Title
GENETIC IMPLICATIONS OF DNA REPAIR

Authors
Haynes, R.H.
Baker, R. M.
Jones, G. E.

Publication Date
1967-04-01

UCRL-17964

cy. 2

University of California

Ernest O. Lawrence Radiation Laboratory

GENETIC IMPLICATIONS OF DNA REPAIR

R. H. Haynes, R. M. Baker, and G. E. Jones

April 1967

DISCLAIMER

Presented at the NATO Advanced Study Institute,
Portmeirion, Wales, April, 1967

UCRL-17964

UNIVERSITY OF CALIFORNIA

Lawrence Radiation Laboratory
Berkeley, California

AEC Contract No. W-7405-eng-48

GENETIC IMPLICATIONS OF DNA REPAIR

R. H. Haynes, R. M. Baker, and G. E. Jones

April 1967

TABLE OF CONTENTS

GENETIC IMPLICATIONS OF DNA REPAIR

I. Introduction

There are four fundamental biological processes in which DNA, as the primary genetic material, plays a central role: they are replication, transcription, mutation, and recombination. Replication provides for the duplication of each DNA strand prior to cell division; transcription of genetic information into messenger RNA is the initial step in protein synthesis; mutation and recombination give rise within species to the hereditary variation that is the basis of evolution. In the context of these four phenomena we must now consider a fifth basic process involving DNA - namely, repair, a function found in many cells which enables them to correct a variety of structural defects that may be formed in their DNA.

One important mode of repair depends on the fact that the two strands of DNA are complementary to one another according to the well-known base-pairing rules for the nucleotide bases (i.e., the purines, adenine and guanine, are normally paired with the pyrimidines, thymine and cytosine, respectively). Thus, if a portion of one strand is damaged or deleted, the genetic information lost from that segment can be retrieved from the intact complementary strand. The selective advantage arising from the potential for repair inherent in this duplex structure could account for the ubiquity of the double-stranded form of DNA as the genetic material of cells.

One might expect that repair would affect other genetic phenomena, and a number of recent experiments indicate that this is the case. It is our purpose in this paper to explore, in a primarily speculative way, some of these genetic implications of DNA repair. For a more detailed discussion and criticism of experimental results and interpretations, the reader should consult

some of the recent reviews in this field.[1-7] The major part of our discussion will be based upon the results of radiation experiments with bacteriophages and procaryotic cells. This approach does not arise from any whimsical predilection for these creatures but, rather, reflects the fact that most of our knowledge of molecular genetics has been derived from studies of these less complex organisms. Unfortunately, we still know relatively little about the structure and behavior of eucaryotic chromosomes, and, although repair has been observed in many lower eucaryotes, it remains uncertain whether such processes occur in mammalian cells.

The main hypotheses to emerge from this work concerning the relation between repair and replication are: first, that mechanisms exist for the repair of both intra- and interstrand crosslinks in DNA that would otherwise block normal replication;[3,8] second, that repair mechanisms may serve as "quality control" devices for monitoring the fidelity of the DNA information content;[7] and third, that repair enzymes could close the single-strand breaks that may be introduced into DNA to allow local unwinding of its helical secondary structure during normal replication.[9] It has also been suggested that single-strand breaks are introduced into DNA in the course of messenger RNA synthesis; the subsequent repair of these breaks would indicate an association between repair and transcription.[10]

Induced premutational changes in the DNA structure are also subject to repair, although this is apparently not true for spontaneous mutations.[11-14] While it is unlikely that mutational changes are introduced into the DNA of bacteria as a result of inaccurate functioning of repair enzymes, such a mechanism has been suggested as a possible source of somatic mutations in mammalian cells which could account for the wide range of specific antibody proteins that can be formed.[15] Finally, it has been argued that there must

be an optimal mutation frequency in natural populations to provide simultaneously for the genetic stability and evolutionary plasticity of each species.[16-18] Since most mutations in well-adapted organisms are deleterious, DNA repair mechanisms might serve to maintain genetic stability in the face of mutagenic agents in the environment. Nevertheless, if repair should become too efficient it could reduce the mutation frequency to such a low level that a species might become trapped in an evolutionary dead-end. Thus, the efficiency of DNA repair processes may be the product of selection for a low but optimal mutation frequency in natural populations.[7]

Genetic recombination is now thought to occur by a process involving breakage and reunion of the two participating DNA molecules.[19] Since certain of the steps that may be required for such a process are at least "topologically" similar to some of those involved in repair, it has been suggested that repair enzymes might also be involved in recombination.[20] This argument can be extended to implicate similar enzymes in a number of other phenomena related to recombination, e.g., prophage integration and lysogenic induction, bacterial transformation, and gene conversion.

It is the far-reaching nature of the genetic implications of DNA repair that has caused so much excitement in this field today. Let us now examine in more detail the experimental and theoretical basis of these ideas.

II. GENERAL ASPECTS OF REPAIR

It is useful at the outset to distinguish among three words which are often used somewhat ambiguously by different authors. The word repair is used to denote the actual enzymic steps that restore damaged segments of DNA to a potentially functional form. The qualification "potentially functional" is necessary because the repair of any particular defect need not be effective in restoring cell viability or in preventing the occurrence of a mutation. In fact, it is known that even if the vast majority of defects formed by a moderate dose of radiation are repaired, those few escaping repair might be sufficient to inactivate the cell. Clearly, there can be substantial biochemical repair in cells that would be counted dead in a biological assay for viability. Furthermore, in some circumstances all defective segments of the DNA might be repaired, and yet the cell could still fail to multiply because of some other concomitant metabolic failure caused by the inactivating agent.

The words reactivation and recovery are often used interchangeably to describe the biologically observed outcome of repair (remembering, of course, that there need not be a one-to-one relation between repair and recovery). However, there is some advantage in utilizing these two words to distinguish between distinct experimental situations.[21] We use the word recovery to denote an increase in viability resulting from post-irradiation treatments other than those normally used in some standard assay for viability (e.g., liquid holding recovery in UV-irradiated yeast (Fig. 1)).[22] On the other hand, we use the word reactivation to denote the enhanced viability of a repair-proficient strain of cells compared with that of repair-deficient mutants derived from the same experimental stock[21] (e.g., the relative resistance of Escherichia coli B/r compared with E.coli B_{s-1} to inactivation by 2537 Å UV or nitrogen

mustard (Fig.2)). Thus, recovery is measured by comparing the viability of a given strain of cells under two different sets of experimental conditions; reactivation is measured by comparing the viability of two related strains under a fixed set of experimental conditions. (The major exception to this usage is the term photo-reactivation.)

The four major elements involved in any enzymic repair process are: the segment of DNA to be repaired, some localized structural defect in this segment, the repair enzyme(s), and an energy supply. The essential features of repair are: first, the recognition of the defect - those enzyme-substrate interactions involved in the initial binding of the repair enzyme or enzyme complex to the defective DNA segment; and, second, the execution of the repair steps themselves. On the basis of the known structure of DNA, three distinct categories of repair can be visualized, as sketched in Fig. 3: (1) reversal of the defect in situ; (2) removal of the defect, accompanied or followed by replacement of the defective nucleotides through de novo DNA synthesis (now called "repair replication"); and (3) physiological bypass which leaves the defect intact but makes it possible for the cell to multiply and carry on those biosynthetic activities essential for survival.[22] Photoreactivation of UV-induced pyrimidine dimers[23] and "ligase"[24] or "sealease"[25] rejoining of broken phosphodiester bonds are well-established examples of the first type of repair; excision of pyrimidine dimers or guanine cross-links accompanied by repair replication is the prototype of the second.[26-30] It is convenient to refer to the latter as "excision-repair" or "dark repair". There is at present no documented example of in vivo repair based on a "bypass" mechanism, although liquid-holding recovery in diploid yeast may represent such a case,[31] and in vitro DNA synthesis can be diverted around pyrimidine dimers in parental strands.[32]

Enzymes that effect repair in situ are presumably specific for some

unique characteristic of the defects to be corrected, and this mode of repair probably is a one-step process. Enzymological studies of the photoreactivation and break-rejoining enzymes support this idea.[24,25,33] In photoreactivation "recognition" would consist in the specific binding of photoreactivating enzyme to dimerized nucleotides. One would not expect this enzyme to be capable of repairing other kinds of defects in DNA, and, indeed, defects induced by nitrogen mustard or X-rays are not photoreactivable. On the other hand, repair by removal and replacement of the defective nucleotides appears to be a multi-step process initiated by nuclease attack on DNA. A variety of chemically distinct defects can be repaired in this way: lesions induced by UV, nitrogen mustard, X-rays, mitomycin C, and other agents all appear to be susceptible of excision-repair.[1-3,8] In this mode of repair, it apparently is not the specific form of the primary base damage that is recognized but, rather, some associated secondary structural alteration in the phosphodiester backbone.[30] For example, because of the helicity of DNA, adjacent pyrimidine bases involved in pyrimidine dimer formation must rotate through 36 degrees in the plane perpendicular to the DNA fiber axis and be displaced two angstroms along this axis toward one another. Such a marked change from the normal parallel stacking of the bases must be accompanied by a loss of hydrogen bonding with the opposite strand and a marked distortion of the phosphodiester backbone. Presumably it is this distortion that is recognized by the nucleases involved in excision-repair. Considerations such as these led to the suggestion that a single excision-repair enzyme sequence might constitute a general error-correcting mechanism for DNA.[21]

In precisely what ways might such a multistep enzymic process be said to be "general"? Three simple possibilities are: (1) that a unique set of enzymes, for which the recognition step is relatively non-specific, is capable of repairing a variety of different defects; (2) that different defects can be repaired by

a common enzymic pathway but with a highly specific recognition enzyme for each type of defect; and (3) that the steps involved in the repair of different defects are just "topologically" equivalent, the net result of repair being the removal and replacement of the defective nucleotides.

There is yet another way that a group of chemically distinct defects could be repaired by a common pathway even though all the enzymes involved in the sequence should be highly specific in their action. The recognition and repair of different defects could be effected if each repairable defect could be recognized by some enzyme in the sequence. Consider the repair sequence represented by the first reaction scheme shown in Fig. 4. Here, $(DNA)_n$ denotes a polynucleotide segment containing a defect which requires a sequence of n enzymic steps to repair. The enzymes in the pathway are E_1, E_2, ..., E_n. The recognition step for repair of the defect consists of the initial binding of E_1 to $(DNA)_n$. The intermediate configurations of the DNA segment during repair are $(DNA)_{n-1}$, $(DNA)_{n-2}$, ..., etc. Suppose that each of the enzymes in the sequence functions independently and that any one of the intermediate DNA configurations can be induced independently by some physical or chemical agent. Thus, despite the specificity of E_1 (or any other enzyme in the sequence), induced defects resembling any of the intermediate configurations could be repaired by the partial sequence of enzymes to the "right" of this configuration in the complete repair sequence. Such a repair system could be described as being "general" for the range of defects resembling DNA configurations $(DNA)_n$ through $(DNA)_1$. Furthermore, a mutational block in any intermediate enzyme would give a sequence unable to repair defects resembling configurations to the "left" of the enzyme but still able to repair defects resembling all subsequent configurations. For example, it is known that X-rays and methyl methane sulfonate (MMS) produce single-strand breaks in DNA, and single-strand breaks occur during the repair of

UV-induced pyrimidine dimers.[34,35] Therefore, the finding of UV sensitive mutants of E.coli that are nonetheless resistant to X-rays[36] and UV sensitive mutants of Bacillus subtilis that are resistant to MMS[37,38] was taken to imply that enzymes in a single excision-repair sequence were capable of functioning independently in just this manner.

The situation would be more complicated if the repair enzymes were bound together in a complex. In this case the repair sequence might be represented by the second reaction scheme shown in Fig. 4. Here we assume that, because the enzymes are associated in a complex, they are unable to function independently, and that the complex has only one effective recognition site, E_1.* The specificity of repair is then determined completely by the specificity of the first enzyme. Only if E_1 were a relatively non-specific endonuclease could the complex function as a general error-correcting device. Furthermore, a mutational block of any intermediate enzyme might conceivably abolish the function of the entire complex and yield a phenotype unable to repair defects resembling any of the DNA configurations in the repair sequence. It has, in fact, been suggested that excision-repair enzymes which act on UV-induced pyrimidine dimers and chemically induced cross-links in DNA may be organized in such a complex.[1,39] If this is true, then it is probably incorrect to conclude that an intermediate block in a unique repair sequence is responsible for the formation of the UV sensitive, X-ray (or MMS) resistant mutants described above. Rather, it would be more likely that at least two distinct repair enzyme sequences exist,

---

*This assumption is biochemically reasonable, and if it is not made, then the behavior of the enzyme complex would be operationally indistinguishable from the sequence of independent enzymes just described.

one capable of repairing cross-links, the other, strand breaks. However, according to the third criterion for generality of repair mentioned above, it would remain appropriate to regard these two repair sequences as belonging to the same general category of repair, even though structurally different enzymes may be involved in each process.

III. DARK REPAIR OF DNA STRUCTURAL DEFECTS

A. Repair of Intra- and Interstrand Cross-links

Biological studies of the radiation sensitivity of E.coli mutants, host-cell reactivation of UV-irradiated bacteriophages, liquid-holding recovery of irradiated yeast and bacteria, and mutation frequency decline (coupled with the gradually accumulating evidence that damage to DNA is the primary cause of radiation inactivation) led a number of workers to postulate the existence of DNA repair processes.[22,40-42] These recovery phenomena are to be distinguished from photoreactivation in that they do not require exposure to visible light; for this reason the underlying DNA repair processes are called "dark repair". The demonstration that photoreactivation arises from the enzymic repair of DNA structural defects provided the intellectual paradigm for much subsequent thinking in this field.[43] Here it was shown that UV-induced pyrimidine dimers in DNA cause biological damage and that photoreactivation is based on the specific cleavage of these dimers in situ.[44-46] These findings made it reasonable to suppose that analogous enzymes might exist which could repair inactivating and mutagenic defects "in the dark". The mapping of genetic loci controlling the radiosensitivity of E.coli lent further credence to this hypothesis.[47-49]

The first biochemical results which provided some insight into the possible mechanism of dark repair were obtained in the well-known "excision" experiments reported in 1964 by Setlow and Carrier[26] and by Boyce and Howard-Flanders.[27] These experiments, together with the subsequent discovery of "repair replication" by Pettijohn and Hanawalt,[28] demonstrated that a repair mechanism based on the removal and replacement of UV-induced pyrimidine dimers (i.e., intrastrand cross-links) did indeed exist. The experimental results can be summarized by describing the type of DNA turnover that is found in UV resistant and sensitive strains of E.coli. In these experiments, the intracellular

distribution of radioactively labeled DNA subunits observed immediately after irradiation is compared with that observed about one half hour later. This provides us with a picture of what might be called the "initial state" and the "final state" of the DNA, respectively (see Fig. 5). No turnover (i.e., neither loss of dimers nor incorporation of nucleotides) is observed after irradiation of UV sensitive, presumably repair-deficient strains such as E.coli B_{s-1}. The initial state of the DNA is, in this case, the same as the final state. However, after the same dose of ultraviolet light, substantial DNA turnover is observed in UV resistant, repair-proficient strains such as E.coli B/r. In these cells dimers and nucleotides are lost from the DNA, and new material is incorporated in the form of small segments scattered randomly throughout each original strand. If we accept the inference that this pattern of DNA turnover is a manifestation of repair, then we are faced with the problem of postulating a reasonable sequence of enzymic steps to account for the observations. Two models have been suggested, each of which is compatible with the biochemical results currently available.[26,27,39] They are described colloquially as the "cut-and-patch" and "patch-and-cut" schemes (Fig. 6).[7] As far as possible, they take into account the polarity of action of known nucleases and polymerases. The "cut-and-patch" model postulates that an endonuclease enzyme excises a short, trinucleotide segment containing the dimer (or that a longer segment is exised and subsequently degraded by exonucleases). This initial excision would account for the appearance of trinucleotides in the acid-soluble fraction of irradiated B/r extracted with trichloroacetic acid (TCA). The resulting gap is then enlarged by exonuclease attack on the exposed 3'- hydroxyl terminus, accounting for the solubilization of additional nucleotides derived from the DNA. Following the exonuclease degradation step, repair replication begins. DNA polymerase inserts the proper nucleotides into the gap left after degradation, their correct sequence being determined by

nucleotides present in the opposite, intact strand. This re-synthesis would account for the appearance in the original DNA strands of short segments of newly polymerized material. Since DNA polymerase can not connect the last newly inserted nucleotide to the exposed 5′ terminus of the original strand, a rejoining step must be postulated to avoid leaving a single-strand break in the DNA . Recently, an enzyme called "sealease" (or "ligase") has been identified which is capable of catalyzing this reaction.[24,25]

In the "patch-and-cut" scheme the process is assumed to be initiated by a single incision that cuts the DNA strand near the defect (Fig. 6). Repair replication is assumed to begin immediately, accompanied by a "peeling back" of the defective strand as the new bases are inserted. Repair is completed by a second cut, which releases the original segment, followed by the rejoining step described above. This scheme is attractive because it could conceivably be carried out by a single enzyme complex that moves in one direction along the DNA molecule repairing defects as it goes. Furthermore, it does not involve the introduction of long, vulnerable, single-strand regions into the DNA in the course of repair. Both models are undoubtedly oversimplifications of the steps actually involved in dark repair, but they do indicate that plausible schemes can be devised to account for the biochemical manifestations of repair.

