

DNA REPAIR AND MUTAGENESIS

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ABSTRACT

A number of DNA repair systems have been discovered, at least some of which are *inducible* in response to exposure to low doses of different agents. Some of these are: SOS repair, adaptive response, heat shock response and inducible resistance to hydrogen peroxide. Although, by and large, genetic control of these repair systems is different, at times there is an overlap in induction of proteins like *DnaK* and *GroEL* which are induced by both UV and heat shock in *E. coli*. There is some relationship between DNA repair and mutagenesis but it is not always direct. Thus, whereas SOS repair induced in *E. coli* cells results in higher survival of UV-irradiated phage plated on them, the mutation frequency in the surviving phage is also increased. On the other hand, low doses of MNNG induce an adaptive response in *E. coli* which not only promotes increased survival of the cells exposed to a higher subsequent dose, but also decreases the frequency of mutations induced. Both these systems have a different genetic control. Bacterium *Haemophilus influenzae* is not mutable by UV light. It is conceivable that it lacks the *E. coli umu*-like gene. Theoretical considerations suggest that DNA repair systems inducible by low exposures of mutagens may turn out to be protective in the real life situation.

INTRODUCTION

GENETIC material (DNA) of all organisms is subjected to damage by environmental agents. Repair systems have evolved to cope with a variety of changes in DNA. Since DNA is the substrate during repair by certain enzymes, unrepaired damage could lead to mutations, cell inactivation and may even be cancers. That there might be a connection between DNA repair and mutagenesis became apparent from the early UV-reactivation (later termed W-reactivation) experiments of Weigle¹. Weigle noted that UV-irradiated phage titred higher on *E. coli* cells irradiated with low doses of UV radiation than on unirradiated cells. Moreover, the surviving phage had a higher frequency of mutations from irradiated cells than from unirradiated cells. Subsequently it was shown that W-reactivation peaks some time after irradiation of cells and can be blocked with chloramphenicol treatment of host cells. From the foregoing observations, the idea of *inducible* DNA repair systems was derived the first one of which was given the name of SOS.

SOS repair was found to be under genetic control as were the later-discovered systems. While the early work utilized mainly UV-radiation for DNA repair and studies on mutagenesis, the repair systems were also discovered for lesions induced by alkylating, cross-linking and oxidising agents. Inducible systems are of great interest from the point of view of protection because of their induction at low exposures. Although, these repair systems were initially detected in prokaryotes, the search for these has been successfully extended to eukaryotes as well.

NATURE OF DAMAGE INDUCED IN DNA

Different types of DNA lesions have been reviewed by Bajaj and Notani² and Hanawalt *et al*³. DNA damages can broadly be described as dimer formation of pyrimidine bases, loss of bases, modification of bases by alkylation or deamination or oxidation, double or single-stranded breaks, formation of cross-links, etc.

With UV-exposure the most preponderant

change in DNA appears to be the pyrimidine dimer formation. Mutants defective in coping with these dimers exhibit an increased sensitivity to lethal and mutagenic action of UV in *Escherichia coli*^{4,5}. However, investigations at the molecular level to correlate the site of dimer formation and hotspots for mutation induction revealed the occurrence of an alkali-labile photoproduct⁶, now identified as Thy (6-4)pyo, an essentially pyrimidine (6-4) pyrimidone⁷. There seems to be a good correlation between pyrimidine dimers and lethality in absence of excision repair and between (6-4) photoproduct and mutagenicity. However, occurrence of (6-4) photoproduct is almost 10 times less frequent than that of cyclobutane type pyrimidine dimers⁷ at low UV doses of about 1–100 J/m².

A number of changes in bases are induced by alkylating agents like N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) and ethyl methane sulfonate (EMS). O⁶-alkylguanine is the most studied mutagenic lesion^{8,9}.

