Photochemistry and Photobiology, Vol. 28, pp. 121–129.

0031-8655/78/0801-0121$02.00/0

MULTIPLE PATHWAYS OF DNA REPAIR IN BACTERIA AND THEIR ROLES IN MUTAGENESIS*

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(*Received* 5 *December* 1977; *accepted* 8 *December* 1977)

Abstract—In bacteria, three processes of DNA repair are known: photoreactivation, excision repair, and postreplication repair. *Photoreactivation*, the enzymatic splitting of cyclobutyl pyrimidine dimers *in situ*, is mediated by exposure of the enzyme-dimer complex to near-UV and visible light. This repair process appears to be error free. The *excision repair* of UV-induced DNA base damage has been divided into two major pathways on the basis of both physiological requirements and genetic control. The major pathway requires a functional *polA* gene, does not require complete growth medium, and appears to be largely error-free and to produce short patches during repair. The second pathway requires complete growth medium and functional *recA*, *recB*, *recC*, *lexA*, *uvrD*, and *polC* genes, and appears to be mutagenic and to produce long patches during repair. There exists a second type of excision repair in which the modified base is removed by an *N*-glycosidase, and the chain is then nicked by an apurinic (apyrimidinic) acid endonuclease. Subsequent events are presumed to be similar to the above excision repair process. The *postreplication repair* system has been divided into at least four separate pathways. Three of these are dependent upon functional *recB*, *lexA*, and *uvrD* genes, respectively, and appear to be error free. A fourth pathway depends upon the above gene products, but is blocked by postirradiation treatment with chloramphenicol, and may be the UV-inducible, error-prone, mutagenic pathway of repair ("SOS repair"). A possible fifth pathway depends upon a functional *recF* gene, and is independent of the *recB*$^+$-dependent pathway. *Mutagenesis* appears to be the result of error-prone DNA repair, and there is growing evidence that carcinogenesis is also the result of error-prone DNA repair.

INTRODUCTION

One of the most important discoveries in the field of photobiology is that cells can recover from radiation-induced damage. This discovery has not only provided a major stimulus to the general field of radiation biology, but has also made a major impact on the fields of mutagenesis (Witkin, 1976), carcinogenesis (Trosko and Chang, 1978), and aging (Hart, 1976).

Three modes of DNA repair have been documented.

(1) The damaged part of the molecule is restored to its functional state *in situ*. This may result from the spontaneous 'decay' of the damage to an innocuous form, e.g. dehydration of pyrimidine photohydrates or the recombination of radicals to yield a restored molecule; or it may be accomplished by some enzymatic mechanism, e.g. *photoreactivation*, the enzymatic splitting of cyclobutyl pyrimidine dimers *in situ* mediated by exposure of the enzyme-dimer complex to near-UV and visible light.

(2) The damaged section of a DNA strand is removed (excised) and replaced with undamaged nucleotides to restore the normal function of the DNA. This *excision repair* system was the first 'dark repair' (a term used to distinguish it from photoreactivation, which is mediated by near-UV and visible light) system to be discovered. The excision repair system has been shown to repair a variety of radiation- and chemical-induced lesions in DNA, but was originally observed as a mechanism for the repair of UV-induced cyclobutyl pyrimidine dimers.

(3) The damage itself is not repaired, but rather, it is bypassed during replication, leaving gaps in the daughter strand DNA; the missing genetic information is subsequently supplied by redundant information within the cell. This type of repair is called *post-replication repair*, since it occurs in the DNA synthesized subsequent to UV irradiation.

Our greatest insight into the complexity of DNA repair, i.e. the diverse systems of repair, and the multiple biochemical pathways within these systems, has come from bacterial studies. This is so because of the availability of numerous radiation sensitive mutants (Clark and Ganesan, 1975; Bachmann *et al.*, 1976), and of the techniques of molecular biology that permit the construction of multiply repair-deficient mutants, and permit one to follow the repair of DNA at the molecular level. With the recent isolation of repair deficient human cell lines, such as xeroderma pigmentosum and ataxia telangiectasia, similar genetic studies are now becoming possible with mammalian cells (Hart *et al.*, 1978).

*Presented at the Symposium: DNA Repair and Its Role in Mutagenesis and Carcinogenesis, at the 5th annual meeting of the American Society for Photobiology, San Juan, Puerto Rico, May 11–15, 1977.

The procedures for delineating the multiple pathways of DNA repair in bacteria are straightforward in principle. After isolating a group of radiation sensitive mutants, and mapping the genes conferring this sensitivity, the next step is to determine, at the molecular level, what type of repair event is blocked in these mutant strains. Then strains are constructed containing two or more of these mutant genes affecting radiation sensitivity. Multiple pathways are implicated when, e.g. a double mutant is more sensitive to killing by radiation than are either of the singly mutant parent strains, and this reduced survival capacity is correlated with deficiencies in repair capacity, measured at the molecular level. Using these techniques, it has been shown that both excision repair, postreplication repair, and the repair of X-ray-induced DNA single-strand breaks are composed of multiple biochemical pathways.

