Pakistan Journal of Pharmaceutical Sciences
5(2) 185-198 (1992)

DNA REPAIR, CANCER AND GENE THERAPY

SHEIKH AJAZ RASOOL, RASHID ALI, AAMIR MIRZA, TALAT YASMEEN AND ROOHI MUSHTAQUE

Department of Microbiology, University of Sarachi, Karachi-75270, Pakistan

Fidelity of DNA synthesis is pivotal to our understanding of fundamental biological processes. An organism must replicate and repair its DNA with high accuracy and precision in order to maintain its genetic activity. DNA repair pathways enable cells to offer enhanced resistance to deleterious effects of chemicals and radiations (Lindahl, 1982). A number of pathways have been described e.g. the error-prone SOS repair (that lacks fidelity of repa[i]r process), the error-proof adaptive repa[i]r of al cylated DNA and inducible response to oxygen radical damage in DNA. These different circuits are under positive regulatory controls. However, the biochemical strategies employed to generate specific protein activators differ among the pathways. Although, the universally occuring repair activities seem to serve efficiently to counteract malignancy, error-prone polymerise and plasminogen activator have been instrumental in tumorigenesis, the process that proceeds by cascading of genetic errors (Sancar and Sancar, 1988).

Environmental factors exert persistent and increasing pressure on all the organisms. A major threat to the cellular system is posed by the external agents (e.g. chemicals and radiations) on DNA (Table 1; Rasool, 1987) which can al*ter* the chemical structure and function of DNA, resulting in either mutations or cell death.While mutagenicity and carcinogenicity are highly correlated, effective cell killing is a major objective in cancer treatment with genotoxic agents. Thus, a study of how the cells cope with structural changes or damage in DNA has ramifications for both prevention and treatment of cancer. Indeed, the subject of DNA repair has attracted the clinicians for intensive studies. In the last decade or so, recombinant DNA technology has made it possible to study DNA repair with purified enzymes. Mutations in certain genetic loci may render cells more susceptible to carcinogens. Some of these genes have been isolated, cloned and dick protein products overproduced using the recombinant DNA technology. Thus, an enzyme (involved in DNA repair and normally present in very small quantities) is obtained in high concentrations for detailed structure-function studies (Orren and Sancar, 1987).

The repair mechanisms

There are different mechanisms through which a cell handles the damage. These mechanisms involve complex processes (Table 2; Sancar and Sancar, 1988).

A) Direct DNA repair

Direct repair involves neither removal nor replacement of bases or nucleotides; rather the covalent modification in DNA is simply reversed. Three examples of this *repa*^{i}*r* are (mown :

(1) Photoreactivation (mediated by DNA photolyase; Dulbeco, 1949; Kehler, 1949).

(2) Spore specific repair (Wang and Rupert, 1977).

(3) Adaptation mediated by 0^6 methyl guanine DNA methyltransferase (that removes methyl groups from DNA ; Samson and Cairns, 1977).

DNA photolyase (that breaks the cyclobutane ring of a pyrimidine dimer) has been most extensively characterized. Recent studies have demonstrated that all phototyases contain two intrinsic chromophores. The ubiquitous presence of $FADH_2$ and its crucial role in photolysis by Escherichin coil and yeast enzymes suggest that electron donation to the dimer is a key-common feature of the action mechanism. The second chromophore (pterin or deazaflavin derivatives) is the major light absorbing cofactor and may function by transfer of energy to FADH2 or may directly transfer electron to the dimer (Eker *et al*, 1987).

B) Indirect repair functions

1) Base Excision Repair: An unusual base is removed from DNA by hydrolysis of N-glycosylic bond between the deoxyribose and the base by a DNA glycosylase (Lindahl, 1974). This reaction produces an AP (apurinic or apyrimidinic) site that needs further processing for completion of repair. AP endonucleases hydrolyse the phosphodiester bond adjacent to AP sites generated by DNA glycosylases or by spontaneous and induced depurination / depyrimidination of DNA (Lindahl and Anderson, 1972). The abasic sugar is then removed from the nick and replaced with correct nucleotide by the joint action of DNA polymerase and ligase (Lindahl, 1979).

