Amplified RNase H Activity in *Escherichia coli* B/r Increases Sensitivity to Ultraviolet Radiation

Richard Bockrath,* Lee Wolff,* Abigail Farr* and Robert J. Crouch†

**Department of Microbiology and Immunology, Indiana University School of Medicine, Indianapolis, Indiana 46223, and*
†*Laboratory of Molecular Genetics, National Institute of Child Health and Human Development, Bethesda, Maryland 20205*

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ABSTRACT

Strains of *E. coli* B/r transformed with the plasmid pSK760 were found to be sensitized to inactivation by ultraviolet radiation (UV) and to have elevated levels of RNase H activity. Strains transformed with the carrier vector pBR322 or the plasmid pSK762C derived from pSK760 but with an inactivated *rnh* gene were not sensitized. UV-inactivation data for strains having known defects in DNA repair and transformed with pSK760 suggested an interference by RNase H of postreplication repair: *uvrA* cells were strongly sensitized, wild-type and *uvrA recF* cells were moderately sensitized and *recA* cells were not sensitized; and minimal medium recovery was no longer apparent in sensitized *uvrA* cells. Biochemical studies showed that post-UV DNA synthesis was sensitized and that the smaller amounts of DNA synthesized after irradiation, while of normal reduced size as indicated by sedimentation position in alkaline sucrose gradients, did not shift to a larger size (more rapidly sedimenting) upon additional incubation. We suggest an excess level of RNase H interferes with reinitiation of DNA synthesis on damaged templates to disturb the normal pattern of daughter strand gaps and thereby to inhibit postreplication repair.

SURVIVAL of *Escherichia coli* after exposure to ultraviolet radiation (UV) is influenced by repair or tolerance mechanisms for photoproducts in the DNA genome (HANAWALT *et al.* 1979; FRIEDBERG 1985). We have found the presence of the plasmid pSK760 in certain *E. coli* B/r strains to increase the cellular RNase H activity and to increase the rate at which survival of colony-forming ability is inactivated by UV. In this paper we describe the increased UV sensitivity associated with transformation by pSK760 and consider how this might result from an effect by amplified RNase H activity on repair or tolerance mechanisms.

The plasmid pSK760 contains the entire *E. coli rnh* gene and a fragment of *dnaQ* (KANAYA and CROUCH 1983). The two genes are transcribed in opposite directions from overlapping promoter regions (MAKI, HORIUCHI and SEKIGUCHI 1983). The plasmid or relevant gene insert (760 base pairs) has been used to complement defective *rnh* alleles in the bacterial genome without producing any characteristic different from those of the wild-type phenotype (OGAWA *et al.* 1984; KANAYA and CROUCH 1984).

RNase H activity degrades RNA of RNA-DNA hybrids (STEIN and HAUSEN 1969; HAUSEN and STEIN 1970). It may have several functions (CROUCH and DIRKSEN 1982), but clearly is important in the initiation of DNA replication. Studies with wild-type and defective alleles of *rnh* in *E. coli* have shown that decreases in cellular RNase H activity stimulate initiation pathways for DNA synthesis. For example, *in vitro* initiation of ColE1 plasmid replication at the *ori* site requires polymerase I and processing of the RNA primer by RNase H (ITOH and TOMIZAWA 1980). Yet, ColE1 plasmids replicate in *rnh* defective cells (OGAWA and OKAZAKI 1984); plasmids with a base-pair change near *ori* that do not replicate in wild-type cells do replicate in *rnh* cells (NAITO *et al.* 1984); and the inhibition of ColE1 replication in *polA* cells is suppressed in *rnh* defective cells (KOGOMA 1984). Plasmid replication in the absence of RNase H has been attributed to an *alternative initiation* pathway that may utilize an extended R loop for entry of the replisome (KOGOMA 1984). CHAMPOUX and MCCONAUGHY (1975) have shown RNA polymerase to produce stable RNA-DNA hybrids on supercoiled duplex DNA, and this type of RNA transcript, persisting in the absence of RNase H, could prime DNA synthesis. Alternative initiation also may account for constitutive stable DNA synthesis and suppression of *dnaA* and *oriC* defects by the absence of RNase H activity (OGAWA *et al.* 1984). KOGOMA, SUBIA and VON MEYENBURG (1985) recently have shown that normal levels of RNase H produce specificity for initiation at *oriC* by eliminating alternative RNA primed sites, rather than by actively directing initiation to *oriC*. Specific alternative initiation sites for constitutive stable DNA synthesis have been delineated (DEMASSY, FAYET and KOGOMA 1984).

