B15

Reprinted From:
Advances in Radiation Research, Biology and Medicine
(J.F. Duplan and A. Chapiro, eds.), Vol. 1, pp. 83-93,
Gordon and Breach, London (1973)

# GENETIC CONTROL AND CHEMICAL INHIBITION OF THE REPAIR OF RADIATION DAMAGE TO BACTERIAL DNA*

DANIEL S. KAPP and KENDRIC C. SMITH

*Department of Radiology, Stanford University School of Medicine, Stanford, California 94305, U.S.A.*

Mutations which affect the ability of cells to undergo genetic recombination have been mapped at three loci. These have been termed *recA*, *recB* and *recC*. The *recA* mutants have recombination efficiencies of about $10^{-4}$ compared to a value of 1.0 for "wild-type" cells. The *recB* and *recC* mutants show an intermediate deficiency of genetic recombination of about $10^{-2}$ [12]. An ATP dependent exonuclease has been isolated as the product of the *recB* and *recC* genes [9]. The *recA* gene product is not known but is presumed to act to control the exonucleolytic function of the *recB* and *recC* genes. *RecA* mutants show a high degree of DNA breakdown and are called "reckless" mutants while *recB* and *recC* mutants show a less than normal breakdown and are called cautious mutants [4]. *RecA* strains are UV-stable at doses that are demonstrably mutagenic in *rec*$^+$ derivatives, whereas *recC* strains show a reduced mutability compared with *rec*$^+$ cells [13]. *RecA* strains are sensitive to killing by UV-irradiation and are very sensitive to killing by X-irradiation. The *recB*

*This investigation was supported by USPHS research grant CA-02896, research program project grant CA-10372, and research career development award CA-3709 (to K.C.S.) from the National Cancer Institute and a Medical Scientist Fellowship (to D.S.K.) from the Life Insurance Medical Research Fund.

and *recC* mutants show intermediate sensitivities to radiation killing. These properties are summarized in Table 1.

Table 1. Comparison of *Rec* Mutants of *E.coli* K-12

| | Wild Type | *recA* | *recB* | *recC* |
|---|---|---|---|---|
| Recombination ability [12] | 1 | $\sim 10^{-4}$ | $\sim 10^{-2}$ | $\sim 10^{-2}$ |
| DNA Breakdown [4] | Normal | Reckless | Cautious | Cautious |
| UV Mutability [13] | + | - | ± | + |
| UV Sensitivity (Survival: 63 ergs/mm$^2$) | 79% | 0.06% | | 14% |
| MMR (on *uvr*⁻ mutants) (UV) [3] | + | - | - | - |
| Post-Republication Repair (U.V.) [11] | + | - | + | + |
| X-Ray Sensitivity ($D_{37}$, krd) [6] | 5.7 | 1.1 | 1.7 | 1.7 |
| Repair of X-Ray-Induced Single-Strand Breaks [6] | + | - | ± | ± |

Because *rec* mutants are sensitive to radiation killing it was suspected that certain enzymatic steps in genetic recombination might be common to a system for the repair of radiation damage. Rupp and Howard-Flanders [10] reported that after UV-irradiation cells deficient in

the system for the excision repair of UV-induced DNA lesions (*uvr* mutants) synthesized DNA in short pieces compared with the DNA synthesized in the unirradiated cells during the same time period. With continued incubation, however, these short pieces of DNA assumed the same sedimentation characteristics in alkaline sucrose gradients as those of the control DNA. These observations provided the basis for suggesting that, during DNA synthesis, *rec*$^+$ cells by-passed radiation damage in the parental strands and the resultant gaps in the newly synthesized daughter strands were subsequently repaired by some type of sister strand exchange.

Smith and Meun [11] have investigated the genetic control of the post-replication repair of UV-induced damage. *RecA* mutants show no ability to elongate the short pieces of DNA synthesized after 63 ergs/mm$^2$ (254 nm). *RecB* and *recC* mutants, however, show no deficiency in this respect at this radiation dose. Therefore, the exonuclease coded for by the *recB* and *recC* genes does not appear to be necessary for the gap filling step.