2) Nucleotide Excision Repair: The damaged bases are removed from DNA as oligonucleotide and the resulting gap is patched by repair synthesis. Many nucleotide mono- and diadducts are removed by this repair (Boyce and Howard-Flanders, 1964). Briefly, an ATP-dependent nuclease (made of 3 subunits; the ABC exinuclease) hydrolyses the 8th phosphodiester bond 5' and 4th 5th phosphodiester bond 3' to mono-and dinucleotide adducts to remove a 12-13 mer from DNA and generate a gap of the same size that is filled in by DNA polymerase and sealed by ligase (Sancar and Sancar, 1988) The genes for nucleotide excision repair are uwA,uwB *and uvrC* (Howard - Flanders *et al*, 1966). These genes ar*e* unlinked and *uvrA* and *uvr*B each constitutes a single operon ; while uvrC is cotranscribed with 2 genes (Sancar *et al.*, I982). The genes for the subunits of ABC exinuclease *uvr*A,

*u*vrB and uvrC have been sequenced (Husain *et al,* 1986) and cloned (Sancar, 1987).

3) Recombinational Repair: When polymerase encounters certain nucleotide adducts, for example pyrimidine dimers in DNA, it stops replicating and reinitiates replication about 10' base pairs beyond the adduct, thus, generating a gap in the daughter strand. This discontinuity (Postreplication gap) is filled by the RecA protein which transfers the complementry strand from sister duplex into the gap (Rupp *et at,* 1971). It may be assumed that for complete in *vit*ro reconstitution of recombinational repair, RecA , Poll , "nicking in trans" activity, ligase and Holliday resolvase are needed (West and Korner, 1985). Recombinational repair contributes to cellular survival in two *ways i.e., by* bypassing, adducts that block replication and by altering the adduct containing region so that the modified base may be removed by ABC exinuclease.

4) Repair of Cross Links : A number of carcinogens and chemotherapeutic agents, including mitomycinC, nitrogen and sulphur mustards, formaldehyde, nitrous acid, cisplatin and psoralen plus light do crosslink the two strands of the DNA (Singer and Kusmierek, 1982).Crosslinks pose a special challenge to repair enzymes because they involve both the strands of DNA and, therefore, cannot be repaired using the redundant information in the complementary strand for resynthesis. It appears that crosslinks are repaired by a combined action of nucleotide excision and recombinational repair pathways (Sinden and Cole, 1978). Attempts have been made to reconstitute cross-link repair *in vitro*. Accordingly, a synthetic DNA fragment of 40 base-pairs containing a psoralen crosslink at a unique position was treated with AB exinuclease and reaction products were analyzed. It was found that the enzyme incised the 9th phosphodiester bond 5' and 3rd phosphodiester bond 3' to the thymine residue attached to the furan ring of psoralen. The strand attached to psoralen via pyrone ring was not incised. In the light of this observation, the model (Fig. 1) for repair of crosslinks in *E. coli* was proposed (Cimino *et al,* 1985; Van Houten *et al,* 1986).

5) Regulatory Network Repair : Repair enzymes are usually present at 20 - 100 molecules per cell. Obviously, a cell does not have to (continually) synthesize repair proteins in the absence of life - threatening DNA lesions. The enzyme levels are elevated under genotoxic stress to enable cell to repair DNA before [i]rreversible reactions take place. In fact, the organisms have devised a control mechanism to limit the transcription of DNA repair genes in the absence of danger and do induce transcription in response to DNA damage. In *E.coli,* three well understood regulatory networks modulate the levels of DNA repair proteins according to the cellular needs (Sancar and Sancar, 1988). Examples of such processes are described below:

i) The SOS Response: It involves a number of physiological phenomena observed after exposure of *E. coli* to DNA damaging agents. A regulon, (of about 20 genes) is responsible for this response. This regulatory mechanism involves the interaction of LexA protein with the promoter regions of genes (mvolved in DNA repair). LexA binds to DNA upstream of the coding regions of about 20 genes, directly or indirectly involved in cell survival, thereby repressing their transcription. Upon exposure of cells to DNA damaging agents, the small amount of RecA protein

present is activated by binding to single stranded DNA produced as a result of damage and then promotes the autocatylic cleavage at the LexA protein, depressing DNA repair genes, including recA itself. After damage is removed, RecA ceases to be activated and newly synthesized LexA can repress transcription of the SOS genes (Little and Mount, 1982). The cleavage of LexA turns on the ferA and recA genes (the regulators), the *uvr*A, *uvr*B, and *uvr*D (but not *uvr*C) genes that are responsible for excision repair, *umu*DC genes, responsible for mutagenic damage bypass mechanisms, su/A and sum that are involved in cell division, and a number of *din* (damage inducible) genes whose functions are unknown (Walker, 1984).

