CHAPTER I

I N T R O D U C T I O N

Mechanism by which living organisms are affected by various kinds of environmental stresses have become an important area of research as such a knowledge can have far-reaching advantages in diverse fields of human welfare. The main focus of attention in these pursuits is the manner in which living cells are injured after exposure to deleterious agents as this knowledge forms the basis for our understanding of the step-wise development of injury to higher organisms. Atomic radiations, variety of man-made chemicals of modern age and some natural compounds have been found to cause injury to living cells by specific damage on DNA. This class of stress agents has aroused tremendous concern because exposure of man to them could result in the long run, in somatic changes such as cancer and inheritable deleterious effects. The importance of studies probing the nature of their action on a living cell lies in the two divergent aspects of peaceful uses of atomic energy: (i) in evolving and organizing adequate and economically optimal radiation protection measures in nuclear industry; and (ii) in harnessing this property in diagnosis of various diseases and therapy of malignant tumours, sterilization of medical supplies, and preservation of food products.

The topic of injury to DNA and cellular responses to repair or bypass the lesions created in DNA has therefore become the subject of intensive research. Our knowledge

in this subject has been largely derived from studies with bacteria and other unicellular organisms.

The studies described in the thesis relate to the actions of radiations and other agents on a phenomenally radioresistant microorganism. As a background to the subject current developments in the following topics will be discussed: (i) nature of physical and chemical agents affecting cellular DNA; (ii) cellular DNA repair systems; (iii) alterations in cellular processes due to lesions in DNA and (iv) relevance of research on the mechanisms of injury and recovery mechanisms to fundamental and applied aspects of Life Sciences.

Nature of physical and chemical agents affecting cellular DNA

A voluminous literature has been accumulated to show that diverse types of radiations - ionizing radiations (gamma rays, X-rays, alpha particles, neutrons, etc.) and non-ionizing radiations (ultra violet light) - exert their action by damage to cellular DNA. Likewise, a great deal of information is now available on a variety of chemical agents principally mutagens and carcinogens which also injure cells by interacting with DNA (1, 2). Pertinent recent information on this topic will be summarized in what follows.

Radiation-induced modifications in cellular DNA

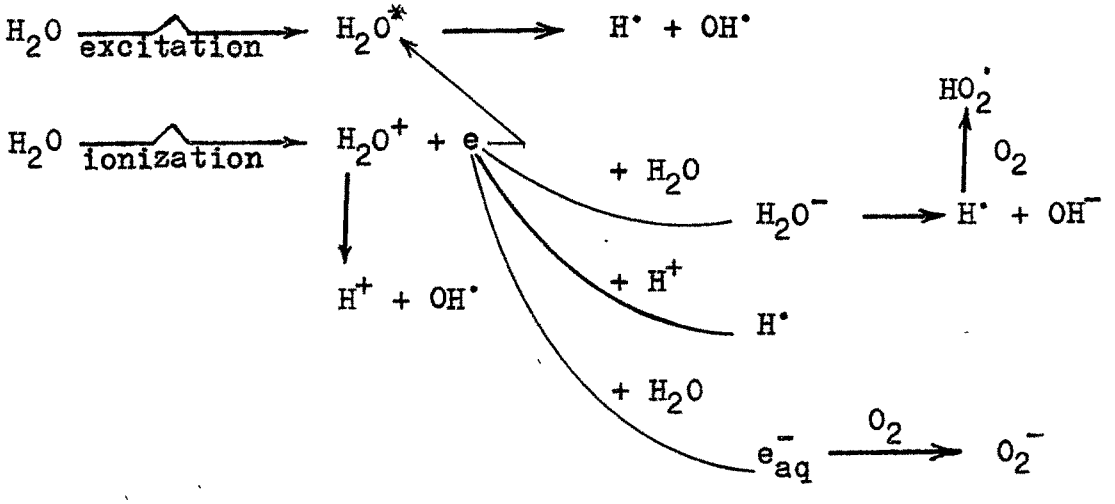
The discovery of X-rays by Roentgen in 1895 and the discovery of radioactivity by Becquerel in 1896 (2) had led many workers to investigate into the events that occur between exposure of an organism to various radiations and manifestation of a permanent biological change. Much progress has been made in the detailed analysis of the two earliest steps, namely, (i) the primary physical process of energy deposition, and (ii) subsequent chemical changes, triggered within microseconds of radiation exposure, which bring about initial biochemical lesions.

Exposure to ionizing radiations

Most of the work carried out so far pertains to effects of ionising radiation exposure *in vivo* on cellular/viral DNA or *in vitro* on aqueous solutions of DNA, nucleotides and bases (3). Ionizing radiations can inflict damage on cellular DNA (as also DNA dissolved in aqueous solution) either by direct hits on DNA itself or by indirect actions *via* the formation of radical species of cellular non-DNA components. Water being the major component of living cells, the aqueous radiolytic products have a major participation in the indirect action. The contribution of direct action of radiation has been studied under various conditions such as frozen conditions, in freeze-dried states, in the presence of cysteine, glutathione and cysteamine (4). These studies have revealed that

the indirect action has a major role in the manifestation of radiation effects in the living systems.

During the past few years, a number of approaches have been attempted to understand the interaction of water with radiations - the nature of aqueous radiolytic products formed and their reactivity. Among these approaches are the use of appropriate radical scavengers (e.g. of OH• and e^-_{aq} radicals), pulse radiolysis and fast-mixing irradiation techniques. Current information on aqueous radiolysis is summarised in the following scheme:



(5)

The above-mentioned chemical processes extend upto 10^{-2} seconds after the primary deposition of radiation energy (6 - 8). Attempts have been made to assess the life-time of some of the aqueous radiolytic products. Thus hydrated electron (or aqueous electron) in neutral water has been found to have the life-time of 2.3×10^{-4} seconds.