UV-induced mutagenesis appears to be due to mistakes made in the repair of damaged DNA. While some of the repair pathways appear to be error free, others appear to be error prone and inducible by radiation damage. In general, postreplication repair appears to be more error prone than is excision repair.

The purpose of this report is to summarize current knowledge on the multiple pathways of the various types of DNA repair in bacteria, and to indicate, where possible, which pathways are responsible for mutagenesis.

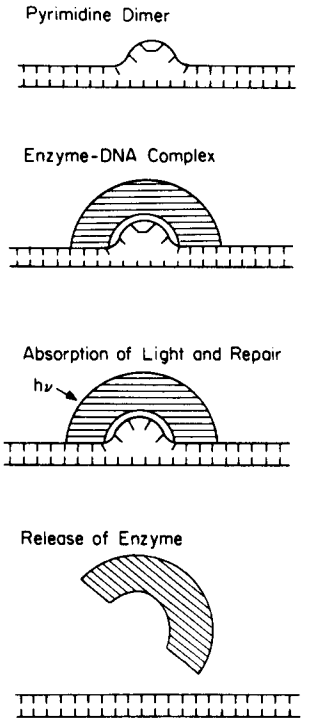

Figure 1. A general model for photoreactivation. In the dark, the enzyme combines with cyclobutyl pyrimidine dimers in UV-irradiated DNA to form an enzyme-substrate complex. The absorption of light between 320–410 nm activates the complex, the cyclobutyl pyrimidine dimers are converted to monomeric pyrimidines, and the enzyme is released.

PHOTOREACTIVATION

Kelner (1949) observed that the survival of UV-irradiated bacteria could be greatly enhanced if the cells were subsequently exposed to an intense source of blue light. In the 1960s, Rupert (for a review see Harm *et al.*, 1971) demonstrated the existence of a photoreactivating enzyme, and established its basic properties (more recently studied by Snapka and Fuselier, 1977).

In the dark, the enzyme combines specifically with cyclobutyl pyrimidine dimers in UV-irradiated DNA to form an enzyme–substrate complex. The complex is activated by the absorption of light between 320–410 nm, the cyclobutyl pyrimidine dimers are converted to monomeric pyrimidines, and the enzyme is released. This is shown schematically in Fig. 1.

Under certain experimental conditions, as much as 80% of the lethal damage induced in bacteria by low fluences of UV radiation at 254 nm can be photoreactivated (for references see p. 226 of Setlow, 1966), thus indicating the importance of cyclobutyl pyrimidine dimers as lethal lesions. Photoreactivating enzymes have been found in a wide range of species from the simplest living cells, the mycoplasmas, to the skin and white blood cells of man (for a review see Friedberg *et al.*, 1977).

EXCISION REPAIR

polA$^+$*-dependent pathway*

Out of the early work on DNA repair (Boyce and Howard-Flanders, 1964; Pettijohn and Hanawalt, 1964; Riklis, 1965; Setlow and Carrier, 1964) came a prediction of the probable steps involved in excision repair; shown schematically in Fig. 2. The first steps in the excision repair system are the recognition of damage, and the introduction of a break in the DNA chain near the lesion (incision step). Resynthesis is then initiated by the action of DNA polymerase (repair replication) using the opposite strand of DNA as the template. The lesion is cut out to complete the excision process, and finally, when the excised region is filled with undamaged nucleotides, the single-strand interruption is closed enzymatically by polynucleotide ligase, yielding repaired DNA. While this has proved to be the general mechanism for the major pathway of excision repair, it is not the only pathway (see below).

The enzyme responsible for making an incision break in the DNA at the site of the damage appears to be coded for by the *uvrA* and *uvrB* genes (Braun and Grossman, 1974). In a strain carrying a mutation at *uvrC*, most of the incisions produced by the *uvrA,B* endonuclease are apparently resealed by polynucleotide ligase (Seeberg and Rupp, 1975), thus thwarting

the excision repair process. Polynucleotide ligase has also been shown to reseal *urvA,B* endonuclease incisions *in vitro* (Braun *et al.*, 1975).

