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CHAPTER 20

DARK REPAIR OF DNA DAMAGE

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1. Introduction

One of the most exciting results to come out of photobiology and radiation biology in recent years is the observation, at the biochemical level, that cells can repair damage to their DNA. The importance of this observation extends to all areas of biological science. The genetic control of repair systems has become a subject of intensive investigation. The enzymes involved in repair are being isolated and characterized. The relationship of repair systems to normal life processes in the absence of radiation is being assessed. The absence of one type of repair process has been correlated with the genetically controlled susceptibility to light-induced skin cancer (xeroderma pigmentosum) and may have far-reaching implications in the field of cancer research. A new repair system, controlled by the genes that control genetic recombination, appears to be the major system by which cells repair X-ray induced to their DNA. Several agents have been found that inhibit this repair system. The use of specific inhibitors for this repair system may find application in the radiation treatment of cancer.

The most recent development in studies on the repair of radiation damage is the observation that there are at least two dark repair systems that differ both in their biochemical mechanism and their genetic control, and there may well be other systems yet to be discovered. The repair systems that operate in the light (e.g., photoreactivation) have been discussed by Dr. J. Setlow [51]. This paper will be largely restricted to those repair systems that do not require light energy to power their biochemical reactions.

The first indication that cells might have the capacity to recover from radiation damage was the observation that minor modifications in the handling of the cells (e.g., growth media, temperature, etc.) had a marked effect upon the ultimate viability of irradiated cells. Thus in 1937, Hollaender and Claus [24] found that higher survival levels of UV-irradiated fungal spores could be obtained if they were allowed to remain in water or salt solution for a period of time before plating on nutrient agar. Roberts and Aldous [45] extended these observations by showing that the

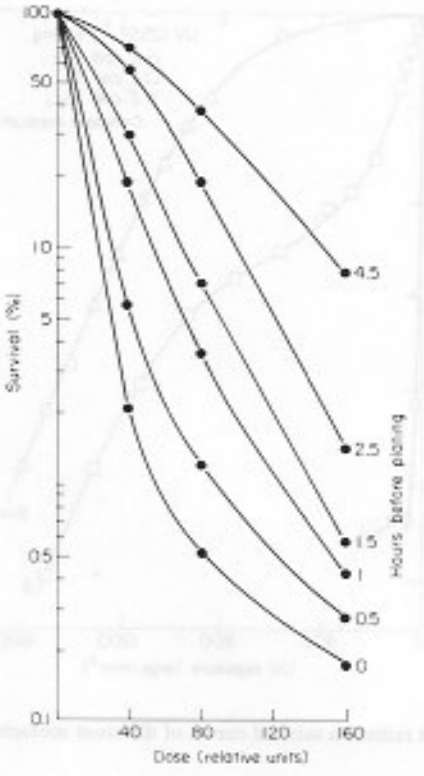

Fig. 1. Ultraviolet radiation survival curves for *E. coli* B. After irradiation the cells were suspended in a liquid medium without an energy source for the times indicated before being plated on nutrient agar. It is evident that both the slopes and the shapes of the survival curves can be altered by the post-irradiation treatment of the cells [45].

shapes of the UV survival curves for *E. coli* B could be changed quite drastically simply by holding the irradiated cells in media devoid of an energy source for various times before plating on nutrient agar (fig. 1). This phenomenon, known as liquid holding recovery, has now been shown to require the presence of intact uvr genes [12], the genes that control the first step in the excision repair system. Thus, holding *E. coli* B (and certain rec strains of *E. coli* K-12 [10]) in non-nutrient media appears to improve the efficiency of the excision repair process.

A second indication of the possible repair of radiation damage came from a study of survival curves. According to classical target theory, a shoulder on a survival curve should indicate either multiple targets or multiple hits on targets. However, very closely related mutants of *E. coli* are not expected to have a markedly different number of targets, yet their survival characteristics are markedly different (fig. 2). The shoulder on the survival curve of the more resistant strains has been

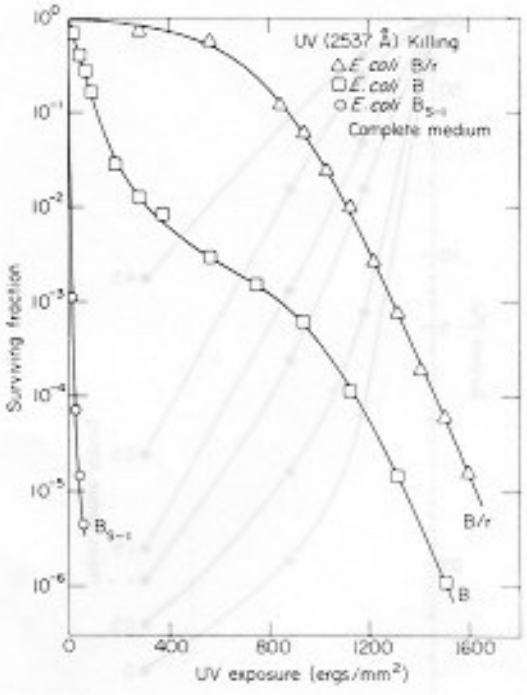

Fig. 2. Ultraviolet radiation survival curves of different mutants of *E. coli* B [21].

reinterpreted as implicating the capacity to repair [22]. The shoulder represents the dose range within which the cells can cope with the damage produced. At higher doses where the survival curve becomes steep, the repair systems have themselves either become inactivated by the radiation or the number of lesions in the DNA exceed the capacity of the repair system to cope with this damage.

Another method for studying or detecting the presence of repair systems in cells is the split-dose technique used by Elkind and co-workers [7, 8]. The rationale for this type of experiment is that if there is no repair of radiation damage then it should have little effect upon the ultimate survival of the cells whether the total radiation dose is given at one time or whether only part of the dose is given at one time and the remainder is given at some later time. However, if the survival of the cells receiving a split dose of radiation is greater than that for cells receiving the same total dose delivered at one time, then it seems reasonable to conclude that the former cells have been able to repair a portion of the first dose of radiation (fig. 3). This split-dose technique has been most widely used with mammalian cells in tissue culture although some work has been done with microorganisms [32].

Perhaps the most conclusive proof of the presence of repair systems in cells is the observation that different mutants of the same strain of bacteria show widely

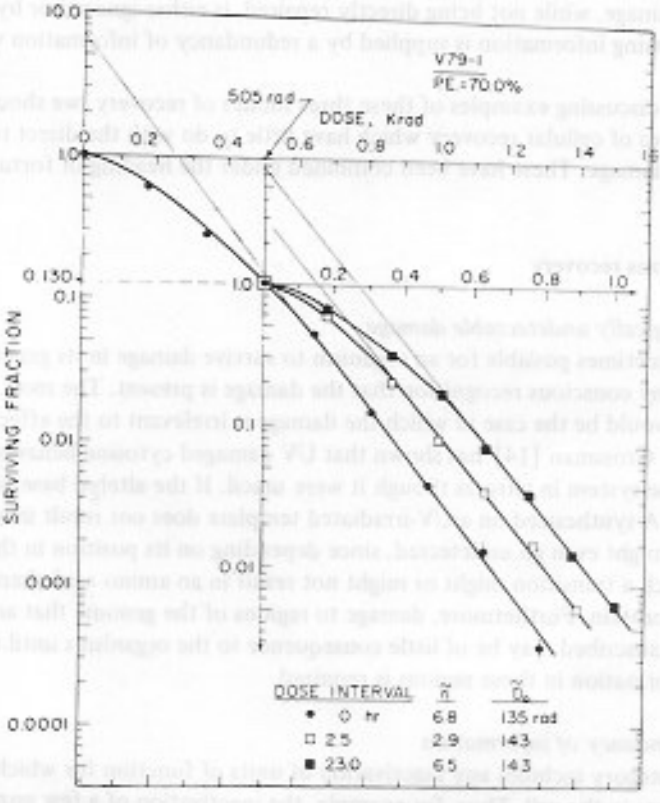

Fig. 3. X-ray survival curves for Chinese hamster cells (V79-1) using fractionated doses. When there was either 2.5 or 23.0 hr between the first dose of 505 rad and the subsequent doses of X rays, the cells were more resistant than if there was no fractionation of the dose. This shift in resistance suggests that the cells have repaired part of the damage produced by the first dose of radiation [7].

differing sensitivities to radiation (fig. 2). The location of several genes that affect the radiation sensitivity of cells have been mapped, and the biochemical deficiencies of several of these mutants have been determined (for reviews see [59, 61, 66]).