Results to be described here are consistent with

inhibition by amplified RNase H activity of the tolerance *postreplication repair* mechanism that normally functions to reconstitute continuous DNA from the discontinuous DNA (gapped daughter strands) newly synthesized after irradiation (RUPP and HOWARD-FLANDERS 1968; FRIEDBERG 1985). The data are of three kinds: UV inactivation curves for different repair-deficient strains with and without the plasmid-borne rnh^+ gene, the effect of pSK760 on post-UV DNA synthesis and the effect of pSK760 on postreplication repair, as determined by sedimentation of cellular DNA in alkaline sucrose gradients. It seems possible that the reinitiation of DNA synthesis integral to the formation of gapped daughter strands, which are the substrate for postreplication repair, is analogous, at least in part, to alternative initiation and is atypical when RNase H activity is amplified.

MATERIALS AND METHODS

Bacterial strains and plasmid DNA: All results described here with *E. coli* B/r strains. Strains WU3610-11 and WU-11 were tyrosine-requiring auxotrophs differing only in that the latter was *urvA* excision repair defective (BOCKRATH, HARPER and KRISTOFF 1980). WP10 and $WP2_s$-RF were tryptophan-requiring auxotrophs derived from WP2 and, respectively, *recA* and *uvrA recF* (E. M. WITKIN, personal communication). Plasmid DNA was prepared from *E. coli* K-12 hosts either by lysing the cells with lysozyme (2 mg/ml), NaOH (0.2 N) and SDS (1%) and purifying the DNA in a cesium chloride-ethidium bromide gradient (MANIATIS, FRITSCH and SAMBROOK 1982), or by treating the cells only with NaOH and SDS and utilizing the supernatant directly for ethanol precipitation (ISH-HOROWICZ and BURKE 1981).

Growth and transformation: Cultures were grown overnight in A-O minimal salts medium (BOCKRATH and PALMER 1977) containing tyrosine (20 μg/ml) and limiting glucose (0.2 mg/ml). These were supplemented to full glucose (4 mg/ml) in the morning, incubated for about 1 hr (the *morning culture*) and diluted at least 30-fold into fresh medium of the same composition. After growth to about 2×10^8 cells/ml, the bacteria were harvested by centrifugation and then resuspended in A-O to original volumes for UV inactivation. For transformation with plasmid DNA, overnight cultures were diluted into ZY broth (see below) and grown to about 10^8 cells/ml, then 5-ml samples were centrifuged to pellet cells for $CaCl_2$ treatment, after the method of MANIATIS, FRITSCH and SAMBROOK (1982) with modification by S. LARSEN (personal communication). The cells were resuspended in ice-cold 50 mM $CaCl_2$ (0.5 ml/tube) and were held on ice for 10 min. They were again pelleted and resuspended in TMC (50 mM $CaCl_2$, 10 mM Tris, pH 7.6, 10 mM $MgCl_2$) at 0.2 ml/tube. Samples of plasmid DNA were added to some of these tubes, and all were held on ice for 20 min. After this, the cells were heat shocked at 42° for 1.5 min, then 1.0 ml of ZY broth was added to each tube, and they were incubated for 60–90 min. To isolate transformants, the cells in each tube were pelleted by centrifugation, resuspended in 0.1 ml of ZY broth and spread on nutrient agar medium containing ampicillin (200 μg/ml). All culture growth was at 37° in a New Brunswick reciprocal water bath shaker.

Irradiation and assays: UV was from a low-pressure mercury discharge lamp. The principal emission was at 254 nm, and the fluence rate was quantitated at this wavelength with an International Light radiometer. Cell suspensions were stirred in large watch glasses during irradiation. One-tenth milliliter samples were diluted in A-O, and 0.1-ml samples of diluted cells were spread on a standard defined agar medium consisting of A-O, glucose, leucine and a small amount of nutrient broth (0.2 mg/ml) (BOCKRATH and PALMER 1977). YENB (SHARMA, SARGENTINI and SMITH 1983) and ZY, which was NZCYM (MANIATIS, FRITSCH and SAMBROOK 1982) without casamino acids or $MgSO_4$ were used as rich media. In some cases, ampicillin solution (40 mg/ml) was added a few hours before use by spreading 0.05 ml per plate (*ca.* 25 ml of agar medium). Viable titers were then calculated from the numbers of colonies appearing after incubations at 37° for 16–24 hr.

Labeling cells with ^{3}H-thymidine: For the incorporation experiments, 0.5 ml of a morning culture (see above) was added to 10 ml of A-O plus 1.0 ml of a premix composed of six solutions: 2.0 ml of tyrosine (0.4 mg/ml), 0.4 ml of glucose (400 mg/ml), 0.4 ml of deoxyadenosine (20 mg/ml), 0.4 ml of thymidine (0.0735 mg/ml), 0.4 ml of ^{3}H-thymidine (about 40 Ci/mmol, 1 mCi/ml) and 0.4 ml of A-O. After growth, centrifugation, resuspension in A-O and irradiation, 1.5-ml samples were incubated with 0.150 ml of the same premix. When cells were pulse-labeled for analysis on alkaline sucrose gradients, cells growing in standard defined medium were transferred directly from the culture to the irradiation watch glass. Samples (1.0 ml) after irradiation (or mock irradiation) were returned to culture tubes, each containing 0.100 ml of ^{3}H-thymidine and 0.010 ml of deoxyadenosine (both as above). Incorporation was measured as material insoluble in cold 5% trichloroacetic acid (TCA) determined of samples soaked into Whatman 3MM discs or paper strips (FONG and BOCKRATH 1977).