The indirect action (but not the direct action) is greatly influenced by the presence of oxygen during irradiation which not only alters the spectrum of degradation products of water but also their yields. Indeed the oxygen-modifying effect of radiation on living cells has been well established; in general, living cells are 2-3 times more sensitive to ionising irradiation in the oxic condition as compared to in anoxic condition.

Among the radiolytic products of water $H^{\cdot}$, $OH^{\cdot}$ and e^{-}_{aq} seem to contribute significantly to cellular damage, $OH^{\cdot}$ being much more effective than e^{-}_{aq} in N_2 atmosphere.

As mentioned earlier, DNA is the primary cellular target of radiation attack and living cells succumb to radiation action mainly on account of the damage to cellular DNA (9 - 13). The principal ionising radiation-induced distortions in DNA are production of strand breaks, formation of 5,6-dihydroxy-5,6 dihydrothymine and extraction of proton from the methyl group of thymine (14, 15). All these seem to arise from the reactions of the $OH^{\cdot}$ radical also implicated to be the main factor responsible for the cell death.

Amongst the various structural changes mentioned above strand breaks are believed to be major distortions brought about in cellular DNA as a result of ionising irradiation (16, 17, 12). The total number of strand breaks in

DNA are normally estimated from the sizes of single-strands in alkaline sucrose density gradient analysis (18). Strand breaks determined from such analysis hence include not only single- and double-strand breaks but also alkali-labile lesions in DNA. There is in fact increasing evidence to suggest that a sizeable proportion of single-strand breaks could be formed in response to alkali-labile lesions (12). A fund of useful information on the radiation-induced strand breaks is available and some representative data are collected in Table I.

As seen in the Table, ionising radiations induce about 10-15 times greater number of single-strand breaks than double-strand breaks in cellular DNA. Irradiation in the presence of growth medium or histidine results in less number of strand breaks presumably due to the blocking of indirect action of radiation. The oxygen effect can be clearly seen from the finding that strand breaks production at a given dose is more under oxic conditions of irradiation than under anoxic conditions. At least in E.coli, it was found that the status of the repair system has not affected the initial strand break production. The existence of fast repair systems during the course of irradiation may nevertheless affect the accurate estimation of the strand breaks formed and some new methods may have to be adopted for eliminating the interference of this repair system. Other results indicate that the number of strand breaks formed

Table I

PRODUCTION OF SINGLE- AND DOUBLE-STRAND BREAKS IN DNA OF IRRADIATED MICROORGANISMS

Organism	Suspension media for radiation exposure	No.of strand breaks/ rad/10^{12} daltons DNA	
		Single	Double
Phage T_7	Buffer	25	2
Phage T_7	Buffer + histidine	3	0.26
E.coli Bs-1	Nutrient broth	0.2	
E.coli B/r	Nutrient broth	0.2	
E.coli B/r	Buffer	0.47	
E.coli B/r	Buffer (O_2)	0.68	
M.radiodurans	Buffer	0.2	

Adapted from References 19, 18 & 20.

per unit radiation dose, immediately after irradiation, in the highly radioresistant microorganism M.radiodurans is the same as that formed in E.coli.

The mechanism of formation of DNA strand breaks after irradiation of DNA solutions has been an active area of radiochemical research. One study provides evidence to suggest that OH· radicals attack the C_1 position of sugar in the purine nucleotide residue, which results in the cleavage of the glycosidic bond and release of the purine base. The apurinic site so formed is alkali-labile and prone to strand break formation (21, 22). Recently another study has suggested that radiation causes radical formation at the C_4 position of the sugar moiety in the DNA strand resulting in the heterocyclic splitting of the sugar-phosphate backbone under anoxic conditions (23).

Ultraviolet (UV) radiation exposure

Recent studies have indicated that the relative contributions of direct and indirect actions of UV radiation on living cells are wavelength-dependent. Direct action strongly predominates in the 240 to 300 nm region where DNA possesses an absorption maximum. Damage in this region is mostly a consequence of the electronic excitation of the heterocyclic DNA bases, the major lesions being cyclobutyl dipyrimidines (24, 25). Other important lesions are hydration of pyrimidines at 5,6 positions, formation of intra- and inter-strand

cross-links, deamination of bases and formation of other less important derivatives of pyrimidines.

Unlike in vegetative cells, in bacterial spores adjacent thymines do not form thymine dimers upon UV irradiation, but some other structurally different lesions. One major photoproduct is identified as 5-thyminyl, 5,6-dihydrothymine termed as 'spore photoproduct'. Formation of such unusual photoproducts in bacterial spores presumably results from the dehydrated environment around spore DNA because similar photoproducts were also identified in UV irradiated frozen nucleoside solutions and in dry films of DNA (26, 27). Studies of Brunk on Tetrahymena pyriformis DNA (28) of Prakash on T_4 DNA (29) and of Narayani et al. on Yoshida ascites tumour DNA (30) indicate that at moderate doses of UV radiation formation of thymine dimers along DNA strands is not probably random; there is preferential formation of thymine dimers in pyrimidine-rich regions in DNA compared to the other regions.

Indirect action of UV radiation gains an importance at shorter and longer wavelengths where active oxygen species are produced by water photolysis, photodynamic action, photodissociation of hydrogen peroxide and related reactions. Exposure to monochromatic radiation in the solar UV range (around 365 nm) results in the formation of several lesions including single-strand breaks, pyrimidine dimers, endonuclease-sensitive sites and thymine glycols (31).