Having established that cells have the capacity to recover from radiation damage, we may speculate on the possible molecular mechanism of this recovery. To date, three modes of repair have been documented.

(1) The damaged molecule or part of the molecule may be restored to its functional state in situ. This may be accomplished by the activity of some enzymatic mechanism (e.g., photoreactivation) or it may simply result from the 'decay' of the damage to an inocuous form.

(2) The damaged section of the DNA may be removed and replaced with undamaged nucleotides to restore the normal function of the DNA.

(3) The damage, while not being directly repaired, is either ignored or bypassed and the missing information is supplied by a redundancy of information within the cell.

Before discussing examples of these three modes of recovery, we should discuss some modes of cellular recovery which have little to do with the direct repair of radiation damage. These have been combined under the heading of fortuitous recovery [59].

2. Fortuitous recovery

2.1. *Biologically undetectable damage*

It is sometimes possible for an organism to survive damage in its genetic material without any conscious recognition that the damage is present. The most obvious example would be the case in which the damage is irrelevant to the effect being measured. Grossman [14] has shown that UV damaged cytosine behaves in an RNA polymerase system in vitro as though it were uracil. If the altered base composition of the RNA synthesized on a UV-irradiated template does not result in a lethal mutation, it might even go undetected, since depending on its position in the codon triplet, such a transition might or might not result in an amino acid change in some resultant protein. Furthermore, damage to regions of the genome that are not being actively transcribed may be of little consequence to the organisms until such time as the information in those regions is required.

2.2. *Redundancy of information*

This category includes any inactivation of units of function for which a redundancy exists in the cell. Thus, for example, the inactivation of a few enzyme molecules would have no detectable effect on cell growth if there were still many undamaged enzyme molecules present in the cell.

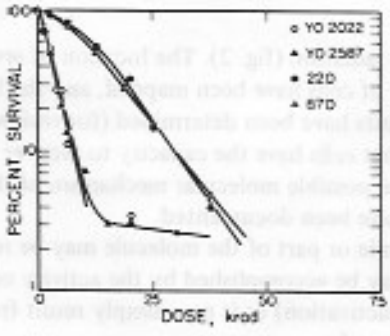

Fig. 4. X-ray survival curves of *Saccharomyces cerevisiae*; haploid cultures YO2022 and YO2587 and homozygous diploid cultures 22D and 87D [37].

2.2.1. *Polyploidy.* The effect of polyploidy on X-ray survival is quite effectively illustrated in a comparison of the survival curves for haploid and diploid strains of yeast (fig. 4). The haploid form initially exhibits the simple exponential survival curve expected for a single target inactivation process, while the diploid strain gives rise to a multi-component inactivation curve with an extrapolation number of roughly 2, consistent with two sensitive targets per cell [37]. It is important to realize that inactivation curves with shoulders may indicate either polyploidy or the presence of repair mechanisms, and it may be quite difficult to ascertain which effect is responsible. The correlation of an apparent polyploidy with cytological observations sometimes can resolve this question.

2.2.2. *Multiplicity reactivation.* This phenomenon, first observed by Luria [34], involves the cooperative effects of UV inactivated bacteriophage to produce some viable phage when the host cell is multiply infected. Multiplicity reactivation has also been demonstrated with animal viruses and it has been speculated that it may even occur between nuclei within uninfected diploid cells. Multiplicity reactivation has also been observed in phage after deleterious treatments other than UV, such as X-rays, nitrous acid, and ^{32}P decay (see Rupert and Harm [47], and references therein). The phenomenon evidently involves genetic recombination in which the random process of molecular rearrangement may result in the production of a viable genome from the undamaged components of otherwise non-viable genomes.

2.2.3. *Cross-reactivation (marker rescue).* The process known as cross-reactivation or marker rescue is essentially the same as the molecular rearrangement aspect of multiplicity reactivation. The bacteria are infected with two genetic types of phage, of which one has been UV-irradiated. Genetic markers from the UV-inactivated phage may be physcially incorporated into the genome of the unirradiated phage. The rescue of genetic markers can be demonstrated even after most of the genetic information 'donor' phage particles has been destroyed by radiation.

2.3. *Suppression of prophage induction*

The UV sensitivity of bacteria may be enhanced by the presence of UV-inducible prophage [47]. Any condition that might inhibit the induction of such a prophage would then fortuitously lead to an increased resistance of the bacteria to irradiation. It is clear that such an apparent recovery factor might bear no relation to the repair of potentially damaging photoproducts in either the prophage or the bacterial genome.

3. Repair of DNA damage in situ

3.1. *Decay of photoproducts*

The simplest mechanism of repair is the one that involves the spontaneous reversion of radiation products to the original undamaged state. Obviously the cell can

have little control over this sort of restoration, but environmental conditions can have a great deal to do with it. The hydration products of the pyrimidines are known to revert spontaneously. Also several of the dimeric thymine photoproducts have been shown to be reversed by acid catalysis (for recent reviews on the photochemistry of the nucleic acids see Smith and Hanawalt [59], Setlow [53] and Smith [55]. For radiation products, with a fleeting existence, to express a biological effect it would seem that they must occur just ahead of the replication or transcription enzymes. This explanation would be consistent with the general observation that cells are more sensitive to UV if they are actively growing (e.g., replicating DNA). The *thermal* reactivation of the viability of irradiated cell (for a review see Rupert and Harm [47], may in part involve the increased decay rate of labile radiation products at higher temperatures.

Little more can be said about the relevance of radiation product decay to biological recovery until we understand more about the kinds of radiation products that can revert spontaneously. Nevertheless, one should be aware of this possible mode of recovery, particularly when considering environmental effects on cellular survival.

3.2. *Enzyme catalyzed photoreactivation*

The most thoroughly characterized cellular recovery mechanism is that of enzymatic photoreactivation, in which illumination with visible light facilitates the direct repair in situ of photoproducts produced by UV in DNA. This subject is covered in the report by J. Setlow [51].

4. Reconstruction of damaged DNA

4.1. *Evidence for excision repair*

The studies of R.B. Setlow and co-workers (for a review see [53]), provided the first experimental evidence leading to a model for the excision repair of UV-damaged DNA. Since it was found that the same number of thymine dimers were produced by a given dose of UV in the UV sensitive strain *E. coli* B_{s-1} and the resistant strain *E. coli* B/r, it seemed evident that the resistant strain must somehow be able to remove or bypass these photoproducts in order to exhibit a higher resistance to killing. The mechanism for this recovery was clarified when it was shown that the resistant strain (but not the sensitive strain) released thymine dimers from its DNA during subsequent incubation in the dark after irradiation (fig. 5). Similar results were soon reported by Boyce and Howard-Flanders [2] for resistant and sensitive strains of *E. coli* K-12. A repair mechanism was postulated in which defective regions in one of the two DNA strands could be excised and then subsequently replaced with normal nucleotides, utilizing the complementary base pairing information in the intact strand. This mechanism (fig. 6), which has come to be known colloquially as 'cut and patch', has turned out to be of widespread significance for the repair of a variety of structural defects in DNA. The existence of this mechanism also provides a logical explanation for the evolution of two-stranded DNA, which comprises a redundancy of information.

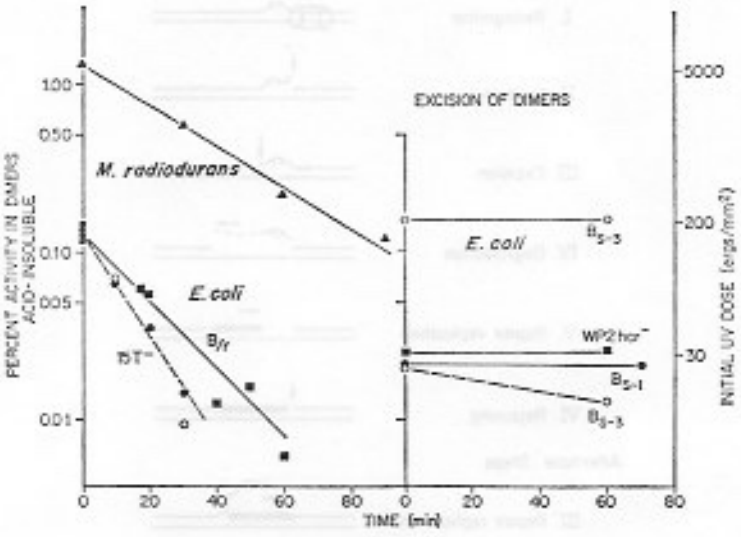

Fig. 5. *Left:* Excision of dimers from the acid-insoluble fraction of ultraviolet resistant cells at various times of incubation in growth medium after irradiation. For *Escherichia coli* 15 T⁻; ○, incubation with thymine; ●, incubation without thymine. *Right:* Lack of extensive excision, even at low doses, in hcr strains of *E. coli*. The initial doses at 265 nm are given on the right-hand ordinate [58].