Alkaline sucrose gradient centrifugation: The procedures were from RUPP and HOWARD-FLANDERS (1968) as described previously (FONG and BOCKRATH 1977), but with two modifications. First, a three-quarter milliliter of labeled cells was centrifuged, and the cells were resuspended in 0.75 ml of the chilled Tris buffer; 0.45 ml of this was discarded, leaving 0.3 ml for treatment as previously described. Smaller volumes of pelleted cells frequently dissociated as the supernatants were removed. Second, the gradient tubes were spun at 10,000 rpm in an SW 50.1 rotor for 16.5 hr.

RESULTS

Transformants of strains with the plasmid pSK760 had elevated levels of RNase H activity. RNase H activities were determined by procedure B of CARL, BLOOM and CROUCH (1980), but polyrA:polydT was used as substrate. The averages of results of three independent determinations showed WU-11, WU-11/pBR322 and WU-11/pSK760 to have relative activities of 1.00, 0.78 and 12.2, respectively. The increased synthesis of RNase H when pSK760 was present was similar to that seen with *E. coli* K-12 strains transformed with pSK760 (KANAYA and CROUCH 1983; BIALY and KOGOMA 1986). Thus, transformants carrying pSK760 will be denoted here as rnh^{++}. Control cells with normal levels of RNase H activity consisted of the original strains without plasmids and transformants carrying the vector pBR322 (without inserted DNA) or the plasmid pSK762C, a derivative of pSK760 in which the *rnh* gene was inactivated by

deletion of internal bases. This deletion was by S1 nuclease digestion at the *Bam*HI site and eliminated expression of RNase H activity. No differences among control strains were noted in any of the following tests.

Effects on survival after UV exposure: The shape of the survival curve for WU3610-11 rnh^{++} was biphasic, showing single-hit exponential inactivation for a majority of the cell population and multi-hit inactivation for a small portion of the population. In contrast, the survival curve for control cells showed the typical multi-hit inactivation (Figure 1). Therefore, at small fluences the plasmid pSK760 sensitized WU3610-11 to a considerable extent, although in the exponential regions of inactivation at larger fluences (regions of straight lines in a semilogarithmic plot) there was little difference between WU3610-11 rnh^{++} and the control cells in the rate of inactivation.

The biphasic nature of the survival curve for WU3610-11 rnh^{++} would result if the plasmid pSK760 caused single-hit exponential inactivation, but some of the cells had lost the plasmid. Samples of colonies on the titer plates were tested for growth on medium with ampicillin to determine the proportion carrying the plasmid. Samples from surviving titers of the control cells were 95–100% ampicillin-resistant at all points, but samples of WU3610-11 rnh^{++} showed a decreasing proportion of ampicillin-resistant colonies on titer plates for cells receiving larger UV fluences. This suggested that cells in the WU3610-11 rnh^{++} culture were heterogeneous and that cells carrying the plasmid were relatively more sensitive to UV. Using the decreasing fractions resistant to ampicillin times the indicated titers at every point produced a measure of survival for ampicillin-resistant WU3610-11/pSK760 cells, which was a straight, single-hit line (dashed line, Figure 1). Thus, the biphasic survival curve seen for a suspension of cells from a WU3610-11 rnh^{++} culture could be resolved: about 90% of the initial viable population was ampicillin-resistant (carried pSK760) and showed simple exponential inactivation, and 10% was essentially nontransformed WU3610-11 showing a shouldered inactivation curve as seen with control cells.

Inactivation of the strain WU-11 rnh^{++}, which was analogous to WU3610-11 rnh^{++} but defective in excision repair, again suggested biphasic survival, and a single-hit component could be determined of ampicillin-resistant colonies as described above (Figure 2, circle symbols with broken or dashed lines). In the *uvrA* strain, however, pSK760 caused elimination of the shoulder and a substantial increase in the exponential rate of inactivation (about threefold). Thus, the effect of pSK760 to sensitize cells was not removed in *uvrA* cells and therefore seemed unlikely to result