Action of chemicals on cellular DNA

Numerous studies during the past several years have revealed that a variety of chemicals have the property of specifically interacting with DNA *in vitro* or cellular DNA *in situ* (32, 33). Some of the classes of chemical compounds which may show the attribute to interact with DNA are: alkylating agents, polycyclic hydrocarbons, aromatic amines, azo dyes. Quite a few of these chemicals elicit responses similar to radiations by virtue of their interactions with cellular DNA. It now appears that most DNA-acting chemicals (which include carcinogens) fall into two distinct categories: compounds that react directly with cell constituents and compounds that require preliminary metabolic conversion to a reactive metabolite. The biological alkylating agents, such as sulphur and nitrogen mustards, are examples of the first group, whereas the second category comprises some polycyclic hydrocarbons, aromatic amines, azo dyes, nitrosamines. Covalent binding of these various chemicals or their metabolites to DNA has now been demonstrated and in many instances the nature of the products with DNA have been characterized. All these chemicals or their reactive metabolites are electrophilic reagents capable of reacting with a whole spectrum of nucleophilic contents in DNA. The chemicals, especially the biological alkylating agents, have been extensively investigated in many biological systems and shown to produce several damaging effects in DNA. From studies, mainly *in vitro*, the following reactions

have been shown to occur following direct interaction with DNA: single-base substitutions, depurination reactions, single- and double-strand breaks, esterification of phosphate groups, inter- and intra-strand cross-links, DNA-protein cross-links. Table II gives available information on the reactions of some representative chemical compounds with DNA.

Cellular processes involved in the restitution of DNA damage

Radiation microbiology has been instrumental in bringing to light one of the fascinating cellular phenomena, namely, the property of living cells to repair its damaged DNA. The topic of DNA repair has assumed great importance and several repair pathways have been unravelled. DNA repair seems to be a major contributory factor in the susceptibility of cells to radiation. Repair-deficient mutants of microorganisms, plant and animal cells are sensitive to radiations and other DNA damaging agents. Considerable differences in the susceptibility of microorganisms to radiations are believed to be due to their varying capabilities to repair damaged DNA. Most of our current notions on cellular DNA repair processes have been formulated on the basis of the knowledge gained from the pyrimidine dimer-bearing DNA in UV-irradiated cells of Escherichia coli. These are further revised from investigations of different types of DNA damage, particularly lesions produced by various mutagenic and carcinogenic chemicals. Some impressive advances in our understanding of cellular repair mechanisms in bacteria

Table II

REACTION OF SOME CHEMICAL COMPONENTS OF THEIR METABOLITES WITH DNA

Alkylating agents	Defined or proposed sites of reaction with DNA
Nitrogen and sulphur mustards	N-7;N-3;N-1; O^6 guanine
Alkyl sulphonates	N-7;N-3;N-1 adenine N-1;N-3; O^2 cytosine N-3; O^4 - thymine Phosphate esterification Inter- and Intra-strand Cross-links in DNA : DNA protein cross-links
Polycyclic hydrocarbons	
Benzo(a)pyrene (Reactive intermediate in mammalian cell : dihydro-diol epoxide)	N^2-guanine (to 10 position of hydrocarbon)
Aromatic amines	
2-Aceto aminofluorene (Probable metabolic activation : N hydroxylation followed by esterification (sulfuric acid ester ?)	C-8 guanine (to N atom of AAF) N^2-guanine (to 3 position of AAF)
Other chemicals	
Mitomycin (Probable metabolic activation: reduction to semi-quinone radical)	Cross-links in DNA; of guanine (?)
Aflatoxin B_1 (Reactive intermediate-2, 3-oxide of aflatoxin)	O-6 or N-7 of guanine; N-1, N-3 Adenine

Adapted from Reference 34.

were mainly the outcome of penetrating probes of bacterial genetics. Responses to DNA damage seem to fall into two categories: (i) responses that result in the removal of damage and the restoration of the normal DNA structure; and (ii) tolerance responses that enhance the capacity of cells to survive in spite of unremoved damage. The three well-documented repair systems are: photoreactivation, excision repair and post-replication repair. Recently various kinds of inducible repairs have also been identified. The current status of research in these areas will be briefly reviewed in this section.

Photoreactivation:

The simplest mechanism for repairing pyrimidine dimers is the direct reversal by photoreactivation. Pyrimidine dimers in DNA can be monomerized by three distinct processes, namely, direct photolysis, sensitized photodestruction and enzymatic photoreactivation (35).

Direct and sensitized photolysis of dimers: Direct absorption of UV radiation energy results in the formation of pyrimidine dimers but the absorption of UV photons can as well cause monomerization of the dimers formed. Direct photolysis occurs only if the radiation is absorbed by the dimer, i.e. radiation in the range 220 - 300 nm; it does not occur in the wavelength range 300 - 600 nm, the region

of biological photoreactivation, since the dimer does not absorb light at these wavelengths appreciably (36 - 38).

Sensitised dimer photodestruction depends on light absorbed by a sensitizer. Tryptophan-containing proteins or oligopeptides have been found to serve as the sensitizers (39, 40). Photolysis of dimers by troptophan-containing proteins occurs only in wavelength regions where tryptophan absorbs. This photolysis cannot hence account for biological photoreactivation phenomena occurring at wavelength greater than 300 nm.

Enzymatic photoreactivation: This phenomenon has been known for many years (41) and is extensively studied (42 - 46). The enzymatic process requires light in the wavelength range 300 - 500 nm where neither DNA nor dimers absorb: whether the absorption bands corresponding to the action spectrum of the photoreactivating enzyme are due to an intrinsic chromophere or produced only by interaction of the enzyme with its substrate, i.e. DNA-containing pyrimidine dimers, is still a matter of much debate (47, 48). The photoreactivating enzyme is believed to act as follows: (i) it binds to regions of DNA containing cis-syn pyrimidine dimers - this reaction can occur in the dark; (ii) the enzyme-DNA complex absorbs photoreactivating light; and (iii) the dimers break to yield two pyrimidine monomers. Photoreactivating enzymes from many sources have been described (49-52).