After the initial incision event, excision repair can be divided into two pathways on the basis of both physiological requirement and genetic control (Fig. 3) (Youngs *et al.*, 1974). In one pathway, repair can occur in buffer, and requires DNA polymerase I (*polA*$^+$); it probably proceeds according to the scheme shown in Fig. 2. In the absence of DNA polymerase I (i.e. in a *polA* strain), DNA polymerase III (*polC*$^+$) and/or DNA polymerase II (*polB*$^+$) can partially substitute (about 25% as efficiently) (Youngs *et al.*, 1974). In permeabilized cells of a *polA polC* strain, the action of *polB*$^+$ (DNA polymerase II) has been shown to function in repair replication (Masker *et al.*, 1975). A single mutation at *polB*, however, does not seem to affect cellular sensitivity to either UV (Campbell *et al.*, 1972; Hirota *et al.*, 1972) or X-irradiation (Youngs and Smith, 1973a), suggesting that DNA polymerase I and III do most of the repair synthesis *in vivo*. Polynucleotide ligase is required to reseal the repaired strands of DNA *in vivo* (Youngs and Smith, 1977).

Since a *polA uvrA* strain is only slightly more sensitive to UV radiation than is a *uvrA* strain (Monk *et al.*, 1977), it suggests that most of excision repair is probably accomplished via the *polA*$^+$-dependent pathway. This pathway appears to produce short patches of repair replication (less than 30 nucleotides long) (Cooper and Hanawalt, 1972a, b). The enzymology of excision repair has been reviewed recently (Grossman *et al.*, 1975; Friedberg *et al.*, 1977).

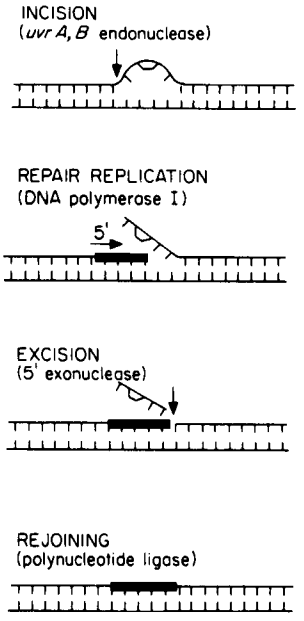

Figure 2. A general model for the major pathway of excision repair. An enzyme recognizes the lesion, shown here as a cyclobutyl pyrimidine dimer, and makes an incision cut in the DNA strand. Repair replication (heavy line) commences using the opposite strand of DNA as the template. Finally, the damaged section of the DNA is excised, and the break in the DNA strand is sealed. Quite a different model will probably be required to describe the growth medium-dependent pathway of excision repair (see text).

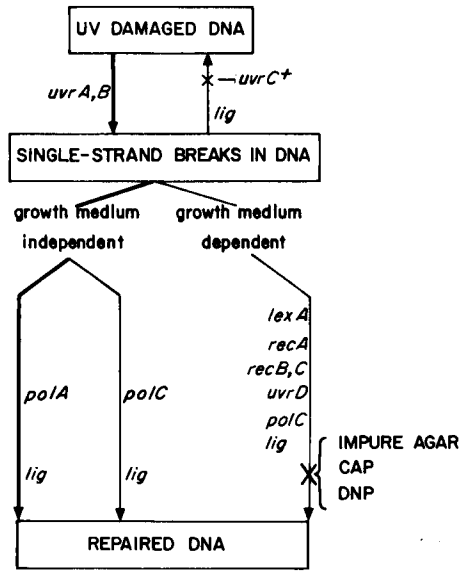

Figure 3. The genetic and physiological control of the different pathways of excision repair. While these pathways have been delineated on the basis of the requirements of only a few gene products, it is clear that many more gene products than are shown will be required to complete the repair in each pathway. The precise functions of the *uvrD*, *lexA*, and *recA* gene products are not known. The gene product of *polA* is DNA polymerase I, of *polC* is DNA polymerase III, of *lig* is DNA ligase, of *recB,C* is exonuclease V, and of *uvrA,B* is 'UV endonuclease'. Symbols: CAP, chloramphenicol; DNP, dinitrophenol. (Modified from Youngs *et al.*, 1974). Recent evidence (D. A. Youngs, E. Van der Schueren and K. C. Smith, unpublished observations) indicates that the growth medium-dependent pathway of excision repair can be subdivided into a *uvrD*$^+$-independent pathway, and a *uvrD*$^+$-dependent pathway.

The possibility of a new *polA*$^+$-dependent excision repair process that works on a photoproduct other than cyclobutyl pyrimidine dimers has been reported (Youngs and Smith, 1976a). DNA single-strand breaks are observed in *uvrA* and *uvrB* strains of *E. coli* after high fluences of UV radiation, and their yield is *not* affected by photoreactivation. These breaks are repaired by a process that requires DNA polymerase I and DNA ligase, but does not require the *recA*, *recB*, *recF*, *lexA* or *uvrD* gene products (Youngs and Smith, 1976a). It is of interest, therefore, that an endonuclease (Endo III) that recognizes UV-induced lesions, but is not the *uvrA*-gene dependent endonuclease, has been isolated from *E. coli* (Radman, 1976; Gates and Linn, 1977). An endonuclease that recognizes nondimer lesions in UV-irradiated PM2 DNA has also been isolated from calf thymus (Bacchetti and Benne, 1975).