The SOS response tends to increase the cell survival by inducing excision (*uvr*A, *uvr*B, *uvr*D), recombination *(rec*A,*recN*, *recQ*, *uvrD,ruv*) and mutagenic (*umu*C, *rec*A) - repair mechanisms as well as by inhibiting cell division to allow more time for repair. Upon recovery of cells from DNA damage the activating signal (single strand DNA) disappears and autodigestion slows down. LexA accumulates and the status *quo ante* is established, where there is the tow level of constitutive expression of all the SOS genes (Orren and Sancar, 1987).

ii) Adaptive Repair: The term adaptation refers to the phenomena resulting from the activation of *Ada* regulon, which is responsible mainly for prevention of mutation and killing by alkylating agents (Samson and Cairns, 1977).Ada is a positive regulator that binds to the Ada box, A3 - N3 - G6 - GCA upstream of the constituent promotors and turns the corresponding genes on (Sancar and Sancar, 1988). Alkylating agents, of which methylating agents appear to be widespread environmental mutagens, act through covalent modifications of the cellular genome to generate miscodinn base derivatives and lesions that block DNA replication. All oxygens and nitrogens in DNA can be modified by methylating agents. However, the most relevant adducts include O^6 methylguanine (O^6meG, a miscoding base) and 3 - methyladenine (3meA, a cell killing lesion). Main function of the adaptive response is to improve the repair of the two harmful base derivatives (Lindahl and Sedgwick, 1988). To remove 3-methyladenine, cell employs the same strategy as for the excision of anomalous bases such as uracil from DNA. The base - sugar bond is cleaved by a DNA glycosylase to release the altered base residue in free form and generates a repairable AP site (Lindahl, 1982). In contrast, O^6meG is corrected by direct reversal of damage accomplished by transfer of methyl group to a cysteine residue in the repair of enzyme itself. The protein is not regenerated and undergoes suicidal inactivation as a consequence of the DNA *rep*air event. Similar repair functions for these damaged purine residues are present in mammalian cells, but the responsible enzymes appear to be constitutive in higher cells (Lindahl and Sedgwick, 1988). Methylating agents trigger the adaptive response in *E. co*il by generating an intracellular signal for its induction. This signal has been identified as one of the minor DNA methylation products, one of the two stereoisomers of a methly phosphotriester (Teo et *at,* 1986). Inducible genes of the adaptive response to alkylating damage are shown in Table 3. A wild type recF gene is also required for normal induction of adaptive response genes (Volkert, 1989). On exposure of *E. coil* to simple methylating agents, the cellular DNA is alkylated at many sites. The Ada protein, present at low level in uninduced cells, transfers a methly group from a methylphosphotriester in DNA to its own N-terminal domain. This covalent

modification, resulting in a conformational change, converts the Ada protein from a weak to a strong transcriptional activator of the inducible genes. Enhanced repair of methylated DNA by the increased cellular levels of these gene products conveys cellular resistance to alkylation mutagenicity and toxicity (Lindahl and Sedgwick, 1988). Several microorganisms have been tested for their ability to acquire enhanced resistance to MNNG toxicity and mutagenicity by treatment with low MNNG doses (Table 4 presents the list of different organisms that have been searched for adaptive repair response). Mammalian, fish and *Drosophila melanogaster* cells also show an activity with biochemical properties similar to that of the *E.* coil methyltransferase. The ada gene was cloned into *E. coli ada* mutant resulting in conferring the resistance to MNNG mutagenesis (Sedgwick, 1983).Recently, the isolation of an *ngs* mutant MR 0192 of *Saccharomyces cerevisiae* has been reported *(Mina,* 1992). Integration and expression of the ada gene in mammalian cells stably conferred increased resistance to mutation induction and killing by a variety of simple alkylating agents (ICataoka *et al,* 1986). Attempts have been made to express the Ada protein in transgenic mice (Brennand and Margison, 1986) which could be useful for evaluating various aspects of carcinogenesis by methylating agents (Zarbl *et al,* 1985). It is expected that the current availabilty of relatively large amounts of pure Ada protein from expression vector systems and the clarification of the domain structure of this protein will encourage work using physico-chemical techniques such as X-ray diffraction measurements to provide a detailed three dimensional structure for the interaction between damaged DNA and this unusual antimutagenic activity and regulatory factor (Lindahl and Sedgwick 1988).

iii) Adaptive Response to Oxidative Stress : *E. coli* cells exposed to 60 uM H_2O_2 for 60 minutes acquire resistance to killing by 10 mM H_2O_2. This adaptation is independent of *lex*A, *rec*A and *ada,* but is inhibited by chloramphenicol (Demple and Halbrook, 1983). The phenomenon has several regulatory components and involves the production of ApppA and related adenylated dinucleotide alarmones that may be responsible for the induction of about 30 proteins (Bochner et a1., 1984).

