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Molecular Mechanisms of DNA Damage and Repair: Progress in Plants*

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ABBREVIATIONS: 6-4 pps, 6-4 photo products; AOS, activted oxygen species; AP, apurinic/apyrimidinic; APXs, ascorbate peroxidases; BER, base excision repair; CPD, cyclobutane pyrimidine dimer; CS, Cockayne syndrome; DNA-PK, DNA-dependent protein kinase; DR, direct reversal; DSB, double strand break; ERCC, excision repair cross complementation; FEN-1, flap endonucelase; GO, 7,8-dihydro-8-oxoguanine; GR, glutathione reductase; GSH, reduced glutathione; GSSH, oxidized glutathione; HDH, human DNA helicases; L·, lipid radicals; LO·, alkoxyl radicals; LOO·, lipid peroxide; LOOH, lipid hydroperoxide; MDA, malondialdehyde; MGMT, methylguanine-methyltransferase; MMR, mismatch repair; MMS, methylmethanesulfonate; NER, nuleotide excision repair; NHEJ, nonhomologous end-joining; O_{2^-}·, superoxide anion radical; ·OH, hydroxyl radical; PARP-1, poly(ADP-ribose) polymerase; PCNA, proliferative cell nuclear antigen ; PI3K, phosphatidylinositol 3-kinase; PUFA, polyunsaturated fatty acids; RAR, repair and recombination; RFC, replication factor C; ROS, reactive oxygen species; RPA, replication protein A; RSH, thiyl radicals ('sulfur' containing functional group); SASP, small acid-soluble protein; SDSA, synthesis-dependent strand annealing; SOD, superoxide dismutase; SSA, single strand annealing; SSBR, single strand break repair; UV, ultraviolet; XP, Xeroderma pigmentosum.

ABSTRACT: Despite stable genomes of all living organisms, they are subject to damage by chemical and physical agents in the environment (e.g., UV and ionizing radiations, chemical mutagens, fungal and bacterial toxins, etc.) and by free radicals or alkylating agents endogenously generated in metabolism. DNA is also damaged because of errors during its replication. The DNA lesions produced by these damaging agents could be altered base, missing base, mismatch base, deletion or insertion, linked pyrimidines, strand breaks, intra- and inter-strand cross-links.

These DNA lesions could be genotoxic or cytotoxic to the cell. Plants are most affected by the UV-B radiation of sunlight, which penetrates and damages their genome by inducing oxidative damage (pyrimidine hydrates) and cross-links (both DNA protein and DNA-DNA) that are responsible for retarding the growth and development. The DNA lesions can be removed by repair, replaced by recombination, or retained, leading to genome instability or mutations or carcinogenesis or cell death. Mostly organisms respond to genome damage by activating a DNA damage response pathway that regulates cell-cycle arrest, apoptosis, and DNA repair pathways. To prevent the harmful effect of DNA damage and maintain the genome integrity, all organisms have developed various strategies to either reverse, excise, or tolerate the persistence of DNA damage products by generating a network of DNA repair mechanisms. A variety of different DNA repair pathways have been reported that include direct reversal, base excision repair, nucleotide excision repair, photoreactivation, bypass, double-strand break repair pathway, and mismatch repair pathway. The direct reversal and photoreactivation require single protein, all the rest of the repair mechanisms utilize multiple proteins to remove or repair the lesions. The base excision repair pathway eliminates single damaged base, while nucleotide excision repair excises a patch of 25- to 32-nucleotide-long oligomer, including the damage. The double-strand break repair utilizes either homologous recombination or nonhomologous endjoining. In plant the latter pathway is more error prone than in other eukaryotes, which could be an important driving force in plant genome evolution. The *Arabidopsis* genome data indicated that the DNA repair is highly conserved between plants and mammals than within the animal kingdom, perhaps reflecting common factors such as DNA methylation. This review describes all the possible mechanisms of DNA damage and repair in general and an up to date progress in plants. In addition, various types of DNA damage products, free radical production, lipid peroxidation, role of ozone, dessication damage of plant seed, DNA integrity in pollen, and the role of DNA helicases in damage and repair and the repair genes in *Arabidopsis* genome are also covered in this review.

I. INTRODUCTION

In general, the genomes of all the living organisms, including plants, are stable. Normally the DNA of genome in the cell replicates during cell division and passes all the genetic information to their progeny. It is very important for all living organisms to ensure proper functioning and propagation of their genetic information. However, due to constant exposure of the genome to various endogenous and environmental agents, the DNA gets damaged and can produce a large variety of DNA lesions. These lesions can affect the fidelity of DNA replication (Painter, 1985), and transcription (Protic-Sabljic and Kraemer, 1985), which can create mutations in important protein coding sequences. As a result, the produced mutated protein can affect various biological processes leading to the genome instability. Proliferating cells are often presumed to be more mutable than quiescent cells because they have less time to repair DNA damage before DNA replication. If these damages occur in the germ cells, it can be heritable and will be harmful to the next generation in passing a heritable disease. Moreover, the damage in somatic cells plays an important role in the development of cancer and aging.

The DNA damage can have genotoxic and cytotoxic effects on the cell. The real

biological consequences of these damaged products usually depend on the chemical nature of the lesion. In plants if this damage are not repaired properly they can induce proliferation as well as play an important role in the aging of seeds stocks and perennial crops. This unrepaired damage can also lead to the general deterioration of cell function and cell death. This damage situation requires constant and accurate excision and the replacement of damaged nucleotide by various DNA repair pathways. In order to maintain the integrity of the genome, the prokaryotic and eukaryotic organisms are well equipped with several DNA repair mechanism pathways (Pieper et al., 1998; Britt, 1999). In general, the lesions in the actively transcribed strand are repaired more rapidly than the lesions in the nontranscribed strand (Selby and Sancar, 1993).

DNA repair is not only a fundamental cellular process for protecting cells against the damage, but it is also essential to ensure the faithful transmission of genetic information from one generation to the next. The biological consequence of defective DNA repair in human has been reported to be the cause of many diseases, such as Xeroderma pigmentosum (XP), Cockayne syndrome (CS), and Trichothiodystrophy (TTD) (Hoeijmakers, 1993a, b; Lehmann, 1998; Winkler et al., 1998; Lindahl and Word, 1999; Tuteja and Tuteja, 2001). However, few inherited diseases are associated with altered processing of double-strand breaks such as Ataxia telangiectasia and Nijmegen breakage syndrome (Shiloh, 1997; Lindahl and Wood, 1999). All the known mechanisms of DNA repair processes have been shown to be complex and diverse and best studied in microorganisms and mammals. In humans it is of great interest because of the role of DNA repair in mutagenesis, in carcinogenesis, and possibly in aging (Ribeiro et al., 1998). In humans, the most important self-inflicted mutagen is tobacco smoke, which is responsible for more cancer deaths. However, the progress in plant genetic engineering and the use of mutagenesis in the creation of genetic diversity for the improvement of crops have now demanded more research in DNA damage and repair. However, DNA repair in plant has not been well studied, but now it is slowly progressing. In this article we are describing the molecular basis of DNA damage and repair and the recent advances of DNA repair in plants. Further, we have also focused on endogenous factors affecting DNA damage during natural desiccation process and the dependence of desiccation tolerance mechanisms on excision repair pathways.

II. DNA DAMAGES

The Earth's atmosphere is getting polluted due to the man-made pollutants such as chlorofluorocarbons. As a result, the stratospheric ozone layer is getting depleted, which causes the increased exposure to solar ultraviolet-B (UV-B, wavelength 280 to 320 nm) on the Earth's surface. A possible increase in UV radiation by ozone depletion is now attracting attention of researchers. This increased exposure of UV-B is harmful to all living organisms, especially to the plants. Plants are constantly being challenged by UV radiation through the sunlight because of their obligatory requirement of sunlight for photosynthesis. This radiation penetrates plant tissues and damages their genome and other cellular targets such as photosystem II and

plasma membrane ATPase (Stapleton, 1992). These ambient and increased solar radiations are responsible for retarding growth and development, morphological and biochemical alteration, and biomass accumulation in the plants (Bornman, 1989; Tevini and Teramura, 1989; Bornman and Teramura, 1993; Teramura and Sullivan, 1994; Jansen et al., 1998; Vonarx et al., 1998).

UV-B light is potentially very damaging to DNA and proteins and also increases the free radical production. Plants have their own way of tackling this problem. They have developed two protective mechanisms to cope with harmful effect of UV. First, the shielding through the production of UV-absorbing compound such as flavonoids and anthocyanins and the reflection of UV radiation by epicuticular waxes and cuticular structures (Bornman et al., 1997; Stapleton et al., 1997). Second, removal and direct reversion by photoreactivation of UV-induced DNA lesions that involve photolyase (Pang and Hays, 1991; Sutherland et al., 1996; Stapleton et al., 1997; Yasui and Eker, 1998; Britt, 1999). Recently, Cathie Martin's group at John Innes Centre, UK, have shown that the production of UV-protecting sunscreens in *Arabidopsis* is controlled through the transcriptional repression by AtMyB4. The mutant *AtMyB4* line is more tolerant of UV-B irradiation than wild type (Jin et al., 2000). However, the light-dependent pathway is the major mechanism of repair of DNA damage in plant parts exposed to sunlight induced by UV radiation. Other pathways such as base excision repair (BER), nucleotide excision repair (NER), postreplication, and recombinational repair may be more important in internal tissues where light penetration is limited (Hoeijmakers and Bootsma, 1990; Hoeijmakers, 1993 a, b; Wood, 1997; Balajee and Bohn, 2000).

