation (1,3). The effect of caffeine in our present study may be explained either as an inhibition of the induction of *de novo* replication proteins, or by competition between the DNA binding properties of caffeine and constitutive rep-

lication proteins. Caffeine is known to inhibit nucleases (26) and to bind to single-stranded or distorted DNA (27), but it is not known to inhibit induced enzyme synthesis; we therefore consider its effect on DNA molecular weights in UV-damaged cells (Fig. 3) to be more readily explained by competition with a constitutive system than by an effect on a hypothetical induced system.

In conclusion, we have shown that DNA replication in UV-damaged human cells depends on the excision repair ability of the cells. There appears to be a delicate interrelationship between repair processes and the number of replicons and their elongation. A thorough understanding of the organization of DNA replication is clearly needed before we can explain all of the effects of UV damage and its repair on replication, mutagenesis, and other sequelae. Many of the phenomena subsumed under descriptive terms such as "post-replication repair" (28), "caffeine-sensitive and resistant pathways of post-replication repair" (29), and "enhanceable post-replication repair" (25) may be explainable as only perturbations in the numbers of replicons and their chain growth caused by damage and excision repair interacting with replication, rather than as specific, unique radiation recovery mechanisms.

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