recA$^+$-*dependent pathway*

A second pathway of excision repair requires complete growth medium and functional *recA*, *recB*, *lexA*

(Youngs *et al.*, 1974), *uvrD* (D. A. Youngs, E. Van der Schueren and K. C. Smith, unpublished observations), *polC* (Youngs and Smith, 1973b), and *lig* (Youngs and Smith, 1977) genes (Fig. 3). This pathway appears to produce long patches of repair replication (~1500 nucleotides long), since long patch repair was not observed in a *recA recB* strain (Cooper and Hanawalt, 1972b). Such repair was enhanced in a *polA* strain (Cooper and Hanawalt, 1972b), which is blocked in the major pathway of excision repair, but was absent in a strain deficient in DNA polymerase II (i.e. *polB*) (K. M. Carlson and K. C. Smith, unpublished observations). From considerations discussed in the previous section, the $recA^+$-dependent pathway probably handles many fewer lesions than does the $polA^+$-dependent pathway.

The growth medium-dependent pathway of excision repair is irreversibly inhibited by an 80–90 min postirradiation treatment with chloramphenicol at 50 μg/ml (Youngs *et al.*, 1974), or 0.01 *M* dinitrophenol, which appears to selectively block a $lexA^+$-dependent repair function (Van der Schueren and Smith, 1974). The growth-medium dependent pathway is also blocked by impurities in all brands of regular agar that have been tested, but is not inhibited by washed Noble agar (Van der Schueren *et al.*, 1974).

The model for excision repair shown in Fig. 2 should be classified as *prereplication* excision repair, since normal DNA replication is not required. There have been some suggestions that the *uvrA* and *uvrB* genes may play a role in postreplication repair (Howard-Flanders, 1973; Sedliakova *et al.*, 1975; Green *et al.*, 1977; R. H. Rothman and A. J. Clark, unpublished observations), and models for postreplication excision repair have been advanced (Howard-Flanders, 1973; Green *et al.*, 1977; R. H. Rothman and A. J. Clark, unpublished observations). If there is an excision repair system that does depend upon DNA replication, then the growth medium-dependent pathway shown in Fig. 3 is the most likely candidate for such a system.

Liquid holding recovery

When UV irradiated cells of certain strains of *E. coli* are held in buffer for a number of hours before plating on nutrient medium, they show a much higher survival than if plated immediately. This phenomenon, now called liquid holding recovery (LHR), was first described for bacteria using *E. coli* B (Roberts and Aldous, 1949), but is also observed in *recA* strains of *E. coli* K-12 (Ganesan and Smith, 1968). Mutations at *uvrA*, *uvrB* or *uvrC* block LHR (Ganesan and Smith, 1969), suggesting that the molecular basis of LHR is excision repair. Since LHR occurs in buffer, it seems logical that the pathway of excision repair that is involved in LHR is the $polA^+$-dependent pathway. Since this pathway proceeds in buffer anyway, what then can be the basis of the enhanced survival? After preparing various double mutants in combination with *recA*, it has been observed (K. C. Smith, unpublished observations) that *uvrD* blocks LHR, *recF* has little effect on LHR, and *lexA* and *recB* mutations in combination with *recA* greatly enhance LHR over that observed in *recA* alone. One interpretation of these results is that some of the excision gaps that are processed by the growth medium-dependent pathway may be modified, especially in *recA lexA* and *recA recB* strains, such that they become a substrate for the $polA^+$-dependent pathway of excision repair. In growth medium these excision gaps would be converted to lethal lesions, but in buffer they may be shunted to the $polA^+$-dependent pathway, be properly repaired, and result in enhanced viability. Other interpretations of these data are possible, and we are continuing our investigation of the genetic control and molecular basis of LHR.

Repair of X-ray-induced DNA single-strand breaks

The first step in the excision repair of UV-induced DNA base damage is the enzymatic production of a single-strand break in DNA. X-irradiation, besides producing base damage, also produces breaks in DNA strands by radiation chemical mechanisms. It has been shown that X-ray-induced DNA single-strand breaks are repaired by the same general pathways described in Fig. 3 (Town *et al.*, 1973). Thus, there is a growth medium-independent pathway (Type II Repair) that requires DNA polymerase I ($polA^+$). In a *polA* strain, DNA polymerase III (i.e. $polC^+$) can substitute in part (Hamelin *et al.*, 1976). Type II repair is very rapid ($\tau_{1/2}$: ~ 10 min at 0°C; ~ 1 min at 37°C), and repairs ~90% of the X-ray-induced DNA single-strand breaks (Town *et al.*, 1973).