Direct physical evidence for the repair replication or 'patch' step in the postulated scheme, was provided by the studies of Pettijohn and Hanawalt [40]. These studies began with attempts to isolate partially replicated fragments of bacterial chromosomes by imposing blocks to replication (i.e., UV-induced damage). Replication was followed by the use of the thymine analog, 5-bromouracil (5BU), as a density label in newly synthesized DNA, and by the subsequent analysis of the density distribution of isolated DNA fragments in a cesium chloride density gradient (fig. 7). This is essentially the method developed by Meselson and Stahl [36] and utilized by them to prove that DNA normally replicates semi-conservatively. When 5BU was used to label the DNA synthesized after UV irradiation of *E. coli* strain TAU-bar to 10^{-2} percent survival, the density pattern observed was not as expected for normal semi-conservative replication. Instead of a hybrid density band in the gradient, the initial incorporation of the 5BU label after UV resulted in no detectable shift in density from the normal parental DNA band.

Proof that the early incorporation of 5BU into DNA that results in little or no shift in density of the DNA is the postulated step of repair replication has come from a number of control experiments as follows:

(1) This mode of replication is not observed if bacteria are illuminated with visible light to allow the in situ photoreactivation of pyrimidine dimers prior to 5BU labeling [40].

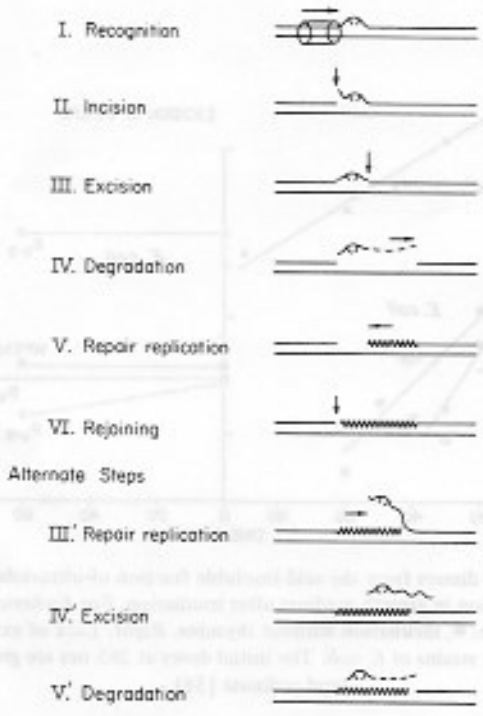

Fig. 6. Schematic representation of the postulated steps in the excision repair of damaged DNA. Steps I through VI illustrate the 'cut and patch' sequence. An initial incision in the damaged strand is followed by local degradation before synthesis of the region has begun. In the alternative 'patch and cut' model, resynthesis step III' begins immediately after 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 strand [59].

(2) It is not observed following UV irradiation of the UV-sensitive strain *E. coli* B_{s-1}, which is unable to perform the excision step in the repair sequence [15].

(3) The non-conservative mode of repair replication can also be demonstrated by the use of D_2O, ^{13}C, and ^{15}N as density labels for newly synthesized DNA to rule out possible artifacts caused by the pathogenicity of 5BU [1, 19].

(4) In low dose experiments (in which viability was as high as 80%) it was demonstrated that DNA which had incorporated 5BU non-conservatively after UV irradiation could then proceed to replicate by the normal semi-conservative model [16].

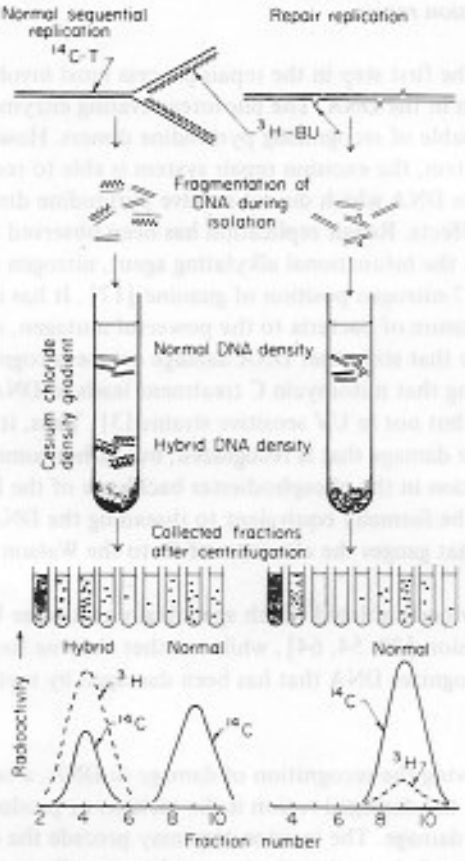

Fig. 7. Protocol for the demonstration of normal replication and repair replication of DNA in growing cells. The DNA is first radioactively labeled (e.g., by the incorporation of ^{14}C-thymine); then the cells are permitted to incorporate another radioactive label at the same time that a 'density label' is being incorporated (e.g., ^{3}H-5-bromouracil). 5-Bromouracil (5BU) is an analog of thymine that can be incorporated into DNA in place of the natural base thymine. Since 5BU is more dense than thymine it has the effect of increasing the density of the DNA fragments that contain it. This density increase is, of course, proportional to the relative amount of thymine and 5BU in the DNA. The density distribution of the isolated DNA fragments is analyzed by means of equilibrium sedimentation in the ultracentrifuge in a density gradient of cesium chloride solution. At equilibrium the DNA fragments will be found in the gradient at positions that correspond to their buoyant densities rather than to their size. This is essentially the method developed by Meselson and Stahl [36] and utilized by them to prove that DNA normally replicates semiconservatively (shown on left half of figure). Parental DNA fragments that contain short regions of repair may differ little in density from those that contain no 5BU (shown on right half of figure) (adapted from Hanawalt and Haynes [18]).

4.2. *The steps in excision repair*

4.2.1. *Recognition.* The first step in the repair process must involve the recognition of the damaged region in the DNA. The photoreactivating enzyme, of course, has been shown to be capable of recognizing pyrimidine dimers. However, unlike the photoreactivation system, the excision repair system is able to recognize a variety of structural defects in DNA which do not involve pyrimidine dimers and which do not result from UV effects. Repair replication has been observed following treatment of bacteria with the bifunctional alkylating agent, nitrogen mustard, which primarily attacks the 7-nitrogen position of guanine [17]. It has also been demonstrated following exposure of bacteria to the powerful mutagen, nitrosoguanidine [5]. Indirect evidence that still other DNA damage can be recognized and repaired comes from the finding that mitomycin C treatment leads to DNA degradation in UV resistant bacteria but not in UV sensitive strains [3]. Thus, it may not be the precise nature of the base damage that is recognized, but rather some associated secondary structural alteration in the phosphodiester backbone of the DNA. The damage recognition step may be formally equivalent to threading the DNA through a close-fitting 'sleeve' that gauges the closeness of fit to the Watson and Crick structure [18].

Enzymes have now been isolated which specifically recognize UV damaged DNA as a template for excision [29, 54, 64], while another enzyme has been isolated which specifically recognizes DNA that has been damaged by methyl methane sulfonate [9, 62].

4.2.2. *Incision.* Following the recognition of damage in DNA, a necessary prerequisite to the excision of the damaged region is the incision or production of a single strand break near the damage. The incision step may precede the excision step, although it has not been ruled out that the two might normally occur as a single enzymatic process.

The incision step has been demonstrated in cell-free extracts of *Micrococcous lysodeikticus* by Rörsch and co-workers [46] in an elegant series of experiments with the double-stranded form of bacteriophage ΦX174. The so-called replicative form of this bacteriophage, when irradiated, can be repaired in spheroplasts of wild-type *E. coli* but not in mutants defective in the recognition (and incision?) step in excision repair. However, a marked increase in biological activity was observed when the damaged DNA was first incubated in an extract of *Micrococcous lysodeikticus* before infection of the excision-defective spheroplasts. Confirmation that the extract was indeed performing the incision step in repair came from studies on the sedimentation behavior (to measure single chain breaks) of untreated and UV-irradiated phage after exposure to the extract. The actual excision of pyrimidine dimers from UV-irradiated DNA by extracts of *M. lysodeikticus* has been shown [4].