Besides UV radiation, the plant genomic DNA is vulnerable to DNA damage from a variety of endogenous reactive metabolites, particularly oxygen-free radicals. The DNA in reproductive structures, pollen, and seed is particularly susceptible to endogenous DNA-damaging agents. In the plants there are a number of developmental stages that are particularly susceptible to the DNA damage. As a part of developmental pathway, seeds and pollens undergo the process of dehydration. This loss of the water triggers several metabolic changes, leading to free radical formations, which are capable of damaging DNA either directly or through lipid peroxidation. The accumulation of DNA lesions has been shown to accompany the seed-aging process (Boubriak et al., 1997). The nature and the role of endogenous metabolites involved in plant seed DNA damage are not clear. However, it is relevant to note that a naturally occurring product of lipid peroxidation, malondialdehyde (MDA), has been shown recently to be a major endogenous DNA damaging agent in mammalian systems (Fink et al., 1997; Marnett, 1999 a, b; Lindahl and Wood, 1999).

A. Types of DNA Damage and Damage Products

The variety of the DNA damage produced by various endogenous and exogenous agents is corrected by different repair pathways. Major DNA damage induced by different DNA damaging agents are shown in Figure 1. The endogenously generated damage to DNA is

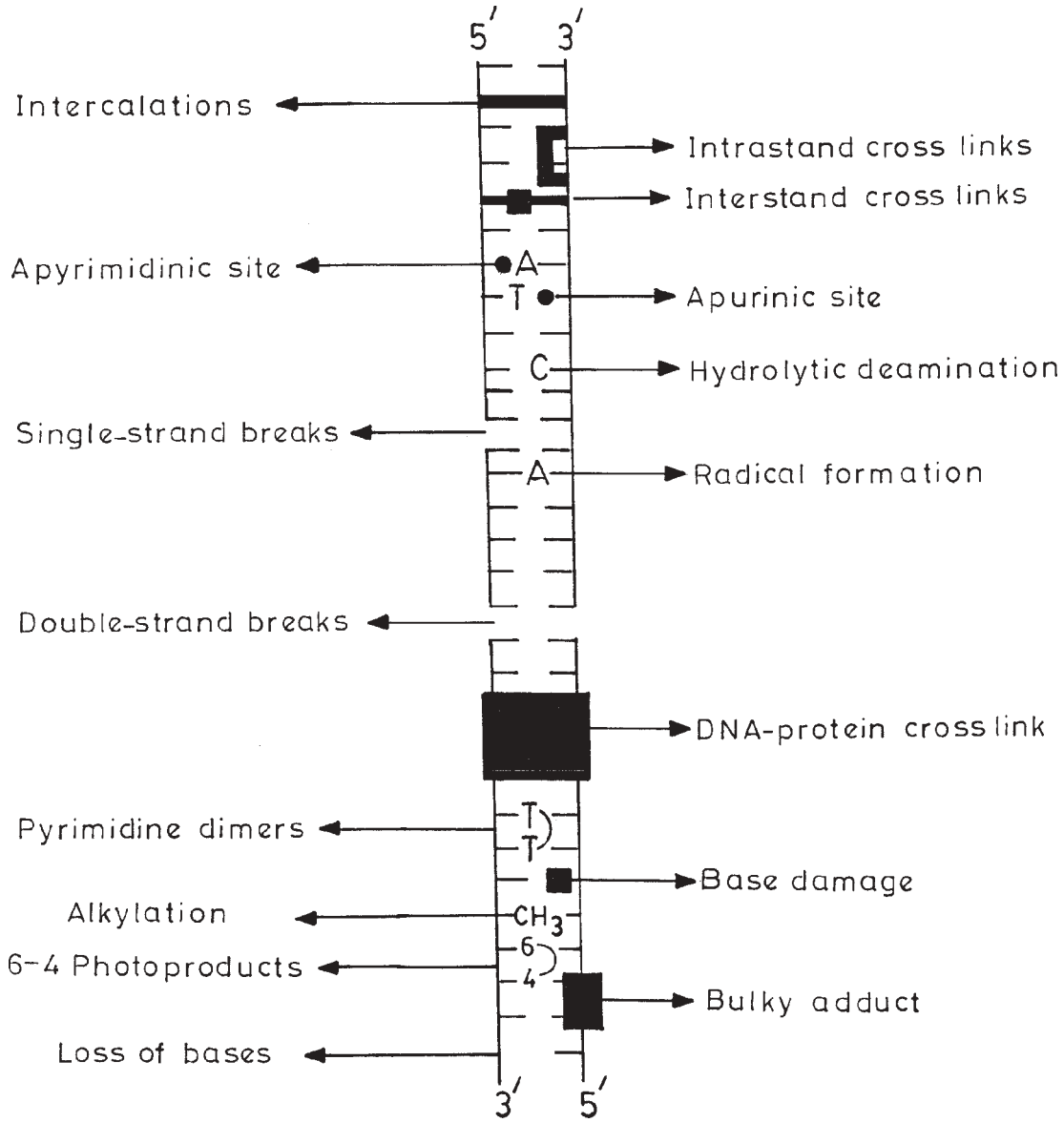

FIGURE 1. Major DNA damage induced by different DNA-damaging agents.

known as "spontaneous DNA damage" (Britt, 1999), which is produced by the reactive metabolites (e.g., oxygen-free radicals, hydroxyl radicals, superperoxide, and nitric oxide) (Demple and Harrison, 1994) and the defect in normal processes of DNA replication or recombination. The environmental DNA-damaging agents could be UV-light, ionizing radiation, chemical mutagens, and cross-linking agents (e.g., mitomycin C and cisplatin), alkylating agents, aromatic compounds, and fungal and bacterial toxins (Plooy et al., 1984; Friedberg, 1985; Doetsch, 1995). The various DNA lesions produced by these agents are mismatches, base alterations, deletion of bases, strand breaks, cyclobutane pyrimidine dimers, 6-4 photo-products, intra- and inter-strand cross-links, alkylated bases, and bulky adducts (Figure 1) (Friedberg et al., 1995).

The most common natural environmental genotoxic agent is the UV component of sunlight. The damage induced by UV radiation to the epidermal cells of a plant is as inevitable as hydrolytic damage induced by the water present in the nucleus (Britte, 1996). Besides UV and hydrolytic damage, other damage could be alkylation damage, oxidative damage and the damage, produced by ionizing radiation (Britte, 1996). The oxidative damage is a major stress to the chilling-sensitive plants (Hodges et al., 1997; Pinehro et al., 1997). Low temperatures together with high light intensities induce the production of activated oxygen species (AOS), such as superoxide ($O_{2^-}^{\bullet}$), hydroxyl radicals (·OH), and hydrogen peroxide (H_2O_2) (Wise and Naylor, 1987). These radicals can react with DNA, proteins, and lipid, causing damage. However, normally the superoxide dismutase (SODs), ascorbate peroxidases (APXs), and catalases play important role as a defense against these radicals (Inze and Van-Montagu, 1995). The environmental stresses to plants, such as drought, heat shock, salt stress, temperature, etc., can generate the production of AOS and are also known to induce plant SODs (Van Camp et al., 1994).

The chemotherapeutic agent Cisplatin forms the intrastrand adduct between the adjacent purines (AG or GG) (Plooy et al., 1984). The alkylating agents such as methylmethanesulfonate (MMS) include *N*-7-alkylguanine, O^6-alkyl-guanine, *N*-3-alkyladenine, and O^4-alkylthymidine induce the major damage (Beranek, 1990; Friedberg et al., 1995). The *N*-3-alkyladenine, a damage product, is highly toxic because it acts as a block to DNA replication by not recognizing the DNA polymerase (Britt, 1999). Alkylating agents can also form the phosphotriesters by alkylating the phosphodiesters (Vaughan et al., 1993). The ligand Bleomycin produces single- and double-strand breaks by intercalating into DNA (Urdea et al., 1988). Overall, we can say that a single DNA-damaging agent can produce lesions that are inconsequential, mutagenic (because of mispairing), or cytotoxic (because it blocks the DNA replication or transcription). The main lesions produced in DNA by hydrolysis, reactive oxygen species, and small reactive intracellular molecules such as *S*-adenosylmethionine have been reviewed by Lindahl (1993).

B. Free Radicals Production and Anti-Oxidant Defense Mechanisms

Free radicals are chemical species (molecules or atoms) possessing an unpaired electron in their outermost orbital.

