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Reprinted from

SUNLIGHT AND MAN

Normal and Abnormal Photobiologic Responses

Consulting Editor: T.B. Fitzpatrick

Edited by: M.A. Pathak
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M. Seiji
A. Kukita

UNIVERSITY OF TOKYO PRESS

1974

4 THE CELLULAR REPAIR OF RADIATION DAMAGE

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ABSTRACT

Since the chemical nature of the radiation damage produced in the nucleic acids varies so greatly in type and complexity, one might predict that systems capable of repairing this damage must be equally complex. The known repair systems do require a multiplicity of enzymes and they have two general functions, i.e., the repair of the different types of DNA base damage and the repair of the different types of DNA chain breaks.

Currently three systems are known for the repair of DNA base damage: (1) *in situ* repair (e.g., photoreactivation of pyrimidine dimers), (2) excision repair (the damaged bases are cut out and replaced with undamaged material), and (3) post-replication repair (the damaged section of DNA is not directly repaired but is bypassed during replication; the missing section of DNA is replaced subsequently by the action of enzymes that also function in genetic recombination).

Currently three systems are known for the repair of DNA chain breaks: (1) an ultra-fast repair system (requiring less than 1–2 minutes at 0°C in buffer) that may require only one enzyme (e.g., ligase), (2) a fast repair system (1–2 minutes at 37°C in buffer) that requires DNA polymerase I, and (3) a slow repair system (~40 minutes at 37°C) that requires complete growth medium and is controlled in bacteria by the *rec* and *exr* genes.

Some of these repair systems are irreversibly inhibited by certain drugs, leading to an increased radiation killing of the treated cells, Such postirradiation sensitizing drugs may be useful adjuncts to radiation therapy.

I. INTRODUCTION

The sensitivity of a cell to radiation is determined largely by its ability to repair radiation damage to its deoxyribonucleic acid (DNA). Since the nature of the damage produced in the nucleic acids by radiation varies so greatly in chemical type and complexity (see Chapter, *Molecular Changes in the Nucleic Acids Produced by Ultraviolet and Visible Radiation*), and since enzymes show great specificity for the substrates upon which they act, one may predict that enzymatic systems capable of repairing radiation damage to DNA must be quite complex. This has proved to be the case. Furthermore, certain chemical types of damage to DNA can be repaired by several separate repair systems, each of which has optimum conditions for its action. For example, one of three systems that repairs the class of damage

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known as cyclobutane-type pyrimidine dimers requires visible light while two others do not. Of the latter class, one system is favored when DNA replication is inhibited while the other system cannot function at all if DNA replication is prevented. One may therefore surmise that the ability to repair DNA under all types of metabolic conditions is so important to the survival of cells that, through evolution, cells have developed overlapping and "back-up" systems of repair. The description of these many repair systems is the subject of this paper.

As may be predicted from the types of damage produced in DNA by radiation (see Chapter, *Molecular Changes in the Nucleic Acids Produced by Ultraviolet and Visible Radiation*), repair systems perform two general functions: (1) they repair the different types of damage to DNA bases, and (2) they repair the different chemical types of DNA chain breaks (for recent reviews on repair see Refs. 1–10).

II. REPAIR OF DNA BASE DAMAGE

Currently three systems are known for the repair of base damage: (1) photoreactivation (the *in situ* enzymatic cleavage of cyclobutane-type pyrimidine dimers mediated by visible light), (2) excision repair (in the absence of light, the damaged bases are cut out of the DNA and are replaced with undamaged material), and (3) postreplication repair (in the absence of light, the damaged section of DNA is not directly repaired but rather is bypassed during replication; the missing section of the newly synthesized DNA is replaced subsequently by enzymatic processes not yet well understood).

A. Photoreactivation

The event that led to the discovery of this repair system was the observation that bacteria which had been inactivated by ultraviolet (UV) radiation could be reactivated by a subsequent exposure to short wavelength visible light (for reviews see Refs. 5, 7, 10). The single enzyme responsible for this process has been isolated in very pure form and appears to act only on cyclobutane-type pyrimidine dimers. In the dark, the photoreactivating enzyme combines with a pyrimidine dimer in DNA, and when this enzyme–substrate complex is exposed to visible light at about 400 nm the dimer is split, yielding the two single pyrimidines in their original state.