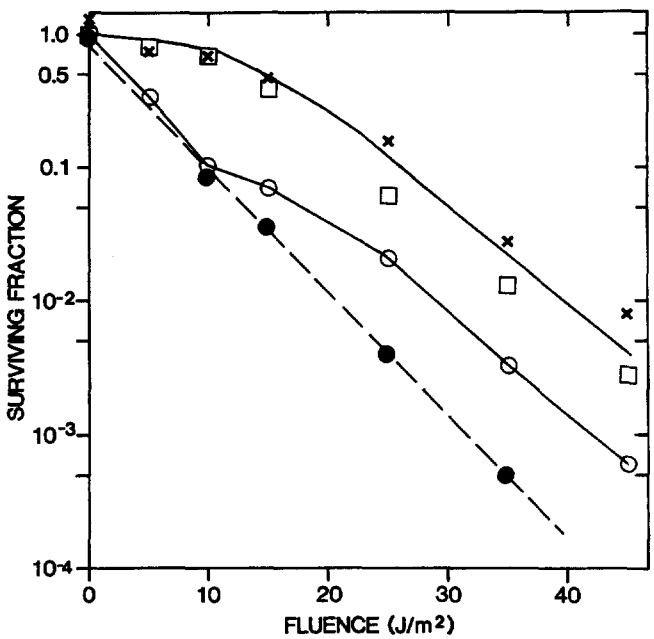

FIGURE 1.—Effect of plasmid pSK760 on the post-UV survival of repair-normal cells. Typical survival curves are indicated as the viable titers for three strains, WU3610-11 (x symbols), WU3610-11/pBR322 (□) and WU3610-11 rnh^{++} (○), plated on the standard defined agar medium after incremental exposures to UV. Colonies from the titer plates for the third strain were isolated and tested on nutrient agar containing ampicillin to determine the fraction ampicillin-resistant at each point. The original surviving titers times these fractions determined the surviving titers for ampicillin-resistant WU3610-11/pSK760 (●). The broken line (– –) through these latter points is a straight line fitted by the method of least squares.

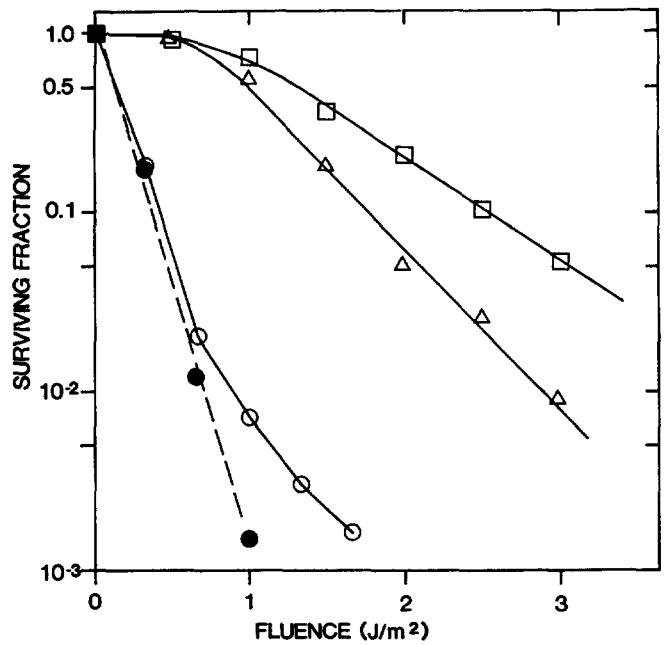

FIGURE 2.—Effect of plasmid pSK760 on the post-UV survival of *uvrA*-defective cells. Typical survival curves are indicated as the viable titers for two strains, WU-11/pBR322 (□) and WU-11 rnh^{++} (○), plated on the standard defined agar medium after incremental exposures to UV. Survival of WU-11/pBR322 plated on rich medium (YENB) is also shown (△). Ampicillin-resistant WU-11/pSK760 were determined as described under Figure 1 (●). The broken line (– –) through these latter points is a straight line fitted by the method of least squares.

from a specific inhibition of excision repair. Moreover, since the effect of pSK760 was greater in *uvrA* cells than in excision proficient cells, the effect of pSK760 may be principally an inhibition of a repair mechanism other than excision repair.

Figure 3 shows inactivation curves for five strains assayed for survival on rich ZY medium and indicates the effects of the plasmid pSK760. To avoid biphasic survival curves, the assay medium contained ampicillin so that only plasmid-carrying ampicillin-resistant cells could grow. For example, survival of cells from WU-11 *rnh*$^{++}$ cultures is no longer biphasic (*cf.* Figures 2 and 3). Survival of a *uvrA recF* derivative of *E. coli* B/r is sensitized by pSK760, but survival of a *recA* derivative is not sensitized.