Ganesan and Smith [2] have observed that cells defective in excision repair still exhibit an ability to recover from radiation damage. These cells show a much higher survival on minimal medium than on complex medium and the response has been called minimal medium recovery (MMR). A mutation at *recA*, *recB* or *recC* completely abolishes this phenomenon [3]. The biochemical basis of MMR is not known except for the finding and although complex medium added to a culture of a *uvr* mutant after UV-irradiation diminishes the viability of the culture, it appears to have no effect on the filling of gaps in the DNA synthesized after UV-irradiation (Smith, K.C., unpublished observations). The complex medium therefore must adversely affect some step in this post-replication repair system other than the gap-filling step or may affect some new recovery mechanism.

Mutations in the *rec* genes produce a 3 to 5 fold increase in X-ray sensitivity (see Table 1) while a *uvrB5* mutation has little if any effect on X-ray sensitivity. We have investigated the effects of these mutations on

the repair of X-ray induced damage [6]. X-irradiation of DNA produces both base damage and chain breaks. Chain breaks are more easy to assay than is base damage and the killing of *E.coli* by X-rays has been correlated with the production of double-chain breaks [5]. Single-chain breaks have been postulated to be of importance in those strains unable to repair them [5]. *Rec*$^+$ cells (including those carrying the *uvrB5* mutation) can efficiently rejoin X-ray induced single-strand breaks in their DNA whereas *recA* mutants show little if any ability to repair these breaks. *RecB* and *recC* mutants show some repair (after 22 krd) consistent with their intermediate sensitivity to killing. The sensitivity of *rec* mutants by X-irradiation thus appears to correlate with their deficiency in ability to repair single-chain breaks [6].

We have found that an unidentified impurity in one brand of hydroxyurea (Nutritional Biochemicals Corp.) is a potent and selective inhibitor of the repair of X-ray induced single-chain breaks [7]. At concentrations of the impure hydroxyurea (0.1 M) that are only slightly toxic to unirradiated *E.coli*, a 1 to 2 hundred-fold additional killing of irradiated (17.6 krd) cells is produced (Fig. 1). Alkaline sucrose gradient studies show that under these conditions there is permanent inhibition of the rejoining X-ray induced single-chain breaks. Chromatographically pure hydroxyurea (E.R. Squibb and Sons; K & K Laboratories) does not show these effects on viability or chain-break repair [7].

While potentiating X-ray induced killing in *rec*$^+$ cells and inhibiting the repair of single-chain breaks, impure hydroxyurea has little or no effect on the viability of X-irradiated *recA* cells (Fig. 2). Thus, for cells already unable to repair single-chain breaks, the administration of a drug that inhibits chain-break repair does not have any additional effects on viability.

The impure hydroxyurea also potentiates the killing of wild-type cells by UV-radiation (Wilcockson, J., Gray, M.W., Kapp, D.S. and Smith, K.C., unpublished observations). Preliminary experiments, however, indicate that after 63 ergs/mm$^2$ the drug does not prevent the gap