Analogous biochemical observations of excision and repair replication have been made in cells treated with the bifunctional alkylating agents nitrogen mustard (HN2) and sulphur mustard.[29,30] Despite the fact that there is no chemical resemblance between the intrastrand pyrimidine dimers induced by UV and the interstrand guanine cross-links formed by HN2 (cf. Figs. 7 and 8), these results provide convincing evidence that both types of defect are repaired in the same general way. The same conclusion has been drawn from similar experiments with mitomycin C[50].

The biochemical results discussed above were obtained in experiments with various strains of E.coli. Evidence for repair has also been derived from physiological experiments with other bacteria and with yeast.[51-59] These results, together with further studies of dimer excision, DNA degradation, and/or repair replication indicate that repair is a phenomenon of apparently wide phylogenetic distribution. For example, excision and DNA degradation have been measured in Micrococcus radiodurans [60] and Bacillus megaterium;[61-64] repair replication has been measured in the pleuropneumonia-like organism, Mycoplasma laidlawii,[65] and in the eucaryotic protozoan, Tetrahymena pyriformis.[66] A discussion of the data on repair in mammalian cells is to be found in section IX of this paper.

There have been a number of attempts to demonstrate repair in cell-free systems. That these experiments have so far met with rather limited success is not surprising in view of the complexity of the process that is envisaged in vivo. However, protein extracts of Micrococcus lysodeikticus are capable of restoring the biological activity of UV-irradiated transforming DNA[67] and of initiating repair in UV-irradiated replicative DNA of phage øX-174 (presumably by inserting single-strand breaks in specific positions near pyrimidine dimers).[68] The actual excision of dimers has also been reported in similar enzyme preparations.[69]

Two important control experiments lend substance to the "repair" interpretation of biochemical results of the kind illustrated in Fig. 5. First, DNA segments that have undergone repair replication are biologically active in the sense that they are capable of subsequently supporting normal semi-conservative replication.[70] This result undermines the criticism that repair replication is some form of aberrant DNA synthesis of no biological significance. Secondly, comparative autoradiographic analyses of X-irradiated E.coli B/r and B_{s-1} show that radioactively labeled DNA components are lost from essentially all the

cells in repair-proficient cultures.[71] This result makes it unlikely that excision or DNA degradation is due primarily to the preferential lysis and degradation of some heavily damaged fraction of the cell population.

B. Repair of Strand Breaks in DNA

Breaks in the phosphodiester backbone of DNA can be produced by ionizing radiation [32,72,73] and also as a consequence of depurination by MMS and other alkylating agents.[1] Both single- and double-strand breaks occur, but it is generally believed that only double-strand breaks are lethal in bacteriophages and procaryotic cells such as E.coli.[34,73] However, even double-strand breaks need not be lethal in cells in which the broken DNA molecules are likely to be protected by complex nucleoprotein structures. This latter situation could account for the apparent ability of M. radiodurans and certain eucaryotic cells to repair double-strand breaks in their DNA.[74] It has also been reported that single-strand breaks— or at any rate, alkali-labile phosphodiester bonds — are present normally, and at presumably well-defined positions, in both viral and cellular DNA.[75-78] So far, no one has thought of a good reason why these breaks should be present, but, if they remain unrepaired throughout the normal biosynthetic processes in which DNA participates, one is again forced to conclude that single-strand breaks are not intrinsically harmful to the cell.

If single-strand breaks are biologically innocuous, it might be argued that cells would have no need of any mechanism for their repair, unless under some circumstances they were to occur in such large numbers that the physical continuity of DNA would otherwise be endangered. Such might be the case if breaks were introduced normally in the course of DNA replication or messenger RNA transcription.[2] At any rate, physico-chemical analysis of the transitory drop in the molecular weight of individual DNA strands after X-irradiation of E.coli[79] and M. radiodurans[74] serves to demonstrate that these cells possess

mechanisms for the repair of broken DNA strands.[80] Recently, enzymes capable of forming phosphodiester bonds at the site of single-strand breaks in duplex DNA molecules have been isolated from both phage-infected and normal cells of E.coli; and the actual repair of single-strand breaks in viral DNA has been demonstrated both by sedimentation and end-group analyses.[24,25] However, it is uncertain whether these enzymes are the same as those that are supposed to rejoin the breaks introduced during excision-repair of pyrimidine dimers. It should also be noted that no nucleotide turnover is associated with the action of these enzymes in vitro.

IV. REPLICATION AND REPAIR

The expectation that there exists a close association between replication and repair has been a major stimulus for much recent work in molecular radiation biology. It is thought that this association is manifested in at least two ways. First, repair serves to remove structural defects from DNA that would otherwise block DNA synthesis, and second, repair could close strand breaks that might be associated with the unwinding of DNA during normal replication. There is a good deal of experimental support for the first of these ideas, but so far only one experiment has been reported which seems to lend substance to the second. Hanawalt measured the background level of DNA turnover, as determined by P^{32} incorporation, in parental DNA strands of E.coli. He found that this turnover was three to five times greater in those DNA regions that had replicated during the labeling period than in those that had not.[2,9] This result is consistent with the notion that single-strand breaks are introduced into DNA in the course of normal replication and that these breaks are repaired as the growing point moves on to the next segment of the parental strands to be replicated. However, the strand-rejoining enzymes that have so far been studied *in vitro* are capable of making phosphodiester bonds between the 3'-hydroxyl and 5'-phosphoryl groups of adjacent nucleotides in the absence of any phosphate turnover.[24,25] On this basis, the detection of P^{32} turnover *in vivo* need not be a necessary correlate of the repair of single-strand breaks *per se*.

It is well established that UV-induced pyrimidine dimers in DNA serve to block DNA synthesis both *in vivo* and *in vitro*.[81,82] (Such blocks may not be absolute; *in vitro* evidence suggests that slow polymerization can occur around them.[32]) Dimers are also responsible for more than 50% of the UV inactivation of the biological activity of transforming DNA.[83] A strong case can thus be

made for the hypothesis that unrepaired dimers inhibit cell multiplication by blocking DNA replication in E.coli.[8] This hypothesis provides a basis for answering one of the classical questions of cellular radiobiology—namely, why are cells inactivated by radiation—and leads us to a new mathematical model for interpreting radiation dose-response curves.

A schematic outline of the main features of this model is given in Fig. 9. It is convenient to distinguish three main steps in the inactivation process: (1) irradiation and the immediate physicochemical absorption processes that give rise to chemically stable structural defects in DNA; (2) the biochemical responses to these defects during the first few hours of incubation of the cells on growth medium after irradiation; and (3) the observation of the net biological effect of the radiation in terms of the clone-forming ability of the cells. It is assumed that in normal, repair-proficient cells the presence of DNA structural defects triggers the excision-repair mechanism, and, if all the initial, potentially lethal defects are repaired, normal replication resumes and a clone is formed. If for any reason normal replication proceeds before repair is complete, replication may be irreversibly blocked by an unrepaired defect "arriving" at the DNA growing point; clone formation is then impossible. Thus, we assert that survival after irradiation is determined jointly by the probabilities of the formation and the subsequent repair of structural defects in DNA.[21] The formation of double-strand breaks in the DNA by the simultaneous excision of neighboring defects in both strands would also lead to inactivation. Yet another route to lethality would be via blocked or erroneous messenger RNA transcription.[84] This interpretation of microbial inactivation by ultraviolet light can also be applied to inactivation by X-rays, nitrogen mustard, and other chemical mutagens. However, it must be realized that, expecially with X-rays, there may be other causes of inactivation that have so far not been identified.

V. REPAIR AND THE INTERPRETATION OF INACTIVATION CURVES

If the DNA inactivation mechanism described above is correct, then we must modify the traditional target theory equations used to describe radiation survival data to incorporate the concept of repair. One particularly simple approach leads to an equation that provides a better description of the UV survival curve of E.coli B/r than does the usual multi-target expression.[8,55] This approach is based on the fact that lethality in a viability assay is an all-or-nothing phenomenon and the assumption that cell-lethal hits are random, independent events distributed throughout the irradiated population according to Poisson statistics. We postulate that cell-lethal hits are, in fact, unrepaired DNA structural defects. We can then write,

$$\text{Surviving fraction of cells} = e^{-[F(x) - R(x)]}$$

where $F(x)$ = number of potentially lethal defects formed initially by the radiation, and $R(x)$ = number of defects removed by repair. The efficiency of repair can be defined as the ratio $R(x)/F(x)$. In the most general case, F should be written as a sum of terms, one for each type of defect contributing to lethality; R should also be a summation, taking into account the possible relevance of several repair mechanisms. We are at present unable to proceed with such a detailed analysis and must be content with a less sophisticated approach. Let us assume, as a first approximation, that the number of potentially lethal defects initially formed increases in proportion to dose (i.e., $F(x) = kx$) and that the number of defects removed by repair at first increases linearly but then reaches a plateau with increasing dose, e.g., $R(x) = \alpha(1-e^{-\beta x})$. This gives us an explicit expression for survival which yields a better fit to many UV dose-response curves than the usual multi-target equation (cf. curves A and B in Fig. 10). The shoulder in curve A results from the assumed reduction in the

efficiency of repair with increasing dose. The asymptotic slope gives the sensitivity of the cells in the absence of repair; clearly, this limiting slope is almost as steep as the exponential slope for the repair-deficient mutant B_{s-1} (Fig. 10).

This exercise in curve-fitting demonstrates an interesting fact about the rate at which the efficiency of repair declines with increasing dose. Using the values of the parameters k, α, and β obtained from the B/r survival curve in Fig. 10, one can construct a plot of R versus F, i.e., the number of hits removed by repair versus the initial number of potentially lethal hits (Fig. 11). If repair were 100% efficient, R would be equal to F for all doses. It is clear from this graph that R is very close to F for small numbers of initial hits and decreases very slowly as the number of potentially lethal hits increases. In this case repair is 100% efficient (i.e., R = F) at vanishingly small UV doses and is reduced only to 88% at a cell survival of 10^{-6}. In Fig. 12 we have plotted both cell survival and the efficiency of repair (R/F); the efficiency of repair falls off much more slowly than survival with increasing UV dose. The slowness of this decline is consistent with our assertion that substantial biochemical repair takes place in cells that are counted dead in viability assays. It is also of interest that the UV dose required to reduce the efficiency of repair by a factor of e^{-1} is about 14,000 ergs/mm^2 at 2537 Å. This dose is comparable to that required for the equivalent inactivation of enzymes at this wavelength.

The average number of DNA defects repaired per cell at any level of radiation dose is described by the term R(x) in the above expression for survival. In order to arrive at an explicit equation which could be used in a preliminary fit of experimental data, we have assumed that R(x) could be represented by a simple, exponential saturation function. That such an expression is not too

unreasonable is indicated by the "goodness of fit" of the theoretical curve to the experimental points shown in Fig. 10. However, it is also possible to determine experimentally the relation between repair and radiation dose by measuring the total amount of repair replication occurring in cells after various UV doses. The results of a preliminary experiment of this kind are shown in Figs. 13 and 14. Here, the Pettijohn-Hanawalt technique was used to determine the amount of nonconservative DNA synthesis[28] taking place following the application of various UV doses to cultures of E.coli TAU-bar. In these cells normal DNA replication was blocked by amino acid starvation (i.e., DNA "completion" cultures were used.[85]) Under these conditions, the amount of newly synthesized, radioactively labeled DNA that bands in a cesium chloride density gradient at the position of normal density DNA can be taken as a measure of the number of defects repaired. The amount of this newly synthesized material for each UV dose is shown in Fig. 13. Assuming that for each dose a constant number of nucleotides is inserted into the parental strands for each dimer removed, we obtain the dose-response curve for repair replication shown in Fig. 14. The amount of repair rises quickly to a plateau but then declines as the dose to the cells is increased still further. (This decline at high doses could also be due to an experimental artifact.) This experiment shows that repair is dose dependent and that the form of this dependence is at least qualitatively similar to that assumed in our analysis of the survival data. However, further experiments in which both survival and repair replication are measured for the same cell cultures will be necessary to establish a quantitative relation between the biological and the chemical measurements of repair.

VI. TRANSCRIPTION AND REPAIR

Messenger RNA transcription takes place along only one of the two DNA strands, and this process probably involves a local unwinding of the DNA double helix.[86-88] It is difficult to see how this unwinding could be accomplished without the introduction of single-strand breaks into the DNA. Presumably these breaks must later be repaired, possibly by the action of sealease or ligase alone, although under certain conditions repair replication, as well as the rejoining steps, may be involved. Pauling and Hanawalt, studying the characteristics of DNA turnover after thymine starvation of a thymine-requiring mutant of E.coli,[10] found that a considerable amount of repair replication takes place in thymine-starved cells upon their being presented with the thymine analogue 5-bromouracil. Neither normal nor repair replication can take place in the absence of thymine, but messenger RNA synthesis does occur.[89] It is conceivable that, in the absence of repair replication during thymine starvation, messenger RNA synthesis introduces single-strand breaks which are subsequently enlarged by exonuclease activity. If a thymine substitute is then restored to the medium, repair replication in the cells would fill the gaps resulting from transcription. This hypothesis accounts for their results and, if true, establishes an important connection between transcription and repair.

VII. MUTATION AND REPAIR

A "mutation" is an inheritable change in the genetic information within a genome. Such changes may range from seemingly trivial structural alterations of single DNA bases to the deletion of hundreds of base pairs from the molecule. It has been argued theoretically that an optimal mutation frequency must exist in natural populations in order to provide simultaneously for evolutionary plasticity and genetic stability of selected genotypes.[16] This argument has been taken to imply that mutation frequencies may be under genetic control, and this expectation has received some experimental support.[18] For example, mutant DNA polymerases of bacteriophages have been found that allow increased frequencies of incorrect base insertions during normal DNA replication,[90,91] and "mutator" genes have been discovered in bacteria which alter mutation rates by some unknown mechanism.[92,93] One may well ask whether DNA repair enzymes might also be involved in the regulation or production of mutations. Two possibilities, not mutually exclusive, are that repair enzymes may correct premutational lesions before their effects can be expressed and that base pairing errors may be introduced into DNA during repair replication. However, before proceeding further we must distinguish between spontaneous and induced mutations.

Although repair processes are capable of correcting mispaired bases in DNA[94,95] and, perhaps, of generating mutations in the absence of normal DNA replication,[96,97] they do not appear to affect <u>spontaneous</u> mutation rates; spontaneous mutation frequencies are similar in repair-proficient and -deficient strains of bacteria.[20] This result is not unreasonable when one considers that many spontaneous mutations arise from the insertion or deletion of nucleotides,[98-100] whereas the known modes of repair might be capable only of eliminating mispaired bases. Furthermore, since the two members of a mis-paired

base pair are excised and corrected with equal frequency,[94] repair could at best reverse only one-half of the potential mutations due to base substitution.

UV-induced mutations, however, presumably arise from premutational lesions associated with radiation-induced structural defects in the DNA. It has been found that these premutational lesions (i.e., mutational lesions which have not yet been expressed or stabilized) are subject to repair. (Recent work in this field has been reviewed by Witkin[13] and by Kimball.[14]) Large numbers of induced mutations to streptomycin resistance, tryptophan independence, and inability to ferment lactose are produced in an excision-deficient strain of E.coli at UV doses far below those at which such mutations are detected in the wild type parental strain.[11,12] These experiments show that at least 99% of the premutational damage is repaired in the wild type strain by a process involving excision. Implicit in this finding is the fact that the repair of premutational lesions is far more efficient than their production as a result of mistakes made during repair replication. These experiments also show that the dose-modifying factor for the repair of premutational damage differs from that for the repair of potentially lethal damage; even mutagenic lesions at different loci are not repaired with equal efficiency.[13] Some 90% of the mutations to streptoymcin resistance and tryptophan independence that are induced in the excision-deficient strain are photoreactivable, indicating that potentially lethal pyrimidine dimers do participate in the induction of a large fraction of these mutations. Mutations to inability to ferment lactose are not photoreactivable, however, and must consist of as yet unidentified photoproducts that can be excised but that are not recognized by photoreactivating enzymes. Moreover, suppressor mutations have been implicated in a phenomenon known as "mutation frequency decline", in which potential mutations to prototrophy are irreversibly lost in repair-proficient strains when growth is inhibited after exposure to UV

light. Witkin[13] has proposed that MFD results from excision of a unique fraction of UV damage at particular loci. The efficiency of repair in such loci might depend on the metabolic state of the cell due to the presence of unusual photoproducts or DNA configurations that vary in their susceptibility to repair enzymes.

Studies such as these demonstrate that DNA repair systems are capable of affecting radiation-induced mutation frequencies and suggest that such systems may be of great evolutionary importance in adjusting induced mutation frequencies to optimal levels.

VIII. RECOMBINATION AND REPAIR

Genetic recombination is the phenomenon in which two chromosomes containing homologous genes "mate" and produce new, intact, chromosomes with associations of genes different from that of either parent. Recombination is observed among the complex chromosomes of eucaryotic cells after both mitosis and meiosis; it is the principal mechanism of genetic reassortment among the simpler chromosomes of procaryotic cells and viruses. Our discussion will center upon results obtained in these latter systems, in which the chromosomes are single DNA molecules.

Two general hypotheses have been advanced to account for the mechanism of genetic recombination:

A) Recombination by breakage and reunion.[101,104] According to this model, homologous chromosomes come together and pair along certain regions, the specificity of the alignment presumably being dictated by the nucleotide sequences within the DNA of homologous genes. The paired strands are then broken at homologous sites, and the broken ends are rejoined so that recombinant chromosomes are produced.