The diversity of their structures and mechanisms of action remains an intriguing problem. Photoreactivating enzymes from baker's yeast, Escherichia coli and Streptomyces griseus have been the subject of much study at the molecular level. Only the S.griseus enzyme appears to contain a chromophore (47) whose absorption spectrum is identical to the action spectrum for photoreactivation (53).

The purified photoreactivating enzymes from E.coli and yeast lack absorption above 300 nm. Presumably the absorption responsible for photoreactiva tion appears only when the enzyme is bound to UV-irradiated DNA. Recently an evidence has been provided for the appearance of such an absorption spectrum in the case of E.coli photoreactivating enzyme (48).

Excision repair:

The most extensively studied repair mechanism is excision repair of pyrimidine dimers, a co-ordinated series of at least four molecular events (54). These include an endonucleolytic cleavage of a phosphodiester bond at or near a dimer, removal of oligonucleotide segment containing the dimer by an exonuclease activity, replacement of the missing nucleotides by a polymerase using a complementary strand as template, and, finally, sealing of the repaired strand to the rest of the strand (55, 56). Our current concepts on excision repair pathway will be briefly reviewed

in the following discussion on the four sequential steps: incision, excision, resynthesis and ligation.

Incision: The recognition and cleavage of a phosphodiester bond at or near a dimer has been found to be achieved in E.coli by an endonuclease comprising the products of three genes, uvr A, uvr B and uvr C (57, 58) and the reaction requires ATP and Mg^{++} (59). Another enzyme with similar properties but independent of ATP and Mg^{++} has also been isolated from E.coli (60 - 62). It appears that uvr A,B,C complex not only recognises thymine dimers but also other structural defects caused by other agents, for example, 4-nitroquinoline-N-oxide, mitomycin C and psoralen plus light. Other endonucleases implicated in the incision step have also been reported (63, 64).

Excision: After the incision step, the dimer and the adjacent nucleotides are removed by exonuclease activity and replaced by repair synthesis. Four exonuclease activities have been suggested to participate in excision: the 5' exonuclease activity of DNA polymerase I (65), the 5' exonuclease activity of DNA polymerase III (66), the single-strand specific exonuclease VII (67) and exonuclease V, the product of the rec B and rec C genes (68, 69). There is some evidence to indicate that dimer excision may occur more efficiently when coupled to polymerization (70).

Resynthesis: Diverse lines of evidence support the idea that all the three DNA polymerases in E.coli may participate in repair synthesis although it is difficult to assess their relative importance in vivo. Polymerase I and III are associated with 5' exonuclease activities and can therefore be implicated in coupled excision and resynthesis (65, 66). DNA polymerase I is more abundant than the other two (71); in fact, mutants deficient in polymerase I are UV sensitive.

The repaired regions in UV-irradiated E.coli are heterogeneous in size (72). Although most lesions result in short regions, or patches, of 20 to 30 nucleotides, a few lead to longer patches of several hundred nucleotides (73). It is suggested that the production of short patches is related to coupled excision and resynthesis of DNA polymerase I, while the production of long patches results from resynthesis that is not directly coupled to excision (73).

Recent results suggest that synthesis of long patches depends upon a functional rec A gene and is sensitive to conditions that inhibit protein synthesis after UV irradiation (74). Evidence has been presented to show that long patch excision repair is the same as the so-called SOS inducible repair or one of the inducible SOS phenomena. The long patch pathway appears to require lex A as well as rec A and protein synthesis, since repair synthesis is reduced in a lex A mutant. Thermal induction of a tif mutant

prior to irradiation overcomes the sensitivity of repair synthesis to post-UV treatment with the inhibitors of protein synthesis (75).

Ligation-Excision repair is completed when the repair patches are covalently linked to the rest of the strand to restore its continuity. In E.coli this is brought about by DNA ligase (76). Studies suggest that the enzyme may be located at the cell membrane (76a).

Variations of excision repair: As mentioned before, excision repair can negotiate not only structural defects like pyrimidine dimers but also defective or incorrect bases. A specific endonuclease may recognize and incise the DNA strand containing the aberrant base (77, 78). In another mechanism, a specific N-glycosylase may remove the base without cleaving the DNA (79). An endonuclease that incises DNA containing uracil (sometimes resulting from incorporation of uracil instead of thymine, or deamination of cytosine) and a glycosylase that removes uracil as the free base have been described (80). Base removal by a glycosylase may lead to an apurinic or apyrimidinic (AP) site. Several AP endonucleases with the property of recognition and incision of DNA containing AP sites have been characterized (81). It is suggested that subsequent to incision all reactions can be mediated by the same set of enzymes involved in excision of dimers.

Recent evidence suggests that simpler pathways for DNA repair may also be present in the living cells. It appears that an AP site may be directly filled with appropriate base by a base insertion enzyme termed as insertase. Two such enzymes have been reported, both of which specifically insert purines into depurinated DNA (82, 83). Possibly a similar enzyme could catalyze the insertion of pyrimidines. The recent notions on these simpler and probably, efficient repair modes are outlined in Figure 1.

It has been proposed that sometimes DNA repair can be accomplished simply by direct reversal of damage. Indeed photoreactivation of thymine dimers can be a well-known example in this category. An enzyme 'demethylase' has been reported and it has been proposed that this enzyme may act on guanine damaged by methylation at the O^6 position and remove the methyl group without otherwise altering the DNA (84). An enzyme of this type might be involved in the phenomenon of adaptation of E.coli to various alkylating agents (85).

Post-replication repairs:

Besides repair responses that enhance survival by eliminating lesions from damaged DNA there are other responses which enable cells to survive inspite of persistent damage. Post-replication repair was the first such mechanism discovered in excision deficient mutants of E.coli(86).