An enzyme with this capability has been found to be widely distributed throughout nature, the most notable exception being the tissues of placental mammals.[7] Since the chances are remote that certain of these tissues that contain the enzyme will ever be exposed to UV or to ~400 nm radiation, one may ponder the teleological reason for the unusual distribution of this enzyme throughout nature. One may also wonder if it has some function in cells other than repairing radiation damage.

The fraction of the UV-induced lethal damage in a cell that is photoreactivable is called the photoreactivable sector. This ranges from 0.1–0.8 for *E. coli*, depending upon the experimental conditions. Since the photoreactivating enzyme has been shown to act only on cyclobutane-type pyrimidine dimers, the photoreactivable

sector then provides an estimate of the relative biologic importance of these lesions under a given set of experimental conditions.

B. Excision Repair

The observations that led to the discovery of this repair system were that radiation-resistant strains of *E. coli* degraded their DNA after exposure to UV radiation while certain very radiation-sensitive strains did not. Furthermore, this breakdown of DNA in *E. coli* after UV irradiation was not just random catabolism, rather, it was found that cyclobutane-type thymine dimers were selectively removed from the DNA.[11-13]

This selective removal of damaged bases from the DNA of radiation-resistant but not of radiation-sensitive strains of bacteria suggested that this excision process might be an important first step in a repair mechanism leading to enhanced cellular survival. A logical second step in this process would be the filling of the resultant gap in the DNA with undamaged material. This process of repair replication of the DNA has been shown to occur in radiation-resistant strains but not in certain-sensitive strains of *E. coli*.[8,14,15]

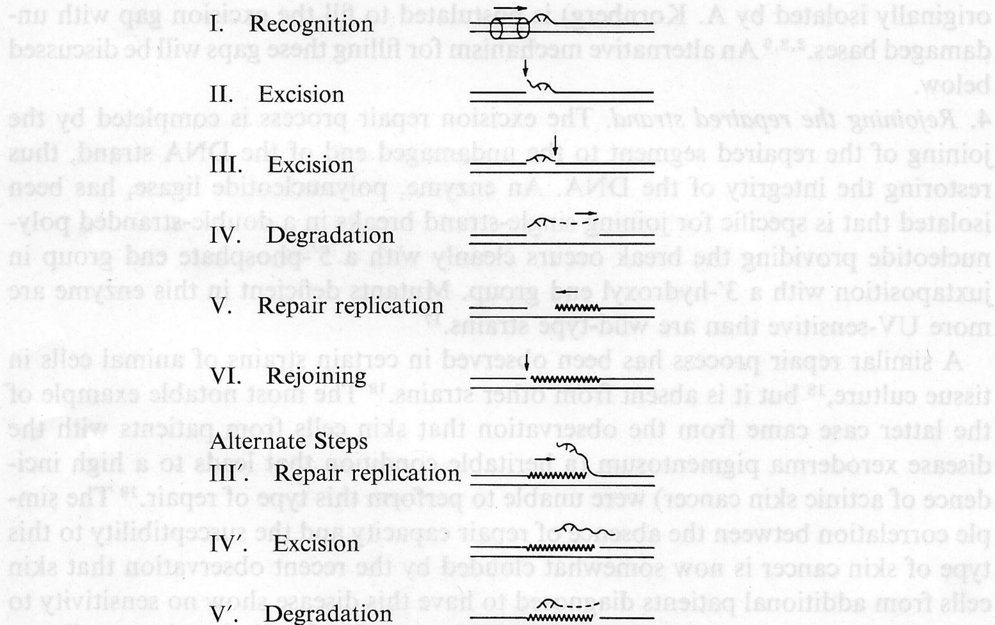

Fig. 1. Schematic representation of the postulated steps in the excision repair of damaged DNA. Steps I through VI illustrate the "cut and patch" sequence. An initial incision in the damaged strand is followed by local degradation before the resynthesis of the region has begun. In the alternative "patch and cut" model the resynthesis step III′ begins immediately after the incision step II and the excision of the damaged region occurs when repair replication is complete. In either model the final step (VI) involves a rejoining of the repaired section to the contiguous DNA of the original parental strand.[4]

Current understanding of the excision repair system suggests that the molecular mechanisms involved in this process are the following:

1. *Recognition of damage.* The first step in any enzymatic process is the recognition by the enzyme of its substrate. Both UV-induced pyrimidine dimers and purine residues alkylated with nitrogen mustard are repaired by the excision repair system.[8,14] It is not known whether separate enzymes are required for the recognition of these two different chemical types of lesions or whether the stimulus for recognition is simply a physical distortion in the DNA helix, as shown schematically in Figure 1.