The growth medium-dependent repair system (Type III Repair) is slow (requires ~50 min at 37°C), and repairs only a very small number of breaks, yet is clearly very important to the survival of X-irradiated cells (Town *et al.*, 1973). This repair pathway is irreversibly inhibited by a 90 min postirradiation treatment with 40 μg/ml of chloramphenicol (Ganesan and Smith, 1972), or 3 m*M* dinitrophenol (Van der Schueren *et al.*, 1973), a lower concentration of dinitrophenol than is required to maximally inhibit this pathway after UV irradiation (Van der Schueren and Smith, 1974). A further difference in this pathway for repair after UV or X irradiation is that, in contrast to the case for UV irradiation, this pathway is *not* inhibited by impurities in the ordinary agar used in growth plates (Van der Schueren *et al.*, 1974). It seems reasonable that there should be some differences in the steps in this pathway for the repair of breaks produced enzymatically after UV irradiation, and the breaks produced by radiation chemistry during X-irradiation.

Excision repair of X-ray-induced base damage

Few data are available on the excision repair of X-ray-induced base damage, primarily due to the lack of an easy assay for this type of damage. Hariharan and Cerutti (1974) have pioneered an indirect assay

that measures one type of thymine damage produced by ionizing radiation.

Endonucleolytic activity that recognizes X-ray-induced nucleotide damage in double-stranded DNA has been detected in extracts of *M. luteus*, HeLa, *B. subtilis*, and *E. coli* cells (Strniste and Wallace, 1975; and references therein).

The elucidation of the mechanisms by which cells repair X-ray-induced DNA base damage is one of the major challenges for the coming years.

Base excision repair

The excision repair system described in Fig. 2 may be classified as a *nucleotide excision process*, since the whole damaged region is excised. A new type of excision repair process has been formulated recently, *base excision repair* (Friedberg *et al.*, 1977; and references therein). In this process, an altered base is recognized and cut off the sugar-phosphate chain by an *N*-glycosidase. An *N*-glycosidase that is specific for uracil residues in DNA, and others specific for alkylated bases have been isolated.

The action of the *N*-glycosidase leaves an apurinic (apyrimidinic) site that is then susceptible to attack by a specific endonuclease. Presumably after this incision event, subsequent excision and resynthesis occurs in a manner similar to that described above for the more usual excision repair system (Friedberg *et al.*, 1977). Much more data are needed to clarify this new mode of excision repair.

POSTREPLICATION REPAIR

The DNA that is synthesized shortly after UV irradiation in cells of *E. coli* K-12 has discontinuities when assayed in alkaline sucrose gradients. In excision deficient cells, the mean length of newly synthesized daughter-strand DNA approximates the average distance between pyrimidine dimers in the parental strands. With further incubation of the cells, however, these discontinuities disappear, and the DNA approximates the molecular size of that from unirradiated control cells (Rupp and Howard-Flanders, 1968). These results suggested a postreplicational mode of repair that is shown schematically in Fig. 4. DNA replication proceeds past the lesions in the parental strands, leaving gaps in the daughter strands. These gaps are then filled with material from the parental strands by a recombinational process. The gaps formed in the parental strands are then filled by repair replication.

The size of the daughter-strand gaps appears to be about 1000 nucleotides long (Iyer and Rupp, 1971). Evidence has been obtained that parental and daughter-strand DNA become covalently attached during postreplication repair (Rupp *et al.*, 1971), as would be predicted by the model in Fig. 4. Perhaps the best evidence for this comes from the observation that about half of the sites in UV-irradiated DNA that are sensitive to the action of the T4 UV endonuclease

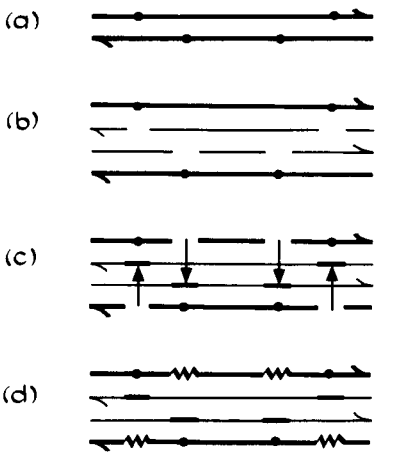

Figure 4. A general model for the postreplication repair of DNA damaged by UV radiation. (a) Dots indicate photochemical lesions produced in the two strands of DNA. (b) DNA synthesis proceeds past the lesions in the parental strands leaving gaps in the daughter strands. (c) Filling of the gaps in the daughter strands with material from the parental strands by a recombinational process (depends upon functional $recA^+$ genes). (d) Repair of the gaps in the parental strands by repair replication. The reader is cautioned that steps (c) and (d) are highly schematized, and will probably be modified as additional data become available.