Photosensitising agents like acridines and psoralens used in the treatment intercalate between DNA strands and induce cross-links¹⁰. Antitumour drug *cis*-platinum diamine dichloride (*cis*-DDP) is cytotoxic and its cytotoxicity is due to the interaction with DNA at G C rich regions resulting in intrastrand cross-links¹¹. Major changes in DNA induced by X- or gamma rays are single and/or double strand breaks, base damage, loss of base(s) resulting in apurinic or apyrimidinic (AP) site(s).

DNA REPAIR SYSTEMS

To repair DNA damage, cells have evolved a variety of systems which may be error-prone or error-free. Several of these are induced in response to the insulting agent. However, some damage may be repaired by mechanisms constitutively present in the cells. The damage in DNA may be directly reversed as described later. Indirect repair constitutes removal of the damaged bases from DNA by excision mechanism and then resynthesis of the missing segment using opposite strand as template. Both the above-

mentioned modes are error-free. Post-replication or daughter strand gap repair involves recombinational exchange of strands in replicating DNA and this mode may be mutagenic¹².

Some of the inducible DNA repair systems studied are the so-called SOS repair, the adaptive response, the heat shock response and the recently-reported repair of H₂O₂-induced lesions.

SOS Repair

Defais *et al*¹³ first clearly articulated the 'SOS Hypothesis' to account for the fact that a variety of diverse physiological responses induced in the wake of damage to DNA are under the coordinate control of two genes *recA* and *lexA*. Witkin¹² defined SOS repair as "*recA*⁺-and *lexA*⁺-dependent error-prone repair activity induced in *E. coli* by UV-irradiation or other agents having in common the ability to damage DNA or interrupt its synthesis".

Apart from W-reactivation and W-mutagenesis, inhibition of cell division (filamentation), prophage induction, etc are some of the other manifestations of the SOS repair. In *E. coli* the SOS responses are induced by a variety of agents like UV, alkylating agents, 4-nitroquinoline-1-oxide, aflatoxin B1, nalidixic acid etc. Treatment with UV or nalidixic acid also induce other proteins which are not under the control of *recA-lexA* genes. Two such proteins of Mr 61 K and 73 K have turned out to be similar to the two heat shock proteins coded by the *E. coli* genes *dnaK* and *groEL*. These genes are under the control of *htpR* gene¹⁴. This indicates that *E. coli* cells when exposed to UV or nalidixic acid not only turn on the SOS but also some proteins of other systems like heat shock.

In *Haemophilus influenzae* W-reactivation of UV-irradiated phage HP1c1 could be induced with either UV or mitomycin C treatment of the host cells. W-reactivation peaks after about 20–30 min following UV or mitomycin C treatment and could be blocked by chloramphenicol¹⁵. However, W-mutagenesis¹⁵ or UV-induced mutagenesis of cells¹⁶ is absent in *H. influenzae*. Like UV, PUVA (psoralen plus UVA radiation) treat-

ment of *H. influenzae* does not induce mutations¹⁷. However, prophage induction in *H. influenzae*, although dependent on functional *recI* gene could not be blocked by chloramphenicol treatment¹⁸.

Adaptive Response

Alkylating agents like MNNG and EMS can induce adaptive response. In *E. coli* brief exposure to a low concentration of MNNG (adaptive dose-1 µg/ml) conferred resistance to the lethal and mutagenic action of subsequent challenge by higher concentration (challenge dose-100 µg/ml). Maximum resistance was observed in about 1½ hr following exposure to adaptive dose¹⁹. This repair was found to be distinct from SOS in that UV or nitroquinoline-1-oxide (NQO) could not induce it²⁰. It was independent of *recA* and *lexA* genes but under the control of *ada* gene²¹. Although EMS and MNNG are known to be mutagenic in *H. influenzae*²², adaptive response has not been detected yet.