Minimal medium recovery is a genetically determined mechanism by which a portion of UV-irradiated, excision repair-defective *E. coli* survive on minimal defined media, but not on rich media (SHARMA, SARGENTINI and SMITH 1983). This is shown in Figure 2 in the differences between the two continuous lines drawn for survival of the control cells WU-11/pBR322. Survival is greater on defined agar than on rich YENB agar. However, sensitized inactivation seen with WU-11 *rnh*$^{++}$ shows no enhanced survival on minimal search plates (compare data in Figures 2 and 3). On YENB agar, survival is actually slightly greater than on minimal defined agar medium (data not shown).

Post-UV DNA synthesis: To consider whether the presence of pSK760 affected postreplication repair, first DNA synthesis was measured after various UV exposures to learn what fluence would be effective but not excessive. The excision repair-defective strain carrying pSK760 (WU-11 *rnh*$^{++}$) and control cells were uniformly labeled in DNA with ^{3}H-thymidine, exposed to UV and returned for post-UV metabolism in medium with the same labeling mixture. Incorporated ^{3}H-thymidine increased exponentially in cultures without UV exposure and exponentially, but less rapidly, after increments of UV exposure (Figure

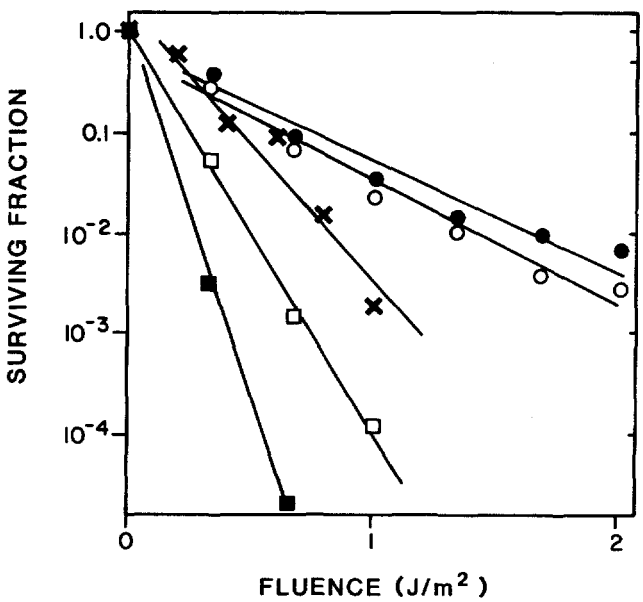

FIGURE 3.—Effect of plasmid pSK760 on the post-UV survival of *recA* and *uvrA recF* defective cells. UV-inactivation is shown for five strains plated on rich ZY agar: WP10(*recA*) *rnh*$^{++}$ (●), WP10(*recA*)/pSK762C (○), WU-11(*uvrA*) *rnh*$^{++}$ (x symbols), WP2$_S$-RF(*uvrA recF*) (□) and WP2$_S$-RF(*uvrA recF*) *rnh*$^{++}$ (■). All strains with plasmids were assayed on agar containing ampicillin (see METHODS). The straight lines are fitted to the data sets (average points from two or three experiments) by the method of least squares.