2. *Incision and excision.* The next two steps involve the introduction of breaks in the DNA chain, first on one side of the lesion (incision step) and then on the other (excision). In *E. coli* K-12, three genes, *uvrA*, *uvrB*, and *uvrC*, are known to control the excision of pyrimidine dimers.[2] A mutant dificient in any one of these loci is unable to excise dimers and is very sensitive to UV radiation (Fig. 2). Certain of the enzymes involved in the excision process have been isolated and their properties are described in a recent review.[16]

3. *Repair replication.* Using the undamaged bases in the DNA strand opposite the excised region as a template, a DNA polymerase (probably DNA polymerase I, originally isolated by A. Kornberg) is postulated to fill the excision gap with undamaged bases.[2,8,9] An alternative mechanism for filling these gaps will be discussed below.

4. *Rejoining the repaired strand.* The excision repair process is completed by the joining of the repaired segment to the undamaged end of the DNA strand, thus restoring the integrity of the DNA. An enzyme, polynucleotide ligase, has been isolated that is specific for joining single-strand breaks in a double-stranded polynucleotide providing the break occurs cleanly with a 5′-phosphate end group in juxtaposition with a 3′-hydroxyl end group. Mutants deficient in this enzyme are more UV-sensitive than are wild-type strains.[17]

A similar repair process has been observed in certain strains of animal cells in tissue culture,[18] but it is absent from other strains.[19] The most notable example of the latter case came from the observation that skin cells from patients with the disease xeroderma pigmentosum (a heritable condition that leads to a high incidence of actinic skin cancer) were unable to perform this type of repair.[19] The simple correlation between the absence of repair capacity and the susceptibility to this type of skin cancer is now somewhat clouded by the recent observation that skin cells from additional patients diagnosed to have this disease show no sensitivity to UV radiation and no deficiency in the excision repair of radiation damage.[20]

The efficiency of the excision repair system has been found to be enhanced by the cessation of normal DNA synthesis (for a discussion of liquid holding recovery see Ref. 21). This seems reasonable since the attempted replication of DNA strands containing excision gaps would be expected to produce double-strand breaks and result in reproductive death.

C. Postreplication Repair

The first indication that the excision mode of repair is not the only mechanism by which cells (in the dark) can repair radiation damage to their DNA, was the observation that cells deficient both in excision repair (*uvr* mutants) and in genetic recombination (*rec* mutants) are much more sensitive to killing by UV radiation than are cells carrying either mutation alone (Fig. 2). This suggested that certain steps in genetic recombination might be important in the repair of radiation damage. Genetic recombination is the process by which DNA that is injected by a male bacterium into a female bacterium during conjugation, is broken and recombined with the DNA in the female bacterium to yield progeny carrying genetic information from both parents.

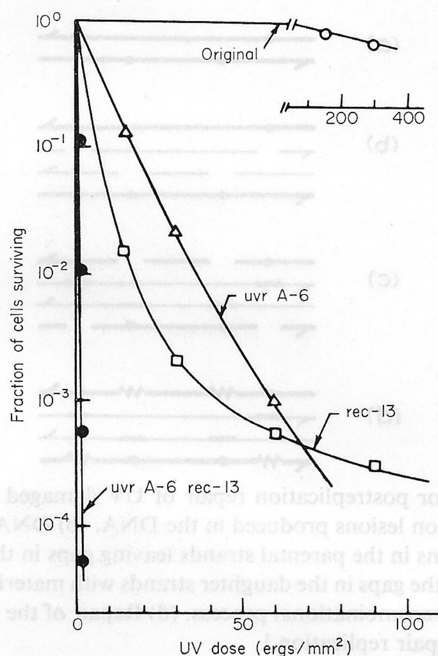

Fig. 2. The sensitivity of colony-forming ability to ultraviolet light in several UV-sensitive bacterial mutants. The mutant strain *uvrA6* is unable to excise thymine dimers. The mutant *rec13* is defective in genetic recombination. The double mutant *uvrA6 rec13* is deficient in both excision and recombination and it is more sensitive than either single mutant.[29]

The second indication of a new repair system came from the observation that UV-irradiated cells carrying the *uvr* mutation show a large recovery of viability when plated on minimal growth medium as compared to plating on complex growth medium. This ability to undergo minimal medium recovery suggested that excision-deficient cells were able to repair radiation damage. This process has been shown to be controlled by *rec* genes (*recA* and *recB*).[1,21]