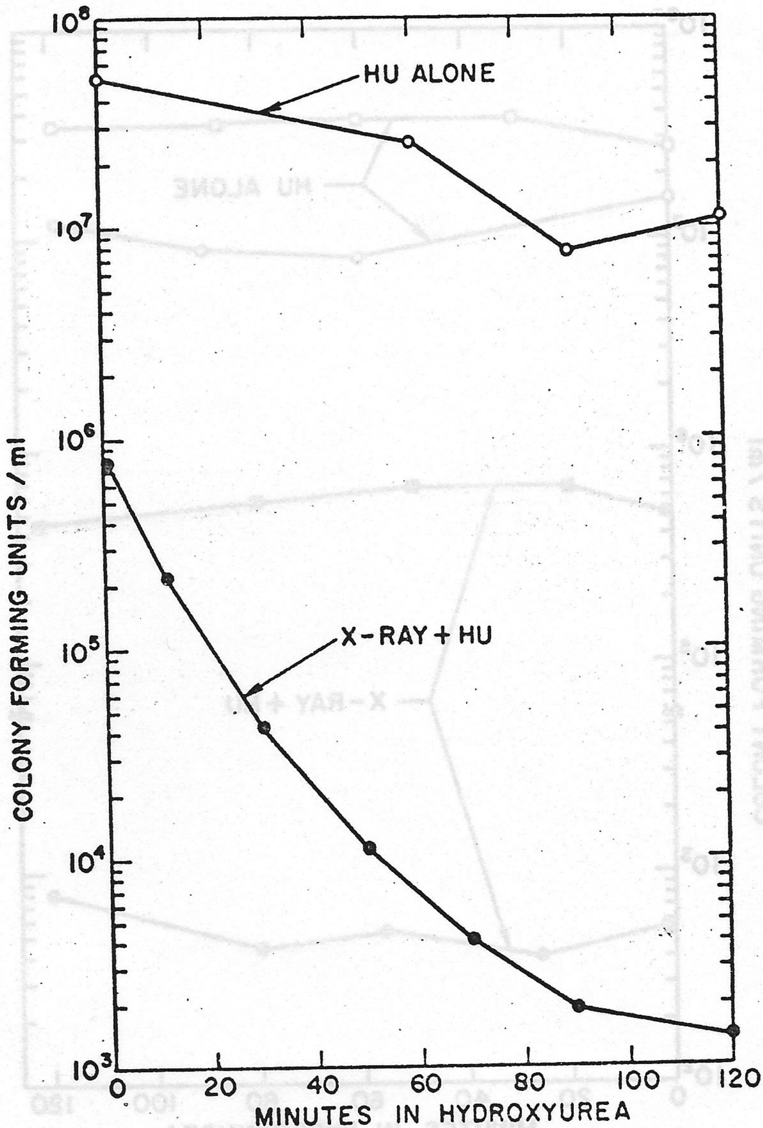


**Fig. 1.** Postirradiation treatment of *E. coli* K-12 *rec*$^+$ (AB2497) cells in exponential growth with an impure sample of hydroxyurea (HU) (Nutritional Biochemicals Corp.) in minimal growth medium at 37°C. The number of viable cells is plotted as a function of time in 0.1 M HU subsequent to X-irradiation. O, HU alone; ●, 17.6 krd irradiation then HU treatment. Chromatographically pure samples of HU (E.R. Squibb and Sons; K&K Laboratories) do not show this effect.

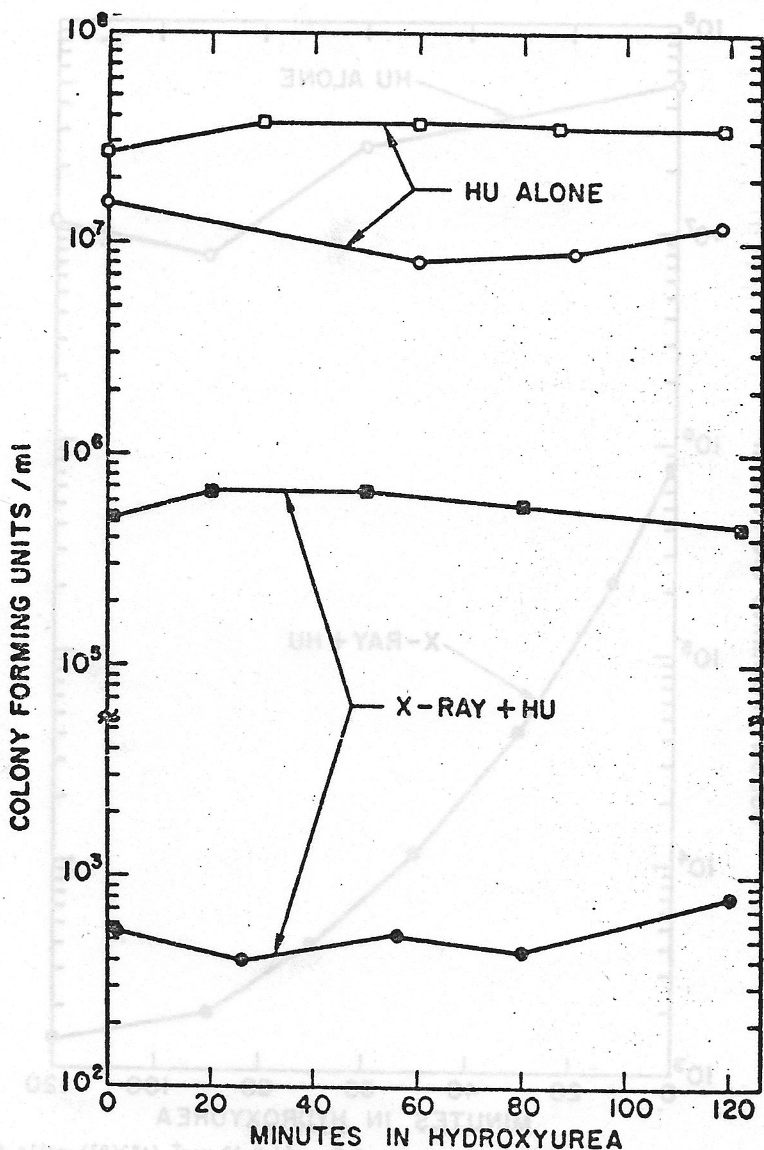


Fig. 2. Postirradiation treatment of *E. coli* K-12 *recA56* cells in exponential growth with an impure sample of HU (Nutritional Biochemicals Corp.) in minimal growth medium at 37°C. The number of viable cells is plotted as a function of time in 0.1 M HU subsequent to X-irradiation. O, HU alone; ●, 11.7 krd irradiation then HU treatment; □, HU alone; ■, 5.1 krd irradiation then HU treatment.