4.2.3. *Excision and repair replication.* The process of excision and replacement of damaged nucleotides may occur as separate steps or they may be carried out concurrently with a pealing back of the defective DNA strand. The fact that there is a close coordination between excision and repair replication is indicated by the fact that relative to the number of lesions produced in a cell by a given dose of UV irradiation, there are only a relatively small number of chain breaks present at any one time during the repair process [52]. This certainly rules out the concept that excision takes place throughout the whole genome before repair replication proceeds.

The known specificities of exonuclease III and the DNA polymerase from *E. coli* make these enzymes attractive candidates for excision and repolymerization, respectively. An in vitro model for the 'cut and patch' process was demonstrated in which a portion of one strand of a transforming DNA was degraded with exonuclease III with the concommitant loss in biological activity. The biological activity was subsequently restored by the action of the DNA polymerase [44].

Kelly et al. [31] have recently demonstrated exonucleolytic activity in highly purified *E. coli* DNA polymerase. A single strand break in a double-stranded DNA template is translated along the structure as nucleotides are released from the 5′ phosphate end of the template, while the polymerase adds nucleotides to the 3′ hydroxyl end. The 5′ → 3′ exonuclease activity of the polymerase also has the ability to excise mismatched sequences, including pyrimidine dimers, by hydrolyzing phosphodiester bonds in the hydrogen-bonded region on the 3′ side of pyrimidine dimers or other distortions in the polynucleotide duplex. Thus, the Kornberg polymerase can perform both the cut and the patch steps in repair replication, requiring only the ligase to close the polynucleotide chain for complete repair. These results, therefore, support the 'patch and cut' model of repair (fig. 6); the simultaneous excision and replacement of nucleotides.

Two lines of evidence support the concept that different enzyme systems are involved in the normal semi-conservative mode and in the repair mode of DNA synthesis in *E. coli*. Firstly, the repair mode of synthesis is essentially unaffected at the restrictive temperature for normal DNA synthesis in temperature sensitive mutants [19]. Such mutants synthesize DNA and grow normally at 35°C but normal replication stops when the temperature is raised to 42°C, presumably because some component in the replicase complex is thermosensitive. Secondly, it has been shown that repair replication exhibits a greater selectivity for thymine over 5BU than does normal DNA synthesis, when both the natural base and its analogue are present in the culture medium [27]. Since it can be presumed that both types of synthesis utilize the same internal pool of nucleotide triphosphate precursors, the repair polymerase seems to have a more stringent requirement for thymine than does the normal polymerase.

4.2.4. *Rejoining.* The excision repair process is completed by the rejoining of the repaired segment to the continuous intact DNA strand to restore the integrity of the two-stranded molecule. Evidence for the occurrence of this step in vivo was

found by an examination of the molecular weights of single stranded DNA by sedimentation in alkaline sucrose gradients following the gentle lysis of bacteria on top of the gradient (method of McGrath and Williams [35]). Thus, large single stranded DNA fragments were obtained from unirradiated cells and smaller pieces were seen shortly after irradiation. A subsequent reduction in the number of strand breaks with time during incubation after irradiation could be followed by this method [52]. It has been shown that the breaks occur only in the damaged strand [54].

An enzyme has been isolated that is specific for joining single strand breaks in a double stranded polynucleotide providing the break occurs such that there exists a 5′-phosphate in juxtaposition with a 3′-hydroxyl group [38]. This enzyme also requires a divalent cation (Mg^{++} or Ca^{++}) and DPN. This enzyme rejoins the chains through the formation of 3′–5′-phosphodiester linkages. This enzyme has been shown to repair single chain breaks in DNA produced by pancreatic DNase [67]. An ATP-dependent enzyme system with related activity has been purified from *E. coli* infected with T4 bacteriophage [13, 65]. This enzyme (polynucleotide ligase) may well be the rejoining enzyme responsible for the proposed last step in the excision repair of radiation damage. Certainly, mutants deficient in this enzyme are appreciably more radiation sensitive than wild-type strains [39].

4.3. *Generality of excision repair*

We have already discussed that this repair system is not specific only for UV-induced damage, but can also repair chemical damage resulting from the treatement of cells with alkylating agents and certain antibiotics.

Three genes are known to control the excision step in the repair of UV damage [25]. It is not known whether these three genes specify three different enzymes or whether two of the genes function only to control one enzyme. A cell that is mutant at any one of these three loci is just as UV sensitive as a cell that is mutant in all three [25].

This repair system is found in a wide variety of microorganism and in certain strains of mammalian cells in tissue culture. Excision repair has also been demonstrated in the smallest living cells, the mycoplasma [19]. The presence of a DNA repair mechanism in these cells attests to the general importance of such mechanisms for the maintenance of viability in even the simplest organisms.

The preferential removal of thymine dimers has been observed from three human cell lines (RA, RAX-10 and HeLa) in tissue culture [42] but not in mouse L-cells [33]. Rassmussen and Painter [41] found an 'unscheduled' DNA synthesis stimulated by UV in cultures of HeLa cells. Their analysis of the replicated DNA, using the 5BU density labeling method, has provided support for the interpretation that repair replication occurs in mammalian cells.

In studies on repair replication in human skin fibroblasts, Cleaver [6] made an important discovery that correlates carcinogenesis with defective repair of DNA. Fibroblasts from patients with *xeroderma pigmentosum* were found to exhibit much reduced levels of repair replication. It was suggested that the failure of DNA repair

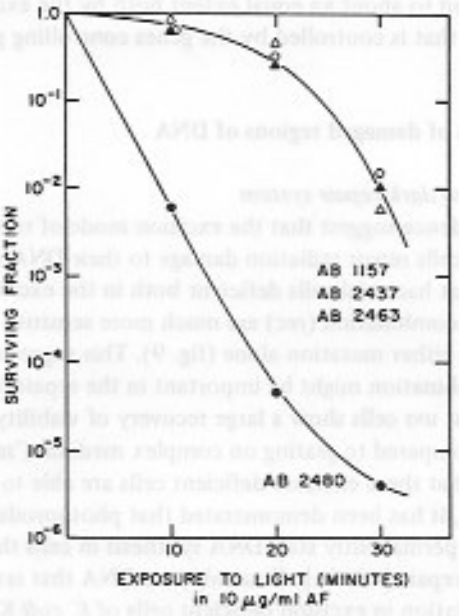

Fig. 8. Survival of mutants of *E. coli* K-12 after exposure to visible light (G.E. 'Daylight' fluorescent lamps) in the presence of acriflavine (AF). AB1157 ('wild-type'), AB2437 (uvrA6), AB2463 (recA13) and AB2480 (uvrA6, recA13) [20].

might be related to the fatal skin cancers that patients with this hereditary disease develop upon exposure to sunlight.

Since bacterial strains that are deficient in the excision process for ultraviolet-induced damage are not particularly sensitive to killing by X-irradiation, it may by hypothesized that either the excision repair system does not play a major role in the repair of X-ray induced damage in the DNA of bacteria or that the incision step is not required for the repair of radiation damage since X-rays themselves produce breaks in the DNA backbone. The repair of X-ray induced damage in DNA will be more thorroughly discussed when we turn to the dark repair system that appears to be controlled by the genes that control genetic recombination.

Numerous investigations have attempted to demonstrate the repairability of the deleterious damage produced when cells are exposed to visible light in the presence of certain dyes (photodynamic action). These attempts have largely proved unsuccessful. However, Harm [20] has recently shown that cells that are deficient in excision of UV damage and cells that are deficient in genetic recombination are just as resistant to the deleterious effects of visible light and acriflavine as is the wild-type strain, whereas the double mutant, deficient both in excision and genetic recombination, is very sensitive to killing by acriflavine and visible light (fig. 8). These results may be interpreted to suggest that the lesions produced by acriflavine and

visible light are repaired to about an equal extent both by the excision repair system and the repair system that is controlled by the genes controlling genetic recombination.