(i.e. cyclobutyl pyrimidine dimers) are transferred from the parental strands to the daughter strands during postreplication repair (Ganesan, 1974). This implies that it may take several rounds of replication and of postreplication repair before the DNA lesions are 'diluted out', and a viable strand of DNA is obtained. This idea is consistent with the observation that about 5 h is required to eliminate the effect of cyclobutyl pyrimidine dimers on viability in an excision repair-deficient strain after a UV fluence of $6.3\ J\ m^{-2}$ ($\sim 65\%$ survival) (Ganesan and Smith, 1971).

On the basis of genetic and molecular biological studies (Youngs and Smith, 1976b), postreplication repair in bacteria has been divided into several independent biochemical pathways (Fig. 5). The filling of the gaps in newly synthesized daughter strands of DNA is inhibited in *recA* mutants (Smith and Meun, 1970; Sedgwick, 1975a). The *recB*, *uvrD* (Youngs and Smith, 1976b), and *lexA* (Sedgwick, 1975b; Youngs and Smith, 1976b) strains are only partially deficient, and additive (Youngs and Smith, 1976b), in their ability to repair the gaps. Chloramphenicol partially blocks the filling of gaps, but not if the cells are mutant at either *lexA* (Sedgwick, 1975b; Youngs and Smith, 1976b), *uvrD*, or *recB* (Youngs and Smith, 1976b), suggesting that, in addition to their independent functions, there must be another pathway that requires the cooperation of all three gene products, and also requires the synthesis of protein after UV irradiation (Youngs and Smith, 1976b).

A *uvrB lexA uvrD recB* strain is not quite as UV sensitive as is a *uvrB recA* strain, suggesting that at

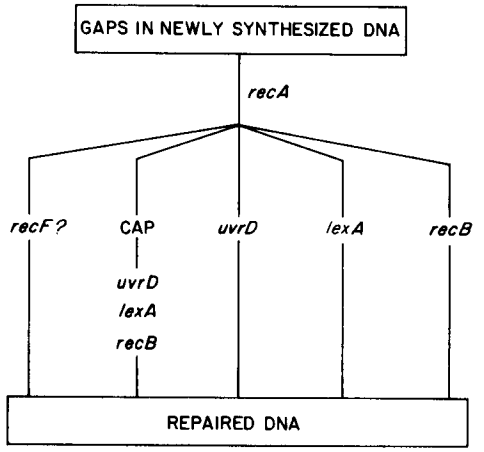

Figure 5. The genetic control of the different pathways of postreplication repair. While these pathways have been delineated on the basis of the requirements of only a few gene products, it is clear that many more gene products than are shown will be required to complete the repair in each pathway. The gene product of *recF* is not known. The other symbols are explained in Fig. 3. (Modified from Youngs and Smith, 1976b).

least one other pathway of postreplication repair must exist (Youngs and Smith, 1976b). The *recF* gene may code for such a pathway since the *recF* mutation has been shown to act independently of *recB* in its effect on postreplication repair (Rothman *et al.*, 1975). Whether or not *recF* is independent of *lexA* and *uvrD* has yet to be determined.

It has been demonstrated that efficient postreplication repair can occur in either *polA* or *polC*ts strains (Sedgwick and Bridges, 1974; Tait *et al.*, 1974), but is blocked in *polA polC*ts strain at the restrictive temperature (Sedgwick and Bridges, 1974; Tait *et al.*, 1974; Tomlin and Svetlova, 1974). This result does not tell us which of the two DNA polymerases (i.e. I or III) is the preferred enzyme in wild-type cells. Polynucleotide ligase is required for the completion of postreplication repair (Youngs and Smith, 1977).

MUTAGENESIS

The *uvrA* and *uvrB* strains appear to be more mutable than wild-type strains. This led to the suggestion that postreplication repair, *apparently* the only type of dark repair remaining in a *uvrA,B* strain, produces mutations due to inaccurate repair (Bridges, 1969; Witkin, 1969a). In support of this concept are the observations that *recA* (Miura and Tomizawa, 1968; Witkin, 1969b) and *lexA* (Witkin, 1967) strains, which are deficient to various degrees in postreplication repair (Fig. 5), cannot be mutated by UV (254 nm) radiation,. It should be recalled, however, that the *recA* and *lexA* strains are also deficient in the growth medium-dependent pathway of excision repair (Fig. 3), which has been implicated in UV-induced mutagenesis (Nishioka and Doudney, 1969, 1970; Bridges and Mottershead, 1971). Taken together, these data suggest that, (1) the *polA*$^+$-dependent pathway of excision repair must be largely error-free, (2) the *recA*$^+$ *lexA*$^+$-dependent pathway of excision repair produces mutations, and (3) postreplication repair must be partially error-prone (i.e. mutagenic).

It would seem easy enough to test these concepts: one simply needs to compare the UV-induced mutability of the various repair-deficient strains with that of the wild-type strain, using 'classical' techniques. Unfortunately, the interpretation of such results is often not easy (Smith, 1976).