B) Recombination by copy choice.[104,107] This model envisions recombination as occurring during the replication of paired DNA molecules. Thus, it is suggested that a point of replication advancing along one chromosome might somehow switch to the other, so that the newly synthesized DNA molecule contains genetic information derived from each of the paired homologues. A second recombinant molecule may be produced by replication along the opposite course.

Without dwelling on the complex genetic evidence that has been advanced to support one or the other of these hypotheses or their variants,[101-108] we may note that two fundamental distinctions may be drawn between them. The copy

choice model requires that recombination occur at the time of DNA replication, while recombination by breakage and reunion could occur in the absence of normal DNA replication. A recombinant formed by breakage and reunion would contain DNA derived from each parental molecule, while a recombinant formed by a copy choice mechanism would contain no parental material. It is on the basis of these distinctions that the copy choice model of recombination has been largely abandoned in recent years.

Meselson and his coworkers[19,109,110] working with prelabeled phages have established that some recombinant particles are composed almost entirely of parental DNA and that there is a correspondence between the amount of DNA and the extent of genetic information which a parental phage contributes to a recombinant. More recently, Tomizawa and Anraku have used labeled parental T4 phages to study the sequence of events in the formation of recombinant T4 chromosomes.[111-113] They have demonstrated (1) that the first step in recombination is the formation of a "joint molecule" consisting of portions of the parental DNA molecules united end to end by means of hydrogen bonds; (2) that these joint molecules are then transformed into intact recombinant molecules in which the components derived from parental structures are covalently bonded; and (3) that while prior protein synthesis is required for the formation of joint molecules, the entire process of recombinant formation can occur in the absence of detectable DNA replication. A T4 mutant defective in the formation of the initial joint molecule has been identified.[114] These experiments provide conclusive evidence that recombination among bacteriophages can occur by a mechanism closely related to breakage and rejoining.

The implications of DNA repair for the problem of genetic recombination are twofold. First, recombination according to a mechanism of breakage and rejoining would seem to involve certain steps which are topologically indistinguishable

from those envisioned for excision-repair. Second, repair may be invoked to account for certain irregular genetic phenomena, such as gene conversion, that are apparently related to recombination but are not easily explained on the basis of a simple breakage and reunion model.

Howard-Flanders and Boyce[20,115] have proposed that the terminal steps of recombination by breakage and reunion include steps also involved in DNA repair. Thus the primary steps of recombination are envisioned to be: (1) the pairing of homologous DNA molecules, followed by the introduction of single strand breaks and the association of complementary single strands from the two parents in some general way such that a "joint molecule" of the type depicted in Fig. 15 - "H" is generated; (2) the degradation of unpaired single strands to eliminate the regions of overlap between the recombined components of the parental structures (Fig. 15 - "I"); (3) repair replication to fill in any single-strand gaps in the recombinant molecule; and (4) the rejoining of the phosphodiester backbones of the molecule so that the recombinant components are united by covalent bonds (Fig. 15 - "J"). This repair sequence would be consistent with the observed progression from a hydrogen-bonded joint molecule to an intact, covalently bonded, recombinant molecule. Moreover, there are indications that recombination in phage λ may be accompanied by the removal and resynthesis of small portions of the molecule such as would result from repair in the region of overlap connecting the recombining components.[19]

The possibility that the excision enzymes themselves might be essential for recombination was eliminated by the finding that bacterial mutants which are defective in excision are able to support normal recombination.[20] The primary evidence in support of common pathways in DNA repair and genetic recombination has been the isolation of "REC$^-$" mutants of E.coli bacteria which are both deficient in recombination and extremely sensitive to ultraviolet light

and X-rays.[116] Several varieties of REC⁻ mutants have been found; they exhibit somewhat different phenotypes corresponding to mutations in different regions of the bacterial chromosome.[117-119] It was at first thought that these mutants might be deficient in the repair replication step that was presumed to be common to both DNA repair and recombination, but subsequent studies have failed to support this interpretation. The REC⁻ strains apparently do possess some repair capacity. One reckless mutant is capable of successfully repairing approximately 95% of UV-induced pyrimidine dimers even though relative to the wild type it is several hundred times more sensitive to UV and its efficiency in recombinant formation is only about 10^{-4}.[120] In fact, repair replication has been observed in these mutants by the Pettijohn-Hanawalt technique.[121] Moreover, although the extent of DNA turnover in the class of REC⁻ mutants known as "cautious" appears to be normal, a "reckless" class of mutants exhibits a high degree of DNA turnover and degradation even when they have not been irradiated.[20,120] Since the nature of the biochemical defect in the REC⁻ mutants is not understood, it remains possible that the joint occurrence of recombination-deficient and radiation-sensitive phenotypes may be attributable to the disruption of basically independent DNA repair and recombination mechanisms by an irregularity of DNA metabolism. Radiation-sensitive mutants that are defective in mitotic or meiotic recombination have been found in the fungus Ustilago maydis, but here too the nature of the altered functions is unknown.[122]

The possible relation between repair and recombination is also being studied in bacteriophages. A T4 mutant, known as T4x, has been found to be about 70% more sensitive to UV than the wild type.[123] The recombination frequencies are reduced about threefold in crosses of T4 in which both parents carry the x mutation, but the number of progeny phage produced per infection is also greatly reduced under these circumstances;[124] again the specific nature of

the defective function is unclear. Whereas T4 is an "autonomous" bacteriophage, whose intracellular development is independent of the integrity of the host cell genome, the smaller phage λ is "dependent" in that it requires the participation of the host's genes and metabolic machinery for its development. UV damage to dependent phages such as λ can be repaired in wild type strains of bacteria by a process known as host cell reactivation (HCR) (Fig. 16), which requires the host-cell excision enzyme and is thought to be similar in nature to the excision-repair of UV-damaged bacterial DNA.[5,125] One may inquire, then, whether host cell functions are also required for recombination among dependent bacteriophages. Normal vegetative recombination and lysogenization occur when λ phages infect an excisionless or recombinationless bacterial host.[118,126,127] However, the principal mode of recombination among S13 (a small coliphage containing only about 1.7×10^6 daltons of single-stranded DNA) is blocked when it is grown in REC⁻ host bacteria.[128]

In an extension of this line of inquiry, we have studied the efficiency of UV-stimulated recombination among λ phages in host bacteria that are defective in DNA repair.[127] It has been known for some time that low doses of ultraviolet light enhance recombination,[129] as do many other agents which produce specific, repairable lesions in DNA. This concurrent stimulation of repair and recombination suggested to us the possibility that a common mechanism might be involved in the two processes. We found, however, that the UV stimulation of recombination is even <u>greater</u> in bacterial hosts that are unable to excise pyrimidine dimers from phage DNA than it is in the wild type strains (Figs. 16 and 17). Moreover, the recombination-promoting UV lesions in the phage DNA are repaired by host cell reactivation with the same efficiency as are potentially lethal UV lesions (Fig. 18). Thus, for a particular UV dose, the number of recombination events is inversely correlated with the number of excision-repair events. These results

eliminate the possibility that host cell excision enzymes might be required for UV-stimulated phage recombination. On the other hand, the UV enhancement of recombination is the same in recombination-deficient bacterial hosts (which are capable of HCR) as it is in the wild type hosts. These results are consistent with the idea that genes controlling λ recombination are contained in the phage genome and that not all (if any) of the host recombination apparatus is essential for λ recombination. The later stages of DNA repair in λ may also be controlled by phage genes, for all known bacterial mutants which are HCR⁻ are defective in only the initial, excision, step of repair.[20,130]

Several λ mutants that are defective in recombination have recently been isolated.[131] It is reported that these mutants have a somewhat greater sensitivity to UV than wild-type λ and that their recombination-deficient phenotype is most pronounced in REC⁻ host bacteria, but more complete data from these studies are not yet available.

Mutants of λ and P22 phages have also been isolated which are "int" (integration-deficient).[132,133] These mutants appear to participate normally in vegetative phage recombination but are defective in their ability to recombine with the bacterial chromosome during the process of lysogenization. It is conceivable that the enzymes required for the integration of temperate phage are also necessary for their induction, since such a mechanism is intact in REC⁻ host bacteria even though no spontaneous or UV induction is observed in "reckless" strains.[126 134]

We are thus confronted with the possibility that at least three different recombination systems operate during temperate phage infection -viz., that which mediates bacterial recombination, that which mediates recombination among vegetative phages, and that which is responsible for lysogenization and perhaps

also induction. Two of these processes and certain analogous steps of DNA repair may be under the control of phage genes, but the precise way in which these mechanisms of recombination are related to repair has yet to be defined.

In the phenomenon of bacterial transformation, competent cells of a recipient strain may acquire genetic characteristics of a donor strain by incorporation of donor DNA which is present in the medium. There is good evidence that in this case a different mechanism of recombination operates to effect the insertion of only single strands of the donor DNA into the recipient chromosome, and it has been proposed that repair processes also participate in this type of recombination.[135,136] Strains of B. subtilis have been isolated which are sensitive to chemical mutagens and radiation and also exhibit impaired transformability.[137,138] It has also been suggested that the differing efficiencies with which various allelic mutations are integrated and preserved in recipient DNA are a reflection of the differential operation of repair processes.[139]

An irregular genetic phenomenon that may be closely related to the involvement of DNA repair processes in recombination is gene conversion.[140,141] This phenomenon, most extensively studied in fungi, consists in the aberrant segregation of two homologous alleles, so that rather than the normal 2:2 Mendelian ratio a 3:1 ratio is observed among the products of meiosis or mitosis. Since this conversion is frequently accompanied by recombination for outside markers, it may be considered an example of recombination which is non-reciprocal for closely linked sites. Holliday has proposed a model in which gene conversion is attributed to the repair of mispaired bases that may arise during the association of complementary strands of the parental chromosomes in a breakage and rejoining recombination event.[142] Whitehouse has advanced a somewhat more elaborate theory which suggests that DNA repair processes are also implicated in the formation of the initial "joint" recombinant molecule, as in Fig. 13,

"A" - "H".[143,146] He has further proposed that the polarity often observed in the frequency of conversion events from one end of an allele to the other may reflect predetermined points of initial strand breakage at the ends of a "polaron." In any case, strong evidence for the involvement of DNA repair and recombination in gene conversion is provided by Holliday's identification of mutations to radiation-sensitivity in Ustilago maydis that have a marked effect on gene conversion as well as on mitotic or meiotic recombination.[122]

Another phenomenon relevant to repair and recombination is the formation of bacteriophage heterozygotes, in which a region of the phage chromosome carries information for different alleles on the complementary DNA strands.[104,147] As with points of gene conversion, most of these heterozygous regions are found in molecules which are recombinant for outside markers.[148-150] It should be noted from Fig. 15 that the generation of heterozygote molecules could be a natural consequence of recombination by a breakage and reunion process. As has been mentioned in earlier sections, mispairing of bases such as must occur in genetically heterozygous regions of DNA can be repaired.[94,95] Such repair has been demonstrated by Hogness during a study of the relative activity of the two strands of λ phage DNA in messenger RNA transcription.[94] He first constructed "heteroduplex" λ DNA molecules by annealing complementary single strands of phage chromosomes differing by a single point mutation. When the template activity of these heteroduplex molecules was observed in normal host cells, the strands appeared to be equally active because the mispaired bases in half of the transcribing strands were repaired to reflect the sequence in the complementary strand. This effect could be suppressed, however, by pre-irradiation of the host cells with a heavy dose of UV in order to "trap" excision-repair enzymes on lesions in the bacterial DNA. Variation in the efficiency of this type of repair may account, in part, for the widely differing

frequencies of heterozygotes found among various kinds of phage.

It is conceivable that repair could also introduce heterozygosity for a particular allele into a DNA molecule. A hypothetical scheme for this process is depicted in Fig. 19, where a heterozygote is represented as resulting from the repair of mis-matched bases in "mated" parental strands during an abortive recombination event. If a second repair event corrects this heterozygosity, there is a 50% chance of converting one parental allele to the other. The net result would thus be an information transfer unaccompanied by any recombination or transfer of physical material. Such a possibility illustrates the versatility of the concept of DNA repair in suggesting explanations for extraordinary genetic phenomena.

IX. THE QUESTION OF REPAIR IN MAMMALIAN CELLS

Having established the existence of DNA repair processes in a number of procaryotic and lower eucaryotic cells, one may well inquire whether such systems are to be found in mammalian cells. Our discussion here will be confined simply to the question of the existence of repair in cultured mammalian cell lines, for at present even this fundamental problem remains unresolved.

Photoreactivation is widely distributed phylogenetically,[151-153] but it has not been found in mammalian cells cultivated in vitro.[154] Why such a ubiquitous phenomenon should be absent from these cells in puzzling, to say the least. There is a good deal of indirect evidence that mammalian cells do possess dark repair mechanisms, but biochemical studies of DNA turnover have not yielded the kind of unequivocal results that have been obtained with bacteria and lower eucaryotes.

One of the earliest indications that mammalian cells possess recovery mechanisms came from the so-called "split-dose" experiments with ionizing radiation.[155] In these experiments, a dose of x-rays is given in two portions separated in time: if recovery occurs between the exposures, fewer cells die than would have if the radiation were given all at once. Such experiments show that cells which survive the first exposure respond to the second as if many of the "sublethal" lesions produced by the first are repaired in the interim.[156] In fact, given sufficient time between the two exposures (about two hours), the surviving cells appear to recover completely from the sublethal effects of the first. (It is important to note that this "recovery" is manifested as a "recovery of radioresistance" rather than the usual recovery of viability in the irradiated population.) Recovery can occur at any time in the cell division cycle and apparently does not depend upon normal DNA synthesis.[156]

The nature of the damage from which recovery occurs between exposures is unknown. One possibility is that lesions must accumulate beyond some "threshold" number before death ensues. If the cells are allowed time to repair or bypass some fraction of the damage caused by the first radiation dose, fewer of them will accumulate the number of lesions necessary for death during the second exposure. Alternatively, the lack of additivity in split-dose experiments might indicate that repair is more efficient in the presence of fewer lesions or that damage to a repair system can be circumvented during the interval between the doses. Whatever model is invoked to account for the quantitative details of these experiments, the results are most easily interpreted on the basis of repair. However, similar split-dose experiments with ultraviolet light give no indication of recovery in mammalian cells.[157]

Further indirect evidence for the existence of repair processes in mammalian cells is the presence of a "shoulder" on the radiation dose-response curves. As discussed above, such shoulders might arise from the progressive inhibition of repair with increasing dose. In addition, caffeine serves to reduce the shoulder on the UV survival curve of mouse L-cells.[158] Caffeine is known to be an inhibitor of dark-repair processes in bacteria, and so it might be concluded that similar processes are inhibited in mammalian cells by this compound.

Numerous attempts have been made to obtain biochemical evidence for dark repair in mammalian cells. However, with few exceptions, attempts to observe the release of pyrimidine dimers from the DNA of UV irradiated cells have yielded negative results. Pyrimidine dimers are formed in mammalian cells with about the same efficiency as in bacteria, and their number increases linearly with dose.[159] But these dimers remain in the acid insoluble fraction of the DNA for several hours after irradiation. It is possible, of course, that the dimers are

initially released in long, single-strand segments which remain insoluble in TCA. Some recent experiments indicate that loss of dimers does occur about 48 hours after irradiation.[160] Similarly, the loss of sulfur mustard lesions from mammalian cells has been reported.[161] Nevertheless, no direct association has been established between the removal of these products and DNA repair.

Experiments analogous to those in which regions of repair replication in bacteria were originally identified have also been carried out with mammalian cells grown in vitro.[162] Autoradiography and density-gradient analysis of their DNA have established that 'unscheduled' DNA synthesis does occur in UV irradiated cells. The synthesis is not semi-conservative, and it takes place in the pre- and post-DNA synthesis periods of the cell division cycle. However, this unscheduled synthesis is not sensitive to caffeine.[163] Similar unscheduled synthesis occurs in X-irradiated mammalian cells.[164]

The experiments discussed here have dealt exclusively with mammalian cells cultured under highly artificial conditions. Almost nothing is known about the ability of in vivo cells to repair their DNA

In summary then, the evidence for the existence of DNA repair in mammalian cells grown in vitro is meager. While some experiments indicate that recovery and DNA repair may occur, most of the available positive evidence is rather ambiguous. Since repair systems are known to occur in other eucaryotes, one might expect to find them in mammalian cells as well. However, the increased complexity of the chromosomes of mammalian cells may frustrate the application of present detection methods.

X. CONCLUSIONS

Having surveyed the evidence for the existence of enzymic DNA repair mechanisms in cells, and having inquired into the possible influence of repair on other genetic processes, we feel constrained to provide an overall assessment of the status and general credibility of these ideas at the present time. The interpretation of the observations summarized in Figs. 2 and 5 in terms of repair appears to be sound and well justified. It might be argued that the very concept of "repair" is distressingly anthropomorphic and that what we take to be "repair" is simply an accidental corollary of some other, unrecognized, metabolic process. One can hardly reply to this sort of philosophical criticism, except to affirm that the notion of repair seems to be at once the simplest and most fruitful working hypothesis presently available. We are satisfied that repair processes are closely related to replication and mutation, because DNA defects capable of inhibiting replication and causing mutations can be corrected by them. The evidence for the involvement of repair in normal DNA synthesis and in messenger RNA transcription is very tenuous, and much more work will be required before any firm conclusions can be reached. The possible relation between repair and recombination remains as tantalizing as ever, and although there are obvious topological similarities between certain steps in each process, there is as yet no direct evidence to support the idea that the enzymes involved are identical.