Evidence at the molecular level for a new repair system came from the observation that DNA synthesized in excision-deficient cells of *E. coli* K-12, immediately after UV irradiation, was shorter than that synthesized in unirradiated cells. The length of the pieces of DNA synthesized after UV irradiation approximated the distance between the pyrimidine dimers in the parental strand (i.e., the size of the DNA was inversely proportional to the dose of UV radiation). With further incubation of the irradiated cells, however, these short pieces of DNA became progressively longer until they approximated the size of the unirradiated DNA. A postreplication repair process was thus implicated that was independent of the excision repair process.[22] This repair system has been shown to be controlled by the *recA* gene but not by the *recB* or *recC* genes,[23] and therefore must share some steps in common with the process of genetic recombination.

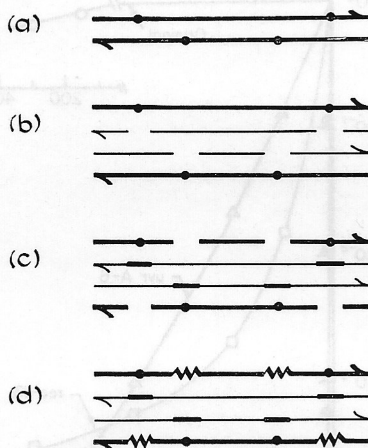

Fig. 3. A model for postreplication repair of UV damaged DNA. (a) Dots indicate radiation lesions produced in the DNA. (b) DNA synthesis proceeds past the lesions in the parental strands leaving gaps in the daughter strands. (c) Filling of the gaps in the daughter strands with material from the parental strands by a recombinational process. (d) Repair of the gaps in the parental strands by repair replication.[1]

A model for postreplication repair is shown schematically in Figure 3. DNA replication must be allowed to proceed before this postreplication repair system can function. Thus, while the excision repair system works on parental strand DNA the postreplication repair system works on daughter strand DNA. There is evidence for the involvement of parental DNA in the gap-filling step[24] (Fig. 3c) and it is presumed that DNA polymerase and ligase (last steps of excision repair) are involved in repairing the resulting gaps in the parental DNA. The enzymology of this complicated repair system remains largely unexplored.

Postreplication repair following UV irradiation has been observed in mammalian cells,[25-27] and shown to be inhibited by caffeine.[25,26]

D. New Repair Systems to be Discovered?

There are several lines of evidence which suggest that there may be modes of repair controlled by the *rec* genes in addition to the postreplication repair process. For example, *recB* mutants are quite sensitive to UV radiation, although not as sensitive as *recA* mutants, yet they show no deficiency in postreplication repair.[23] Either the *recB* gene performs some subtle function in postreplication repair that has thus far not been detected or it may be involved in another, as yet undefined, pathway of repair. Consistent with this latter hypothesis is the observation that complex growth medium inhibits (in *uvr* mutants) a recovery process coded for by both *recA* and *recB* genes (minimal medium recovery), yet complex medium does not inhibit postreplication repair.[1] Quinacrine and chloramphenicol also have an adverse effect on the survival of UV-irradiated *uvr* mutants but these drugs do not permanently inhibit postreplication repair.[1] Thus, we may still expect the discovery of new modes of repair in the coming years.

E. The Possible Involvement of Post-replication Repair Functions in Excision Repair

As mentioned above, it is thought that DNA polymerase is involved in the gap-filling step of excision repair. However, a mutant deficient in DNA polymerase I is not as sensitive to UV radiation as an excision defective mutant.[28] This suggests that there may be alternate pathways for the rapair of excision gaps. Because of the efficiency of the postreplication repair system for filling gaps in DNA, it has been suggested that certain enzymes involved in postreplication repair may also function in the gap-filling step of excision repair.[28]

III. REPAIR OF DNA CHAIN BREAKS

The experimental technique used to follow the formation and repair of chain breaks in DNA involves the gentle lysis of cells on top of a column of alkaline sucrose. The alkali dissociates and separates the two strands of DNA. When such a sample is spun in an ultracentrifuge, the single strands of DNA travel through the column of sucrose towards the bottom of the tube at a rate that is proportional to the molecular weight of the DNA. Thus, unbroken strands of DNA will sediment rapidly and broken strands more slowly. It is therefore relatively easy to determine the number of chain breaks produced in DNA by a given dose of radiation, and to follow the kinetics of their repair. This technique can also be used to determine whether a given radiation-sensitive mutant is capable of repairing chain breaks or whether drugs interfere with the repair of DNA chain breaks.