filling step postulated to be responsible for the shift in molecular weight of the pulse-labeled DNA synthesized on damaged templates. After 850 ergs/mm$^2$ there is a partial inhibition of repair of about half the small pieces of DNA but this partial inhibition can be reversed if the drug is removed (Smith, K.C., unpublished observations).

Thus, preliminary evidence suggests that while the impure hydroxyurea is a potentiator of the killing of bacteria by UV-radiation it does not appear to be doing so by inhibiting the gap filling step which is supposed to be the important step in post-replication repair. These results are consistent with the hypothesis that there may be steps other than the mere filling of gaps that are important in this repair system or there may be yet another new repair system to be described at the molecular level. This concept has already been expressed to explain why *recB* and *recC* mutants appear to show no deficiency in gap filling but are quite UV sensitive, and to explain the effect of complex media on the survival of UV-irradiated *uvr*$^-$ mutants.

The survival of irradiated cells is greatly diminished when grown on agar plates containing acriflavine [1]. We have observed (Kapp, D.S. and Smith, K.C., unpublished observations) that there is a marked potentiation of killing when X-irradiated wild-type *E. coli* K-12 cells are grown in the presence of 10 ug/ml acriflavine for up to 90 minutes before washing and plating (Fig. 3). The results of alkaline sucrose gradient studies suggest that acriflavine may act to potentiate X-ray induced killing by binding to DNA and producing a partially irreversible blockage of single-strand break repair.

In summary, recombination deficient mutants are sensitive to UV-irradiation and very sensitive to X-irradiation. Two events, at the molecular level, that are related to repair have been found to be governed by certain genes controlling genetic recombination: (1) single-strand break repair in *both* parental strands as exemplified after X-irradiation and (2) gap repair in the daughter strand of each DNA duplex as exemplified after UV-ir-