The most obvious and, in retrospect, the most radical idea to be established by these studies is that the genetic material does not stand insulated from the characteristic processes of breakdown and resynthesis of cellular constituents. DNA turnover is a part of the normal metabolism of cells, and we can now see that the stability of the gene should be attributed as much to the balance of dynamic, biochemical processes as to the structural durability of double-stranded polynucleotide chains.

ACKNOWLEDGEMENTS

This work was supported by the U.S. Atomic Energy Commission and U. S. Public Health Service pre-doctoral fellowships Nos. 5-F1-GM-30, 952-03, and 1-F1-GM-36, 437-01.

REFERENCES

1. B. S. Strauss, in "Current Topics in Microbiology and Immunology," Springer-Verlag, New York, 1968.

2. P. C. Hanawalt, in "Photophysiology, Vol. III" (ed. A. C. Giese), Academic Press, New York, 1968.

3. R. B. Setlow, in "Regulation of Nucleic Acid and Protein Biosynthesis" (ed. V. V. Koningsberger and L. Bosch), p. 51, Elsevier, Amsterdam, 1967.

4. A. Rörsch, P. van de Putte, I. E. Mattern, H. Zwenk and C. A. van Sluis, in "Genetical Aspects of Radiosensitivity: Mechanisms of Repair," p. 105, I.A.E.A., Vienna, 1966.

5. C. S. Rupert and W. Harm, in "Advances in Radiation Biology, Vol. II" (ed. L. Augenstein, R. Mason and M. Zelle), p. 1, Academic Press, New York, 1966.

6. R. H. Haynes, S. Wolff and J. E. Till (eds.), "Structural Defects in DNA and their Repair in Microorganisms," Radiation Res., Suppl. 6, Academic Press, New York, 1966.

7. P. C. Hanawalt and R. H. Haynes, Scientific American, February, 1967, 216, 36; ibid, Umschau, 1967, H. 17, 553.

8. R. H. Haynes, Radiation Res., 1966, Suppl. 6, 1.

9. P. C. Hanawalt, in "Genetical Aspects of Radiosensitivity: Mechanisms of Repair," p. 97, I.A.E.A., Vienna, 1966.

10. C. Pauling and P. C. Hanawalt, Proc. Nat'l. Acad. Sci. U.S., 1965, 54,1728.

11. R. F. Hill, Photochem. Photobiol., 1965, 4, 563.

12. E. M. Witkin, Science, 1966, 152, 1345.

13. E. M. Witkin, Radiation Res., 1966, Suppl. 6, 30.

14. R. F. Kimball, in "Advances in Radiation Biology, Vol. II" (ed. L. Augenstein, R. Mason and M. Zelle), p. 135, Academic Press, New York, 1966.

15. S. Brenner and C. Milstein, Nature, 1966, 211, 242.

16. M. Kimura, Genet. Res., Camb., 1967, 9, 23.

17. S. Zamenhof, L. H. Heldenmuth and P. J. Zamenhof, Proc. Nat'l. Acad. Sci. U.S., 1966, 55, 50

18. S. Zamenhof, in "Advances in Nucleic Acid Research," 1967, Vol. 6, 1.

19. M. Meselson, J. Mol. Biol., 1964, 9, 734.

20. P. Howard-Flanders and R. P. Boyce, Radiation Res., 1966, Suppl. 6, 156.

21. R. H. Haynes, Photochem. Photobiol., 1964, 3, 429.

22. M. H. Patrick, R. H. Haynes, and R. B.Uretz, Radiation Res., 1964, 21, 144

23. C. S. Rupert, S. H. Goodgal, and R. M. Herriott, J. Gen. Physiol., 1958, 41, 451.

24. B. Weiss and C. C. Richardson, Proc. Nat'l. Acad. Sci. U.S., 1967, 57, 1021.

25. M. L. Gefter, A. Becker, and J. Hurwitz, Proc. Nat'l. Acad. Sci. U. S., 1967, 58, 240.

26. R. B. Setlow and W. L. Carrier, Proc. Nat'l. Acad. Sci. U. S., 1964, 51, 226.

27. R. P. Boyce and P. Howard-Flanders, Proc. Nat'l. Acad. Sci. U. S., 1964, 51, 293.

28. D. Pettijohn and P. C. Hanawalt, J. Mol. Biol., 1964, 9, 395.

29. B. Papirmeister and C. L. Davison, Biochem. Biophys. Res. Comm., 1964, 17, 608.

30. P. C. Hanawalt and R. H. Haynes, Biochem. Biophys. Res. Comm., 1965, 19, 462.

31. J. T. Lyman and R. H. Haynes, Radiation Res., 1967, Suppl. 7, 222.

32. F. J. Bollum and R. B. Setlow, Biochim. Biophys. Acta, 1963, 68, 599.

33. A. Muhammed, J. Biol. Chem., 1966, 241, 516.

34. D. Freifelder, Radiation Res., 1966, Suppl. 6, 80.

35. R. B. Setlow, W. L. Carrier, and R. W. Williams, Biophysical Society Abstracts, 1967, 83.

36. B. A. Bridges and R. J. Munson, Biochem. Biophys. Res. Comm., 1966, 22, 268.

37. T. Searashi and B. S. Strauss, Biochem. Biophys. Res. Comm., 1965, 20, 680.

38. H. Reiter and B. S. Strauss, J. Mol. Biol., 1965, 14, 179.

39. "General Discussion" (led by M. Delbrück), in Radiation Res., 1966, Suppl. 6, 227.

40. M. Lieb, Z. Vererbungsl. 1961, 92, 416.

41. E. M. Witkin, J. Cellular Comp. Physiol., 1961, 58, Suppl. 1, 135.

42. W. Harm, Z. Vererbungsl., 1963, 94, 67.

43. C. S. Rupert, J. Cellular Comp. Physiol., 1961, 58, Suppl. 1, 57.

44. R. B. Setlow and J. K. Setlow, Proc. Nat'l. Acad. Sci. U. S., 1962, 48, 1250.

45. D. L. Wulff and C. S. Rupert, Biochem. Biophys. Res. Comm., 1962, 7, 237.

46. J. K. Setlow and R. B. Setlow, Nature, 1963, 197, 560.

47. H. I. Adler and J. C. Copeland, Genetics, 1962, 47, 701.

48. P. Howard-Flanders, R. P. Boyce, E. Simson, and L. Theriot, Proc. Nat'l. Acad. Sci. U. S., 1962, 48, 2109.

49. P. van de Putte, C. A. van Sluis, J. van Dellewijn, and A. Rörsch, Mutation Res., 1965, 2, 97.

50. R. P. Boyce and P. Howard-Flanders, Z. Vererbungsl., 1964, 95, 345.

51. R. B. Roberts and E. Aldous, J. Bacteriol., 1949, 57, 363.

52. T. Alper and N. E. Gillies, J. Gen. Microbiol., 1958, 18, 461.

53. T. Alper and N. E. Gillies, J. Gen. Microbiol., 1960, 22, 113.

54. R. H. Haynes and W. R. Inch, Proc. Nat'l. Acad. Sci. U. S., 1963, 50, 839.

55. R. H. Haynes, in "Physical Processes in Radiation Biology" (ed. L. Augenstein, R. Mason and B. Rosenberg) p. 51, Academic Press, New York, 1964.

56. J. Jagger, W. C. Wise, and R. S. Stafford, Photochem. Photobiol., 1964, 3, 11.

57. A. Castellani, J. Jagger, and R. B. Setlow, Science, 1964, 143, 1170.

58. M. H. Patrick and R. H. Haynes, Radiation Res., 1964, 23, 564.

59. W. Harm, Mutation Res., 1966, 4, 93.

60. M. E. Boling and J. K. Setlow, Biochim. Biophys. Acta, 1966, 123, 26.

61. H. Reiter and B. S. Strauss, J. Mol. Biol., 1965, 14, 179.

62. J. E. Donnellan, R. S. Stafford, and R. B. Setlow, Biophysical Society Abstracts, 1966, 112.

63. H. Reiter, B. S. Strauss, R. Marone, and M. Robbins, Biochem. Biophys. Res. Comm., 1966, 24, 892.

64. R. Shuster, J. Bacteriol., 1967, 93, 811.

65. D. W. Smith, Doctoral Dissertation, Stanford University, 1967.

66. C. F. Brunk and P. C. Hanawalt, Science, 1967, 158, 663.

67. R. Elder and R. F. Beers, J. Bacteriol., 1965, 90, 681.

68. A. Rörsch, C. van der Kamp, and J. Adema, Biochim. Biophys. Acta, 1964, 80, 346.

69. W. L. Carrier and R. B. Setlow, Biochim. Biophys. Acta, 1966, 129, 318.

70. P. C. Hanawalt, Nature, 1967, 214, 269.

71. C. R. Shaffer and R. A. McGrath, Exptl. Cell Res., 1965, 39, 604.

72. A. R. Peacocke and B. N. Preston, Proc. Roy. Soc. London, 1960, B153, 90.

73. G. S. Stent and C. Fuerst, Advances in Biol. and Med. Phys., 1960, 7, 1.

74. C. J. Dean, P. Feldschreiber, and J. T. Lett, Nature, 1966, 209, 49.

75. P. F. Davison, D. Freifelder, and B. W. Holloway, J. Mol. Biol., 1964, 8, 1.

76. D. Freifelder and A. K. Kleinschmidt, J. Mol. Biol.,1965, 14, 271.

77. J. Abelson and C. A. Thomas, J. Mol. Biol., 1966, 18, 262.

78. R. A. McGrath and R. W. Williams, Biophys. J., 1967, 7, 309.

79. R. A. McGrath and R. W. Williams, Nature, 1966, 212, 534.

80. H. S. Kaplan, Proc. Nat'l. Acad. Sci. U. S., 1966, 55, 1442.

81. R. B. Setlow, P. A. Swenson, and W. L. Carrier, Science, 1963, 142, 1464.

82. P. A. Swenson and R. B. Setlow, J. Mol. Biol., 1966, 15, 201.

83. R. B. Setlow and J. K. Setlow, Proc. Nat'l. Acad. Sci. U. S., 1962, 48, 1250.

84. E. C. Pollard, Science, 1964, 146, 927.

85. O. Maaløe and P. C. Hanawalt, J. Mol. Biol., 1961, 3, 144.

86. B. D. Hall, M. Green, A. P. Nygaard, and J. Boezi, Cold Spring Harbor Symp. Quant. Biol., 1963, 28, 201.

87. M. Hayashi, M. N. Hayashi, and S. Spiegelman, Proc. Nat'l. Acad. Sci. U. S., 1963, 50, 664.

88. G. P. Tocchini-Valentini, M. Stodolsky, A. Aurisicchio, M. Sarnat, F. Graziosi, and S. B. Weiss, Proc. Nat'l. Acad. Sci. U. S., 1963, 50, 935.

89. P. C. Hanawalt, Nature, 1963, 198, 286.

90. J. Speyer, J. Karam, and A. Lenny, Cold Spring Harbor Symp. Quant. Biol., 1966, 31, 693.

91. E. Freese and E. Freese, Proc. Nat'l. Acad. Sci. U. S., 1967, 57, 650.

92. J. Treffers, V. Spinelli, and N. Belser, Proc. Nat'l. Acad. Sci. U. S., 1954, 40, 1064.

93. C. Yanofsky, E. Cox, and V. Horn, Proc. Nat'l. Acad. Sci. U. S., 1966, 55, 274.

94. D. S. Hogness, J. Gen. Physiol., 1966, 49, No. 6 part 2, 29.

95. J. Weigle and W. Bode, Biology Annual Report, Calif. Inst. Technol., 1965, 42.

96. F. Ryan, J. Gen. Microbiol., 1959, 21, 530.

97. G. Grigg and J. Stuckey, Genetics, 1966, 53, 823.

98. S. Brenner, L. Barnett, F. H. C. Crick, and A. Orgel, J. Mol. Biol., 1961, 3, 121.

99. E. Freese, Proc. 5th Int'l Cong. Biochem, Moscow, 1961, 204.

100. E. Freese and E. Freese, Radiation Res., 1966, Suppl. 6, 97.

101. T. H. Morgan, Science, 1911, 34, 384.

102. C. D. Darlington, J. Genet., 1935, 31, 185.

103. M. Meselson, in "Heritage from Mendel" (ed. R. A. Brink), p. 81, Univ. of Wisconsin Press, Madison, 1967.

104. W. Hayes, "The Genetics of Bacteria and Their Viruses," John Wiley and Sons, New York, 1964.

105. J. Belling, Univ. Calif. Publs. Botany, 1931, 16, 153.

106. J. Lederberg, J. Cell. Comp. Physiol., 1955, 45, Suppl. 2, 75.

107. C. Levinthal, in "The Viruses" (ed. Burnet and Stanley), Vol. II, p. 281, Academic Press, New York, 1959.

108. A. Sturtevant, "A History of Genetics," Harper and Row, New York, 1965.

109. M. Meselson and J. Weigle, Proc. Nat'l. Acad. Sci. U. S., 1961, 47, 857.

110. G. Kellenberger, M. L. Zichichi, and J. J. Weigle, Proc. Nat'l. Acad. Sci. U.S., 1961, 47, 869.

111. J. Tomizawa and N. Anraku, J. Mol. Biol., 1964, 8, 508, 516.

112. N. Anraku and J. Tomizawa, J. Mol. Biol., 1965, 11, 501.

113. N. Anraku and J. Tomizawa, J. Mol. Biol., 1965, 12, 805.

114. J. Tomizawa, N. Anraku, and Y. Iwama, J. Mol. Biol., 1966, 21, 247.

115. P. Howard-Flanders and R. P. Boyce, Genetics, 1964, 50, 256.

116. A. J. Clark and A. D. Margulies, Proc. Nat'l. Acad. Sci. U. S., 1965, 53, 451.

117. P. Howard-Flanders and L. Theriot, Genetics, 1966, 53, 1137.

118. P. van de Putte, H. Zwenk, and A. Rörsch, Mutation Res., 1966, 3, 381.

119. A. J. Clark, J. Cell. Comp. Physiol., 1967, in press.

120. A. J. Clark, M. Chamberlain, R. P. Boyce, and P. Howard-Flanders, 1966, J. Mol. Biol., 1966, 19, 442.

121. C. Pauling and P. C. Hanawalt, personal communication, 1966.

122. R. Holliday, Mutation Res., 1967, 4, 275.

123. W. Harm, Virology, 1963, 19, 66.

124. W. Harm, Mutation Res., 1964, 1, 344.

125. A. Garen and N. D. Zinder, Virology, 1955, 1, 347.

126. K. Brooks and A. J. Clark, J. Virology, 1967, 1, 283.

127. R. M. Baker and R. H. Haynes, Molec. Gen. Genetics, 1967, 100, 166.

128. I. Tessman, Biophys. Biochem. Res. Comm. ... 1966, 22, 169.

129. F. Jacob and E.-L. Wollman, Ann. Inst. Pasteur, 1955, 88, 724.

130. P. Howard-Flanders, R. P. Boyce, and L. Theriot, Genetics, 1966, 53, 1119.

131. E. Signer, personal communication, 1967.

132. J. Zissler, Virology, 1967, 31, 189.

133. H. O. Smith and M. Levine, Virology, 1967, 31, 207.

134. I. Hertman and S. E. Luria, J. Mol. Biol., 1967, 23, 117.

135. M. Fox and M. Allen, Proc. Nat'l. Acad. Sci. U. S., 1964, 52, 412.

136. W. Bodmer, J. Mol. Biol., 1965, 14, 534.

137. S. Okubo and W. R. Romig, J. Mol. Biol., 1966, 15, 440.

138. J. A. Hoch, M. Barat, and C. Anagnostopoulos, J. Bacteriol., 1967, 93, 1925.

139. H. Ephrussi-Taylor and T. C. Gray, J. Gen. Physiol., 1966, 49, No. 6 part 2, 211.

140. C. C. Lindegren, J. Genet., 1953, 51, 625.

141. M. B. Mitchell, Proc. Nat'l. Acad. Sci. U. S., 1955, 41, 215.

142. R. Holliday, Genet. Res., Camb., 1964, 5, 282.

143. H. Whitehouse, Nature, 1963, 199, 1034.

144. H. Whitehouse and P. Hastings, Genet. Res., Camb., 1965, 6, 27.

145. H. Whitehouse, Sci. Prog., 1965, 53, 285.

146. H. Whitehouse, Nature, 1966, 211, 708.

147. A. D.Hershey and M. Chase, Cold Spring Harbor Symp. Quant. Biol., 1951, 16, 471.

148. C. Levinthal, Genetics, 1954, 39, 169.

149. T. A. Trautner, Z. Verebungsl., 1958, 89, 266.

150. G. Kellenberger, M. L. Zichichi, and H. T. Epstein, Virology, 1962, 17, 44.

151. R. Dulbecco, in "Radiation Biology, Vol. II" (ed. A. Hollaender), p. 455,

McGraw-Hill, New York, 1955.

152. J. Jagger, Bacteriol. Rev., 1958, 22, 99.

153. C. S. Rupert, in "Photophysiology, Vol. 2" (ed. A. C. Giese), p. 283, Academic Press, New York, 1964.

154. J. E. Cleaver, Biochem. Biophys. Res. Comm., 1966, 24, 569.

155. M. M. Elkind and H. Sutton, Radiation Res., 1960, 13, 556.

156. M. M. Elkind and W. K. Sinclair, in "Current Topics in Radiation Research, Vol. 1" (eds. M. Ebert and A. Howard), p. 165, North-Holland Publishing Company, Amsterdam, 1965.