Most of our knowledge concerning the repair of DNA chain breaks comes from investigations using x-rays. Using appropriate radiation-sensitive mutants of *E. coli* and chemical and physical inhibitors, three separate repair systems have thus far been delineated (for a review, see Ref. 3). They have been designated as the Type

I, Type II, and Type III repair systems because of the remarkable differences in the speed with which they act. The major distinguishing features of these systems are summarized in the Table.

Table. Systems for the repair of DNA chain breaks.

TYPE I.	Ultra-fast (<2 min at 0°C); occurs in buffer; does not require DNA polymerase I; repairs 3/4 of the x-ray-induced breaks produced under N_2 but only 1/4 of the breaks produced under O_2.†
TYPE II.	Fast (1–2 min at 37°C; $T_{1/2} \sim 10$ min at 0°C); occurs in buffer; requires DNA polymerase I; repairs 6/7 of breaks presented to it whether produced under O_2 or N_2.
TYPE III.	Slow (20–60 min at 37°C); requires complete growth medium; controlled by *rec* and *exr* genes; does not require DNA polymerase I; repairs two (2) breaks per single-strand genome whether produced under O_2 or N_2.

†At least part of Type I repair now appears to be due to chemical restoration rather than enzymic repair. Smith, K. C., Unpublished observations

The chemistry of the radiation-induced DNA chain breaks can vary from simple to complex (see Chapter, *Molecular Changes in the Nucleic Acids Produced by Ultraviolet and Visible Radiation*). Correspondingly, the number of enzymes required to repair a given chain break may be one or many. It is assumed that the large differences in the speed of these three repair systems reflects the complexity of the damage that they have to repair. Thus, the Type I process may require as few as one enzyme. If the chain is broken such that a 5′-phosphate group and a 3′-hydroxy group are formed at the break, this could be repaired by polynucleotide ligase. It may be recalled that this enzyme has also been implicated in the excision repair process. If the recriprocal configuration were formed, the 3′-phosphate group would have to be removed by a 3′-phosphatase and the 5′-phosphate group would have to be restored by a polynucleotide kinase before the ligase could work—a total of three enzymes.

Since the Type II process requires the action of DNA polymerase I and takes longer to complete than does Type I repair, we may presume that the damage repaired by the Type II process is chemically more complicated than that repaired by the Type I process. Similar reasoning would suggest that the damage repaired by the Type III process is the most complicated of all.

Since the Type I and Type II processes repair a fixed percentage of the chain breaks, it suggests that x-radiation produces a constant proportion of breaks with differing chemical structures. Why the Type III process repairs only 2 chain breaks per single-strand genome regardless of the number presented to it remains unanswered.

A. Drug Inhibition of the Repair of Chain Breaks

Drugs with quite diverse pharmacologic properties such as dinitrophenol, quinacrine, chloramphenicol (or amino acid starvation), acriflavine, and an impure

sample of hydroxyurea have been found to sensitize wild-type bacteria to x-irradiation when supplied to the culture after irradiation. These agents irreversibly inhibit the Type III repair of chain breaks. In confirmation, they do not sensitize x-irradiated *recA* mutants which are devoid of the Type III repair process (for reviews, see Refs. 1,3).

The effect of drugs on the inhibition of the Type I and Type II systems have received some study but their effects have yet to be correlated with survival.[3]

Since the survival of cells exposed to radiation is so critically dependent upon their ability to repair damage to their DNA (compare the survival curves of the repair deficient mutants in Figure 2), repair inhibitor drugs may prove to be a potent adjunct to various forms of radiation therapy.

IV. CONCLUSIONS

One cannot help but be impressed by the large number and diverse types of enzymatic systems that cells have for the repair of damage to their DNA. This is true even for cells that are normally not exposed to solar radiation (e.g., *E. coli*). Chemical damage to DNA is also repaired by these systems.

Apparently normal metabolic processes produce damage to DNA that must be repaired in order for cells to continue to proliferate. Two observations support this concept. (1) A small amount of repair replication is found in unirradiated control cells.[8] (2) A cell deficient in either DNA polymerase I (*polA*) or in genetic recombination (*recA*) is viable, but a double mutant (i.e., *polA recA*) is not.[30] Since the repair of DNA appears to be essential for normal growth, the importance of repair processes thus transcends radiation biology.

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