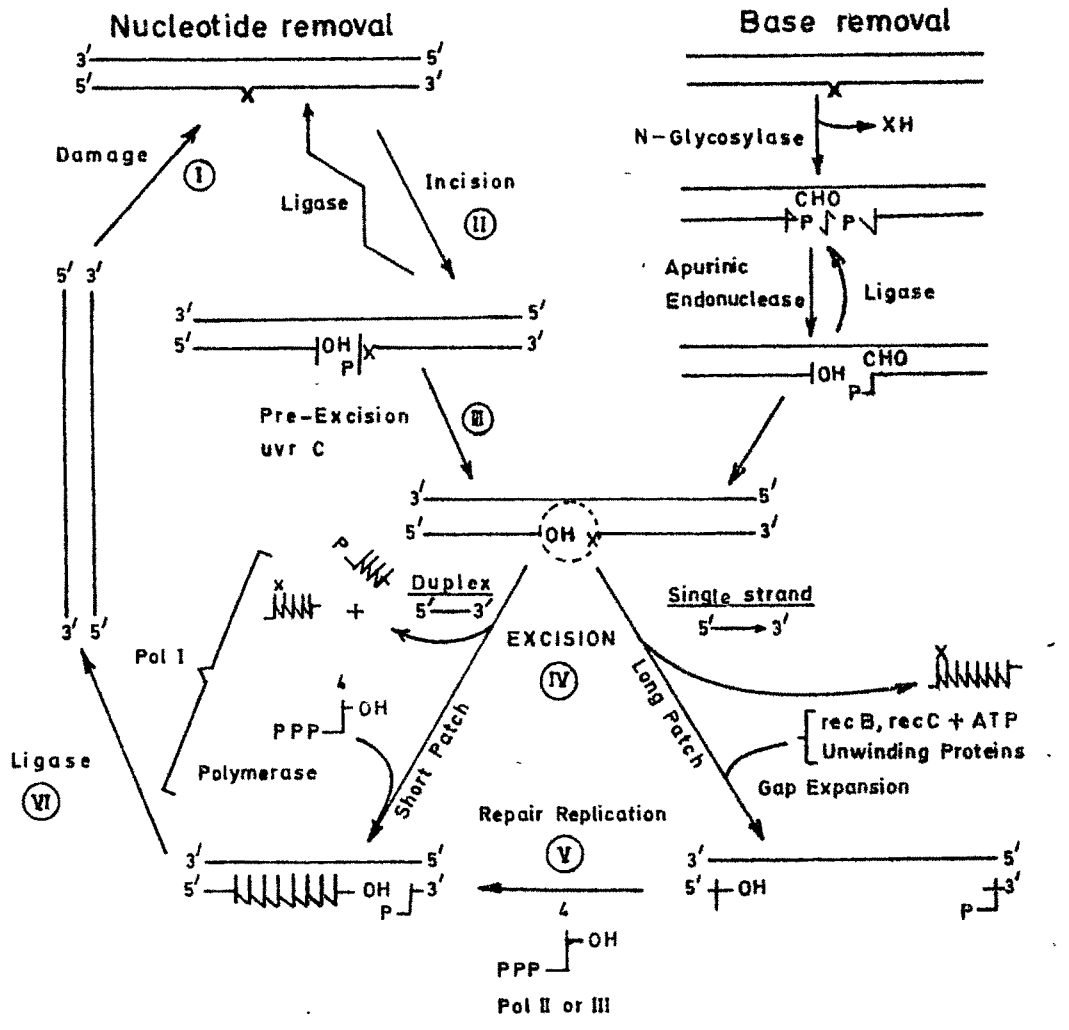


Figure 1- Nucleotide Excision Repair Mechanisms

Adopted from Grossman, L. and Riazuddin, S. DNA Repair Mechanisms (1978) (eds. Hanawalt, P.C. et al.).

In this mechanism, DNA synthesized after UV irradiation leaves a gap opposite each pyrimidine dimer present in the template strand (Fig. 2-1). The gaps are formed when strand elongation is blocked at a dimer but the DNA polymerase oversteps it and resumes its progression beyond it presumably at the next site for the initiation of a replicative intermediate (Okazaki fragment). The repair (also termed as daughter strand gap repair) seems to occur by strand exchanges that fill each gap with undamaged DNA regions from the isopolar parental strand (i.e. the parental strand containing the same sequence as opposed to the complementary strand). The sequential events in the post-replication repair are schematically shown in Fig.2. According to this scheme, the daughter strand is extended by polymerization from a 3'-OH terminus at one end of the gap, it then invades the neighbouring duplex and uses the complementary daughter strand as template. The isopolar parental strand assumes a D-loop form which is eventually cut at one end (Fig. 2-2). The subsequent steps may follow the same steps as envisaged in the current models of genetic recombination (87). Thus the structure resulting at the step 3 in Fig.2 may undergo branch migration, ligation and strand isomerization as proposed by Radding in his model on genetic recombination. Cutting and rejoining of strands may produce a structure in which a limited region of parental DNA has become covalently linked to its isopolar daughter strand (Fig. 2-6 a). Cutting and rejoining could also produce more extensive regions of