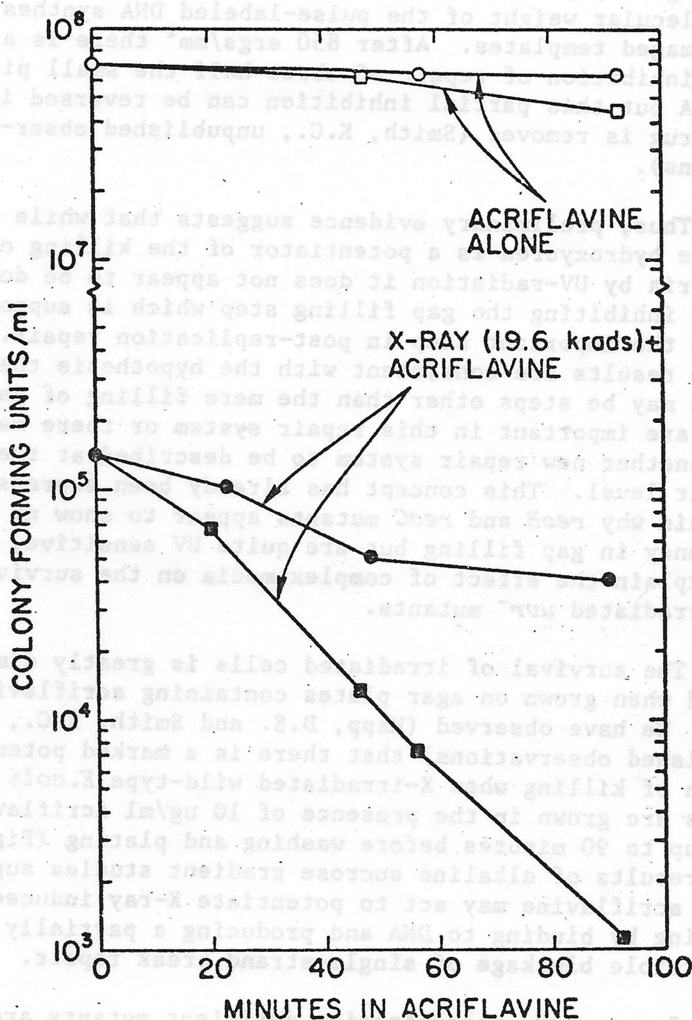


Fig. 3. Postirradiation treatment of *E. coli* K-12 *rec*$^+$ (AB2497) cells in exponential growth with acriflavine in minimal growth medium at 37°C. The number of viable cells is plotted as a function of time in acriflavine subsequent to X-irradiation. O, 5 μg/ml acriflavine alone; ●, 19.6 krd irradiation then treatment with 5 μg/ml acriflavine; □, 10 μg/ml acriflavine alone; ■, 19.6 krd irradiation then treatment with 10 μg/ml acriflavine.

radiation. It is not certain whether quantitatively these are two modes of the same repair system or whether while having certain enzymatic steps in common additional enzymes are required for one system that are not required by the other. For example, one can imagine that nuclease action might be necessary to remove fragmented nucleotides from X-ray induced chain breaks before the chain breaks could be repaired [8]. Such a cleaning step would be predicted to be unnecessary for the repair of daughter strand gaps after UV-irradiation.

*RecA* strains appear to be deficient in both types of repair functions. *RecB* and *recC* mutants are less sensitive to radiation and show an intermediate ability to repair parental strand gaps after high doses of X-rays but show wild-type ability to repair daughter strand gaps after moderate doses of UV-radiation.

Chemical inhibitors of the repair of radiation damage have been described. Impure preparations of hydroxyurea potentiate the killing of wild-type *E.coli* K-12 cells but not the killing of repair deficient *recA* cells after either UV- or X-irradiation. The active agent in these impure preparations of hydroxyurea irreversibly inhibits the repair of chain breaks after X-irradiation but preliminary results indicate that only a partial inhibition of daughter strand repair is observed after high doses of UV-radiation and this partial inhibition is reversible.

Acriflavine also potentiates the killing of X-irradiated wild-type *E.coli* and inhibits the repair of X-ray induced single-chain breaks. Only part of this inhibition can be reversed by washing and transferring the cells to normal medium.

Radiation therapy has long been one of the most effective methods for the treatment of certain types of cancer. Considerable effort has gone into optimizing the physical and chemical efficiency of the radiation and of sensitizing cells to radiation by such procedures as the incorporation of base analogs into the DNA of cells. The recent description, at the molecular level, of systems

for the repair of radiation damage provide another approach for the radiation therapist. Drugs that specifically inhibit the repair of radiation damage should greatly improve the effectiveness of the radiation. Since many of the agents used in cancer chemotherapy are "radiomimetic", the selective inhibition of the repair of DNA lesions has importance to the field of cancer chemotherapy as well as radiation therapy.

REFERENCES

1. ALPER, T., *Nature*, *200*, 534, 1963.

2. GANESAN, A.K. and SMITH, K.C., *Cold Spring Harbor Symp. Quant. Biol.*, *33*, 235, 1968.

3. GANESAN, A.K. and SMITH, K.C., *J. Bacteriol.*, *102*, 404, 1970.

4. HOWARD-FLANDERS, P. and BOYCE, R.P., *Radiation Res. Suppl. 6*, 156, 1966.

5. KAPLAN, H.S., *Proc. Nat. Acad. Sci. U.S.*, *55*, 1442, 1966.

6. KAPP, D.S. and SMITH, K.C., *J. Bacteriol.*, *103*, 49, 1970.

7. KAPP, D.S. and SMITH, K.C., *Int. J. Radiat. Biol.*, *19*, 255, 1971.

8. KAPP, D.S. and SMITH, K.C., *Int. J. Radiat. Biol.*, *14*, 567, 1968.

9. OISHI, M., *Proc. Nat. Acad. Sci. U.S.*, *64*, 1292, 1969.

10. RUPP, W.D. and HOWARD-FLANDERS, P., *J. Mol. Biol.* *31*, 291, 1968.

11. SMITH, K.C. and MEUN, D.H.C., *J. Mol. Biol.*, *51*, 459, 1970.

12. WILLETTS, N.S. and MOUNT, D.W., *J. Bacteriol.*, *100*, 923, 1969.

13. WITKIN, E.M., *Mutation Res.*, *8*, 9, 1969.