Due to the presence of one or more unpaired electrons, these species are paramagnetic, which makes them highly reactive. These are conventionally represented by a superscript dot $R^{\cdot}$ (Dormandy, 1980). Free radicals can be formed in a molecule by gaining an additional electron, for example, the reduction of molecular oxygen (O_2) to the superoxide anion radical ($O_2^{-\cdot}$).

$$O_2 + e^- \rightarrow O_2^{-\cdot}$$

Transition metals contain unpaired electrons and so act as free radicals, with the sole exception of zinc. The most important feature of transition metals, from the free radical point of view, is their variable valency, which allows them to undergo changes in their oxidation state involving one electron; for example, Fe (iron) has two common valencies. If a solution of ferrous salt (Iron II) is left in contact with the air, it slowly oxidizes to the ferric (Iron III) state. This is a one-electron oxidation and oxygen dissolved in solution is reduced to the superoxide radical (Halliwell and Gutteridge, 1986).

$$Fe^{2+} + O_2 \Leftrightarrow Fe^{2+}\ O_2 \Leftrightarrow Fe^{3+}\ O_2^- \Leftrightarrow Fe^{3+}\ O_2^{-\cdot}$$

(Intermediate Complexes)

Copper (Cu) has two common valencies Cu^+ and Cu^{2+}. Similarly, under oxidation conditions copper salts can receive and donate electron to superoxide radicals $O_2^{-\cdot}$

$$Cu^{2+} + O_2^{-\cdot} \rightarrow Cu^+ + O_2$$
$$H_2O_2 + Cu^+ \rightarrow Cu^{2+} + {}^{\cdot}OH$$

The variable valency of the transition metals helps them to be effective catalysts in many oxidation reduction reactions, and they are present for this purpose at the active sites of many enzymes catalyzing such reactions. The most important free radicals in biological systems are a derivative of oxygen. The complete reduction of O_2 by univalent pathway results in the formation of superoxide anion, hydrogen peroxide (H_2O_2), and other products as shown below (Naqvi et al., 1986; Pryor, 1986):

$$O_2 + e^- \longrightarrow O_2^{-\cdot}$$
(superoxide)

$$2\ O_2^{-\cdot} + 2H^+ \xrightarrow{\text{SOD catalysed}} H_2O_2 + {}^3O_2$$
(peroxide) (triplet O2)

$$2\ O_2^{-\cdot} + 2H^+ \xrightarrow{\text{spontaneous}} H_2O_2 + {}^1O_2$$
(peroxide) (singlet O2)

$$O_2^{-\cdot} + H_2O_2 + H^+ \xrightarrow{\text{metal catalyst}} O_2 + {}^{\cdot}OH + H_2O$$
(hydroxyl radical)

$$O_2^{-\cdot} + {}^{\cdot}OH + H^+ \longrightarrow H_2O + {}^1O_2$$

Hydrogen peroxide is an oxidizing agent but not especially reactive, and its main significance lies in it being a source of hydroxyl radicals. This radical is an extremely reactive oxidizing radical that reacts with most biomolecules at diffusion-controlled rates. It will not diffuse a significant distance within a cell before reacting and has an extremely short half-life but is capable of causing a great damage within a small radius of its site of production. Under normal circumstances, the major source of free radicals in cells is electron leakage from electron transport chain, such as those in mitochondria. These react with

molecular oxygen to generate superoxide ion. Other enzymes, such as flavin oxidases located in the peroxisome, can also produce superoxide or hydrogen peroxide. Autooxidation of certain compounds, including ascorbic acid (vitamin C), thiols (e.g., Glutathione, cysteine), adrenaline, and flavin coenzymes are yet other sources of superoxide anions in the cells. These autooxidation reactions can be greatly enhanced by the transition metal ion involvement.

Various potential sources that are responsible for the production of superoxide anions are as follows:

1. Enzymes: Various enzymes are involved in catalyzing the oxidation reactions, as a result there is univalent reduction of O_2 to $O_{2^-}^{\bullet}$, for example, xanthine oxidase, aldehyde oxidase, dihydrocerotic dehydrogenase, flavin dehydrogenase (Fridovich, 1976), and peroxidases (Halliwell, 1990).
2. Autooxidation: a large group of compounds, including epinephrine, flavins, and ferrodoxin (Misra and Fridovich, 1972), reduced forms of riboflavin and thiol compounds such as cysteine and glutathione, generate superoxide radicals by autooxidation.
3. Subcellular organelles: the mitochondrial electron transport chain, endoplasmic reticulum substrate hydroxylation, chloroplasts, and neutrophils are all capable of producing superoxide radicals.
4. The oxidation of hemoglobin in erythrocytes is a very rich source of oxygen free radicals (Misra and Fridovich, 1972).
5. Irradiation: irradiation of living tissues also produce superoxide anion and other free radicals.

Superoxide anion and hydrogen peroxide react together to generate highly reactive hydroxyl ($^{\bullet}OH$) radical (Beuchamp and Fridovich, 1973). Peroxides also react with metal cations to produce hydroxyl radicals in the living system. Some important mechanisms responsible for generation of hydroxyl radicals are briefly underlined.

1. Harber-Weiss reaction: in the presence of copper and/or iron (metal catalyst), H_2O_2 reacts with $O_{2^-}^{\bullet}$ and forms highly reactive $^{\bullet}OH$ (Harber and Weiss, 1934; Kehrer, 2000).

$$O_{2^-}^{\bullet} + H_2O_2 \xrightarrow[Fe/Cu]{\text{(metal catalyst)}} {}^{\bullet}OH + {}^{-}OH + O_{2^-}^{\bullet}$$

2. Fenton reaction: damage to DNA by oxygen radicals (oxyradicals) is mediated by metal ions, especially iron, as in the classic Fenton reaction, which is the major source of highly reactive $^{\bullet}OH$ radicals (Barb et al., 1957). This reaction was first observed by its inventor H. J. H. Fenton in 1894.

$$O_{2^-}^{\bullet} + Fe^{3+} \rightarrow O_2 + Fe^{2+}$$
$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + {}^{\bullet}OH$$
$$Fe^{2+} + {}^{\bullet}OH \rightarrow Fe^{3+} + OH^-$$
$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + HO_2^{\cdot} + H^+$$
$$H_2O_2 + {}^{\bullet}OH \rightarrow HO_2^{\cdot} + H_2O$$
$$HO_2^{\cdot} \Leftrightarrow O_{2^-}^{\bullet} + H^+$$
$$Fe^{3+} + O_{2^-}^{\bullet} \rightarrow Fe^{2+} + O_2$$

Halliwell and Gutteridge (1984) discuss the significance of Fenton reaction in free radical production in a biological system. An analogue of this reaction, where hydroperoxides replace H_2O_2, is also important in biological systems, and is

considered to be a Fenton type reaction.

$$Fe^{2+} + ROOH \rightarrow Fe^{3+} + RO\cdot + OH^-$$

However, both the Harber-Weiss and Fenton reactions produce ·OH, but the difference is that in the Harber-Weiss reaction, metal ions are used as catalyst, while in Fenton reactions the metal ions are involved in the reaction.

3. Hydroxyl radicals are also formed during the exposure of high-energy radiations such as X-rays or γ-rays to the living tissues. Most of the energy is absorbed by the cell sap having very high water content. It may result in splitting of one of the covalent bonds of water.

$$H\text{-}O\text{-}H \xrightarrow{\text{radiation}} \text{Intermediate step} \longrightarrow \underset{\text{(hydrogen radical)}}{H\cdot} + \underset{\text{(hydroxyl radical)}}{\cdot OH}$$

Generally, free radicals in biological systems are extremely reactive and unstable. Most of these radicals exist only at a low concentration and they do not move far from their site of formation. Many free radicals are formed by the reaction of a free radical with a nonradical compound, so a chain reaction is started that may be several thousand events long, for example, lipid peroxidation involving polyunsaturated fatty acids. The primary free radical produces only local effects at the site of its production but the secondary radicals derived from it and degradation products of primary reaction cause more damaging effects at sites distinct and distant from the site where the primary free radical was formed. Two free radicals after reacting with each other form a stable molecule, which explains the eventual termination of free radical-induced chain reaction. Reactive oxygen species (ROS), such as superoxide, H_2O_2, and the ·OH radical, are now known to play an important role in programmed cell death in both plants and animals (Jobs, 1999; Bethke and Jones, 2001).

Free radicals are capable of reacting with almost every known molecule of biological system in their vicinity. Free radicals damage proteins, cause breakdown of DNA strands, and initiate the peroxidation of various compounds (Rosen et al., 1993). Almost all the vital components of cells are susceptible to damage by free radicals. The damage caused by free radicals to different vital macromolecules and the mechanism involved is summarized in the Table 1.

7,8-dihydro-8-oxoguanine (GO) is a common oxidative DNA lesion generated by direct modification via reactive oxygen species. GO lesions are mutagenic and can mispair with adenine during DNA replication (Yang et al., 2001). If the resulting A/GO is not repaired before the next round of DNA replication, a C/G→A/T transversion occurs and the opportunity for repair, is lost. The A/GO is repaired via base excision repair which is initiated by the DNA repair enzyme adenine-DNA glycosylase (Yang et al., 2001).

As a defense against the deleterious actions of free radicals, cells contain several enzymes for removing free radicals and their products. These are known as the antioxidant defense system or as free radical scavengers (Sies, 1991). Antioxidant means "against oxidation". Antioxidants work to protect lipids from peroxidation by radicals. Antioxidants are effective because they give up their own electrons to free radicals and thereby break the oxidation chain reaction. Some of the important ones are superoxide

TABLE 1
Biological Roles of Oxy Free Radicals

Radical species	Damaging roles	Protectors
$O_{2^-}^{\cdot}$	RSH damage Enzyme activation and inactivation Fenton reaction	SOD
$O_{2^-}^{\cdot}$	Lipid peroxidation Fenton-type reaction	Vit. C and E β-carotene
·OH	Breaking of DNA strand Lipid peroxidation Secondary radical peroxidation	RSH antioxidants DNA repair process
RO^-	Secondary radical peroxidation	RSH antioxidants
ROOH	Peroxidation Fenton reaction	Glutathione peroxidase
H_2O_2	Peroxidase action Fenton reaction	Catalase

dismutase (SOD), catalase, and glutathione peroxidase.