There is a considerable overlap between various stress response networks, viz the SOS response, adaptation, the adaptive response to oxidative damage and the heat-shock response, indicative of a concerted cellular efforts to protect and repair DNA (Walker, 1984).

Role of repair processes in tumor progression

In humans, the study of DNA repair has been assisted by the existence of genetic disorders (Table 5) in which cells are deficient in some aspects of repair (Orren and Sancar, 1987). There are a number of cell lines established from patients with genetic disorders e.g. cell lines from xeroderma pigmentosum (XP), ataxia telangiectasia (AT), Fanconi's disease and Bloom's syndrome have been subjected to extensive investigation in order to look for relationship between the gene defects and their phenotypic effects, usually observed as radiation sensitivity and predisposition to develop cancer. It is believed that cell lines derived from XP patients lack excision repair functions. An increased spontaneous mutation rate was demonstrated in cell

lines from patients with Bloom's syndrome (Warren et *at,* 1981). Bloom's syndrome cells exhibit a variety of chromosome aberrations, increased sister chromatid exchanges (SCE) and chromosome instability. It is interesting that genetic defects cause Bloom cells to exhibit SOS type functions constitutively (Shiraishi *et al,* 1985). However, most of the agents that are efficient SOS inducers are also known to cause transmissible mutations and cancers in mammals. This is particularly true for long term mutagens/carcinogens as UV and ionizing radiations and allcylating agents (Rasool, 1987).

Plasminogen activator (PA) is a serine protease which is believed to be associated with cellular transformation, neoplasia and tumor promotion. Certain hormones, oncogenic viruses and tumor promotors induce PA. Various agents which damage DNA, including UV irradiation, induce PA in embryonic fibroblast cells of humans, rodents and chicken. PA is also induced in adult skin fibroblasts following UV irradiations if the cells are derived from XP patients (Miskin and Ishai, 1981). However, no PA was induced from normal cells. Furthermore, intracellular level of PA induced by UV was correlated with the extent of repa[i]r deficiency of XP cells used. The more severe the repair deficiency, the higher the level of PA induced at even lower UV doses. The UV induced PA induction is cycloheximide sensitive and takes more than 48 hours after UV irradiation for full induction in contrast to PA induction by hormones and other known PA inducing agents that take only 6-8 hours after the treatment. Thus, impaired excision repair capacity in XP cells results in a DNA structure following UV [i]rradiation which triggers PA induction. This is reminiscent of a very similar situation observed in SOS induction in uvr mutants of *E. coli.* It is not clear, however, why normal adult skin fibroblasts lack PA inducing capacity *even* at very high UV dose, whereas, embryonic and XP cells are able to induce PA. In any case, the PA induction in response to DNA damage is one manifestation of SOS type reaction in mammalian cells (Miskin and Reich, 1980).

Possible role of inducible process in tumorigenesis is interesting but still controversial. A number of arguments favouring the significance of the SOS response to carcinogenesis and evolution have been given by Echols (1981). According to a later hypothesis, the methylation hypermutability results from the effects of recF mutations on the induction of both the SOS and the adaptive response (Volkert, 1989).

Prospects of gene therapy for correction of genetic disorders

A long term goal of DNA repair studies is to correct the defects in human cells by introducing the appropriate genes or proteins exogenously (Table 5). The first attempt towards this goal was made by Tanaka *et al* (1975). Accordingly, Ti endonuclease V restored excision repair to permeabilized XP-D mutants. Similarly, microinjection of *M. luteus* UV endonuclease or yeast DNA photolyase into XP cells enables them to repair pyrimidine dieters (de Jonge *et al,* 1985). However, microinjection of ABC exinuclease or introduction of uvrA, *uvrB* and uvrC genes into

human cells failed to elicit any repair response in the recipient (Zwetsloot *et al,* 1986). Bacterial repair genes have also been introduced into mammalian cells to correct specific repair deficiencies. Thus, Ts endonuclease V was spliced into a mammalian enhancer-promotor and introduced into XP cell line. The gene became stably integrated and was expressed efficiently as evidenced by increased UV resistance of the host cells (Valerie *et al,* 1985). Likewise, mutant cell lines from both humans and hamsters have been successfully transfected with *ado* gene from E. *colt,* thereby rendering them less susceptible to 0^6-alkylation damage (Katoka *et al,* 1986; Brennand and Margison, 1986; Samson *et al.,* 1986; Ishizaki *et a1.,* 1986).