5. Biochemical bypass of damaged regions of DNA

5.1. *Evidence for a new dark repair system*

Several lines of evidence suggest that the excision mode of repair is not the only mechanism by which cells repair radiation damage to their DNA in the dark. The first indication was that bacterial cells deficient both in the excision repair mode (uvr) and in genetic recombination (rec) are much more sensitive to killing by UV than are cells carrying either mutation alone (fig. 9). This suggested that certain steps in genetic recombination might be important in the repair of radiation damage [25]. The fact that uvr cells show a large recovery of viability when plated on minimal medium as compared to plating on complex medium ('minimal medium recovery') suggested that these excision deficient cells are able to repair radiation damage [11]. Thirdly, it has been demonstrated that photoproducts such as pyrimidine dimers do not permanently stop DNA synthesis in cells that are deficient in the excision mode of repair [49, 56]. Fourthly, the DNA that is synthesized immediately after UV irradiation in excision deficient cells of *E. coli* K-12 has discontinuties when assayed in alkaline sucrose density gradients. The mean length of the

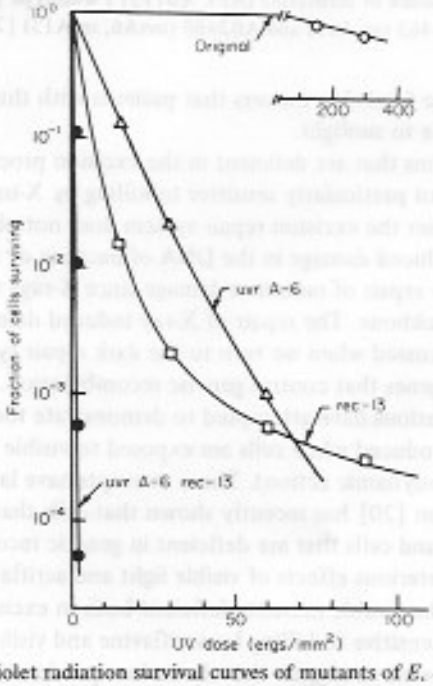

Fig. 9. Ultraviolet radiation survival curves of mutants of *E. coli* K-12 [25].

newly synthesized DNA approximates the distance between pyrimidine dimers in the parental strand. With further incubation of the cells, however, these discontinuities disappear and the DNA approximates the molecular size of that from the unirradiated control cells [49, 26]. A post-replication repair mode is thus indicated which appears to be mediated by some of the enzymes involved in genetic recombination [11, 26, 49].

5.2. *Steps in post-replication repair*

5.2.1. *DNA synthesis can occur on radiation damaged templates.* The effect of UV radiation upon DNA synthesis kinetics has been recently reinvestigated, and it has now been shown that pyrimidine dimers do *not* permanently inhibit DNA synthesis in cells that are deficient in the excision repair of pyrimidine dimers [49, 56]. Because of the stability of UV-induced pyrimidine dimers in excision-defective mutants, it is possible to investigate the replication of bacterial DNA containing a known number of damaged bases. Rupp and Howard-Flanders [49] have measured the molecular weight of the DNA synthesized upon damaged templates in UV-irradiated bacteria, using the technique of McGrath and Williams [35]. In this technique, bacterial protoplasts containing radioactive DNA are lysed on the top of an alkaline sucrose gradient and then sedimented in an ultracentrifuge. The distance that the DNA moves in the gradient under these conditions is proportional to the molecular weight of the single-stranded pieces of the DNA.

The DNA from excision deficient cells that have been labeled prior to UV irradiation with radioactive thymidine exhibits essentially the same sedimentation characteristics whether the cells are irradiated with UV and immediately banded in the centrifuge or whether they are allowed to incubate in growth medium for about 70 min before banding in the ultracentrifuge (fig. 10). This indicated that the parental DNA in these excision-deficient strains was not broken down nor were a significant number of single chain breaks introduced into the parental DNA during this time period. The parental DNA therefore appears to be a stable template on which DNA can be synthesized [26, 49, 60].

However, DNA synthesized by UV irradiated cells during a 10-min pulse is of lower molecular weight than DNA synthesized during 10 min in unirradiated cells [49]. Fig. 10 shows a typical alkaline sucrose density gradient sedimentation for radioactive DNA from cells that were labeled for 10 min after exposure to 0 or 63 erg/mm^2 of UV at 254 nm. The DNA synthesized during a 10 min labeling pulse in the untreated cells sediments nearly as fast as the parental DNA. In contrast, DNA synthesized after UV irradiation sediments much more slowly, indicating that it is of a lower molecular weight.

The factors that are presently known that affect the sedimentation characteristics of the DNA that is synthesized after UV irradiation are as follows:

(a) The molecular weight of the DNA synthesized after UV is a function of the dose of UV (fig. 11). The higher the dose of UV the slower the sedimentation rate

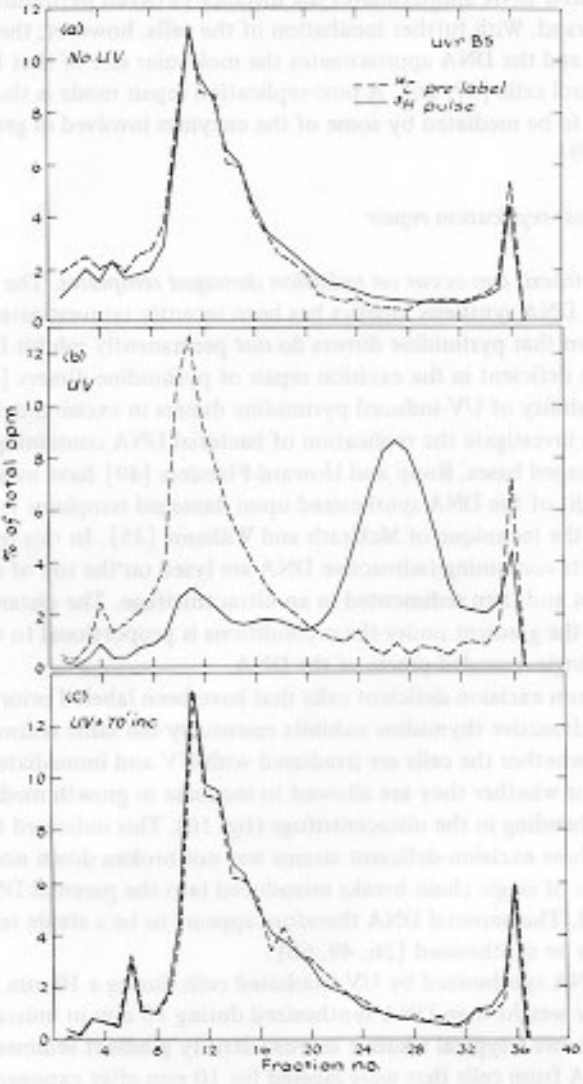

Fig. 10. Sedimentation in alkaline sucrose gradients of DNA labeled before and after UV irradiation. *E. coli* K-12 (uvrB5) were grown for several generations on ^{14}C-thymine and the ^{14}C-thymine was removed from the medium. The cells were then pulsed with ^{3}H-thymidine for 10 min (a) before or (b) after 63 erg/mm^2 (254 nm) and (c) after 70 min of further incubation in non-radioactive medium. The cells were protoplasted and lysed on an alkaline sucrose gradient and spun in rotor SW 50.1 at 30,000 rpm for 105 min at 20°C in a Spinco L2-65B centrifuge [60].

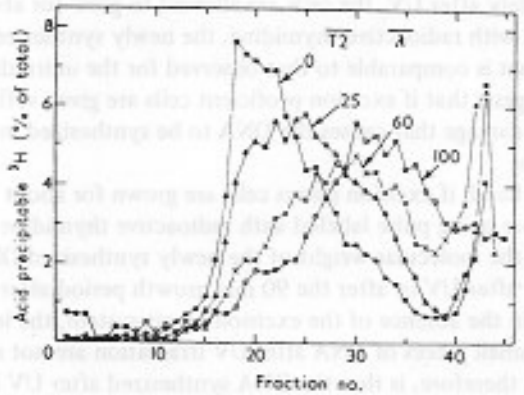

Fig. 11. Effect of several doses of UV on the sedimentation characteristics of the DNA synthesized by *E. coli* K-12 AB2500 uvrA6 after UV irradiation. The conditions are similar to those described in fig. 10 except that the DNA was not prelabeled. Centrifugation was in a SW 50 rotor for 120 min at 30,000 rpm at 20°C in a Spinco L2 centrifuge. The positions of intact strands of phages T2 and λ are indicated. The numbers by the arrows refer to dose of UV (254 nm) in erg/mm² [49].

and therefore smaller the molecular weight of the DNA synthesized after UV irradiation [49].

(b) Knowing the number of pyrimidine dimers produced per erg of UV radiation and the molecular weight of the *E. coli* chromosome, one can calculate the mean distance between pyrimidine dimers in the *E. coli* chromosome for a given dose of UV. The average molecular weight of the DNA synthesized after UV irradiation was found to be in close agreement with the average molecular weight of the pieces of parental DNA that were present between the pyrimidine dimers. The obvious conclusion therefore was that the DNA was synthesized along the undamaged section of the parental DNA but the polymerase skipped the section containing the pyrimidine dimers, thus leaving a gap in the daughter strand DNA [49].