157. A. Han, B. Meletic', and D. Petrovic, Int. J. Radiation Biol., 1964, 8, 187.

158. A. M. Rauth, Radiation Res., 1967, 31 121.

159. J. E. Trosko, E. H. Y. Chu, and W. L. Carrier, Radiation Res., 1965, 24, 667.

160. J. D. Regan and J. E. Trosko, Radiation Res., 1967, 31, 548.

161. A. R. Crathorn and J. J. Roberts, Nature, 1966, 211, 150.

162. R. E. Rasmussen and R. B. Painter, J. Cell Biol., 1966, 29, 11.

163. J. E. Cleaver, Radiation Res., 1967, 31, 607.

164. R. B. Painter and J. E. Cleaver, Nature, 1967, 216, 369.

Figure Captions

Figure 1. Liquid-holding recovery of stationary-phase diploid yeast (Saccharomyces cerevisiae) after irradiation with 2537 A ultraviolet light. The lower curve shows the survival observed upon immediate plating on Wort agar. The upper curve shows survival after four days storage of the irradiated cells in distilled water at 30°C in the dark. The survival curve obtained upon delayed plating is related to that for immediate plating by a constant dose-modifying factor of 1.6.

Figure 2. Comparative survival curves of stationary-phase E.coli B/r and B_{s-1} for inactivation by 2537 A light and the bifunctional alkylating agent, nitrogen mustard [methyl bis (β-chloroethyl)amine hydrochloride]. The cells were grown overnight in peptone broth, washed and resuspended for irradiation in phosphate buffer, and plated on nutrient (salt) agar. Note that the relative resistance of the two strains is similar for both inactivating agents.

Figure 3. Schematic diagram indicating the essential features of repair: (1) the recognition of the defect inherent in the initial binding of the repair enzymes, and (2) the three principle ways in which repair might be executed.

Figure 4. Two models of DNA repair sequences. $[DNA]_n$, $[DNA]_{n-1}$, ... represent sequential DNA configurations during repair involving: I, independent repair enzymes E_1, E_2, ..., E_n; or II, an enzyme complex symbolized by $E_2 \cdots E_n$ / E_1. Biological consequences of these two models are discussed in the text.

Figure 5. The experimentally observed "initial" and "final" states of DNA in UV-irradiated cells of sensitive and resistant strains of E.coli. No DNA turnover occurs in the sensitive strain B_{s-1}; in the resistant strain B/r, DNA breakdown and re-synthesis result in the release of dimers and nucleotides, coupled with the incorporation of new material into short segments distributed randomly throughout the two original strands.

Figure 6. The explicit hypotheses regarding the enzymic steps in repair that can account for the experimental observations illustrated in Figure 5. The main difference between the two is that, in the "Patch-and-Cut" model, repair replication occurs concurrently with the removal of the defect, whereas in the "Cut-and-Patch" model, the defect is removed prior to the initiation of repair replication.

Figure 7. The most common type of pyrimidine dimer formed in DNA by ultraviolet light. Adjacent thymine moieties in the same strand are linked by a cyclobutane ring.

Figure 8. Guanine-guanine cross-linking by nitrogen mustard attack on DNA. The 7-nitrogen atoms of the two guanine moieties in opposite strands of DNA are alkylated by the two "arms" of the bifunctional HN2 molecule.

Figure 9. A schematic outline of the general features of alternative bacterial responses to the formation of DNA structural defects by radiation or chemical treatment. It is assumed that DNA is one of the principal targets for damage relevant to cellular inactivation; that chemically stable defects (represented by asterisks) result from the fast, physico-chemical reactions associated with the initial attack on DNA; that the presence of these defects triggers repair replication; and that, if all the potentially lethal defects are repaired, normal replication resumes and a clone may be formed. Repair and normal replication are regarded as competitive processes, and, if for any reason, normal replication proceeds before repair is complete, its subsequent irreversible blockage by an unrepaired defect makes clone formation impossible. A possible alternatate route to lethality would be via blocked or erroneous messenger RNA transcription.

Figure 10. A, the fitting of a set of UV survival points for E.coli B/r to the new dose-response function discussed in the text. B, a fit of the same data to the classical one-hit, multitarget equation is shown for comparison. The asymptote of curve A is shown displaced to the left so that it passes through the origin; this curve is to be compared with the B_{s-1} survival curve.

Figure 11. A plot of R(x), the number of hits removed by repair, versus F(x), the number of initial potentially lethal hits, obtained from the UV dose-response curve shown in Figure 10.

Figure 12. A plot of percentage survival and efficiency of repair (R/F) calculated from the UV dose-response curve shown in Figure 10. Note that the UV dose axis is greatly compressed compared with that of Figure 10 and that the efficiency of repair declines much more slowly with increasing dose than does survival.

Figure 13. The amount of newly incorporated material appearing in cesium chloride density gradient analysis of the DNA extracted from UV-irradiated cultures of E.coli TAU-bar. The cultures were starved of their required amono acids for 90 minutes prior to irradiation. Such starvation produces a population of cells that have completed one round of DNA replication and are unable to initiate a second round. Incorporation of tritiated thymidine observed after irradiation of this DNA-completion culture arises from repair replication. The amount of label incorporated provides a measure of the number of lesions repaired after each dose of UV.

Figure 14. A plot of the amount of tritiated thymidine incorporated as a result of repair versus UV dose. The ordinate gives the peak counts per OD_{260} unit of DNA extracted from the cells on the basis of the data shown in Figure 13. The resulting curve is, in effect, a dose response relation for DNA repair.

Figure 15. A hypothetical scheme for genetic recombination, illustrating the possible role of DNA repair processes in the breakage and reunion of parental molecules. The sequence A through H is an adaptation from that proposed by Whitehouse in his "polaron hybrid" DNA model of crossing-over.[1,2] H is

FOOTNOTES

1. H. Whitehouse and P. Hastings, Genet. Res., Camb., 1965, 6, 27.

2. H. Whitehouse, Sci. Prog., 1965, 53, 285.

a form suggested by Tomizawa and Anraku for the T4 "joint molecule."[3] The steps in the transition from H through J are those proposed by Howard-Flanders and Boyce.[4] J is the structure suggested by Meselson for the recombinant molecule.[5] Note that the recombinant molecules contain regions of structural heterozygosity (HR_1 and HR_2), in which the complementary strands are of different parental origin. The scheme dipicted here also results in a region of gene conversion (GCR), or non-reciprocal recombination, in which one of the parental genes is represented on three strands and the other on only one strand of the two recombinant molecules. Subsequent repair of mis-paired bases that might result from genetic heterozygosity in this region could lead to the elimination of one of the parental alleles.

Figure 16. UV survival curves for λ bacteriophage assayed on a wild type host strain of E.coli (AB 1157) and on a mutant strain (AB 1886) that is defective in the excision of structural defects from DNA. The difference in phage survival on the two strains is due to the fact that potentially lethal UV lesions in the phage DNA are repaired by host cell reactivation (HCR) in the AB 1157 but not in the AB 1886 host.

Figure 17. Dose-response curves for the UV enhancement of recombination frequency in crosses of two λ phage mutants in HCR^+ and HCR^- host bacteria. The UV stimulation of recombination is even greater in the strain that is unable to excise UV-induced lesions than in the wild type. Analogous results have been reported for UV stimulation of bacterial recombination[1] and of mitotic recombination in yeast.[2]

FOOTNOTES

FIG. 17

1. P. Howard-Flanders and R.P. Boyce, Radiation Res., 1966 Suppl. 6, 156.

2. R. Snow, Genetics, 1967, 56, 591.

FIG. 15

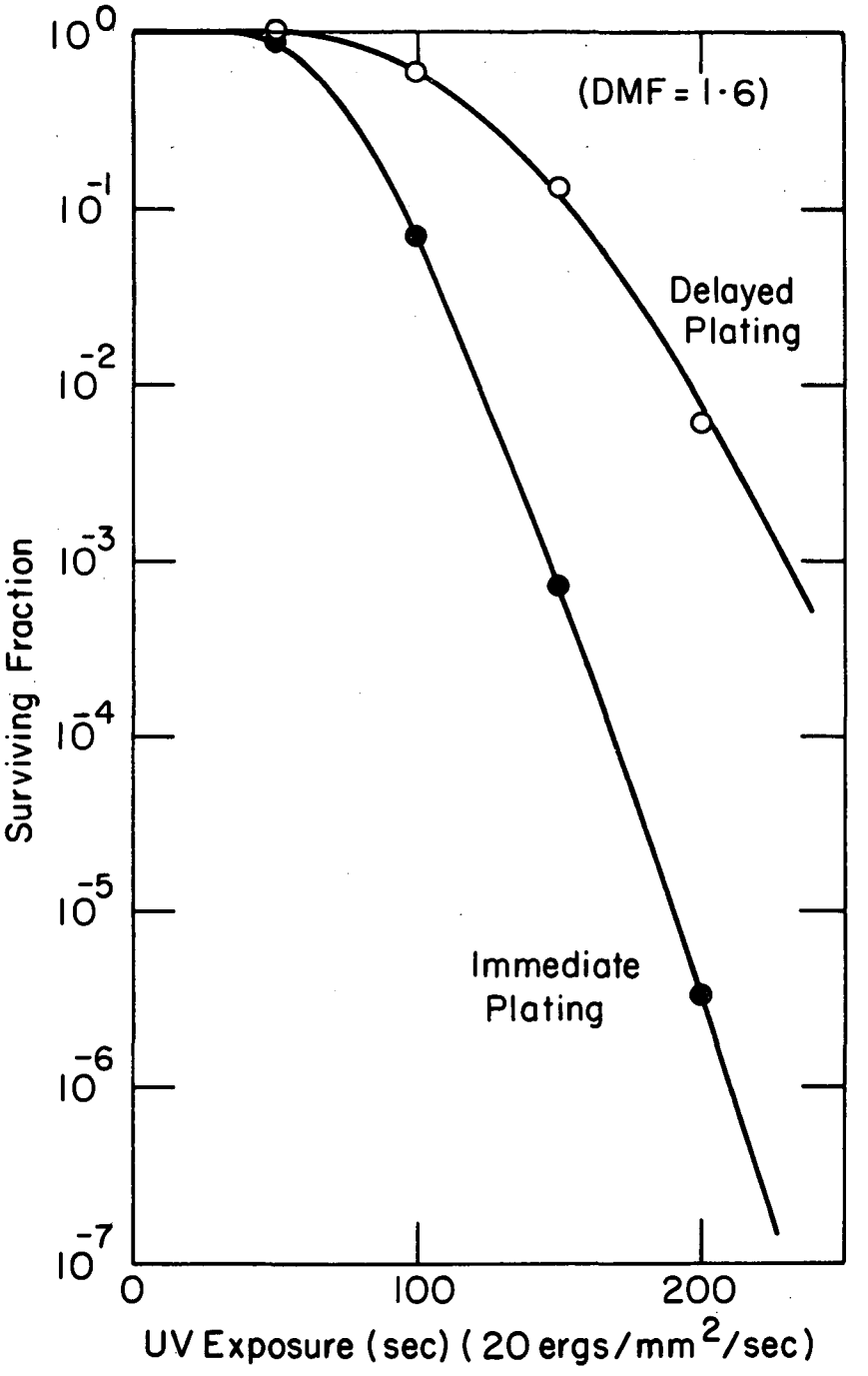
3. J. Tomizawa and N. Anraku, J. Mol. Biol., 1964, 8, 516.

4. P. Howard-Flanders and R. P. Boyce, Radiation Res., 1966, Suppl. 6, 156.

5. M. Meselson, J. Mol. Biol., 1964, 9, 734.

Figure 18. The UV enhancement of recombination frequency for the crosses of phage shown in Fig. 17, but plotted as a function of the fraction of infecting phage which survives in single infection of the host (Fig. 16). This plot gives the recombination frequency in the two hosts as a function of the number of unrepaired UV lesions in the phage DNA rather than as a function of the initial number of lesions as in Fig. 17.

Figure 19. A hypothetical scheme for transfer of information between homologous DNA molecules.



XBL 6712-1962

Fig. 1

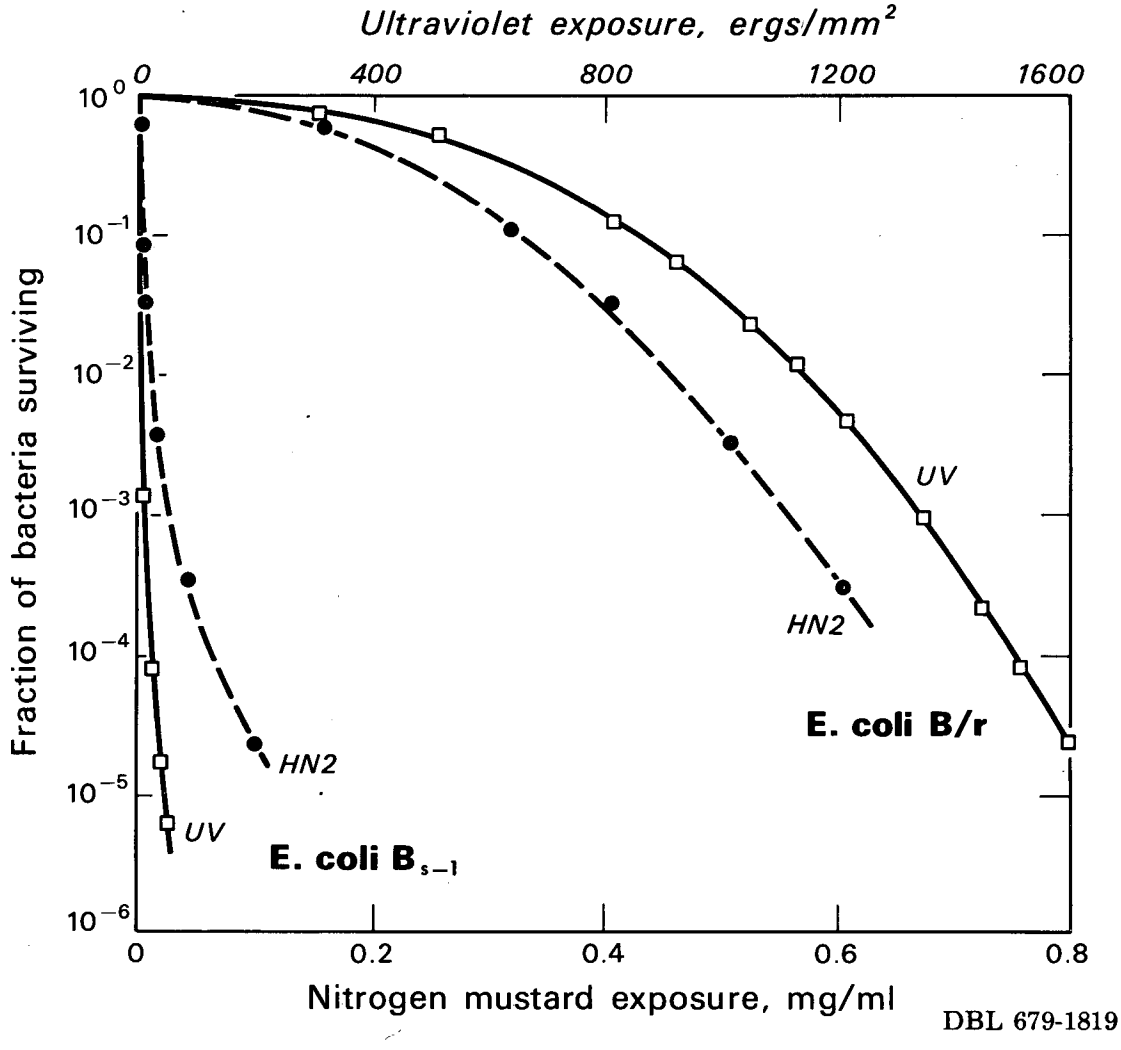
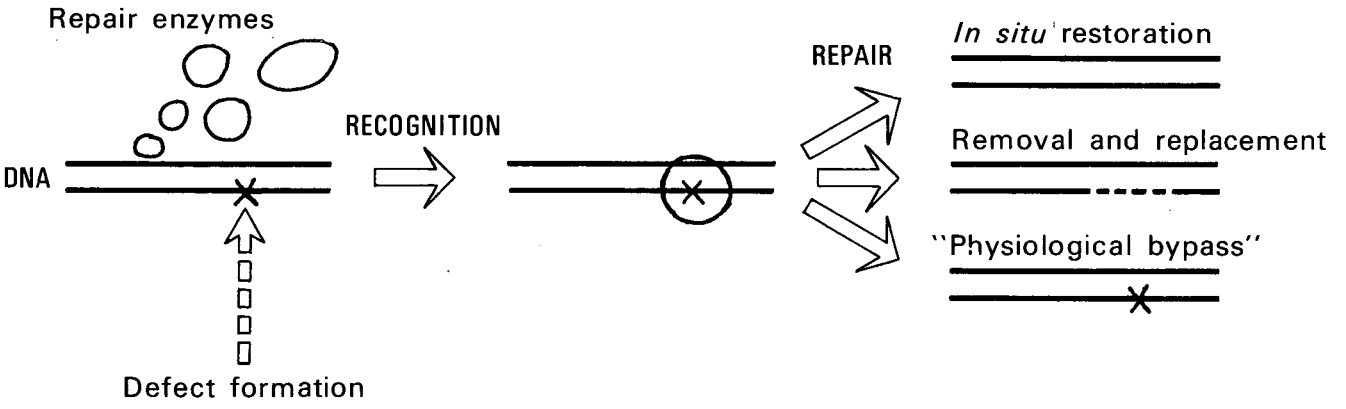
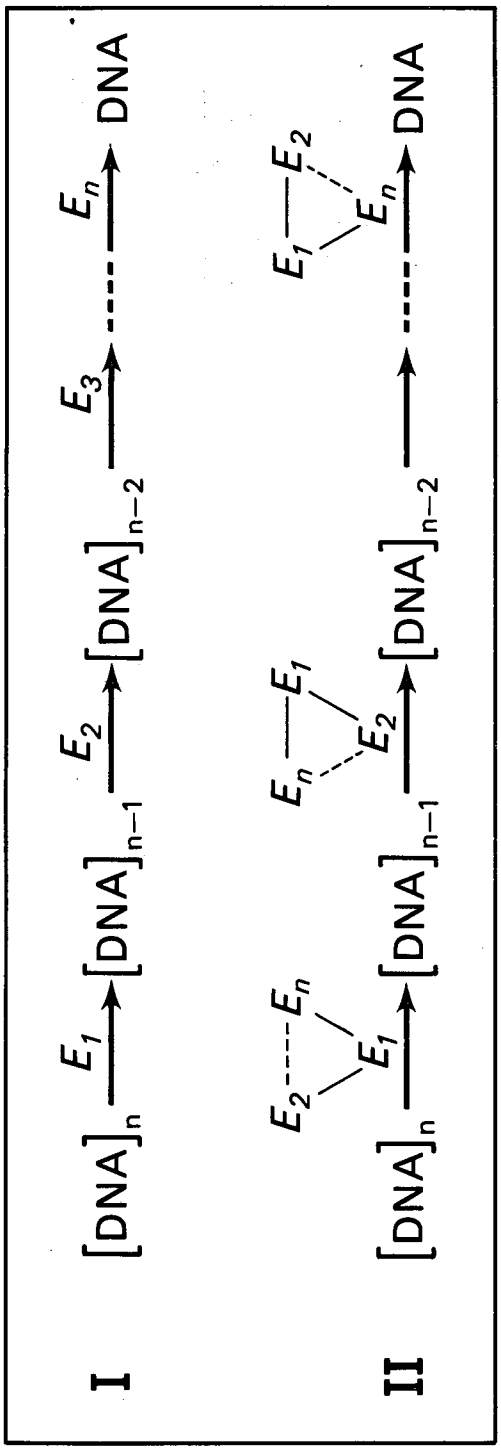