Heat Shock Response

A brief treatment (shift from 28°C to 42°C) causes changes in DNA that result in altered sedimentation of DNA in neutral sucrose gradient, indicating unfolding of the DNA²³. A number of genes including *dnaK*, *groEL* etc respond to the 'thermal' damage in DNA in *E. coli*. These genes are under the control of *htpR* (high temperature production) gene. At least a few of the heat shock or 'stress' proteins are induced by a variety of different treatments like UV, nalidixic acid¹⁴, ethanol, coumermycin, and λ (phage) infection²⁴.

Inducible Resistance to Hydrogen peroxide (H₂O₂)

Inducible resistance to H₂O₂ is observed in *E. coli*. Exposure to 10 µM H₂O₂ conferred resistance to lethal action of 2–5 mM H₂O₂. Surprisingly, adaptation to H₂O₂ also induced resistance to gamma-radiation, supporting the notion that both H₂O₂ and gamma rays induce similar DNA damage. However, induction of

H₂O₂ resistance could not induce resistance to alkylating agents or UV-treatment indicating a specific and novel inducible DNA repair system²⁵.

GENETIC CONTROL OF DNA REPAIR

SOS Repair

Regulation of the SOS repair has been reviewed recently by Walker²⁶. The present model of the regulation of SOS repair may be described as follows: In an uninduced cell the product of *lexA* gene acts as repressor for a number of unlinked genes (*uvr A, B, C, umu D, C, sulA, dinD*, etc.). SOS inducing signal reversibly activates specific protease activity of the *recA* protein which then cleaves the *lexA* repressor (and λ-repressor in lysogen) allowing expression of various SOS genes. Following DNA repair, as the inducing signal is removed the *recA* protein level returns to proteolytically inactive state. However, a recent report suggests that the *lexA* protein is autolytically cleaved at the same alanine-glycine bond that is cleaved by the *recA* protein implying that *recA* protein in some way enhances the self-digesting activity of *lexA* protein²⁷.

The number of genes under the control of *recA-lexA* gene circuit seems very large. Kenyon and Walker²⁸ using *Mu-lac* fusion phage discovered the existence of damage inducible (*din*) loci, the function of all of these is not quite clear. Among the vast number of physiological responses, induced by SOS signals are the excision of bulky adducts and pyrimidine dimers (mediated by the products of *uvrA, B, C*)²⁹; filamentation, the result of *sulA* division inhibitor³⁰; and UV-mutagenesis due to functional *umuD, C* gene products³¹.

Nature of inducing signals of SOS repair is not very clear yet. *recA* protein of *E. coli* is a DNA-dependent ATPase³² that is involved in homologous recombination as well as proteolytic cleavage of *lexA* and λ-repressors. *In vitro*, *recA* is activated when it forms a ternary complex with single-stranded DNA and a nucleoside triphosphate³³. Thus, it seems reasonable that single-stranded regions generated by SOS inducing

treatments form at least part of *in vivo* signal for SOS induction. Boiteux *et al*³⁴ showed that 3-methyladenine residues in DNA induce filamentation – an SOS function, in *sulA* mutants in the absence of repair of alkylated DNA.

In *H. influenzae*, the W-reactivation of phage HP1c1 and UV-induction of prophage are under the control of *rec1* gene (gene involved also in recombination)¹⁵. At least 2 genes have been identified in *H. influenzae* which act in sequence to remove the UV-induced pyrimidine dimers^{35,36}. This is unlike in *E. coli* wherein products of *uvrA*, B, C act simultaneously to remove the pyrimidine dimers and bulky adducts²⁹. Differences between *E. coli* and *H. influenzae* regarding DNA repair are listed in table 1. We have cloned *uvr1* gene and by locus-directed mutagenesis isolated 3 new mutations. One of these mutations imparted UV-resistance while the other two were UV-sensitive. One of the UV-sensitive mutants, *uvr1*^{sens1} is also filamentous. The 7.5 megadalton chromosomal splice of the chimeric plasmid pKuvr1 contains the *uvr1* gene and flanking DNA sequences. Potentially it has information for about 10 genes and directed mutagenesis may reveal other DNA repair loci linked to the *uvr1* gene³⁷.