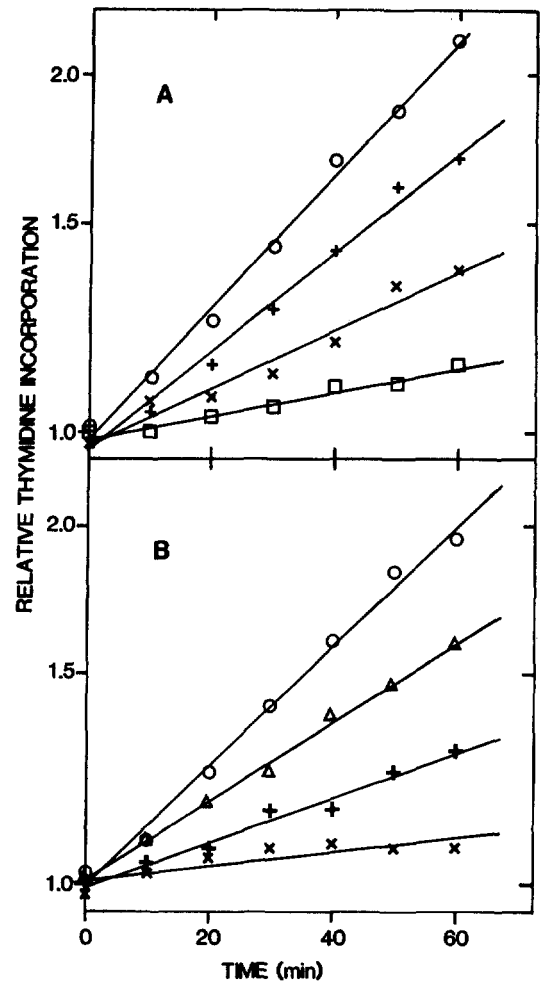

FIGURE 4.—Post-UV DNA synthesis in WU-11/pBR322 and WU-11 *rnh*$^{++}$ cells. Cells were labeled for 3–4 generations with ^{3}H-thymidine, collected by centrifugation and resuspended in A-O buffer for UV exposure, and incubated again in the presence of ^{3}H-thymidine to produce the relative increases recorded here (see METHODS). Points representing the average of two experiments are shown: A, WU-11/pBR322, after zero (○), 1 (+), 2 (x) and 4 (□) J/m^2; B, WU-11 *rnh*$^{++}$, after zero (○), 0.5 (△), 1.0 (+) and 2.0 (x) J/m^2. The value 1.0 on the ordinate represents the average of the four duplicate radioactivity measurements for 0-time incorporation (each >10,000 cpm), and the measurements at 10–60 min for each sample culture are then plotted relative to this value. All lines are straight lines fitted by the method of least squares to the data for 0–60 min. The slopes for these lines (top to bottom) are k = 0.0128, 0.00949, 0.00562, 0.00228, 0.0115, 0.00767, 0.00425 and 0.00137 min^{-1}, using an exponential function with the natural log base.

4). Similar exponential lines resulted when control cells were exposured to fluences twice those used for WU-11 rnh^{++}. Data not given here showed 0.5 J/m^2 to have very little effect on DNA synthesis in control cells and 4.0 J/m^2 to stop DNA synthesis in WU-11 rnh^{++}, with larger fluences yielding degradation. DNA synthesis measured by incorporated ^{3}H-thymidine after irradiation was about twice as sensitive to UV in WU-11 rnh^{++}, the cells having elevated levels of RNase H.

The same conclusion was apparent in a more quantitative analysis. UV exposure seemed to decrease a first-order rate constant for post-UV DNA synthesis. If the log of the rate constants (see slopes k in legend of Figure 4) were plotted against UV fluence, simple exponential inactivations could be determined with mean lethal fluences of 2.4 and 1.0 J/m^2 for WU-11/pBR322 and WU-11 rnh^{++}, respectively (not plotted here). This indicated that post-UV DNA synthesis was at least twofold more sensitive in cells with elevated RNase H activity.

The strain WU-11 was not auxotrophic for thymidine; therefore, differences in incorporation of ^{3}H-thymidine in control and rnh^{++} strains may have resulted because synthesis of endogenous precursor was affected differently by UV in the two strains. If the internal pools were different after irradiation, the magnitudes of the differences in post-UV incorporation should change with changes in the exogenous concentration of thymidine. However, when the concentration of total thymidine present in the labeling cultures was reduced fivefold (from 0.725 to 0.145 μg/ml), the results were still congruent with those of Figure 4 (data not shown). Thus, the differences in incorporated radioactivity seemed to reflect differences in DNA synthesis and not differences in the endogenous specific activities.

To study postreplication repair, DNA synthesized immediately after a UV fluence of 1.5 J/m^2 was labeled with ^{3}H-thymidine, and the relative size of this labeled DNA was estimated from a profile of radioactivity after sedimentation in an alkaline sucrose gradient. Small DNA synthesized in WU-11 rnh^{++} immediately after UV-irradiation sedimented less rapidly than large genomic DNA (Figure 5A) and was found in a position similar to that of small DNA synthesized in control cells (compare Figure 5A and B). The amount of labeled material synthesized after UV in WU-11 rnh^{++} cells was less than the amount in control cells as anticipated from the thymidine incorporation data.

When cells were labeled immediately after UV and then allowed additional incubation for recombinational repair before harvest (a pulse-chase experiment), labeled material previously positioned as small molecular weight DNA was found shifted toward the