Ultraviolet exposure, ergs/mm²
0
400
800
1200
1600
10⁰
10⁻¹
10⁻²
10⁻³
10⁻⁴
10⁻⁵
10⁻⁶
Fraction of bacteria surviving
UV
HN2
E. coli B/r
HN2
UV
E. coli B s-1
0
0.2
0.4
0.6
0.8
Nitrogen mustard exposure, mg/ml
DBL 679-1819

Fig. 2



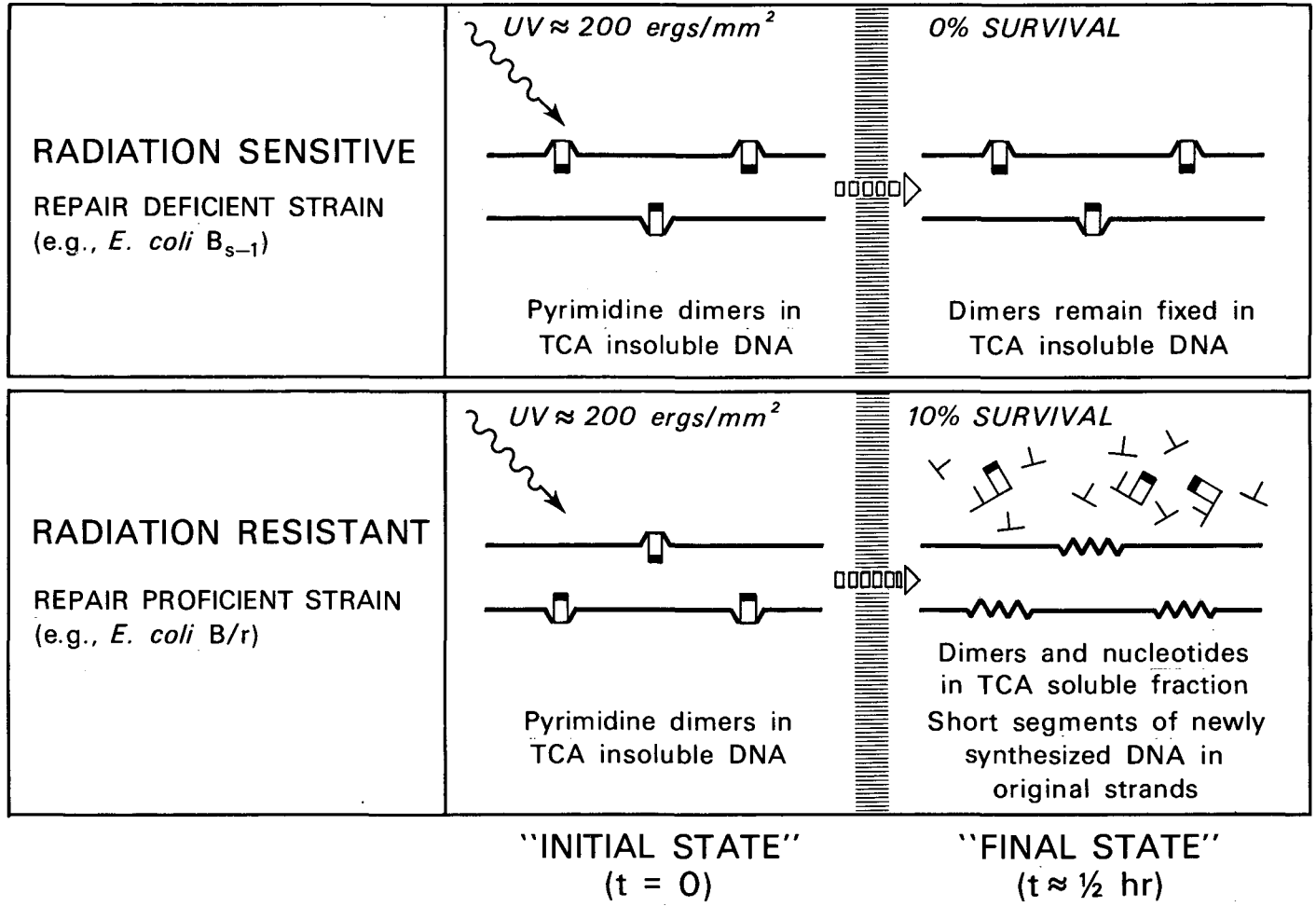
DBL 679-1821

Fig. 3



DBL 679-1820

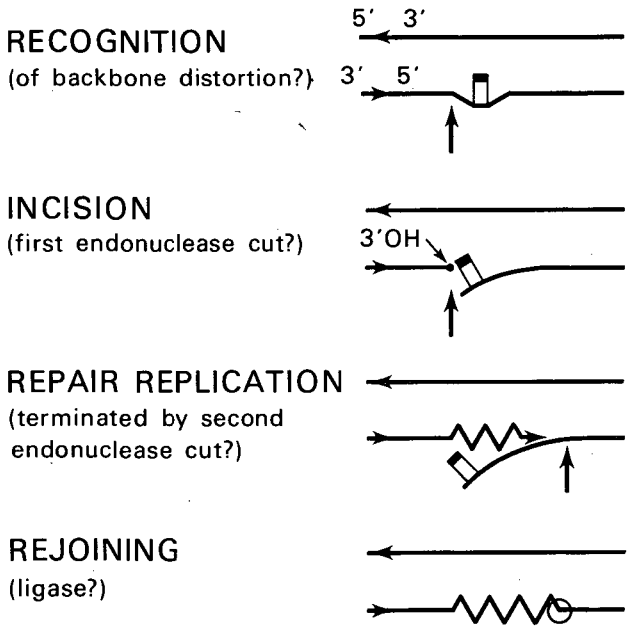
Fig. 4



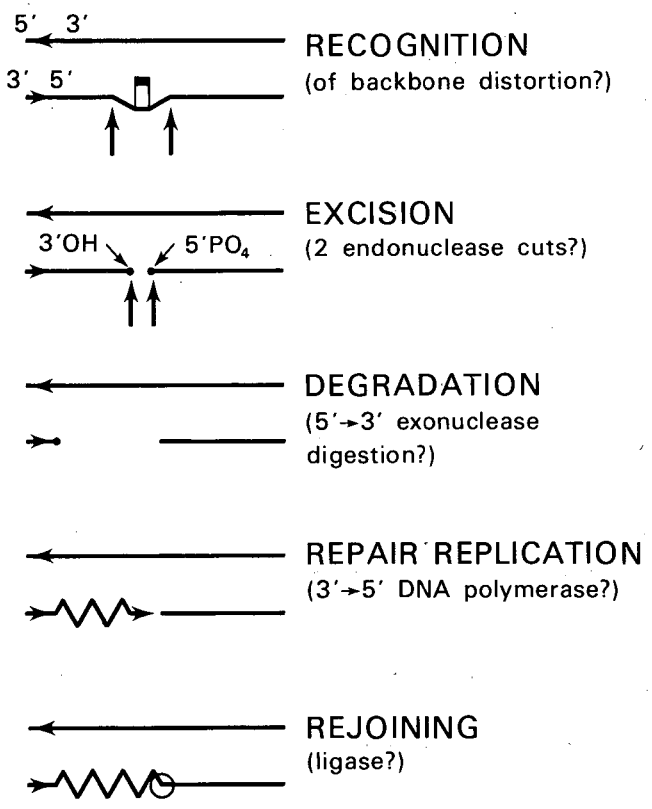
DBL 679-1822

Fig. 5

"PATCH AND CUT" REPAIR

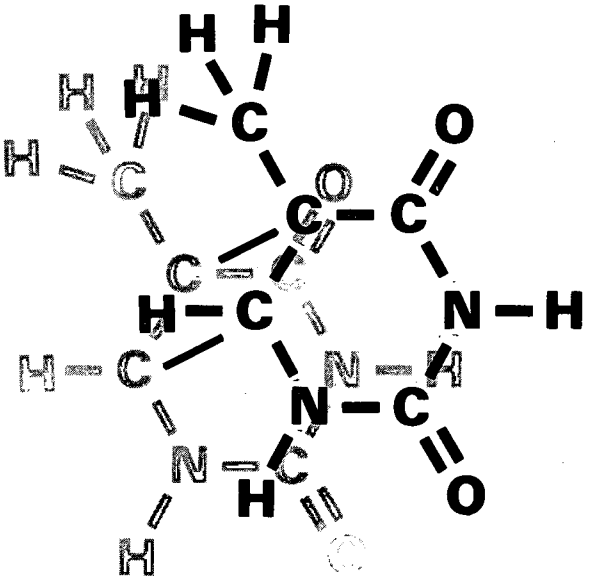


"CUT AND PATCH" REPAIR



DBL 679-1824

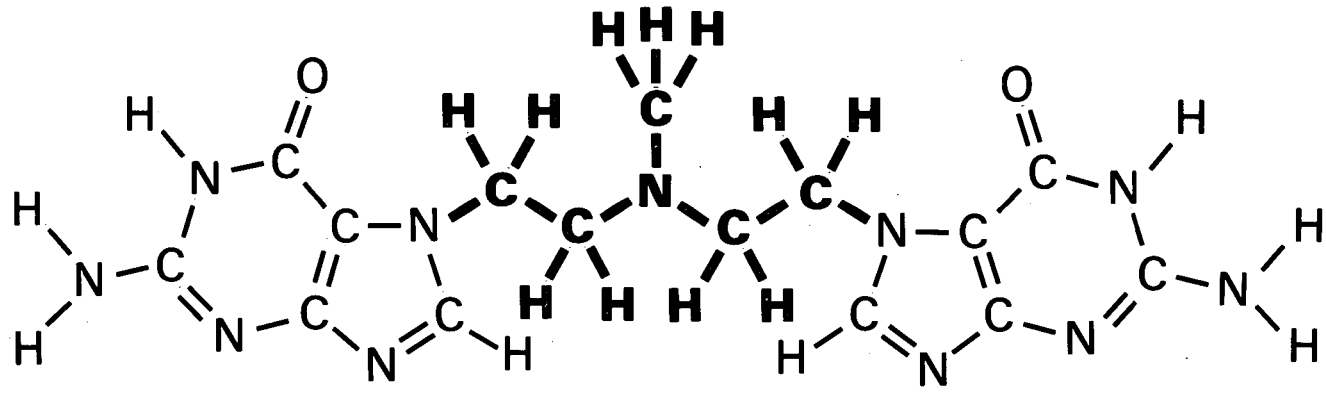
Fig. 6



THYMINE DIMER

DBL 679-1818

(Fig. 7)



GUANINE **HN2** GUANINE

Fig. 8

DBL 679-1823

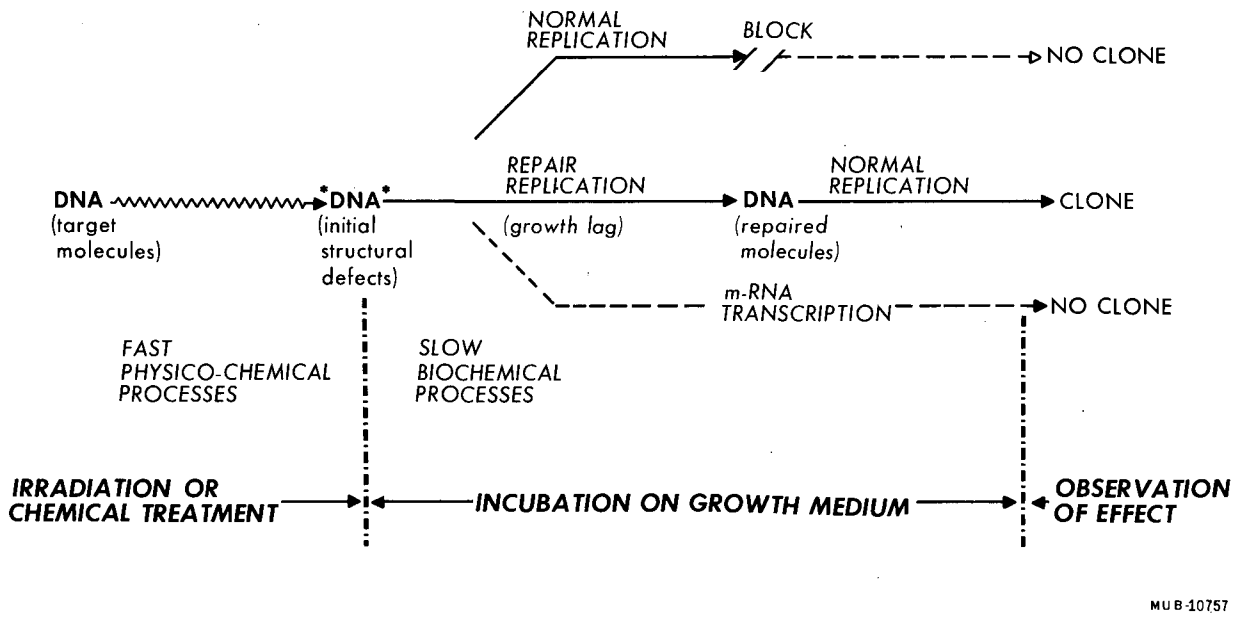

NORMAL REPLICATION
BLOCK
NO CLONE
DNA
(target molecules)
DNA
(initial structural defects)
REPAIR REPLICATION
(growth lag)
DNA
(repaired molecules)
NORMAL REPLICATION
CLONE
m-RNA TRANSCRIPTION
NO CLONE
FAST PHYSICO-CHEMICAL PROCESSES
SLOW BIOCHEMICAL PROCESSES
IRRADIATION OR CHEMICAL TREATMENT
INCUBATION ON GROWTH MEDIUM
OBSERVATION OF EFFECT
MUB-10757