(c) When irradiated cells were exposed to visible light under conditions that favor photoreactivation and then pulsed with radioactive thymidine, it was found that the size of the pieces synthesized was much larger than for the same cells prior to the photoreactivation treatment [48, 60]. Since photoreactivation is known to repair pyrimidine dimers in situ [51], it follows that pyrimidine dimers are important in determining the length of the DNA pieces that are synthesized after UV irradiation.

(d) If a cell that is capable of excision repair is UV irradiated and then pulsed for 10 min with radioactive thymidine, small pieces of DNA are observed to be synthesized as they are in the cells that are excision deficient [48, 60]. This indicates that the excision repair system does not in itself interfere with the post-replication repair process.

(e) However, if an excision proficient cell is irradiated with UV and, then instead

of pulsing immediately after UV, the cells are allowed to grow for about 60 min before being pulsed with radioactive thymidine, the newly synthesized DNA is of a molecular weight that is comparable to that observed for the unirradiated control cells [60]. This suggests that if excision proficient cells are given sufficient time they can repair the damage that causes the DNA to be synthesized in short pieces after UV irradiation.

(f) On the other hand, if excision minus cells are grown for about 90 min after UV irradiation before being pulse labeled with radioactive thymidine, there is little difference between the molecular weight of the newly synthesized DNA whether pulsed immediately after UV or after the 90 min growth period after UV [49, 60]. This indicates that in the absence of the excision repair system, the lesions leading to the synthesis of small pieces of DNA after UV irradiation are not repaired.

The implication, therefore, is that the DNA synthesized after UV irradiation contains real gaps that are presumed to be opposite the pyrimidine dimers present in the parental template strands of the DNA [49]. Since the experiments are performed in strong alkali, it could equally be argued that the newly synthesized DNA contains some kind of alkaline labile bond opposite each pyrimidine dimer which then is cleaved when it is placed in the alkaline sucrose gradients. Howard-Flanders and co-workers [26] have performed one experiment to directly test this hypothesis in the absence of alkali. Labeled DNA was isolated from control and irradiated cells with phenol. It was denatured by heating for 5 min at 100°C and then centrifuged in a neutral sucrose gradient. The pulse labeled DNA from the UV irradiated cells sedimented more slowly in a neutral sucrose gradient than did the DNA from the control cells. These authors concluded that it appears unlikely that the low molecular weight chains synthesized upon the damaged template are joined by alkali labile bonds. However, since the formulation of subsequent steps in the current working hypothesis for post-replication repair assumes the presence of real gaps in the newly synthesized DNA, independent confirmation of the presence or absence of real gaps is desirable.

5.2.2. *Repair of defects in the daughter strands of DNA*

5.2.2.1. *The chemical nature of post-replication repair.* If instead of assaying the UV irradiated cells immediately after pulse labeling with radioactive thymidine the cells are incubated in the presence of non-radioactive thymidine for various periods of time before sedimentation in alkaline sucrose, the lifetime of the low molecular weight material synthesized on the damaged template can be investigated. With a 60 min incubation in non-radioactive medium, after the post-radiation pulse, the slow sedimenting material is converted to a fast sedimenting form that is comparable in its sedimentation with that of the control DNA (fig. 10).

This increase in molecular weight cannot be due to degradation of the pulse labeled DNA and a reutilization of the label for the synthesis of high molecular weight DNA. If this were true, then the radioactivity in the small molecular weight piece should simply disappear and radioactivity should appear directly in the high

molecular weight fraction. What is observed, however, is that there is a progressive shift in the molecular weight of the pulse labeled material with time towards the molecular weight of the unirradiated DNA [59]. This suggests that the lower molecular weight material is enzymatically joined during incubation into high molecular weight DNA. This change may reflect the action of a genetic recovery mechanism.

5.2.2.2. *Genetic control of post-replication repair.* In the introduction to this section, we listed several lines of evidence that suggest that the excision repair mode is not the only mechanism by which cells repair radiation damage to their DNA. Among these observations was the indication that certain steps in genetic recombination might be important in the repair of radiation damage. If this post-replication repair of radiation damage is mediated by some of the enzymes involved in genetic recombination [11, 26, 49] then one would expect to find recombination deficient mutants unable to repair the discontinuities in the newly synthesized DNA. This hypothesis has been investigated and it has been observed [57] that recA mutants synthesize the small pieces of DNA after UV irradiation but they are unable to repair the discontinuties in the newly synthesized DNA (fig. 12). This result lends support to the hypothesis that this post-replication repair system is mediated by the genes that control genetic recombination. However, it has been observed [58] that two other recombination deficient mutants, namely recB (fig. 13) and recC are capable of repairing the discontinuities in the DNA synthesized after UV irradiation. It is not known at this time whether these results mean that there are steps in this repair process beyond the mere repair of the discontinuties in the DNA or whether the deficiencies of the recB and recC mutants are quantitative rather than qualitative.

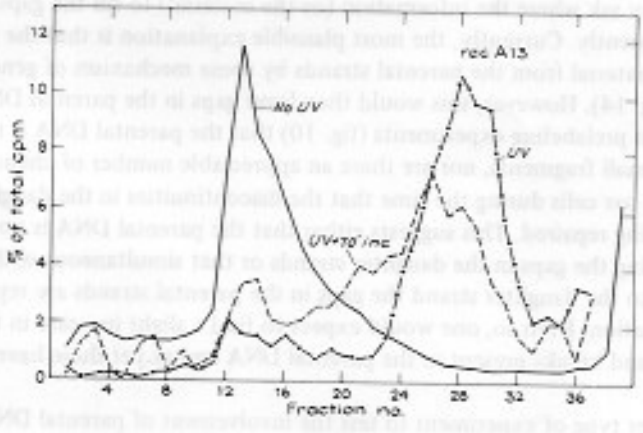

Fig. 12. The absence of post-replication repair in *E. coli* K-12 (rec A13). The conditions are similar to those described in fig. 10 except that the DNA was not prelabeled [60].

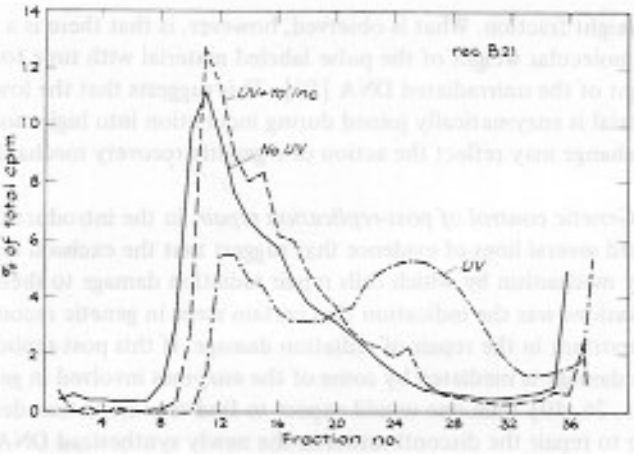

Fig. 13. The presence of post-replication repair in *E. coli* K-12 (recB21). The conditions are similar to those described in fig. 10 except that the DNA was not prelabeled [60].

Experiments in progress in this laboratory are designed to investigate this problem. Specifically, we are attempting to determine if at still higher doses of UV radiation the kinetics of repair of the discontinuities are the same in the rec$^+$ cells and in the recB and recC cells. If the rates prove to be different then the deficiency would appear to be quantitative rather than qualitative. We already have evidence that this is the case for the repair of single strand breaks produced in these strains by X-irradiation [30]. This point will be discussed more fully below.

5.2.2.3. *The role of parental DNA in filling the gaps in the daughter strands.* If information was not available during the time of DNA synthesis to fill in these gaps then one may ask where the information (or the material) to fill the gaps comes from subsequently. Currently, the most plausible explanation is that the gaps are filled with material from the parental strands by some mechanism of genetic recombination (fig. 14). However, this would then leave gaps in the parental DNA and it appears from prelabeling experiments (fig. 10) that the parental DNA is not broken down into small fragments, nor are there an appreciable number of chain breaks produced in uvr cells during the time that the discontinuities in the daughter strand DNA are being repaired. This suggests either that the parental DNA is not being utilized for filling the gaps in the daughter strands or that simultaneous with the transfer of material to the daughter strand the gaps in the parental strands are repaired by repair replication. Even so, one would expect to find a slight increase in the number of single strand breaks present in the parental DNA and as yet these have not been observed.