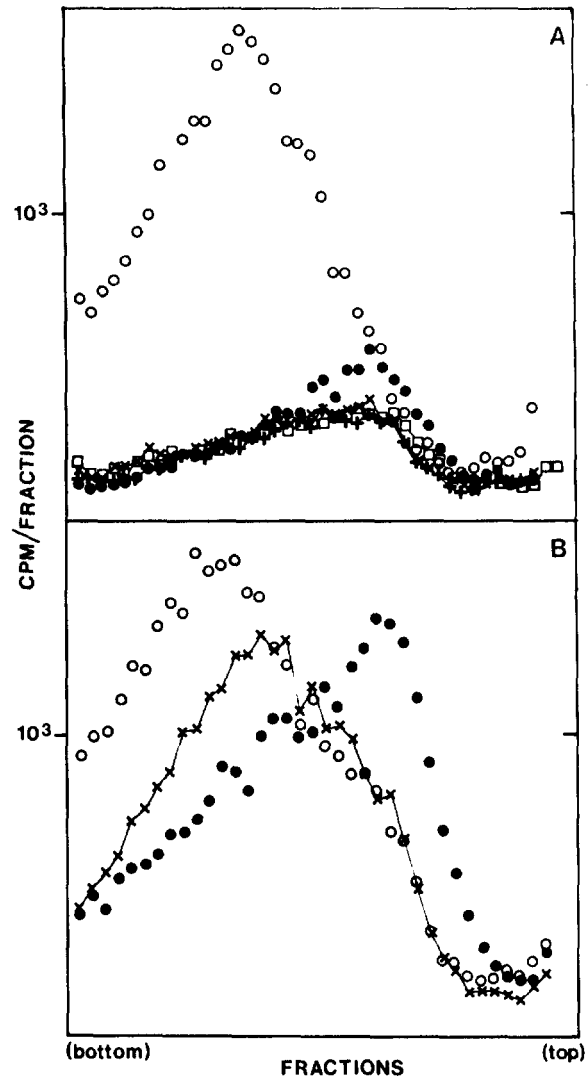

FIGURE 5.—Distributions in alkaline sucrose gradients of DNA labeled after UV of 1.5 J/m^2. Cells were labeled for 20 min with ^{3}H-thymidine after no UV exposure (○), immediately after UV (●) and after UV with an additional incubation (*chase*) (see METHODS). The chase incubations (data points with connecting lines) were for 10 min (+), 15 min (x) or 20 min (□). A, Data with WU-11 rnh^{++}: the summation of primary counts per minute data for open circles, fractions 1–32, was 27,425 cpm. B, Data with WU-11/pSK762C: the summation of primary counts per minute data for open circles, fractions 1–32, was 20,803 cpm. To accommodate small differences in the cell densities of the two experimental cultures, the plotted counts per minute values in each panel were adjusted by respective factors (approximately 1.0) to make the integrals of the open circle peaks (no UV) the same value.

position of large genomic DNA in control cells (Figure 5B) but this shift was not found with WU-11 rnh^{++} cells (Figure 5A). When longer chase periods were used than those shown here the amount of labeled DNA recovered on the gradient was insufficient to define a peak. Control data with WU-11/pBR322 (not shown) were similar to control data with WU-11/pSK762C shown here.

When the cells were exposed to 1.0 rather than 1.5 J/m^2, the amount of post-UV-labeled material in the gradient was relatively greater and it sedimented

closer to the position of large genomic DNA (Figure 6). With WU-11 *rnh*$^{++}$, the post-UV-labeled DNA again did not shift toward the position of genomic DNA during a 15- or 20-min chase, whereas with control cells the shift was evident (compare connected points, upper and lower regions of Figure 6). Thus, in cells sensitized to UV by the plasmid pSK760, there was less post-UV DNA synthesis, but the size of the DNA as indicated by position in the gradient was similar to that appearing in normal cells. The small molecular weight DNA synthesized after UV in *rnh*$^{++}$ cells, however, did not shift toward the size of genomic DNA during a chase period as is normal during post-replication repair.

DISCUSSION

Following our first observation of UV sensitization in wild-type cells by the plasmid pSK760 (Figure 1), we sought to localize the effect in some aspect of explicit DNA repair or damage tolerance. A brief survey of inactivation of colony-forming ability with different repair-deficient strains, with or without the plasmid pSK760, was undertaken to determine whether sensitization might be eliminated when a particular aspect of repair was genetically defective. There was a large sensitization effect in excision-defective cells (Figure 2) and no effect in *recA* cells (Figure 3), suggesting that amplified RNase H activity might inhibit one or more of the survival mechanisms dependent on wild-type *recA* function.