Superoxide dismutase (SOD): This enzyme directly interacts with oxy free radical to convert it to hydrogen peroxide.

$$O_{2^-}^{\cdot} + 2H^+ \xrightarrow{\text{SOD}} H_2O_2 + O_2$$

Cytosolic enzyme contains copper and zinc (Cu/Zn-SOD) (Fee, 1982), whereas mitochondrial enzyme contains Mn (Mn-SOD) (Weisigner and Fridovich, 1973). In plant cells the active oxygen species (AOSs) are also known to be produced under stress conditions. Recently, in rice seedling the responses of SOD genes to environmental stresses, such as drought, salinity, and chilling, were analyzed by Kaminaka et al. (1999). They observed that the expression of abscisic acid (ABA)-inducible genes, Mn-SOD gene (*sodA1*), and one of the cytosolic Cu/Zn-SOD gene (*sodCc2*) were strongly induced under the drought and salinity stresses. While Fe-SOD gene (*sodB*) and the other cytosolic Cu/Zn-SOD gene (*sodCc1*) were also reported to be induced by ABA. However, the mRNA level of *sodB* was decreased under drought stress, and *sdCc1* gene did not induce under drought and stress conditions. The plastidic Cu/Zn-SOD gene (*sodcp*) altered under salinity stresses in the light but not in the dark. The above results clearly showed that phytohormone and AOSs are associated with the regulation of SOD genes under environmental stresses (Kaminaka et al., 1999).

Catalase: The enzyme catalyses the transformation of less toxic hydrogen peroxide to water

$$2\ H_2O_2 \rightarrow 2\ H_2O + O_2$$

It is a heme-containing enzyme present in the peroxisomes and the other cell organelles.

Glutathione peroxidase: This enzyme catalyzes the oxidation of reduced glutathione (GSH) to oxidized form (GSSG) at the expense of H_2O_2 thereby remov-

ing toxic H_2O_2. Furthermore, glutathione peroxidase also metabolizes lipid hydroperoxides to hydroxyl fatty acids

$$H_2O_2 + 2\ GHS \xrightarrow{\text{glutathione peroxidase}} GSSG + 2H_2$$

The supply of reduced glutathione for continuous removal of H_2O_2 is maintained by the glutathione reductase

$$GSSG + 2NADPH \xrightarrow{\text{glutathione reductase}} 2GSH + 2NADP^+$$

In plants free radicals are known to be present *in vivo* in the young roots enriched with oxygen (Goodman et al., 1986), in the germinating seeds subjected to dehydration (Senaratna and McKersie, 1986), in ungerminated seed and pollen, and also accumulate during artificial aging (Priestley et al., 1985). As a result of the drought-induced impairment of the electron transport system, the superoxide radical is generated, which damages the membrane in droughted wheat (Halliwell, 1987; Hendry et al., 1989; Price et al., 1989). It was known that through Fenton reaction, the superoxide is reduced to the highly reactive hydroxyl radical, which causes severe peroxidative damage to lipid membranes (Price and Hendry, 1989; Price et al., 1989). The extracellular sources of activated oxygen could be air pollutants such as ozone (Mehlhorn et al., 1990; Kanofsky and Sima, 1991) and the radicals produced by neighbouring cells during the hypersensitive response (Levine et al., 1994).

Recently, Luxford et al. (1999) demonstrated conclusively that Histone H1 and other protein and amino acid hydroperoxides can give rise to free radicals, which might result in oxidative damage to associated DNA. The hydroperoxides can decompose to oxygen- and carbon-centred radicals (detected by electron paramagnetic resonance spectroscopy) on exposure to Cu^+ and other transition metal ions. These hydroperoxide-derived radicals react readily with pyrimidine DNA bases and nucleosides to give adduct species (i.e., protein-DNA base cross-links). Product analysis has demonstrated that radicals from histone H1-hydroperoxides and other protein and amino acid hydroperoxides can also oxidize both free 2´-deoxyguanosine and intact calf thymus DNA to give the mutagenic oxidized base 8-oxo-2´-deoxyguanosine (8-oxo dG). 8-oxo dG is considered to be a precise and sensitive biomarker of oxidative DNA damage (Liu and Wells, 1995). Bialkowski and Olinski (1999) studied 8-oxo dG levels in DNA as a measure of oxidative DNA damage in Cardamine pratensis plant as affected by different environmental parameters such as light, water accessibility, and low temperature. No significant difference in 8-oxo dG level between DNA of etiolated and light-exposed plants and between DNA of drought and control plants was found. However, cold temperature (1^0C for 28 h)-treated plants showed an increase of 8-oxo dG in their DNA, indicating oxidative damage to plant DNA under low-temperature conditions.

C. Lipid Peroxidation and DNA Damage

Lipid peroxidation is a highly destructive process that causes DNA damage and alters RNA transport from nucleus to cytoplasm. It also alters the structure and function of cellular membrane (Agrawal and Kale, 2001). All

the major classes of biomolecules may be attacked by the free radicals, but lipids are probably the most susceptible. The cell membranes are rich sources of polyunsaturated fatty acids (PUFA), which are readily attacked by oxidizing radicals. The oxidative destruction of PUFAs, known as lipid peroxidation (Tappel, 1973), causes a loss of fluidity and a breakdown of the membrane's secretory functions and transmembrane ionic gradient chain reaction (Barber and Berheim, 1967). They can further oxidize PUFA molecules and initiate new chain-producing lipid hydroperoxides (LOOH) that can break down to yet more radicals and to a wide range of compounds, notably aldehydes (Esterbauer et al., 1990a). Therefore, lipid peroxidation can be used as a measure of oxidative damage.

$$LH + R^{\cdot} \rightarrow L^{\cdot} + RH$$
$$L^{\cdot} + O_{2}^{-\cdot} \rightarrow LOO^{\cdot}$$
$$LOO^{\cdot} + LH \rightarrow LOOH + L^{\cdot}$$
$$LOOH \rightarrow LO^{\cdot}, LOO^{\cdot}, \text{aldehydes}$$

The end products of lipid peroxidation processes are aldehydes, hydrocarbon gases, and various chemical residues (del Maestero et al., 1981a). These degradation products can diffuse away from the site of chain reaction and give rise to cell edema, influence vascular permeability, and cause inflammation and chemotaxis in animals. These products may also alter the activity of phospholipids and induce the release of arachidonic acid with the subsequent formation of prostaglandins (Hemler and Lands, 1980) and various endoperoxides (del Maestro et al., 1981a, b).

The $O_{2}^{-\cdot}$ can indirectly influence lipid peroxidation *via* Fenton/Harber-Weiss reaction.

$$O_{2}^{-\cdot} + Fe^{3+} \rightarrow O_2 + Fe^{2+}$$
$$2O_{2}^{-\cdot} + 2H^{+} \rightarrow H_2O_2 + O_2$$
$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^{-} + {}^{\cdot}OH$$
$$Fe^{2+} + LOOH \rightarrow Fe^{3+} + OH^{-} + LO^{\cdot}$$

The generation of $O_{2}^{-\cdot}$ by any source, in the presence of metal ions, particularly iron ions, can lead to the formation of ${}^{\cdot}OH$ radical and in turn may initiate lipid proxidation. However, lipid peroxidation has no intrinsic metal ion requirement. Nonetheless, iron compounds can increase the rate of propagation of lipid peroxidation dramatically by decomposing lipid hydroperoxides (LOOH) to reactive alkoxyl ($LO^{\cdot}$) or peroxyl ($LOO^{\cdot}$) radicals, which can initiate new reactions (Agrawal and Kale, 2001).

Lipid peroxidation causes the endogenous lesions by inducing the formation of exocylic DNA base adducts (Lindahl and Wood, 1999). A major recent advancement has been the detection of number of lesions in DNA that can be attributed to metabolic sources (Fink et al., 1997). Lipid peroxidation, for example, leads to the formation of a complex variety of nucleophiles capable of reacting with proteins and DNA (reviewed by Marnett, 1999a,b). The reaction of nucleophiles with DNA leads to formation of mutagenic adducts. The lipid peroxidation product malondialdehyde (MDA) reacts with G residue in DNA to form a pyridimidopurinone called M_1G, an exocyclic adduct that can block the Watson-Crick base pairing of DNA bases and thus have the potential to block DNA replication (Marnett, 1999b). Site-directed mutagenesis studies have shown that M_1G is an efficient premutagenic lesion (Fink et al., 1997). The M_1G adduct is not very stable and can decompose to a secondary ring-

opened derivative. In addition to MDA, lipid peroxidation can also produce acrolein and crotonaldehyde, which are readily metabolized to epoxides. This resulted in the generation of exocylic etheno modifications of DNA bases (Lindahl and Wood, 1999). Two such bases, etheno-A and etheno-C, are removed by DNA glycosylases (Hang et al., 1997; Saparbaev and Laval, 1998). These strongly suggest that the generation of such adducts occurs at high rate (Lindahl and Wood, 1999).

Several leading laboratories around the world are currently addressing the identification of endogenous DNA lesions in mammalian DNA and their role in genetic disease. As lipid peroxidation and DNA damage individually have been considered major determinants of seed viability loss; however, no linkage between lipid peroxidation product and DNA damage in seed deterioration has been contemplated so far.