Adaptive Response

Adaptive response is under the control of *ada* gene. *ada*⁻ mutants could not be adapted for EMS or MNNG treatment. Analysis of 5 *ada*⁻

mutants revealed that the adaptation to killing and adaptation to mutagenesis are not strictly correlated, since the order of decreasing mutation frequencies was different from the order of decreasing cell sensitivity for the 5 mutants²¹. Extracts from *E. coli* cells adapted for MNNG treatment could repair the phosphotriesters on DNA backbone resulting from the action of MNNG. It was proposed that this activity accounts for much of the increased resistance to killing and is found to be mediated by a methyltransferase distinct from the one demethylating O⁶-methylguanine³⁸. Surprisingly 37K *ada* gene product thought to be a positive regulator generates the structural gene product O⁶-methylguanine-DNA-methyltransferase (18K) on proteolytic processing³⁹.

Volkert and Nguyen⁴⁰ identified 2 genes *aid* (alkylating agent inducible) A and B under the control of *ada-5* mutation with the help of *Mu-lac* phage insertion mutagenesis. *aidA* appears to be *alkA* but *aidB* harbors no previously identified gene involved in the repair of alkylation damage. *aidB* fusions are characterised by increased resistance to alkylating agents but are without a significant effect on biological adaptation and increased expression of B-galactosidase in growth-phase-dependent fashion.

GENETIC CONTROL OF MUTAGENESIS

There seems to be a connection between DNA repair, replication, recombination and mutagenesis.

Table 1 Repair of UV or PUVA damage by *E. coli* and *H. influenzae*

	<i>E. coli</i>	Ref.	<i>H. influenzae</i>	Ref.
PUVA sensitivity	1	55	4	55
Mutagenicity with				
a) UV	Present	60	Absent	16
b) PUVA	Present	61	Absent	17
DNA repair genes	<i>uvrA</i> , B, C <i>recA</i> etc.	26 (review)	<i>rec1</i> , <i>uvr1</i> , <i>uvr2</i> etc.	62, 63
a) Mode of action	<i>uvrA</i> , B, C act simultaneously	29	<i>uvr1</i> , <i>uvr2</i> act sequentially	35, 36
b) UV, PUVA sensitivity	<i>recA</i> is more sensitive than <i>uvr</i>	10 64	<i>uvr1</i> is more sensitive than <i>rec1</i>	55
Photoreactivation	Present	65	Absent	66

nesis. Some of the gene products that interact with replicating enzymes (DNA polymerases) leading to mutations are reviewed by Cox⁴¹. A number of genes like *mutD*, *S*, *R*, *L* induce transversions and frame shift mutations by interacting with DNA polymerase I.

The most striking discovery regarding UV-induced mutagenesis was the isolation of mutants (*umuD*, *C*) defective in UV-induced mutagenesis⁴². These mutants are deficient in W-reactivation and W-mutagenesis and are UV-sensitive, but could still be mutated by alkylating agents⁴³. Obtaining a null mutation of *umu* gene using *Mu-lac* fusion suggests that the DNA damage in itself is not mutagenic but the cellular processing of the damage is responsible for mutations³¹. This is also supported by the fact that a number of bacteria could not be mutated by UV, e.g. *H. influenzae*^{15,16}, *Streptococcus pneumoniae*⁴⁴, *Micrococcus radiodurans*⁴⁵ and these may therefore be devoid of *umu* gene(s).

Natural analogs of *umu*⁺ are also detected on some plasmids. *mucA*, *B* loci of the plasmid pKM101 are similar to *umuD*, *C* genes of *E. coli* as regards the proteins coded and their ability to complement for the mutant phenotype⁴⁶. This plasmid has been included in Ames' Salmonella Mutagenicity test as it increases the mutations spontaneous, UV and chemical induced reversions and confers increased UV-resistance on the bacteria under the control of *recA* and *lexA* genes⁴⁷.