If the *polA*$^+$-dependent pathway is largely error-free, it leads to the prediction that mutagenesis should be greatly enhanced in a *polA* strain. However, the mutagenesis data for *polA* strains are confusing, and do not entirely support this hypothesis. The mutability (i.e. mutants per survivor as a function of UV fluence) of *polA* strains has been reported to be greater than wild-type strains up to a fluence of $10\,J\,m^{-2}$ ($\sim 50\%$ survival for *polA*), but the same as wild-type strains at higher fluences (Witkin and George, 1973). However, if these data are compared at equal survival rather than at equal fluence, then a *polA* strain shows *less* mutability than a wild-type strain (Clark and Shankel, 1973). If the data are plotted yet another way, as a function of UV fluence but on linear coordinates, they indicate that a *polA* strain is only very slightly more mutable at fluences up to $10\,J\,m^{-2}$, and thereafter is markedly *less* mutable than the wild-type strain (Doudney, 1977). Thus, *polA* strains seem to be more mutable than wild-type strains at fluences where the *polA* strain shows greater than $\sim 50\%$ survival, but at higher fluences the *polA* strain is interpreted to be either the same or less mutable, depending upon how the data are plotted.

While the results at the lower fluences are consistent with the idea that the *polA*$^+$-dependent pathway of excision repair is largely error free, the data at the higher fluences are not. These results emphasize that different conclusions can be reached concerning the mutability of repair-deficient strains depending upon how the data are plotted (Smith, 1976), and on the range of fluences of UV radiation used.

Which of the different pathways of postreplication repair are error-prone and which are error-free? One pathway of postreplication repair has been shown to be blocked by postirradiation treatment with chloramphenicol, suggesting that protein synthesis is required after UV irradiation before this pathway can function (Sedgwick, 1975b; Youngs and Smith, 1976b). This pathway has also been shown to require functional *uvrD*, *recB* (Youngs and Smith, 1976b), and *lexA* (Sedgwick, 1975b; Youngs and Smith, 1976b) genes (Fig. 5). UV-induced mutagenesis is blocked by postirradiation treatment with chloramphenicol (Sedgwick, 1975b) suggesting that the *uvrD*, *lexA*, and *recB* gene products are involved in mutagenic repair. The fact that UV-induced mutations are not observed in *lexA* strains (Witkin, 1967) seems to support the idea that the chloramphenicol-inhibitable pathway is the mutagenic pathway.

When compared at equal fluence, the *uvrD* and *uvrA uvrD* strains show the same mutation frequency as their respective *uvrD*$^+$ parent strains, suggesting that the *uvrD* gene product is not involved in mutagenic repair. However, if the same data are plotted as a function of equal survival, then the *uvrD* strains show much less mutability, thus implicating the *uvrD* gene product in error-prone repair. Which interpretation is correct? The question has only been partially resolved (Smith, 1976). A similar dilemma in interpretation of mutagenesis data exists for the *recF* strain (R. H. Rothman and A. J. Clark, unpublished observations).

The data for the *recB* and *recC* strains are also difficult to interpret. Reversions to phototrophy seem to be slightly reduced in *recB* or *recC* strains (Witkin, 1972). Mutations in a *recC* strain that were scored selectively (e.g. Lac$^-$) were not reduced, and it was postulated that lethal sectoring was responsible for the reduced yields of mutations that were scored selectively (Hill and Nestmann, 1973). Thus, a conclusion as to whether or not the *recB* and *recC* gene product (exonuclease V) is involved in mutagenesis depends, in part, on how the experiments are run.*

Regretably, 'classical' studies on the relative mutagenicity of repair deficient strains compared with wild-type strains frequently do not permit an easy answer to the question of which repair pathways are error prone and which are error free. It is hoped that this dilemma can be resolved in the near future.

An area of research in mutagenesis that is currently receiving a considerable amount of attention is based upon the concept (Radman, 1975) that error-prone (i.e. mutagenic) repair is due to a new repair function (the so-called 'SOS' repair system) that is *induced* by damage produced in DNA (for a review see Witkin, 1976). Since UV-induced mutagenesis is prevented by a short postirradiation treatment with the protein synthesis inhibitor chloramphenicol (Sedgwick, 1975b), it suggests that postirradiation protein synthesis is required in order for UV-induced mutations to be expressed. Since *recA* and *lexA* strains are not UV mutable (Miura and Tomizawa, 1968; Witkin, 1967, 1969b), it suggests that *recA*$^+$*lexA*$^+$ functions [these gene functions are not known, although the *recA* protein has recently been isolated (McEntee *et al.*, 1976)] are essential for expressing this inducible error-prone repair function. It will be exciting to follow the developments in this new field.

*Recent data (N. J. Sargentini and K. C. Smith, unpublished observations) indicate that UV-induced mutagenesis cannot be detected in *uvrD recB* strains of *E. coli* K-12, and suggest that the *uvrD*$^+$ and *recB*$^+$ genes control separate branches of error-prone repair.