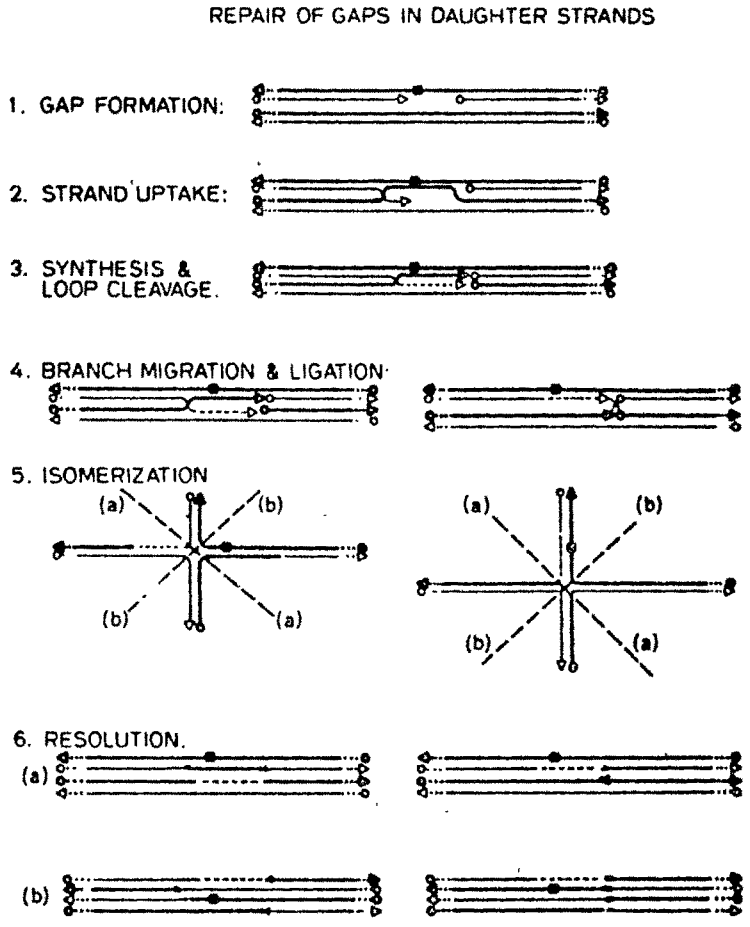


Figure 2.

Adapted from Ganesan, A.K. (1979). Proceedings of 6th International Congress of Rad. Res. (eds. Okada, S. et al.) p. 443.

parental DNA, some of which may contain pyrimidine dimers, covalently linked to daughter strands (Fig. 2-6 b). In E.coli, the probability of any dimer in a template strand becoming linked to the daughter strand during this process can be estimated to be about 0.5 (88).

Daughter strand gap repair is inhibited in E.coli by mutations in several genes like lex A, rec B, rec F, uvr D, and dna G (89 - 95), either singly or in certain combinations. Among these are the ones affecting the structure or the amount synthesized of the rec A protein. Very little however is known on the enzymology of post-replication repair.

Repair through transdimer synthesis: In E.coli, it has been revealed that under certain conditions, DNA synthesis could proceed past pyrimidine dimers without leaving any gaps in the daughter strands though the probability of incorporating incorrect bases is significantly increased. Such process - which enables cells to tolerate persistent DNA damage - has been suggested to occur on account of a modification of the replication apparatus. It has been proposed that an inducible protein can suppress the 3' exonuclease, or "editing" activity, of one or more of the DNA polymerases of E.coli, which facilitates synthesis past pyrimidine dimers (96). This transdimer synthesis may be the underlying mechanism for the inducible (Weigle) reactivation and mutagenesis of UV-irradiated bacteriophages lambda in E.coli (97).

Ultrafast and fast repairs of DNA strand breaks:

Single-strand breaks in DNA have been suggested to be restituted by three operationally distinct types of repair systems (12, 98). These are described in Table III. It would seem that the ultrafast and fast (types I and II) repairs act mainly on breaks which require minimum or no prior processing. There have been conflicting reports on the existence of type I repair. Evidence based on the analysis of strand breaks within a few milliseconds of irradiation with electrons has been advanced to show that such repair does not exist (99). Type III or slow repair could involve initial cleaning action of an exonuclease and subsequent processing by excision repair pathway.

Inducible repair systems (SOS repairs):

The phenomenon that has excited much attention in recent years is error-prone DNA repair activity that is induced in E.coli after exposure to many agents that cause DNA damage (100). A mention of this phenomenon has already been made above under 'Excision repair' and 'Post-replication repair'.

The inducible DNA repair or SOS repair has been the outcome of a study of Weigle reactivation (already mentioned earlier) in which UV-irradiated bacteriophage survives better and is mutated to a greater extent if plated on bacteria which have themselves previously been irradiated(101).

Table III

TYPES OF THE REPAIR OF X-RAY-INDUCED SINGLE-STRAND BREAKS IN E.COLI

Type I	Ultrafast (2 min at 0°C); occurs in buffer; does not require DNA polymerase I; repairs about 75% of breaks produced under anoxic conditions of irradiation but only about 25% of breaks produced under oxic conditions of irradiation.
Type II	Fast (10 min at 0°C, about 1 min at 37°C); occurs in buffer; possibly requires DNA polymerase I; repairs about 90% of the breaks (produced by anoxic as well as oxic irradiation) remaining after type I repair.
Type III	Slow (40 - 60 min at 37°C); requires complete growth medium; controlled by rec, exr, dna B and ror genes; does not necessarily depend on DNA polymerase I; repairs about 2 breaks per single-strand genome in addition to the breaks (produced by anoxic as well as oxic irradiation) repaired by type II repair.

Adapted from references 12, 98.

Manifestations of induced repair of DNA include inductions of lambda prophage and of filamentation besides Weigle reactivation and mutagenesis. All these coordinately expressed diverse functions have been termed as SOS functions (102). The products of the rec A and lex A genes of E.coli are necessary for induction of these SOS functions (103). The rec A gene product has been identified as a protein of molecular weight of about 40,000 termed as protein X (104). The following model has been proposed to explain the inducible SOS repair. The lex A gene is implicated to code for a repressor that binds to the operator of the rec A gene. Induced expression of the rec A gene occurs when the rec A product (protein X), already present in the cell in small amounts, is activated by an effector, possibly a deoxyribonucleotide (dNMP). An increased level of dNMP might result from an idling reaction of the 3' to 5' exonucleolytic (proof-reading) function of DNA polymerase III at a stalled replication fork due to an unexcised pyrimidine dimer or a malfunctioning replication complex or by DNA degradation. The activated form of the rec A protein then removes the lex A^+-coded repressor, possibly by proteolytic cleavage (105). The other important action of protein X may be to bind DNA at damaged site and to protect it against nuclease attack. The binding of protein X to the single-strand DNA is in some manner involved in DNA repair. Evidence has been advanced to suggest that the inducible repair may operate by the inhibition of 3' - 5' exonuclease activity of DNA polymerase

III (presumably due to an interaction with an inducible inhibitor) which may then allow DNA synthesis past pyrimidine dimers (and possibly other lesions) in the parental template strand (106). The role of protein X (if any) in this operation is not known.