In another type of experiment to test the involvement of parental DNA in post-replication repair, excision defective cells were density labeled for several generations in a ^{13}C–^{15}N medium containing ^{14}C-thymine. The cells were then transferred

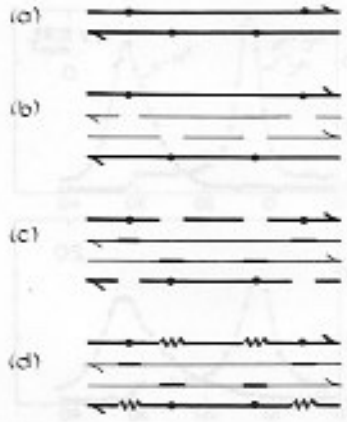

Fig. 14. A model for post-replication repair of UV-damaged DNA. (a) Dots indicate radiation lesions produced in the DNA. (b) DNA synthesis proceeds past the lesions in the parental strands leaving gaps in the daughter strands. (c) Filling of the gaps in the daughter strands with material from the parental strands by a recombinational process. (d) Repair of the gaps in the parental strands by repair replication.

to light medium without radioactive label for 30 min so that the growing point would be incorporating only light label at the time of irradiation. The cells were then exposed to 0, 20 or 50 erg/mm^2 (254 nm) and incubated in the presence of ^{3}H-thymidine in light medium for 30 min. The DNA was isolated and after heat denaturation, the single stranded DNA was centrifuged to equilibrium in a cesium chloride gradient. In the unirradiated cells the heavy ^{14}C-peak and a light ^{3}H-peak were symmetrical and well separated from one another. After irradiation the ^{14}C-peak remained heavy and quite symmetrical whereas the ^{3}H-peak became skewed (as a function of dose) towards the heavy side of the gradient (fig. 15). This indicates that in the UV irradiated cells the newly synthesized DNA has become associated in the same strand with dense label that was synthesized 30 min before irradiation. Rupp and Howard-Flanders [50] have interpreted these results as suggesting that the intermediate density material was produced by recombinational exchanges between sister duplexes, and offer this as a method by which the gaps are repaired in the DNA that is synthesized after UV irradiation.

5.3. *Generality of dark repair controlled by recombination genes*

5.3.1. *Photodynamic action.* As first indicated by Harm [20] and now confirmed by our laboratory [23], the sensitivity to killing by mutants that are deficient in either excision or in recombination are not grossly different from rec$^+$ uvr$^+$ cells in their sensitivity to killing by acriflavine and visible light. However, cells whose genotype is rec uvr are very sensitive to killing by acriflavine and visible light. This sug-

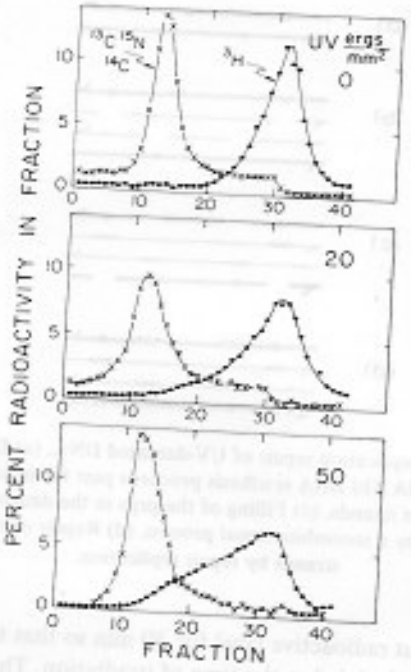

Fig. 15. Evidence for sister strand exchanges in UV irradiated but not in unirradiated *E. coli* K-12. Cells were prelabeled with ^{13}C, ^{15}N and ^{14}C-thymine; grown 30 min in ^{13}C, ^{14}N, non-radioactive medium; UV irradiated and then grown in ^{3}H-thymidine for 30 min; DNA isolated and denatured and banded in CsCl gradients [43].

gests that the excision repair system and the repair system controlled by the recombination genes are of about equal efficiency in the repair of the damage produced in bacteria by the action of visible light in the presence of acriflavine. The photodynamic lesion that leads to the inactivation of bacteria cells is not known. However, our laboratory is currently investigating the possibility that these lethal lesions may be the cross-linking of DNA and protein, a process that is known to occur with a high efficiency with several different photodynamic dyes [59].

5.3.2. *X-rays.* Cells that are deficient in the excision repair of UV damage are only slightly more sensitive to killing by X-rays than are wild-type cells. However, cells that are deficient in genetic recombination are very easily killed by X-rays. This suggests that the repair of X-ray damage may be mediated by the genes that control genetic recombination. We have attempted to test this hypothesis. The major lesion produced by X-irradiation appears to be the chain break produced in DNA. Single chain breaks have been shown to be repaired in resistant but not sensitive strains of bacteria [35], whereas double chain breaks do not appear to be repaired in *E. coli*

[28]. We have therefore studied the production and the repair of X-ray induced single chain breaks in various mutants of *E. coli* K-12.

The excision deficient cells are able to repair X-ray induced single chain breaks with about the same efficiency as the wild-type cells. The recA mutants appear to be unable to repair single chain breaks. RecB and recC mutants can repair single chain breaks but not with the same efficiency with which the wild-type cells perform this function [30].

We have observed that when cells starved for amino acids for 90 min, to allow them to complete their DNA synthesis, are irradiated with X rays and incubated in the absence of amino acids, no repair of single chain breaks occurs. However, if amino acids are added back to the cultures immediately after X-irradiation, the kinetics of the repair of single chain breaks are comparable to those for cells that have never been deprived of amino acids [D.S. Kapp and K.C. Smith, unpublished observations]. This experiment does not distinguish between the possibility that either active DNA synthesis is required before single chain breaks can be repaired or that some labile protein which is lost in the absence of amino acids is required for the repair of these single chain breaks.

Although it has been reported that hydroxyurea (an inhibitor of DNA synthesis) does not inhibit the repair of X-ray induced single chain breaks in mammalian cells in culture [63] we have found it (and/or an associated impurity) to be a potent inhibitor in bacterial cells [D.S. Kapp and K.C. Smith, unpublished observations]. The addition of impure hydroxyurea not only inhibits the repair of single chain breaks produced in irradiated bacterial cells, but it also has a profound effect upon the survival of the cells after X-irradiation. No such effect of hydroxyurea on the viability of X-irradiated recA-56 cells was observed. Since these cells cannot normally repair single chain breaks, the added insult of an inhibitor of this repair process causes no additional killing of these X-irradiated cells.

If the repair of X-ray induced single chain breaks is mediated by the genes controlling genetic recombination, one may speculate as to the biochemical mechanism by which these single chain breaks are repaired. After UV irradiation, the gaps that need to be repaired are in the newly synthesized daughter strands (fig. 14). The cells therefore contain at least four strands of DNA that can be used for recombinational processes to give one viable genome. In the X-ray case, however, immediately after irradiation the breaks are in the parental strands. One may then ask where the extra DNA required for recombinational events can come from. This may come from additional nuclei within the cells since it has been shown that diploid yeast cells are more resistant to X-ray inactivation than are haploid cells [37].

6. Conclusions

Two dark repair systems are now known, differing both in their biochemical mechanism and in their genetic control.

(1) The excision repair system is controlled by the uvr genes. The steps envisioned in this system are those of recognition of the damaged region in the DNA, the cutting out of the damaged region, the patching of the hole by polymerase action, and the subsequent linking of the repaired section by the polynucleotide ligase. This repair system is not specific for UV damage, but has also been shown to repair certain kinds of chemical damage to bacterial DNA. The excision repair system appears to be of about equal importance with the repair system mediated by the genes that control genetic recombination for the elimination of UV damage in bacterial cells. Using the effects of acriflavine as an example of the photodynamic inactivation of bacterial cells, one may conclude that excision repair and recombinational repair are of about equal importance for repairing photodynamic lesions. The excision repair system appears to be of minor importance for the repair of X-ray damage to bacteria, while the recombinational repair system seems to be of extreme importance.

(2) Post-replication repair appears to be controlled by the genes that control genetic recombination. Growth conditions that favor DNA synthesis are required for this repair system to express itself. Thus, after UV irradiation, it appears that DNA synthesis proceeds past lesions in the parental DNA leaving gaps in the daughter strands opposite these lesions. Upon subsequent incubation, these gaps in the daughter strands are repaired by recombinational events whose mechanisms are yet unknown. If parental DNA is involved in the filling of the gaps in the daughter strands, then it is done by a very efficient process that also includes the filling of the gaps left in the parental strands by this process.

We should be cautioned by the fact that several years ago many people thought that the excision repair system could explain all of the repair phenomena in radiation biology. With further experimentation, however, the new dark repair system mediated by the genes that control genetic recombination has been discovered. Still other mechanisms of repair may yet be discovered.