D. Ozone and DNA Damage

Ozone (O_3) itself is not a free radical, but the damage caused by it is often mediated by free radical production and thereby can stimulate lipid peroxidation. In last 40 years, the trophospheric O_3 concentration has increased 2- to 5-fold (Kley et al., 1999). Exposure of animals to O_3 is known to damage lungs (Mustafa and Tiernery, 1978). However, O_3 poses a 2-fold challenge to plants: photosynthesis and growth can be impaired and it can also produce foliar lesions in sensitive species and cultivars (Kley et al., 1999). O_3 has also been regarded as a "wound stress" that causes necrosis by oxidizing and damaging plasma membranes (reviewed in Heath and Taylor, 1997). O_3 responses are known to resemble components of the hypersensitive response (HR) found in incompatible plant-pathogen interactions (Sharma and Davis, 1997; Sandermann et al., 1998). This resemblance is most likely related to the occurrence of relative oxygen species (ROS), such as superoxide anion radicals ($O_{2}^{-\bullet}$) and H_2O_2 in the apoplast, which trigger an oxidative burst in the affected cells (Pellinen et al., 1999; Rao and Davis, 1999). H_2O_2 has been reported to be accumulated in response to O_3 in tobacco (Schraudner et al., 1998) and birch (Pellinen et al., 1999). O_3 stress in *Arabidopsis* is reported to be responsible for cell death (Rao and Davis, 1999).

O_3 also activates ethylene and salicylate (SA) signal transduction pathways leading to downstream responses, such as antioxidant and antimicrobial defenses (Sharma and Davis, 1997; Sandermann et al., 1998). The toxicity of O_3 is through chemical reaction with ethylene, yielding toxic products that initiate a self-propagating lipid peroxidation (Mehlhorn and Wellburn, 1987). The emission of ethylene is a kind of wounding symptom in O_3-stressed plants (Heath and Taylor, 1997). In pea carpel senescence, maize endosperm development, as well as in root aerenchyma formation, ethylene is reported to regulate the programmed cell death (Orzaez and Granell, 1997; Young et al., 1997; Drew et al., 2000). Recently, Overmyer et al. (2000) have isolated an *Arabidopsis* mutant, *rcd1* (for radical-induced cell death 1), which displayed O_3– and $O_{2}^{-\bullet}$–inducible lesion formation. These authors proposed a model for the relative contribution of the different signaling pathways to ROS-driven lession propagation and the process of lesion containment.

E. DNA Damage Response

Most organisms respond to chromosomal insults by activating a complex damage response pathway that regulates some known responses such as cell-cycle arrest, apoptosis, and direct activation of repair networks (Zhou and Elledge, 2000). DNA damage checkpoints control the ability of cells to arrest the cell cycle in response to DNA damage. The DNA damage response pathway is a kind of signal transduction pathway consisting of sensor, transducers, and effectors. The identities of sensors are not yet known, but the DNA-break binding protein such as poly (ADP-ribose) polymerase (PARP) and DNA-dependent protein kinase (DNA-PK) are the kind of DNA damage sensors. Much is known about signal transducers, which are composed of four sets of conserved proteins with some recognizable motifs. These four classes are

1. Phospho-inositide kinase (PIK)-related proteins, which include ATM and ATM-Rad3-related (ATR) in mammals and their homologs in yeast (Mec1, Rad3, Tel1). Downstream of these proteins are two check point kinases (chk1 and chk2).
2. BRCT-repeat containing proteins, which include budding yeast Rad9 and fission yeast Crb2.
3. RFC-like proteins, for example, Rad17, RFC2.5, in mammals and Rad24, Rad17, RFC2-5 and RFC3 in yeasts.
4. PCNA-like proteins, for example, Rad1, Rad9, Hus1 in mammals and Rad17, Rad1, Ddc1, Rad9, Mec3, and Hus1 in yeasts.

The effectors of damage response could be either activation of DNA repair pathways or cell cycle arrest or apoptosis depending on the severity and type of the damage (Zhou and Elledge, 2000).

III. VARIOUS DNA REPAIR MECHANISMS

For more than 2.5 billion years since the first appearance of living cells on Earth, they were continuously exposed to solar UV radiation and many DNA damaging agents. To protect the DNA from various damage, an intricate network of DNA repair mechanisms has evolved early in evolution (de Laat et al., 1999). Several DNA repair pathways known to date in different living cells are shown in Figure 2. Out of these, the nucleotide excision repair (NER) has been studied extensively. Although the DNA repair mechanisms have been described thoroughly in *E. coli*, *Saccharomyces cerevisiae*, rodents, and human, little is known still about these processes in plants. Any alteration in the DNA double helix, even as small is single base damage, are easily recognized. Such recognition is usually by a change in the physical structure of the DNA double helix. The repair mechanisms protect the genome by repairing modified bases, DNA adducts, crosslinks, and strand breaks. Furthermore, these repair mechanisms are also coordinated with other cellular functions, in particular gene transcription and cell cycle. The various basic repair mechanisms by which damages are eliminated from DNA are described below.

A. Direct Reversal (DR)

DR is a simple mechanism that involves a single-enzyme reaction for the

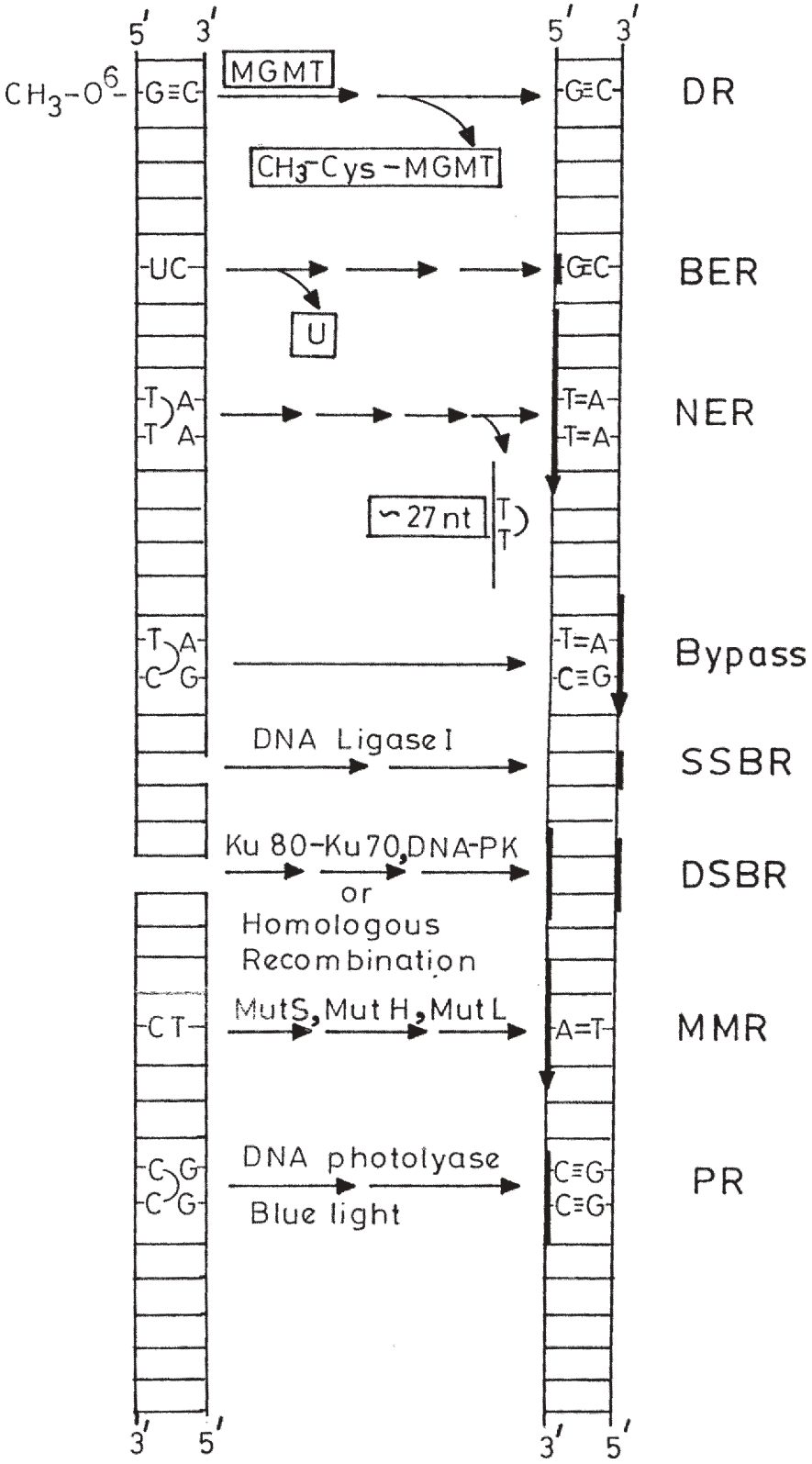

FIGURE 2. Several DNA repair pathways used by different living cells. **MGMT** = methylguanin methyltransferase; **DR** = direct reversal; **BER** =base excision repair; **NER** =nucleotide excision repair; **SSBR** =single strand break repair; **DSBR** = double-strand break repair; **MMR** = mismatch repair; **PR** = photoreactivation.

removal of certain types of DNA damage. Alkyltransferases simply extract the alkyl group from alkylated bases that is transferred to an internal cysteine residue, and thus inactivate themselves (Teo et al., 1984). The best example for DR is the correction of the miscoding alkylation lesion O^6-methylguanine, which is generated endogenously in small amounts by reactive cellular catabolites (Vaughan et al., 1993). DR is carried out by a specific enzyme, called methylguanine-methyltransferase (MGMT), which removes the methyl group from the guanine residue of DNA, and transfers it to one of its own cysteine residues (Figure 2) in a rapid and error-free repair process (Moore et al., 1994). O^6-methyl-guanine can pair with both C and T and thereby cause transition mutations, which are sometime corrected by mismatch repair mechanism (Lindahl and Wood, 1999).