As mentioned earlier, long patch excision repair also requires de novo synthesis of a protein. Protein X may be essential for this process for preventing DNA degradation and facilitating repair synthesis by DNA polymerase I and III.

The overall picture now emerging from accumulating data suggests that the DNA repair is a network of co-ordinated biochemical reactions rather than the operation of several separate repair pathways.

Double-strand break repair:

There is now abundant evidence that double-strand breaks in DNA from bacteria (107 - 110), yeasts (111, 112) and mammalian (113) cells which have been treated with X-rays or other double-strand breaking agentswill under many conditions be repaired if the cells have been incubated after irradiation. Mutants deficient in genetic recombination - such as E.coli rec A mutants, S.cerevisiae rad 52 mutant - lack the capacity to repair double-strand breaks. In bacteria and in yeasts both an active recombination system and an

undamaged DNA homologous to the broken piece seem to be needed for double-strand break repair, implying recombination-like event. Thus, it was shown that S.cerevisiae haploid cells growing synchronously in G_1 phase or grown in stationary phase cannot repair double-strand breaks. The slow-grown E.coli cells (in the medium containing aspartate as the sole carbon source - with essentially one genome per cell, instead of the three to five per cell characteristic of those grown in normal media) failed to repair DNA double-strand breaks. The highly radiation resistant microorganism Micrococcus radiodurans is equally efficient in the repair of both single-strand and double-strand breaks (109). The presence of more than one genome per cell has been suggested as the factor aiding the repair of double-strand breaks in this organism (114). Evidence has been advanced to suggest that restoration of double-strand breaks in E.coli and yeast may require de novo protein synthesis; the newly synthesised proteins may either play an essential role in the actual enzyme repair or may inhibit the action of cellular enzymes in degrading the DNA at the double-strand breaks before repair can take place.

Consequences of damaged DNA on cellular processes:

Any lesion in cellular DNA, unless repaired, can provoke a variety of perturbations in cell biochemistry before the cell losses its viability. A number of studies

have reported the action of different types of radiations and other DNA damaging treatments on the syntheses of DNA, RNA and protein. Generally speaking, one of the first consequences of the injury to DNA is seen on the cellular DNA synthesis. In comparison, inhibition of RNA and protein syntheses have been found to be less sensitive ([redacted], 115). A lesion in DNA may not however result in immediate or gross impairment in DNA, RNA and protein syntheses but may cause differential changes in initiation, elongation and termination depending upon the nature and location of the lesion in DNA. Billen and Hewitt (116) demonstrated that in E.coli after exposure to UV radiation or X-rays, there is a substantial alteration in the normal sequence of DNA replication. It was found that after exposure to UV radiation, a new cycle of DNA replication is initiated from the origin before the cycle already initiated prior to irradiation is completed. Similar effect has been observed in E.coli cells after a period of thymine starvation (thymineless death) (117). The mode of replication in E.coli after exposure to X-rays is somewhat different - random new initiation sites, not restricted to a fixed origin, are induced in response to X-irradiation. The observations of Billen and co-workers are albeit quite old but they emphasize the importance of the quality and sites of lesions created in cellular DNA by the action of different types of agents. These findings merit re-examination in the light of new information that has been accumulated in recent years on the mechanism of cellular DNA replication in bacteria.

Studies on the effects of certain alkylating agents on DNA synthesis in bacteria have indicated that DNA synthesis is impaired at alkylating doses around mean lethal doses. A marked difference in respect of inhibitory effects on DNA synthesis between resistant and sensitive strains can be discerned. In exponentially growing culture of the sensitive strain E.coli Bs-1, DNA synthesis is rapidly and irreversibly inhibited by low doses of mustard gas (118). A resistant strain on the other hand show a different response to this agent. Immediately after exposure to mustard gas, there is a block in DNA synthesis but after a dose-dependent lag period, DNA synthesis is resumed at normal rate.

In recent studies reported from this laboratory it was found that in Saccharomyces cerevisiae cells undergoing transition from anaerobic to aerobic state (which is associated with biogenesis of mitochondria from promitochondrial structures) synthesis of mitochondrial DNA (present in promitochondrial structures) is inhibited by extremely low doses of ultraviolet radiation and many chemical mutagens. These doses however do not affect the survival. Nuclear DNA synthesis is also not affected and it is suggested that the high susceptibility is due to unique base composition of the yeast mitochondrial DNA (119).

Radiations also have a marked inhibitory effect on the synthesis of RNA in a microbial cell. The reduction

in the rate of RNA synthesis following UV radiation exposure has been attributed to the termination of RNA chain growth at the site of UV radiation-induced lesions (120). However evidence from this laboratory indicates that RNA chain initiation may be preferentially affected by UV radiation exposure in bacteria and mammalian cells. The reduction in template activity of cellular DNA after exposure to ionising radiations seems mainly to be due to strand breaks caused in DNA. At the same time, it appears from some reports that strand breaks in DNA can give rise to new non-specific RNA chain initiation sites. The newly synthesised RNAs in gamma-irradiated cells have been found to be shorter in length (121).

As mentioned earlier, RNA and protein syntheses are less susceptible to DNA damage than is DNA synthesis. Thus it has been found that UV radiation doses required for inhibiting /3-galactosidase synthesis are much higher than those required for the inhibition of DNA synthesis or cell killing (117).