We are still quite away from using gene therapy to correct various genetic disorders associated with impaired DNA repair capacity. However, a better understanding of DNA repa[i]r in both microbial and mammalian systems will greatly help in our understanding of carcinogenesis and in adopting proper measures for preventing and treating cancer.

Table 1

DNA modifications by physical and chemical genotoxic agents

Physical agents	Chemical agents
Heat Base deamination ; base loss (by glycosylic hydrolysis).	**Activated oxygen species** generated during oxidative metabolism.
UV radiation Pyrimidine dimers ; 6-4 photo-products.	**Glucose** (a common metabolite).
Ionizing radiation Ring opening ; base fragmentation ;single and double strand breaks.	**Diverse inorganic and organic electrophiles** (including metals, alkylating agents and polycyclic aromatic hydrocarbons).

Table 2

Repair mechanisms

Direct DNA repair	Indirect DNA repair	Regulatory network repair
Chemical change in DNA is simply reversed e.g. DNA photolyase breaks cyclobutane ring of pyrimidine dimers.	Base excision repair : modified base removed from DNA by hydrolysis of N-glycosylic bond between deoxyribose and the base by a DNA glycosylase.	SOS repair
m-O^6G DNA methyltransferase removes methyl groups from DNA.	Nucleotide excision repair.	Adaptive repair
Spore specific repair (e.g. *B. subtilis*).	Post - replicational recombinational repair.	Adaptive response to oxidative stress.

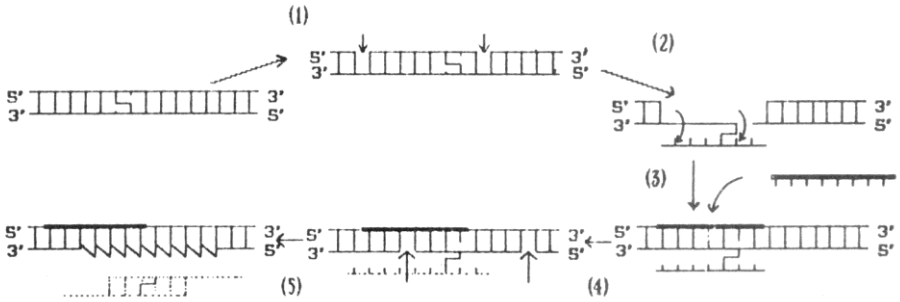

Figure 1: Model for Repair of Psoralen crosslink in *E. coli.*

Step 1: ABC exinuclease incises on both sides of crosslinked base in one strand only.

Step 2 & 3: The excised oligomer is displaced by RecA, which mediates strand invasion by the sister duplex and with the help of RecBCI) or other nucleases and ligasc produces covalent triple-strand intermediate.

Step 4: The ABC exinuclease cuts out the dangling oligomer like a monoadduct.

Step 5: The 2 excision oligomers joined to one another by crosslink are displaced and resulting gap is filled by concerted Pol I, helicase II and ligase action.

Table 5

Repair defective inherited diseases of humans

Name of the disorder	Characteristics of the cells	*In vitro* gene therapy
Xeroderma Pigmentosum (XP)	XP cells lack in photoreactivation, excision repair (incision step) and SOS functions.Predisposition to develop cancer (Plasminogen activator induction in response to DNA damage in XP cells).Sensitive to radiations.	T4 endonuclease V did restore excision repair of permeabilized XP cells (Tanaka *et al.*, 1975). T4 endonuclease V gene spliced to a mammalian enhancer - promotor and introduced into XP cell line. The gene became stably integrated and expressed (Valerie *et al.*, 1985). *M. luteus* UV endonuclease or yeast DNA photoreactivation enzyme into XP cells enables repair of pyrimidine dimers (de Jongs *et al.*,1985 ; Zwetsloot *et al.*, 1986).
Ataxia telangiectasia (AT)	Sensitive to ionizing radiation ; exhibit chromosomal instability ; defective in excision repair and SOS response.	-
Bloom's syndrome	Cells exhibit a variety of chromosome aberrations ; exhibit SOS type functions constitutively ; increased spontaneous mutation rate.	-
Fanconi's anaemia	Lack in crosslink repair and SOS functions.	-
ada mutant cells of humans / hamsters	Cells are sensitive to alkylating chemicals.	*ada* gene from *E. coli* successfully transfected to render *ada* mutants / cells less susceptible to O^6 alkylation damage. (Katoka *et al.*, 1986).

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