Photolyases, on the other hand, revert UV-induced dimers in a light-dependent reaction called photo-reactivation (PR) (Sancar, 1990; Yasui and Eeker 1998; Todo, 1999), which is discussed later.

B. Base Excision Repair (BER)

The BER mechanism is known for the elimination of single damaged base residues in DNA and is considered to be an essential pathway for DNA maintenance. BER mainly removes the DNA damages that are arising spontaneously in a cell from hydrolytic events such as deamination or base loss, fragmented bases resulting from ionizing radiation, and oxidative damages or methylation of ring nitrogens by endogenous agents. BER pathways involve several steps, which include recognition and removal of damaged base, incision, gap filling, and sealing (Britt, 1996; Lehman, 1998; Lindahl and Wood, 1999). A model of various steps in BER is shown in Figure 3. The first step in BER involves the removal of a single damaged base through the action of a specific DNA *N*-glycosylase, which hydrolytically cleaves the base-deoxyribose glycosyl bond of a damaged nucleotide residue, and leaves the sugar-phosphate backbone intact. The resulting abasic sites are then recognized by an apurinic/apyrimidinic (AP) endonuclease that creates an incision on the backbone of the DNA at the 5′ end of AP site (Sakumi and Sekiguchi, 1990). The completion of base excision requires removal of the 5 terminal deoxyribose-phosphate residue, which is catalyzed by the phosphodiesterase (AP lyase) activity of DNA polymerase β (Pol β) (Matsumoto and Kim, 1995). The resulting one-nucleotide gap is filled by DNA Pol β and sealed by either DNA ligase I or DNA ligase III with its accessory protein XRCC1 (Figure 3) in mammalian system (Lehman, 1998).

The BER pathway has been shown previously to depend strongly on the presence of nicotinamide adenine dinucleotide (NAD^+). This dependency was proposed to be mediated through the catalytic activation of the nuclear enzyme poly(ADP-ribose) polymerase-1 (PARP-1) following its transient binding to BER-induced DNA strand break (Satoh and Lindahl, 1992; Satoh et al., 1993). PARP-1 is an abundant nuclear enzyme found in many eukaryotes, with the exception of yeast. This enzyme has high affinity for single- and double-strand DNA breaks. After binding to DNA strand breaks, PARP-1 catalyzes extensive synthesis of poly(ADP-ribose)

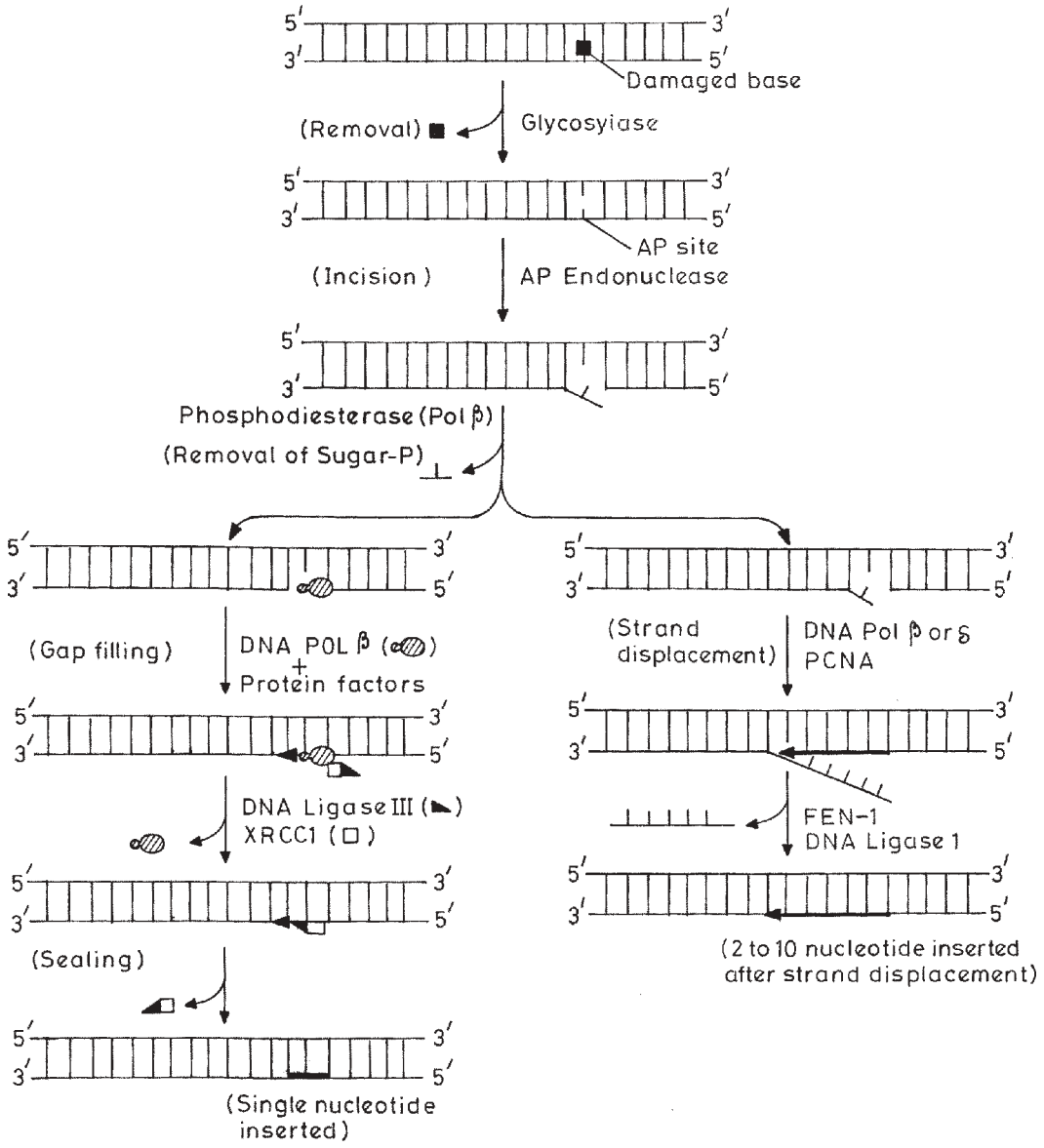

FIGURE 3. A model for base excision repair pathway. **AP endonuclease** = apurinic/apyrimidinic endonuclease; POL.β = polymerase β; **PCNA** = proliferating cell nuclear antigen; **FEN-1** = flap endonuclease-1

from NAD^+ and covalently modifies many nuclear proteins, including itself. The massive automodification of PARP-1 effects its dissociation from DNA strand breaks and inhibition of its catalytic activity. A modulatory role for poly-(ADP-ribose) formation and PARP-1 automodification in BER has been proposed by Satoh and Lindahl (1992). According to this model, the unmodified enzyme binds tightly to DNA strand interruptions formed either by ionizing radiation or through incision of AP sites during BER and interferes with the BER process because the bound PARP-1 molecule hinders access of the repair machinery to the lesion. Automodification and release of PARP-1 from the breaks allow the repair process to proceed. In the absence of NAD^+ or after the inhibition of poly(ADP-ribose) synthesis, PARP-1 persists on DNA breaks and

DNA repair is abrogated. Recently, it has been shown that the cells lacking PARP-1 have normal capacity to repair single-strand breaks inflicted by x-irradiation or breaks formed during the repair of modified bases (Vodenicharov et al., 2000).

Different DNA glycosylases (at least eight) are known to be present in human cell nuclei (Lindahl and Wood, 1999). These glycosylases remove different kinds of damage, and the specificity of repair pathway is determined by the type of glycosylase involved (Seeberg et al., 1995). The mode of action of these glycosylase and their three-dimensional structures have been described earlier (Cunnigham, 1997; Krokan et al., 1997; Parikh et al., 1998). These enzymes have a catalytic domain of ~250 amino acid residues and for additional interactions they also use their amino- and carboxy-terminal region (Lindahl and Wood, 1999). In general, these DNA glycosylases move along the minor groove of DNA until a specific type of damaged nucleotide is recognized. This enzyme then binds to the backbone of a damaged strand and slides out the damaged nucleoside residue to accommodate the damaged base in a specific recognition pocket and mediates cleavage (Parikh et al., 1998). Although these enzymes are essential for BER, in mice the knockouts of various DNA glycosylases have been shown to be viable (Wilson and Thompson, 1997), because the abasic sites are also known to be generated by nonenzymatic depurination.