In studies on the effect of alkylating agents on macromolecular synthesis in E.coli it was found that DNA replication was thousand times more sensitive than transcription and five thousand times more sensitive than translation (34).

DNA degradation:

Bacteria exposed to DNA-acting agents frequently undergo DNA degradation at the doses in the lethal range. It is not clear whether DNA degradation is the cause or consequence of cell death. There is some evidence to indicate that a part of the DNA degradation may be associated with DNA repair. It has been reported that E.coli strains lacking the exonuclease function in the excision repair (e.g. rec B^- and rec C^-) (122) degrade DNA after irradiation to a smaller extent compared to in wild type cells. On the other hand E.coli rec A^- mutants (deficient in recombination or post-replication repair) degrade their DNA extensively after UV radiation exposure (123). Evidence has been reported to suggest that E.coli an induced inhibitor probably a protein could be involved in the protection against DNA degradation caused by gamma irradiation. Similar suggestion has also been made in M.radiodurans (124).

Mutagenesis:

One major consequence of distortions induced in DNA is mutagenesis. Mutations can result either from errors in the replication of DNA bearing structural defects (mispairing) or from errors in repair (misrepair). The Weigle mutagenic phenomenon can be considered as a case of mispairing presumably due to the induction of defective DNA polymerase functions, as described earlier. Excision repair

(excluding perhaps long patch repair) is believed to be error-free. Misrepair has been found to occur during post-replication repair (as this involves genetic recombination step) and various types of inducible repairs discussed earlier. The specificity of mutagenesis seems to be the function and the nature of lesion in DNA. Alkylating agents cause mutagenesis by induced mispairing and misrepair. Certain mutagens can induce transversions and others transitions. Frameshift mutations are often specifically induced by agents that can intercalate between adjacent base pairs (125). The molecular mechanisms of mutagenesis are the subject of several reviews (125A).

Relevance of 'DNA injury and repair' research to the central problems of Biology:

The studies on DNA repair have shed light on the existence of certain complex processes in the living cells which can be expected to have roles in the normal cell cycle. Some, at least, of the repair enzymes may participate in DNA replication. Our ideas developed from studies of bacteria, though subject to reevaluation, are helpful for examining the mechanisms of DNA repair in mammalian (including human) cells. Human beings have been and, will inevitably continue to be, exposed to a variety of environmental agents which may have deleterious effects on cellular DNA leading to genetic disorders or somatic effects resulting in cancer.

A great impetus has therefore been given in recent years to the study of DNA injury and repair in mammalian cells. One of the limitations encountered in studying responses to DNA damage in mammalian cells is the narrow spectrum of mutants available. In studying repair systems in mammalian cells, the complex structure of eukaryotic chromatin structure will have to be taken into account and accessibility of DNA repair enzymes to DNA damage explained. The bacterial models on DNA repair will prove to be inadequate to explain all the responses characteristic of mammalian cells.

Since the first discovery of the repair deficiency in Xeroderma pigmentosum (126) cells by Cleaver, considerable amount of information has been collected on the nature of hereditary repair deficient diseases. An exciting recent development has been the demonstration of the correlation between the repair deficiency and high incidence of cancer as revealed from epidemiological studies on the repair deficient diseases like Xeroderma pigmentosum, Ataxia telangiectasia, Bloom's syndrome, Fanconi's anaemia (127, 128). Most of the repair deficient hereditary diseases are known to be due to autosomal recessive genes. Statistical evidence has been reported to suggest that heterozygotes of at least some mutants (e.g. Atoxia telangiectasia) have an increased liability to develop cancer and their cells exhibit intermediate sensitivity to ionizing radiation in comparison with controls and homozygotes.

Another recent development pertains to the role for at least some repair systems in the differentiation process itself. Evidence from several experimental systems has been interpreted as implying that DNA repair capacity is either reduced or enhanced in certain cell populations in relation to developmental stage in embryo (129). The phenomenon of cellular aging has been known to be associated with reduction in DNA repair capacity (130).

Scope of the thesis:

As will be clear from the foregoing review of DNA injury and repair research, bacterial cells have evolved a variety of modes to correct structural defects in DNA caused by external agents or to live with them if they cannot be repaired. It appears that a network of several repair enzymes is trigerred into action whenever a distortion in DNA structure is created. It is widely accepted that the absence of certain crucial repair enzymes may make a microorganism sensitive to radiations and other DNA-damaging agents. However the role of repair enzymes in extremely high resistance that some microorganisms exhibit to radiations and radiomimetic agents has not been clearly understood. These organisms have also unusually rigid cell surface structures. Whether the cell envelopes or some other cell components contribute in some way to the resistance of the microbial cells to intense onslaughts of DNA-damaging agents has not been well-studied.

Another important aspect of radiation microbiological research that has not so far been satisfactorily understood is the extent to which non-DNA moieties in the cell are affected by radiations and other similar stresses. Can injuries to such components be deleterious to the microbial cells ? Is there any recovery mechanism for such injured cellular components - say proteins ? In bacteria commonly used in DNA injury-repair studies, such as Escherichia coli, DNA is the most susceptible target to radiations and other DNA-acting agents and hence it is difficult to design experiments to ascertain the effects of radiations/DNA-acting agents on non-DNA cell constituents. The solution to these problems could be possible if studies are conducted in a highly radiation resistant microorganism.

It would be of interest to know whether the cell components, such as proteins, of a radiation resistant microorganisms are also highly radiation resistant as compared with their opposite ~~numbers~~ ones in the common bacteria having moderate resistance to radiations. The studies to be presented in the thesis relate to the actions of radiations and other physical-chemical treatments on a phenomenally high radiation resistant microorganism, Micrococcus radiophilus and aimed at seeking answers to some of the questions enlisted above. The salient findings of the thesis have already been outlined in the synopsis.

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