Addendum

Since this talk was presented (September 1969), significant progress has been made in elucidating several systems for the repair of X-ray-induced DNA single-chain breaks. While rec-gene-controlled repair takes about 40 min in growth medium, a new repair system that takes only about 5 min in buffer gas been discovered which requires the action of the Kornberg DNA polymerase. There is also preliminary evidence for an ultrafast system (less than 2 min in buffer; ligase only?) for the repair of X-ray-induced single-chain breaks.

These recombination and DNA polymerase controlled repair systems have recently been reviewed (K.C. Smith in Photophysiology *6* (1971) 209). In addition, the irreversible inhibition of the rec repair system by certain drugs is described.

References

[1] D.R. Billen, R. Hewitt, T. Lapthisophon and P.M. Achey, J. Bacteriol. *94* (1967) 1538.
[2] R.P. Boyce and P. Howard-Flanders, Proc. Nat. Acad. Sci. US, *51* (1964) 293.
[3] R.P. Boyce and P. Howard-Flanders, Z. Vererbungsl. *95* (1964) 345.
[4] W.L. Carrier and R.B. Setlow, Biochim. Biophys. Acta *129* (1966) 318.
[5] E. Cerdá-Olmedo and P. Hanawalt, Mutation Res. *4* (1967) 369.
[6] J.E. Cleaver, Nature *218* (1968) 652.
[7] M.M. Elkind, in: Radiation Research, ed. G. Silini (North-Holland, Amsterdam, 1967) p. 558.
[8] M.M. Elkind and W.K. Sinclair, in: Current Topics in Radiation Research, Vol. 1, eds. M. Ebert and A. Howard (North-Holland, Amsterdam, 1965) p. 165.
[9] E.C. Friedberg and D.A. Goldthwait, Cold Spring Harbor Symp. Quant. Biol. *33* (1968) 271.
[10] A.K. Ganesan and K.C. Smith, J. Bacteriol. *96* (1968) 365.
[11] A.K. Ganesan and K.C. Smith, Cold Spring Harbor Symp. Quant. Biol. *33* (1968) 235.
[12] A.K. Ganesan and K.C. Smith, J. Bacteriol. *97* (1969) 1129.
[13] M.L. Gefter, A. Becker and J. Hurwitz, Proc. Natl Acad. Sci. US *58* (1967) 240.
[14] L. Grossman, Photochem. Photobiol. *7* (1968) 727.
[15] P.C. Hanawalt, in: Recent Progress in Photobiology, ed. E.J. Bowen (Blackwell, Oxford, 1965) p. 82.
[16] P.C. Hanawalt, Nature *214* (1967) 269.
[17] P. Hanawalt and R.H. Haynes, Biochem. Biophys. Res. Commun. *19* (1965) 462.
[18] P.C. Hanawalt and R.H. Haynes, Scientific American *216* (1967) 36.
[19] P.C. Hanawalt, D.E. Pettijohn, E.C. Pauling, C.F. Brunk, D.W. Smith, L.C. Kanner and J.L. Couch, Cold Spring Harbor Symp. Quant. Biol. *33* (1968) 187.
[20] W. Harm, Biochem. Biophys. Res. Commun. *32* (1968) 350.
[21] R.H. Haynes, Photochem. Photobiol. *3* (1964) 429.
[22] R.H. Haynes, in: Physical Processes in Radiation Biology, eds. L. Augenstein, R. Mason and B. Rosenberg (Academic Press, New York, 1964) p. 51.
[23] O. Hidalgo-Salvatierra and K.C. Smith, unpublished observations (1969).
[24] A. Hollaender and W. Claus, Bull. Nat. Res. Council, Nat. Acad. Sci. US *100* (1937) 75.
[25] P. Howard-Flanders and R.P. Boyce, Radiation Res. Suppl. *6* (1966) 156.
[26] P. Howard-Flanders, W.D. Rupp, B.M. Wilkins and R.S. Cole, Cold Spring Harbor Symp. Quant. Biol. *33* (1968) 195.
[27] L. Kanner and P.C. Hanawalt, Biochim. Biophys. Acta *157* (1968) 532.
[28] H.S. Kaplan, Proc. Nat. Acad. Sci. US *55* (1966) 1442.
[29] J.C. Kaplan, S.R. Kushner and L. Grossman, Proc. Nat. Acad. Sci. US 63 (1969) 144.
[30] D.S. Kapp and K.C. Smith, Radiation Res. *42* (1970) 34.
[31] R.B. Kelly, M.R. Atkinson, J.A. Huberman and A. Kornberg, Nature *224* (1969) 495.
[32] J. Kiefer, Photochem. Photobiol. *11* (1970) 37.
[33] M. Klimek, Neoplasma *12* (1965) 599.
[34] S.E. Luria, Proc. Nat. Acad. Sci. US *33* (1947) 253.
[35] R.A. McGrath and R.W. Williams, Nature *212* (1966) 534.
[36] M. Meselson and F.W. Stahl, Proc. Nat. Acad. Sci. US *44* (1958) 671.
[37] R.K. Mortimer, Radiation Res. *9* (1958) 312.
[38] B. Olivera and I.R. Lehman, Proc. Nat. Acad. Sci. US *57* (1967) 1426.
[39] C. Pauling and L. Hamm, Proc. Nat. Acad. Sci. US *60* (1968) 1495.
[40] D. Pettijohn and P. Hanawalt, J. Mol. Biol. *9* (1964) 395.
[41] R.E. Rasmussen and R.B. Painter, J. Cell Biol. *29* (1966) 11.
[42] J.D. Regan, J.E. Trosko and W.L. Carrier, Biophys. J. *8* (1968) 319.

[43] D. Reno and P. Howard-Flanders, (1969) unpublished observations kindly made available to this author.
[44] C. Richardson, R. Inman and A. Kornberg, J. Mol. Biol. *9* (1964) 46.
[45] R.B. Roberts and E. Aldous, J. Bacteriol. *57* (1949) 363.
[46] A. Rörsch, P. van de Putte, I.E. Mattern and H. Zwenk, in: Radiation Research, ed. G. Silini (North-Holland, Amsterdam, 1967) p. 771.
[47] C.S. Rupert and W. Harm, Advan. Radiat. Biol. *2* (1966) 1.
[48] W.D. Rupp, Abstracts of the Fifth International Congress on Photobiology (1968) p. 143.
[49] W.D. Rupp and P. Howard-Flanders, J. Mol. Biol. *31* (1968) 291.
[50] W.D. Rupp and P. Howard-Flanders, Abstracts of the Biophysical Society Meeting (1969) A-20.
[51] J.K. Setlow (1972) chapter 19 of these proceedings.
[52] R.B. Setlow, in: Radiation Research, ed. G. Silini (North-Holland, Amsterdam, 1967) p. 525.
[53] R.B. Setlow, Prog. Nucleic Acid Res. Mol. Biol. *8* (1968) 257.
[54] R.B. Setlow, W.L. Carrier and J.K. Setlow, Biophys. J. *9* (1969) A-57.
[55] K.C. Smith, Radiation Res. Suppl. 6 (1966) 54.
[56] K.C. Smith, Mutation Res. *8* (1969) 481.
[57] K.C. Smith and A.K. Ganesan, Abstracts of the Biophysical Society Meeting (1969) A-20.
[58] K.C. Smith and A.K. Ganesan, Abstracts of the Pacific Slope Biochemical Conference (1969) p. 13.
[59] K.C. Smith and P.C. Hanawalt, Molecular Photobiology: Inactivation and Recovery (Academic Press, New York, 1969).
[60] K.C. Smith and D.H.C. Meun, J. Mol. Biol. *51* (1970) 459.
[61] B.S. Strauss, Current Topics in Microbiology and Immunology *44* (1968) 1.
[62] B. Strauss, M. Coyle and M. Robbins, Cold Spring Harbor Symp. Quant. Biol. *33* (1968) 277.
[63] S. Swada and S. Okada, Abstracts of the Biophysical Society Meeting (1969) A-201.
[64] Y. Takagi, M. Sekiguchi, S. Okubo, N. Nakayama, K. Shimada, S. Yasuda, T. Nishimoto and H. Yoshihara, Cold Spring Harbor Symp. Quant. Biol. *33* (1968) 219.
[65] B. Weiss and C.C. Richardson, Proc. Nat. Acad. Sci. US *57* (1967) 1021.
[66] E.M. Witkin, Brookhaven Symp. Biol. *20* (1968) 17.
[67] S.B. Zimmerman, J.W. Little, C.K. Oshinsky and M. Gellert, Proc. Nat. Acad. Sci. US *57* (1967) 1841.