Structurally, the AP endonuclease belongs to the superfamily of nucleases that also contains pancreatic DNaseI (Gorman et al., 1997). This enzyme flips out the base free deoxyribose residue from the DNA before chain cleavage and also recruits the Pol β to the site of repair (Bennett et al., 1997). The Pol β contains two distinct domains, the larger domain is the polymerase domain, which helps in gap filling and the small basic amino-terminal domain that contain AP lyase (Phosphodiesterase) activity that excises the abasic sugar-phosphate residue at the strand break (Matsumoto and Kim, 1995; Sobol et al., 1996). A knockout mutation of Pol β in mice has been shown to cause embryonic lethality (Gu et al., 1994). This finding suggests that either the single-patch mode of BER is essential for maintaining normal viability or that Pol β has an additional role in the cells such as in chromosomal DNA replication. The XRCC1 plays important role in bringing the Pol β and ligase III together, and it is also known to directly bind to the DNA single-strand break (Kubota et al., 1996; Marintchev et al., 1999).

Sometimes longer repair patches of 2 to 10 residues have also been observed (Matsumoto et al., 1994; Frosina et al., 1996). This might happen when the terminal sugar-phosphate residue has a more complex structure, which is resistant to cleavage by AP lyase, then the DNA strand displacement may occur. These longer repair tracts are thought to result from a nick translation reaction accompanied by strand displacement in the 5′ to 3′ direction, thereby generating a flap type of structure. The produced displacement flap structure is removed by flap endonuclease FEN-1 with the help of PCNA (Figure 3) (Harrington and Lieber 1994; Wu et al., 1996; Klungland and Lindahl, 1997). In this case the gap of few nucleotides long is filled by either Pol β or Pol δ (Fortini et al., 1998; Dianov et al., 1999) and sealed by DNA ligase I (Figure 3).

The BER in plants still has not been well studied when compared with mammalian systems, but this mechanism does

exist in plant (Talpaert-Borle and Liuzzi, 1982). During the early germination of *Zea mays*, the formation of AP site has been reported (Dandoy et al., 1987), which also suggested that plants do have BER pathway. A specific glycosylase enzyme (uracil-DNA glycosylase) was found in cultured cells of carrot that play a role in BER (Talpaert-Borle and Liuzzi, 1982). A cDNA clone encoding 3-methyl adenine glycosylase was reported from *Arabidopsis* and the encoded protein shown to contain glycosylase activity (Santerre and Britt, 1994). This gene was shown to be expressed in growing tissue in *Arabidopsis* (Shi et al., 1997). The gene encoding DNA ligase has also been cloned from *Arabidopsis* and sequence data are available in Genebank (Taylor et al., 1996b).

C. Nucleotide Excision Repair (NER)

NER is one of the most versatile DNA repair pathways operating in both prokaryotes and eukaryotes. Unlike other DNA repair pathways that are repair specific, NER is capable of removing various classes of DNA damage, including those induced by UV radiation (pyrimidine dimers) and chemicals (bulky DNA adducts) such as cisplatin, 4 NQO, benzpyrene, and alfatoxin (Lindahl and Wood, 1999; Balajee and Bohr, 2000).

The major difference between NER and BER is the way the DNA damage is removed. Basically, NER cuts out the damage as a part of an oligonucleotide fragment, while BER excises only one nucleotide. From 24 to 32 nucleotides can be removed by NER in eukaryote (Huang et al., 1992; Moggs et al., 1996). The repair of lesions over the entire genome is called as global genome NER, while the repair of transcription blocked lesions present in transcribed DNA strands is called as transcription coupled-NER (TC-NER) (Mu and Sancar, 1997).

NER pathway in mammalian system involves product of at least 30 genes and most of them have been cloned (de Laat et al., 1999). In bacteria, mainly four proteins (UvrA, B, C, and D) carry out NER process. The UvrA, B, and C are required for incision reaction. The UvrA first dimerizes and then binds to UvrB, which recognize the damage on the DNA and causes local melting at 3′ to the damage (Mazur and Grossman, 1991; Myles and Sancar, 1991). UvrB then makes a 3′ incision (Lin et al., 1992), preceding the 5′ incision by UvrC (Lin and Sancar, 1992). Incision is made at seventh nucleotide from 3′ side and at fourth or fifth positions form 5′ side of the damaged DNA (Sancar and Rupp, 1983). The UvrD helicase releases ~12 or ~13 bases long oligonucleotide containing the damage, DNA polymerase I fills the gap, and finally DNA ligase seals the repair patch. The NER processes in yeast and mammalian cells are very much alike. The molecular mechanisms of NER in a mammalian system have been described in detail in several reviews (Lehman, 1998; de Laat et al., 1999; Lindahl and Wood, 1999; Balajee and Bohr, 2000).

Most of the NER genes have been isolated through transfection of repair-deficient rodent mutant cell lines and the recovery of the excision repair cross complementing (ERCC) human genes. Nearly all ERCC proteins subsequently appeared to be involved in the human repair disorders *Xeroderma pigmentosum* (XP) and Cockayne syndrome (CS) and therefore are called XP and CS factors, respectively. The *XPA*, *XPB*, *XPC*, *XPD*, *XPF*, and *XPG* have been cloned from

the mammalian system. The various XP and ERCC factors take part in the different steps of NER. The NER genes isolated from human, yeast, and plant show significant sequence similarity, suggesting that the NER mechanism is conserved throughout the evolution (Hoeijmakers, 1993a,b; Xu et al., 1998). Unlike bacteria, yeast, and mammalian systems where NER has been studied extensively, in plants it is not well characterized yet. Although biochemical evidence suggests that such a DNA repair mechanism is present (McClennan, 1987; Britt, 1996; Xu et al., 1998). Molecular characterization of gene products involved in DNA repair in plants has so far been limited to photolyases, the enzymes involved in photoreactivation (Ahmad et al., 1997), which is described later. Although NER has a very broad substrate specificity, it is primarily involved in the removal of UV-induced damage from the DNA. UV radiation induces the formation of two major UV photoproducts, the cyclobutane pyrimidine dimers and 6-4 photoproducts. The structure of these photoproducts is shown in Figure 4. These pyrimidine

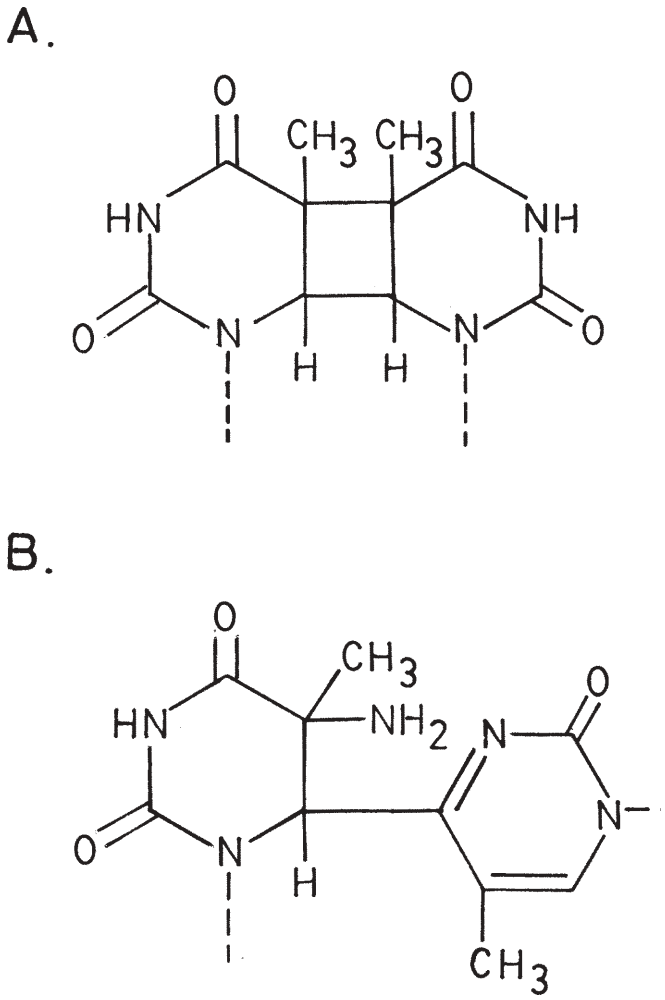

FIGURE 4. Structure of two main UV-induced photoproducts. (A) A cyclobutane pyrimidine dimer (TpT). (B) 6-4 photoproduct (pyrimidine [6-4] pyrimidine dimer) (TpC). The dimers are formed between adjacent (5′ and 3′) bases on the same DNA strand.

dimers are highly toxic because they have effect on transcription. Their efficient removal is an essential function for any living cells that is exposed to sunlight. The cyclobutane pyrimidine dimers can be formed between any two adjacent pyrimidines (Figure 4A) (Protic-Sabljic et al., 1986; Doetsch, 1995). The 6-4 photoproducts are formed by covalent bond between the carbon 6 and carbon 4 of adjacent pyrimidines (Figure 4B). These are the most frequent occurring UV photoproducts and occur at 5′-T-C-3' (Figure 4B), 5′-C-C-3′, and 5′-T-T-3' but not at 5′-C-T-3′ sites in DNA (Doetsch, 1995). These photoproducts can be repaired by NER; enzymatic photoreactivation, recombination repairs, and by postreplications repair (Friedberg et al., 1995).