Fig. 9

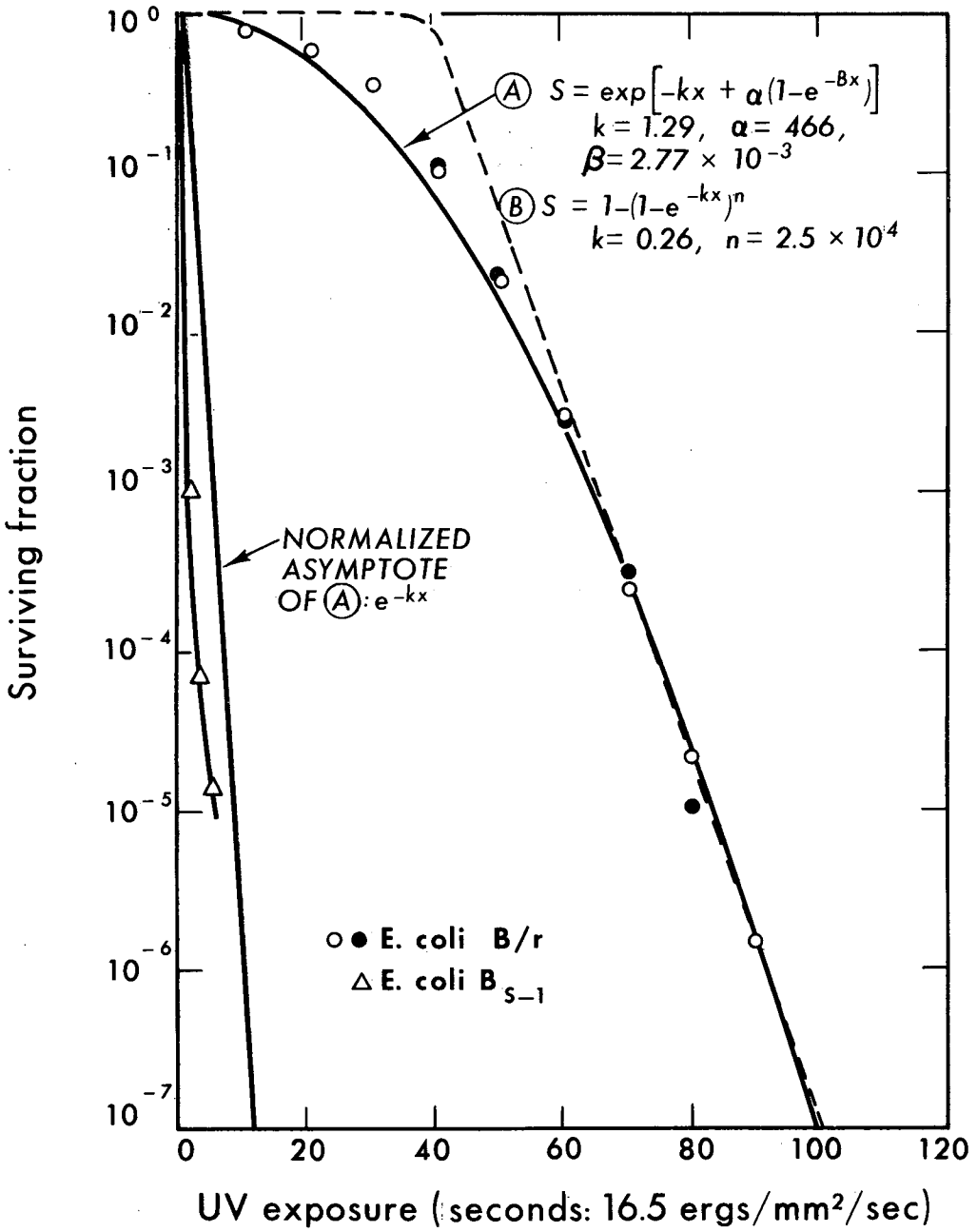
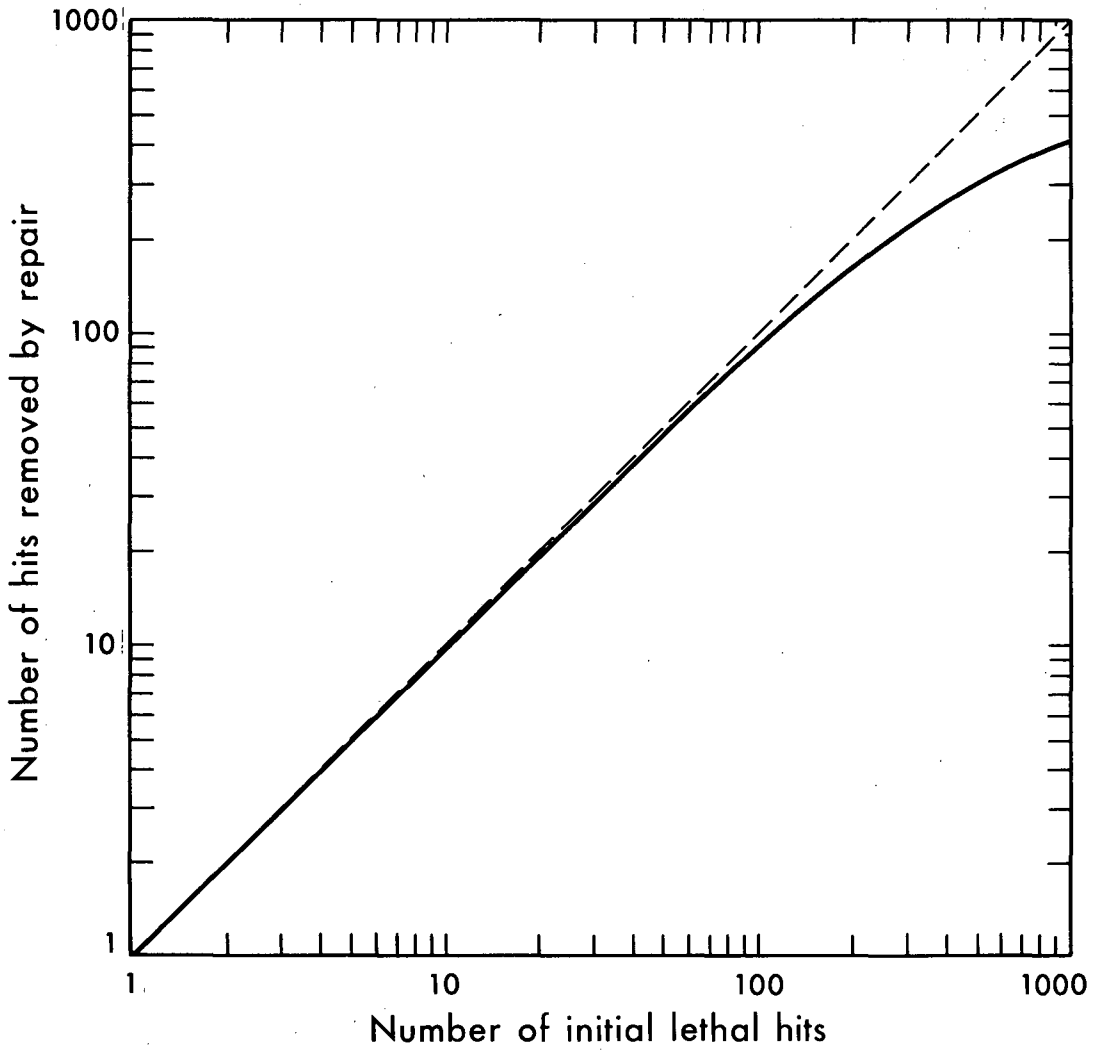

Surviving fraction
UV exposure (seconds: 16.5 ergs/mm²/sec)
Ⓐ S = exp[-kx + α(1-e^-Bx)]
k = 1.29, α = 466,
β = 2.77 × 10^-3
Ⓑ S = 1-(1-e^-kx)^n
k = 0.26, n = 2.5 × 10^4
NORMALIZED ASYMPTOTE OF Ⓐ: e^-kx
E. coli B/r
E. coli B s-1
MUB-10755

Fig. 10



XBL 679-4929

Fig. 11

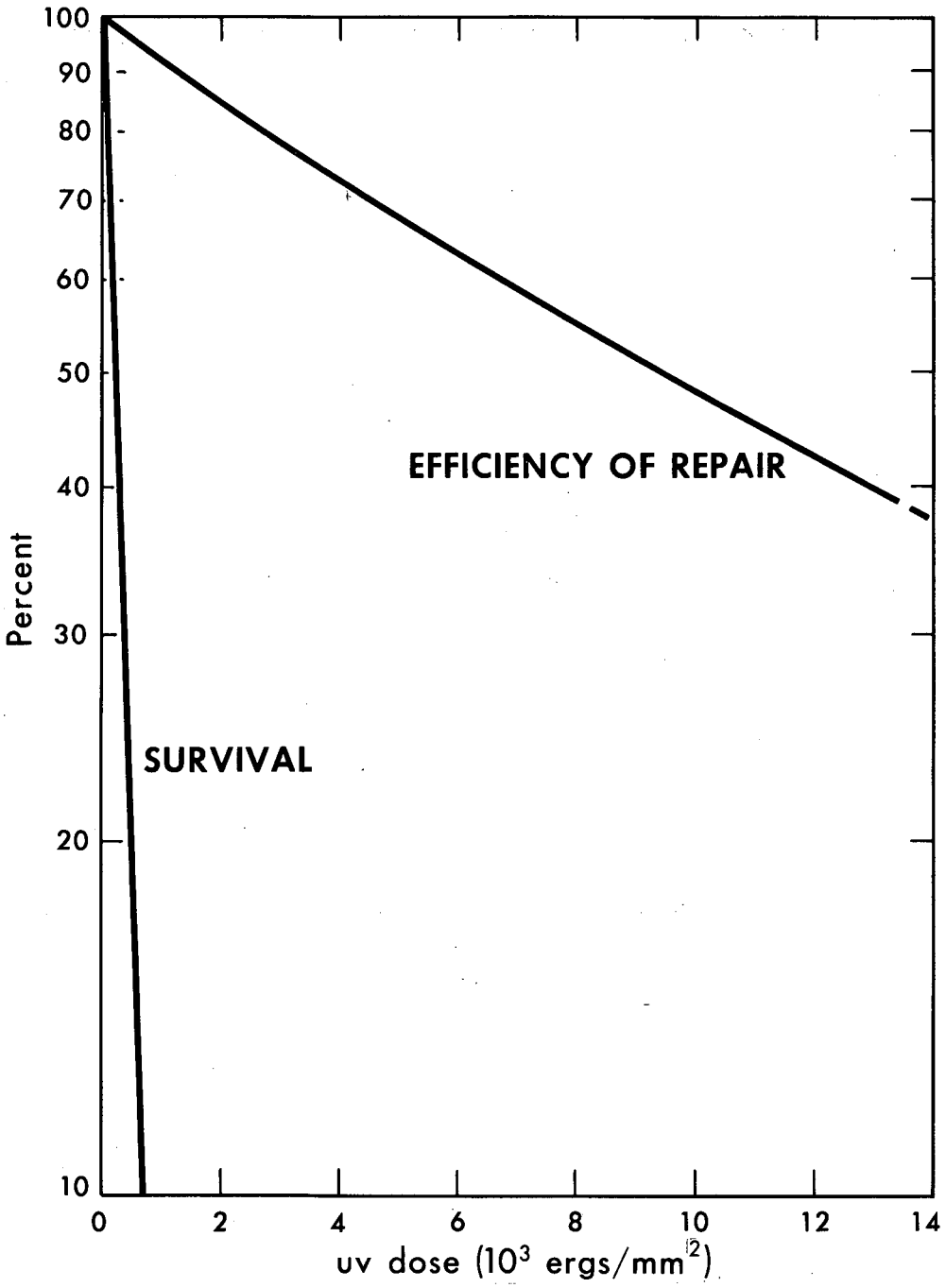

100
90
80
70
60
50
40
30
20
10
Percent
EFFICIENCY OF REPAIR
SURVIVAL
0
2
4
6
8
10
12
14
uv dose (10^3 ergs/mm^2)
XBL 679-4931

Fig. 12

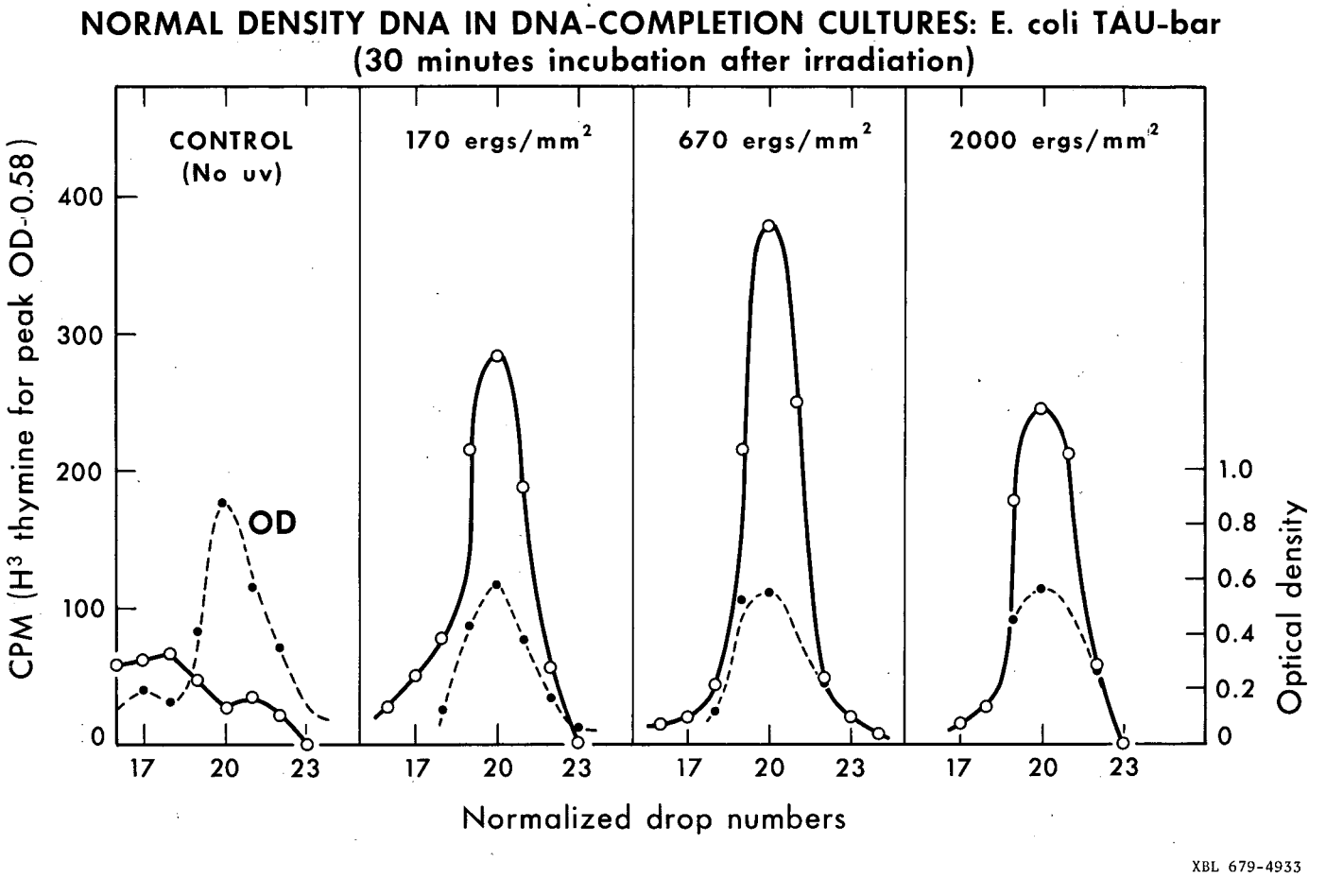
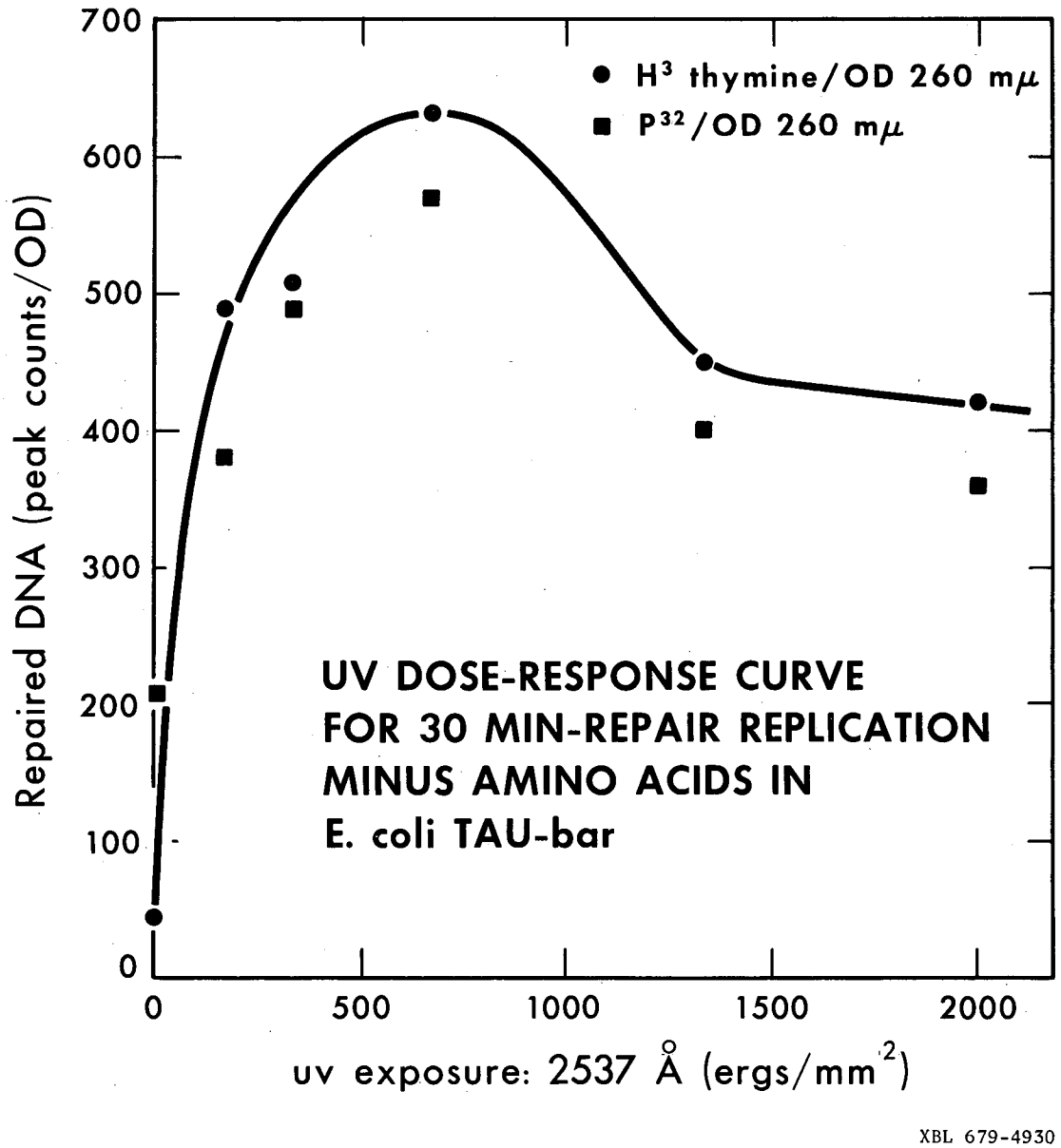
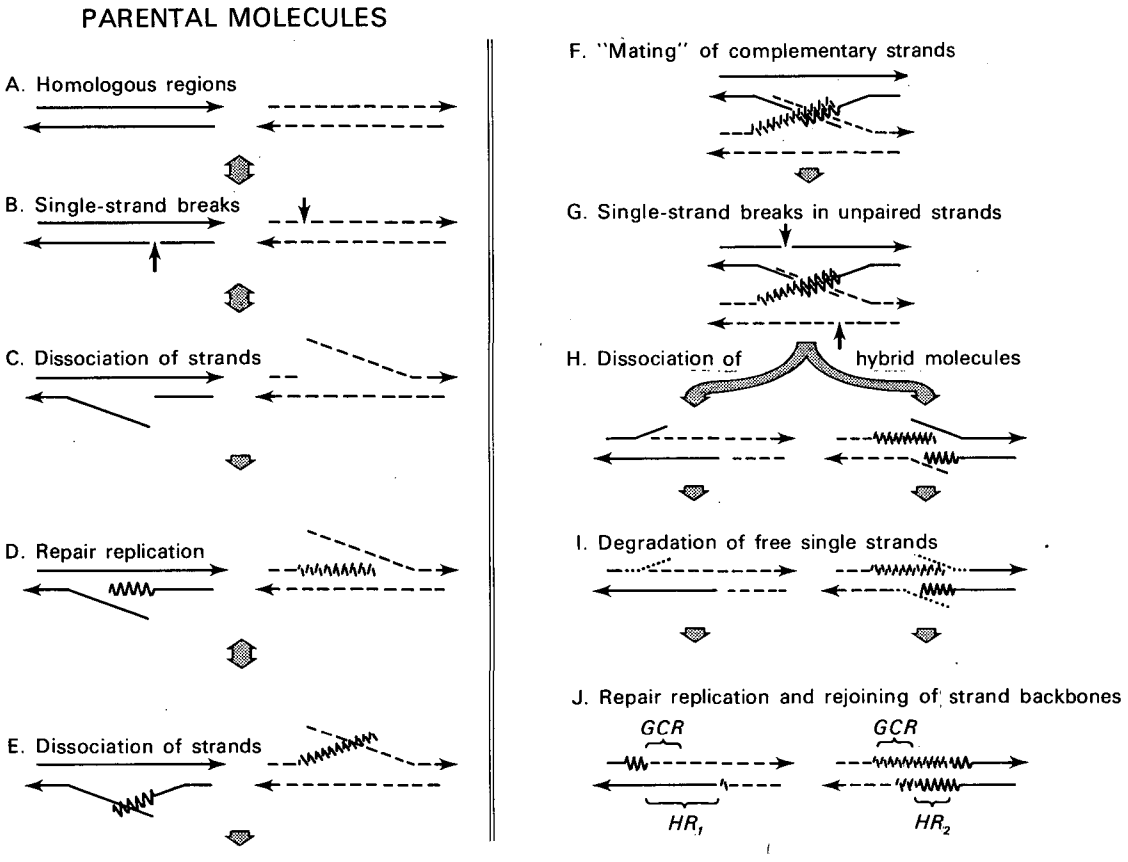