CONCLUSIONS

One cannot help but be impressed with the multitude and sophistication of the repair pathways possessed by bacteria. One system (excision repair) can repair damage in parental strands of DNA under nongrowth conditions, while another repairs gaps in daughter strands that can only be produced in complete growth medium (postreplication repair). Yet another system, which is mediated by near-UV and visible light (photoreactivation), repairs cyclobutyl pyrimidine dimers *in situ*. It thus appears that a significant percentage of the energy of a cell is spent in synthesizing enzymes to repair and maintain the integrity of the genetic code in its DNA.

It is therefore somewhat surprising to find that a cell that is so devoted to accuracy in repair, should also possess an inducible repair system that produces mutations. One could spend considerable time discussing the teleology of this observation.

At first glance it would appear that the mechanisms for DNA repair are well established in *E. coli*, an almost ideal cell system with which to study the molecular biology of repair. However, this is far from the truth. For example, although the photoreactivating enzyme (DNA photolyase) has been known since the early 1960s, there still is no clear understanding of how it uses light energy to split cyclobutyl pyrimidine dimers.

Although models for excision and postreplication repair are glibly presented (and therefore are too often accepted as fact), we know very little about the enzymatic steps involved. While these repair systems have been divided genetically into different pathways, we do not know any of the gene products involved in some of these pathways, or all of the gene products involved in any of the pathways.

The field of mutagenesis is entering an exponential phase of growth. Many 'established' concepts in mutagenesis are now being challenged as new data on DNA repair and replication become available.

It is an exciting time for doing research on DNA repair and mutagenesis, but it is also an extremely frustrating time. It seems that each new 'breakthrough' serves mainly to emphasize the extreme complexity of the problem, and how little we actually know about DNA repair and mutagenesis even in the relatively simple system of bacteria.

Acknowledgement—The author's research has been supported by U.S. Public Health Service grants CA-02896 and CA-06437, and research project grant CA-10372 from the National Cancer Institute, DHEW.

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Photochemistry and Photobiology, Vol. 28, p. 119.

0031-8655/78/0801-0119$02.00/0

SYMPOSIUM ON DNA REPAIR AND ITS ROLE IN MUTAGENESIS AND CARCINOGENESIS*

INTRODUCTION

The discovery that cells can repair radiation-induced damage to their deoxyribonucleic acid (DNA) has not only provided a major stimulus to the general field of radiation biology, but has also made a major impact on the fields of mutagenesis, carcinogenesis and aging.

Our greatest insight into the complexity of DNA repair, i.e. the diverse systems of repair, and the multiple biochemical pathways within these systems, has come from bacterial studies. This is so because of the availability of numerous radiation sensitive mutants, and of the techniques of genetics that permit the construction of mutants that are multiply deficient in repair, and techniques of molecular biology that permit one to follow the repair of DNA at the molecular level.

Even though our knowledge about mechanisms for the repair of DNA in bacteria seems, at least on the surface, to be quite sophisticated, we still do not have a complete understanding of even the simplest repair system, a one enzyme process called photoreactivation. Many hypotheses exist concerning the more complicated repair systems (i.e. excision repair and postreplication repair), but a thorough understanding of these systems is many years away.

Our knowledge about repair in mammalian cells is very rudimentary. However, with the recent isolation of human cell lines that are deficient in repair, such as those from patients with xeroderma pigmentosum and ataxia telangiectasia, genetic and biochemical studies similar to those performed on bacteria can now be done on mammalian cells. Many more repair deficient human cell lines need to be isolated and studied, however, before knowledge about DNA repair in mammalian cells will even approach the sophistication of current knowledge about bacterial systems.

There is good evidence that UV-induced mutagenesis in bacteria is the consequence of mistakes made during the repair of damaged DNA. Which pathways of repair are mutagenic, and are they constitutive or induced by the radiation damage? Attempts to answer these questions constitutes a current and very active area of research. For mammalian cells, it has been observed that xeroderma pigmentosum cells are more UV mutable than normal cells, suggesting that their deficiency may be in repair pathways that are normally error-free.

The observation that greater than 90% of all tested chemical carcinogens are mutagenic, supports the hypothesis that chemical carcinogens act by damaging DNA, leading to mutagenesis via error-prone repair, and these mutations are ultimately expressed as cancer.

Because of the strong implication that carcinogenesis has its roots in the error-prone repair of damaged DNA, it seems appropriate and timely to review what is known about the different types and pathways of DNA repair in bacteria and mammalian cells, and the role of DNA repair in mutagenesis and carcinogenesis. To this end the present symposium was organized.

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*Held at the 5th Annual Meeting of the American Society for Photobiology May 11–15, 1977, San Juan, Puerto Rico.