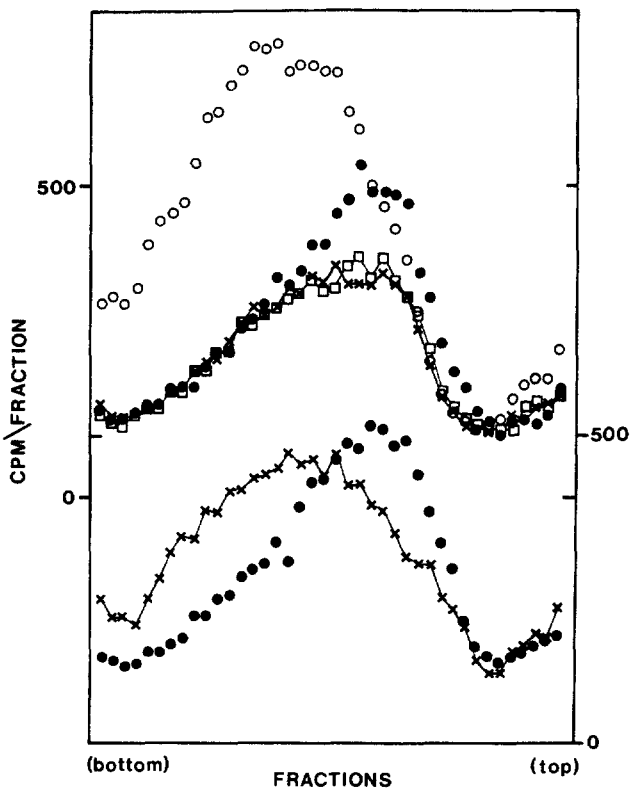

FIGURE 6.—Distributions in alkaline sucrose gradients of DNA labeled after UV of 1.0 J/m^2. Distributions of pulse-labeled DNA in alkaline sucrose gradients were determined as described under Figure 5, but the UV fluence was 1.0 J/m^2. The symbols are the same as in Figure 5: no UV (○), post-UV (●), post-UV with 15-min chase (×) and post-UV with 20-min chase (□). The upper four data sets and left ordinate are for WU-11 *rnh*$^{++}$; the lower two data sets and right ordinate are for WU-11/pBR322 (genomic DNA is not shown).

Two features of the survival data were consistent with an impairment of postreplication repair. The sensitized survival curves were distinctly single-hit (without the typical shoulder), which is a characteristic of reduced postirradiation recombination (POLLARD, BRONNER and FLUKE 1986), and minimal medium recovery was no longer apparent. Minimal medium recovery is known to result from a recombination activity that is blocked by amino acids in rich medium (SHARMA and SMITH 1983). The fact that *recF*-defective cells were still sensitized (Figure 3) suggests that the *recBC* rather than the *recF* pathway for recombination is affected by excess RNase H (HORII and CLARK 1973; WANG and SMITH 1983).

The data about DNA synthesis (Figure 4) were the first to show an effect by the plasmid pSK760 and the accompanying overproduction of RNase H on post-UV DNA metabolism. UV more efficiently inhibited post-UV DNA synthesis in WU-11 *rnh*$^{++}$ cells than in control cells. The data on post-UV recombinational repair, as visualized in the profiles for pulse-labeled DNA in alkaline sucrose gradients (Figures 5 and 6), then confirmed that relatively smaller amounts of DNA were synthesized in WU-11 *rnh*$^{++}$ cells after UV and indicated two features about this DNA. It sedimented more slowly than genomic DNA, as though of smaller molecular weight, being similar to the analogous DNA in control cells in this respect. However, it did not shift to a more rapidly sedimenting material after additional cell incubation, which is characteristic of postreplication repair (RUPP and HOWARD-FLANDERS 1968; FRIEDBERG 1985) and was evident in control cells.

Together, these details seem consistent with the following idea. Normal DNA replication is semidiscontinuous, with the *leading* strand synthesized continuously in the direction of replication fork migration and the *lagging* strand constructed by retrograde synthesis of short fragments that are subsequently ligated to form a continuous strand (KORNBERG 1980). Possibly, the recovery of DNA synthesis during replication with damaged template strands, which must occur frequently to produce gapped daughter strands, utilizes dissimilar mechanisms for the leading and the lagging strands (SARASIN and HANAWALT 1980). While reinitiation of the lagging strand would be a recurring feature in retrograde synthesis and might without great difficulty accommodate a lesion in the template for that strand, reinitiation in the leading

strand may require special functions at least in part similar to the alternative initiations associated with the first initiation of DNA synthesis on a replicon (see Introduction). HILLENBRAND and STAUDENBAUER (1982) found that large concentrations of RNase H eliminated the *in vitro* nonselective stimulation of DNA synthesis on plasmids primed by RNA and removed RNA primers at sites other than those at the origin. If reinitiation of the leading strand past damage in the template for this strand utilized R loops in which the RNA was unaffected by normal cellular concentrations of RNase H, perhaps protected in equilibrium with other proteins (LINDAHL and LINDAHL 1984), amplified levels of RNase H activity might destroy these initiation points. In consequence in *rnh*$^{++}$ cells some template damage may be more frequently a block beyond which no synthesis follows, and the distribution of daughter strand gaps between the leading and lagging strands may be asymmetrical to the norm, thereby disrupting postreplication recombination.

Regardless of a satisfactory model, the data described here suggest an effect by excess levels of RNase H activity on cell survival after DNA damage and on postreplication repair. S. CASAREGOLA, M. A. KHIDHIR and I. B. HOLLAND (personal communication) have noted repression of an *rnh*::*lacZ* fusion after UV exposure (but, see also BIALY and KOGOMA 1986) that suggests a decrease in *rnh* expression is part of the mechanism for DNA synthesis recovery (KHIDHIR, CASAREGOLA and HOLLAND 1985). This observation again implicates RNase H activity in post-UV DNA repair and is consistent with our idea that excess RNase H activity can interfere with aspects of post-UV DNA synthesis essential to postreplication repair.

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