MOLECULAR EVENTS DURING REPAIR

Enzymes of DNA repair.

A number of DNA repair enzymes and their possible mode of action have been reviewed by Lindahl⁴⁸. Major modes of enzyme action can be categorised as direct reversal of damage *in situ* or excision of damaged nucleotide(s) or base(s). Monomerisation of thymine dimers by photo-reactivating enzyme and demethylation of O⁶-methylguanine by O⁶-methylguanine-DNA-methyltransferase are examples of direct reversal of some of DNA damage. The enzyme O⁶-methyl-

guanine-DNA-methyltransferase catalyzes the transfer of methyl group from O⁶ position of guanine to its own cysteine residue and subsequently gets inactivated⁴⁹. Gene coding for the enzyme is yet to be identified but it seems to be the product of *ada* gene as Ada protein on proteolysis generates this enzyme.

Excision of Nucleotides

This may be accomplished in at least two ways: i) by endo- and exonucleases² or ii) by excision nuclease. Repair enzyme UVRABC Excision Nuclease complex (*in vitro* reconstituted UvrA, B and C proteins) cuts DNA strand on both sides of damaged region; 8th phosphodiester bond on 5' side of damaged DNA and 4th or 5th phosphodiester bond on 3' side. The enzyme also releases the oligonucleotide generated by the two cuts. The enzyme specifically acts on DNA damaged by UV, PUVA or *cis*-DDP⁵⁰. Recently, Thy (6-4) pyo product has been shown to be removed by the product of *uvrA*, *B*, *C* genes⁵¹.

Excision of Bases

DNA glycosylases catalyze cleavage of glycosyl bond between base and sugar in DNA. Number of DNA glycosylases are known which specifically act on certain type of altered bases, e.g. 3-methyladenine-DNA glycosylase (removes 3-methyladenine); uracil DNA glycosylase (removes uracil), urea DNA glycosylase, thymine glycol DNA glycosylase, etc. Pyrimidine dimer DNA glycosylase coded by T4 phage gene has been detected in *E. coli*. Similar enzyme has been detected in radiation resistant *M. luteus*. T4 phage-coded endonuclease V (pyrimidine dimer DNA glycosylase with associated AP endonuclease activity) has been shown to restore unscheduled DNA synthesis (UDS) and repair in UV-damaged Xeroderma Pigmentosum cell lines when introduced along with Sendai virus⁵².

Repair of AP sites

AP sites in DNA are cleaved by AP endonucleases. With class II AP endonucleases, DNA glycosylase activity is often associated.

Enzymatic activity that inserts purines into depurinated DNA was detected in a soluble extract of *E. coli* cells. This activity brings about the insertion of adenine and guanine into appropriate site using corresponding dNTPs as purine donors and may represent a mechanism of 'Base Insertion Repair' for repair of apurinic sites⁵³. Missing nucleotides can be replaced by resynthesis of DNA using other strand as template.

Recombinational Repair

This allows cells to tolerate the unrepaired DNA damage and hence is very essential from the point of view of survival but is somewhat less well characterised. It could be imagined to fill, by recombinational events gaps created post-replication opposite the lesions in daughter strands (reviewed by Hanawalt)⁵⁴.

In *H. influenzae* repair events with PUVA damage were that immediately after the treatment the chromosomal DNA sedimented much faster than normal. Some of the fast-sedimenting material was cut and appeared as slow-sedimenting material. After longer incubation the normal-sedimenting material reappeared in wild type. Release from fast sedimenting was slow in the mutants *pso7* (mutant selected directly for PUVA sensitivity) and *uvr2* (second gene in excision repair mechanism). Removal of crosslinks was normal only in wild type⁵⁵.