NORMAL DENSITY DNA IN DNA-COMPLETION CULTURES: E. coli TAU-bar
(30 minutes incubation after irradiation)
CONTROL
(No uv)
170 ergs/mm²
670 ergs/mm²
2000 ergs/mm²
OD
CPM (H³ thymine for peak OD-0.58)
Optical density
Normalized drop numbers
XBL 679-4933

Fig. 13



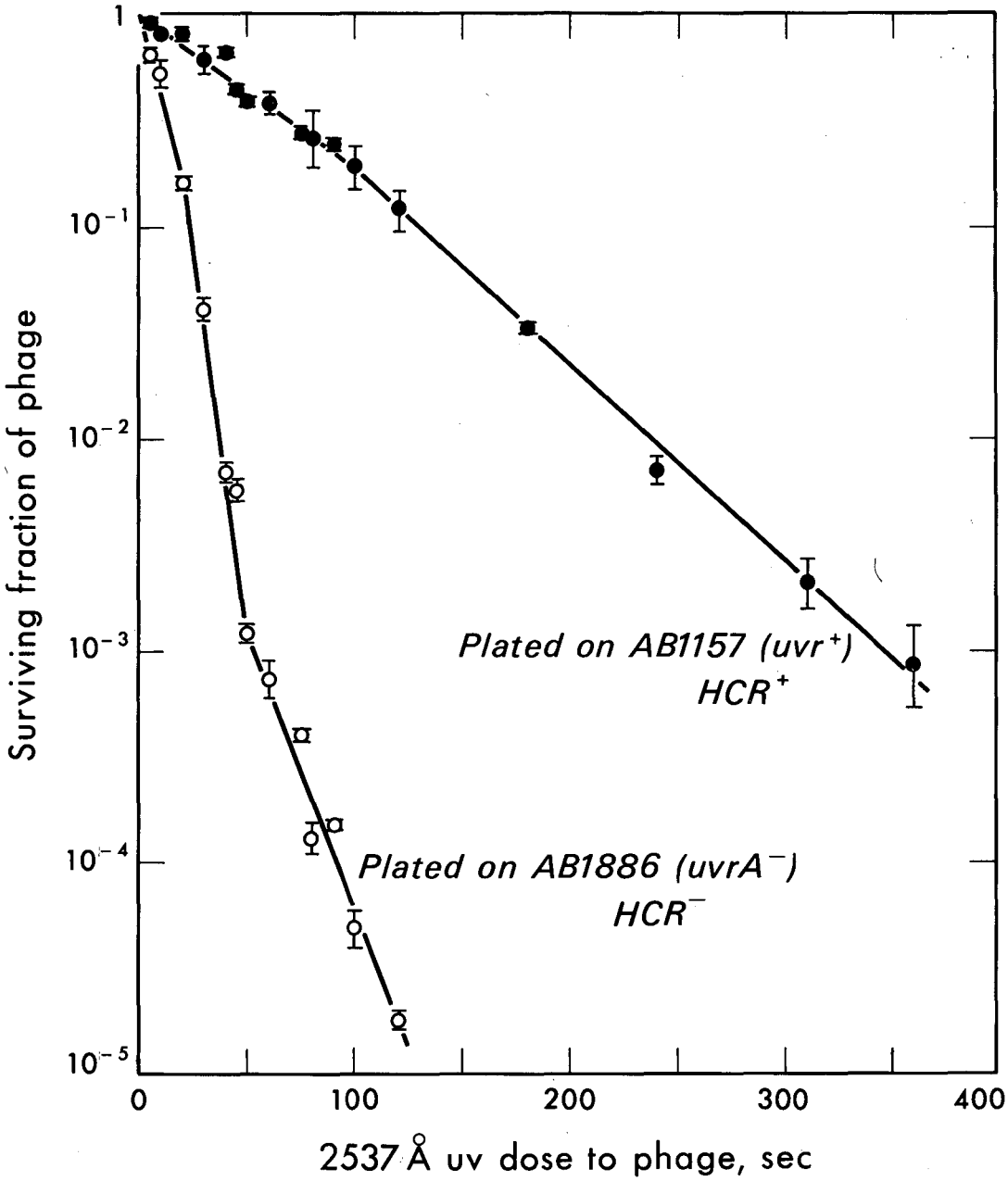
XBL 679-4930

Fig. 14



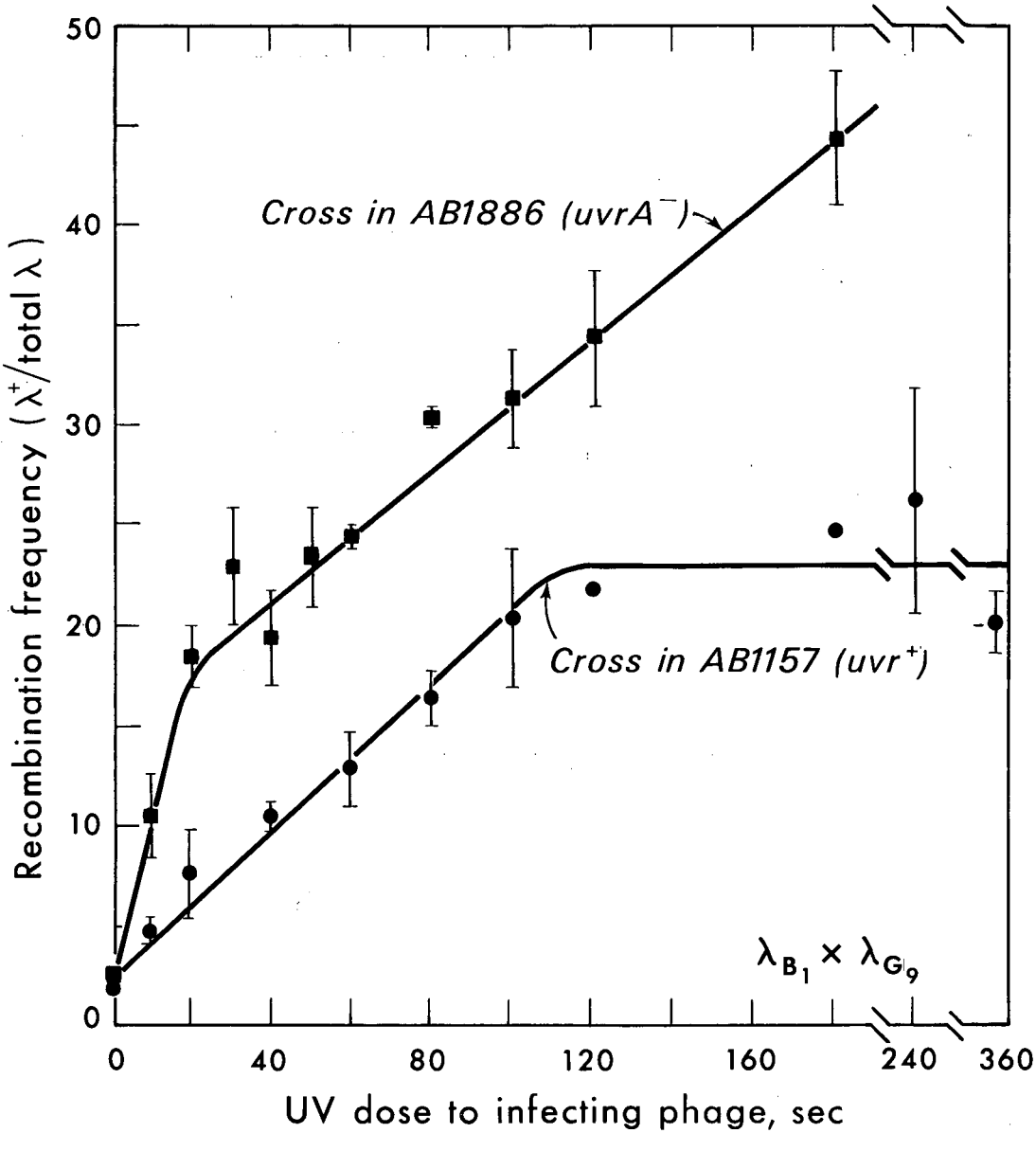
DBL 670-1837

Fig. 15



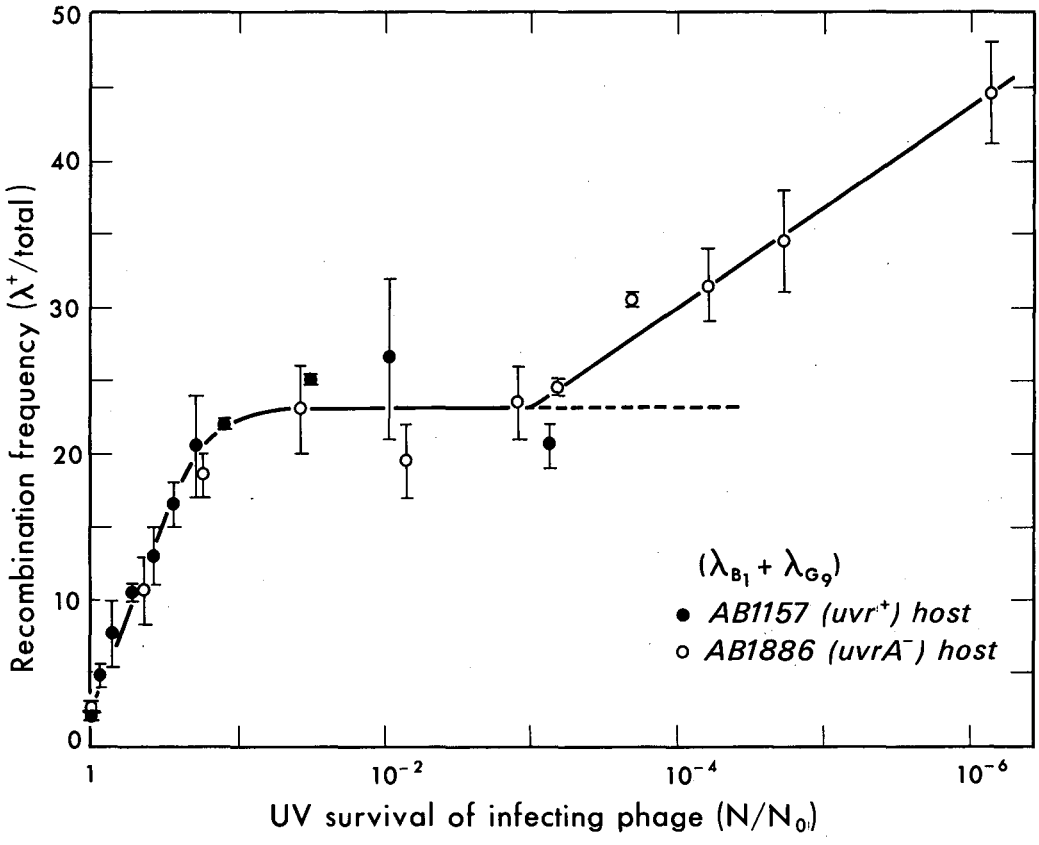
DBL 675-1621

Fig. 16



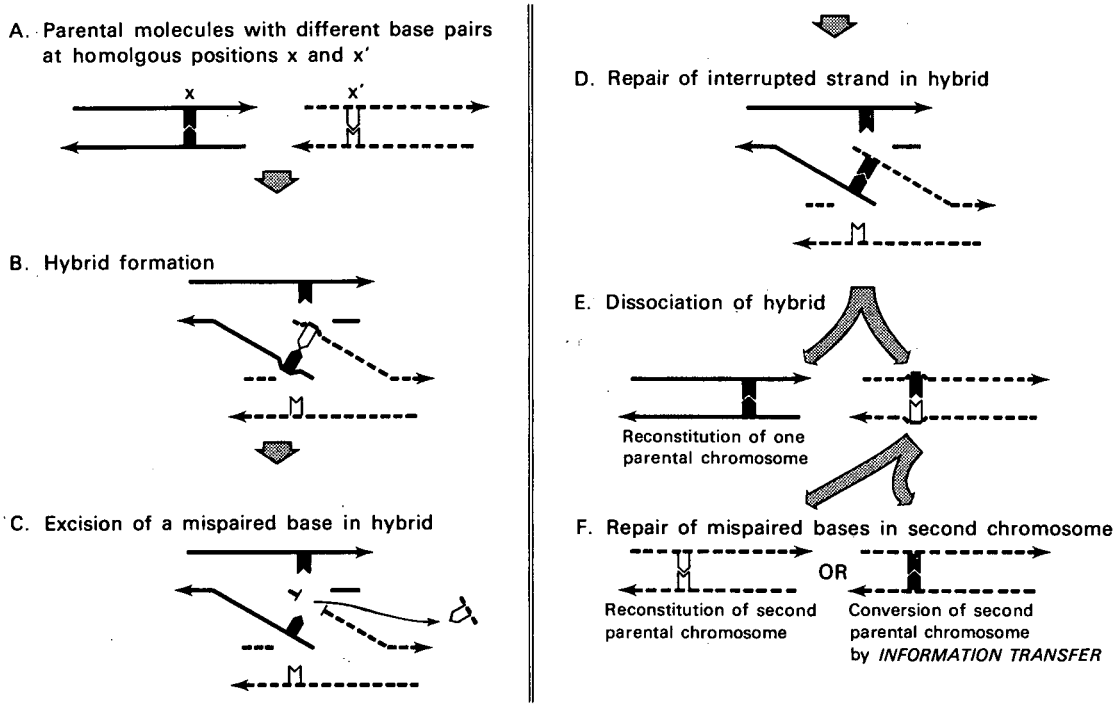
DBL 672-1522

Fig. 17



DBL 675-1620

Fig. 18



DBL 670-1836

Fig. 19