In *E. coli* repair of double strand breaks was shown to be induced by UV exposure of cells⁵⁶. It was also shown that this repair inhibits the DNA degradation after X-irradiation. Picksley *et al*⁵⁷ suggest that *recN* gene product may be involved in repair of double-strand breaks by UV-inducible mechanism. *recN* mutations block conjugational recombination and are mitomycin C and ionizing radiation sensitive but UV-resistant.

DISCUSSION

From the foregoing review, some generalizations are possible. First and foremost is the inducibility at low exposures of at least four different systems which offer some protection to a subsequent higher exposures. From the point

of view of protection these may turn out to be important. Secondly, some species cannot be mutated even with very well-known mutagenic agents such as UV or MMS. The best explanation seems to be that they lack an *umu*-like gene. As a matter of fact *H. influenzae* which is not mutated by UV, is about four-fold more sensitive than *E. coli*. It is also known to lack photoreactivating enzyme and therefore presumably *phr* gene. Thirdly, DNA repair events have an overlap with mutagenicity but these can be uncoupled. Finally, even the repair systems amongst themselves may have some overlap as for example in the *dnaK*, *groEL* gene products which are induced by both heatshock as well as UV. As a matter of fact even some teratogens induce some proteins analogous to heat shock proteins⁵⁸. There are several examples of a connection between mutagenesis and carcinogenesis of prime support for which comes from the *Salmonella* test⁵⁹, in which induction of mutations by an agent has been empirically correlated with its carcinogenicity. But the problem of carcinogenicity is quite complex and the role of oncogenes, transposable elements and enhancers is not completely clear. However, further research in all these related areas will certainly improve our understanding of these DNA processes.

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21 March 1985

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NEWS

GEOGRAPHY: THE FORGOTTEN SUBJECT

... "Name the country drained by the Amazon River, American college students were asked in a 1950 survey, and 77.5% of them correctly said Brazil. The same question was posed last fall in a statewide college exam in North Carolina, and this time only 27% of the students got it right. The apparent deterioration of geographic knowledge evident in the North Carolina survey and in similar tests around the nation has prompted [the Natl. Council for Geographic Education and the Assn. of American Geographers] to devise a set of guidelines for the teaching of geography in elementary and secondary schools. . . . The North Carolina exam, which involved 2,200 students in eight schools of the state's university system, used about 25% of the questions given in 1950. Others were no

longer applicable because of changes in world geography. When asked the approximate 1980 census population the US within a margin of error of 5 million, only 8.4% of North Carolina college students came close to the correct figure—226 million, the report said. Answers ranged from 100,000 to 236 billion. Asked to name the two largest states in area, less than 50% came up with Alaska and Texas; only 21% knew that Rhode Island and Delaware were the two smallest."

[(Theodore Shabad in *Geotimes* 30(4):6, Apr 85 (From *New York Times*) (Reproduced with permission from Press Digest, *Current Contents*®, No. 22, June 3, 1985, p. 14. Published by the Institute for Scientific Information®, Philadelphia, PA, USA.)]

NEW WAY TO PREVENT GONORRHEA?

... "A potential vaccine against the contagious venereal disease gonorrhea has been developed by a group of investigators at the Stanford U. Sch. of Medicine. The candidate substance, a synthetic protein fragment, appears to work by blocking the first step in the process by which gonococcal bacteria infect the cells lining the human urogenital tract; the adhesion of the bacteria to the cell walls by means of the hairlike filaments called pili. According to Gary K. Schoolnik [Stanford U.], the potential vaccine stimulates the immune system to seek out and inactivate the pili, thereby preventing the bacteria from binding to the cells. . . . Previous attempts to develop a vaccine to protect against gonorrhea have failed because the

protein molecule that makes up the pili, called pilin, has a tendency to change its configuration continually, enabling the gonococcal bacteria to elude the body's highly specific immunological defenses. The key to the new vaccine's preliminary success, Schoolnik and his co-workers report, is that it stimulates immunity to a part of the pilin molecule that remains unchanged, even in different bacterial strains."

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