The molecular model for NER in mammalian system is shown in Figure 5. The NER sequentially involves recognition of DNA damage, dual incision on damaged strand, excision of damage containing oligonucleotide, DNA synthesis (gap-filling), and ligation to replace an excised oligonucleotide (Figure 5) (Wood, 1997; de Laat et al., 1999). The initiation of DNA repair is the first step in the NER. The XPC recognizes the damage and binds strongly to the damage and then recruits the entire repair protein apparatus to the damage (Drapkin et al., 1994; Masutani et al., 1994; Reardon et al., 1996; Sugasawa et al., 1998). The XPC protein (125 kDa) exists in a complex with hHR23B (58 kDa), a human homolog of the *Saccharomyces cerevisiae* repair protein Rad23 that enhances XPC-dependent excision repair *in vitro* (Sugasawa et al., 1998). The DNA damage is also recognized by the XPA protein in association with replication protein A (RPA) and forms a XPA-XPC-RPA complex on the damage (Figure 5) (Matsuda et al., 1995; Saijo et al., 1996). The RPA is a heterotrimeric single-stranded DNA-binding protein that is required for both the incision reaction as well as repair synthesis (Coverley et al., 1992; Aboussekhra et al., 1995; He et al., 1995; Mu et al., 1995). RPA also binds to the nondamaged strand to prevent it from incision of the strand (which serves as a template for repair synthesis) and stabilizes the open structure (Kim et al., 1992; Seroussi and Lavi, 1993). RPA is also known to stimulate the interaction of XPA and ERCC1 (Li et al., 1994; Park and Sancar, 1994; Saijo et al., 1996), and on the other hand can bind to the endonuclease XPG (He et al., 1995) and possible XPF (Bessho et al., 1997). The XPA binds to basal transcription factor, TFIIH, and thereby recruits it to the damage area (Figure 5). The TFIIH is a 6-9 subunit complex containing XPB (ERCC3) and XPD (ERCC2) DNA helicases (Winkler et al., 1998; de Laat et al., 1999; Lindahl and Wood, 1999).

The action of TFIIH complex resulted in the formation of a open duplex structure around the damaged site that enables the structure-specific nucleases (XPG and ERCC1-XPF) for the dual incision (Evans et al., 1997; Mu et al., 1997a,b; de Laat et al., 1999; Lindahl and Wood, 1999). TFIIH and RPA can both bind to XPG, which makes the first incision while it cuts the damaged DNA strand on the 3′ side of the damage (Figure 5) (O'Donovan et al., 1994; Matsunaga et al., 1995; Mu et al., 1996). The incision by XPG is made at 5 to 6 phosphodiester bonds away from the 3′ side of the damage (Figure 5) (Svoboda et al., 1993). The XPG protein is a member of the FEN-1 family of structure-specific endonucleases and cuts at the junctions of du-

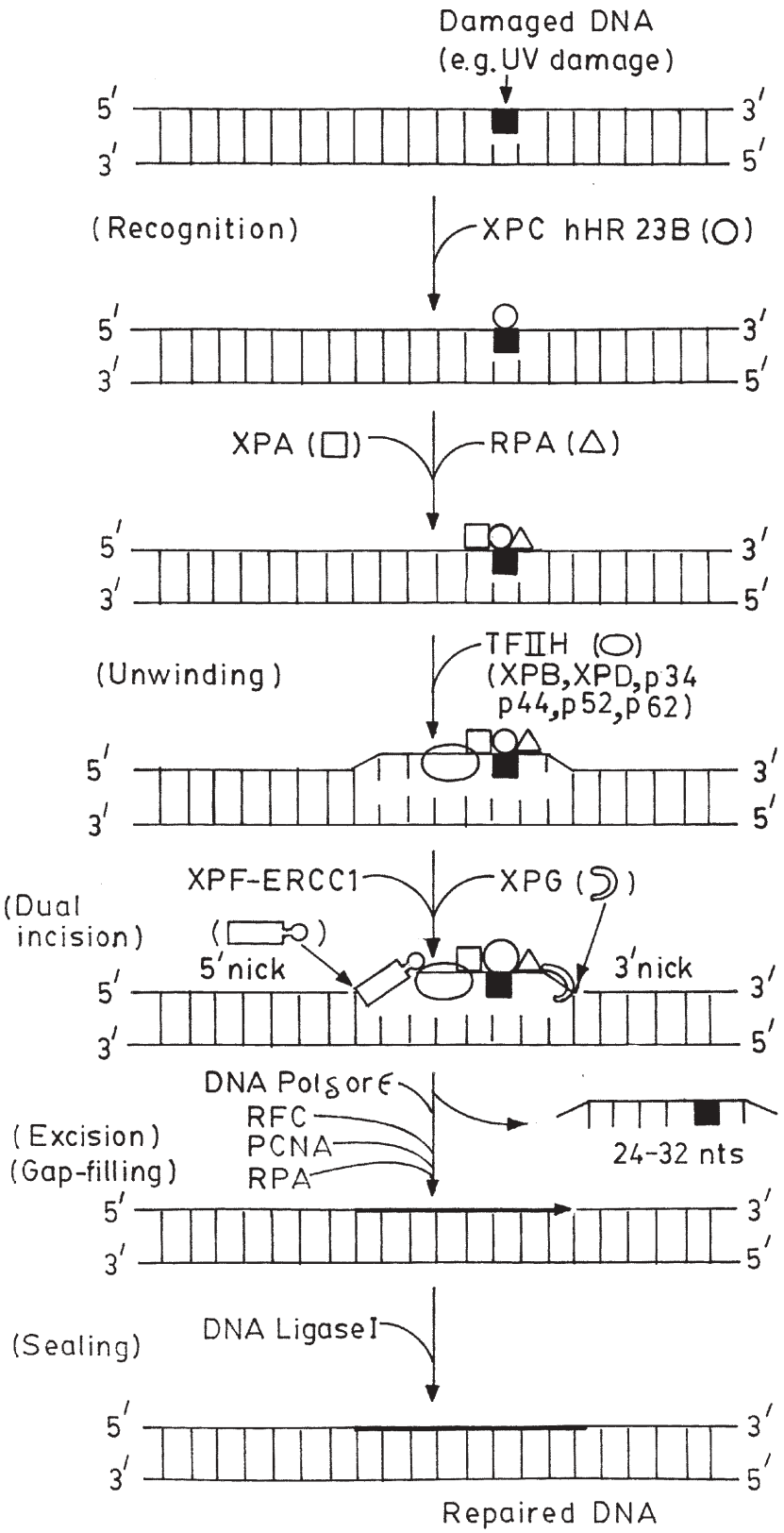

FIGURE 5. A mdoel for nucleotide excision repair. The figure shows several steps, including damage recognition, unwinding of damage area, dual incision, excision of damage peace, gap filling, and the sealing (for details see the text).

plex and unpaired DNA (Lieber, 1997; de Laat et al., 1999). XPG is also known to interact with proliferating cell nuclear antigen (PCNA) (Gary et al., 1997), which is involved in DNA repair synthesis but dispensable for the incision stage of NER (Shivji et al., 1992). Next, the ERCC1-XPF complex makes the incision at 22 to 24 phosphodiester bonds away from the 5′ side of the damage (Figure 5) (Svoboda et al., 1993; Mu et al., 1996). The dual incision is absolutely dependent on ATP hydrolysis (Svoboda et al., 1993). The specificities of these two nucleases make them ideally suited to cleave the opened up damaged site on either side of the damage. This results in the removal of a 24- to 32-residue oligonucleotides in length (Figure 5) (Huang et al., 1992; Moggs et al., 1996; Mu et al., 1997a,b; de Laat et al., 1999).

The final step in the NER pathway is the gap-filling or repair synthesis of the excised patch, which is performed by common DNA replication factors. An *in vitro* reconstituted repair reaction showed that efficient repair synthesis required the mammalian replication factors RPA, RFC, PCNA, and DNA polymerase δ or ε (Hubscher and Thommes, 1992; Shivji et al., 1995). RFC is needed to load PCNA onto the DNA (Podust et al., 1994), and PCNA is required for the initiation of repair synthesis. PCNA also stimulates polymerase activity. The newly synthesized repair patch, which exactly matches the excision patch, is then sealed by ATP-dependent DNA ligase, most likely ligase I (Figure 5) (Lindahl and Barnes, 1992; Petrini et al., 1995).

In plant, the NER system is still not well characterized, although biochemical evidence suggests that such a DNA repair mechanism is present (McCleannan, 1987; Britt, 1996; Vonarx et al., 1998; Britt, 1999). An UV-specific endonuclease resembling UVrABC nuclease, which is known to be involved in NER in bacteria, was reported and characterized from spinach (Doetsch et al., 1989). Molecular characterization of gene products involved in DNA repair in plants so far has been limited to photolyases (Ahmad et al., 1997). The UV products CPDs and (6-4) photoproducts are also known to be repaired in plant through a light-independent repair pathway that supports the existence of a pathway equivalent to NER mechanism in yeast and mammal (Howland, 1975; Quaite et al., 1994; Taylor et al., 1996). NER was also suggested to occur in carrot protoplasts (Howland, 1975, Eastwood and McLennan, 1985), *Arabidopsis* and alfalfa seedlings (Pang and Hays, 1991; Quaite et al., 1994), soybean chloroplasts and leaves (Cannon et al., 1995; Sutherland et al., 1996), and in different rice cultivars (Hidema et al., 1997). It was also reported in wheat leaf tissue (Taylor et al., 1996). The *Rad23* gene of yeast is involved in NER and transcription coupled repair (Mueller and Smerdon, 1996). Mutation of the yeast *Rad23* gene resulted in moderate UV sensitivity that was due to the defect in NER pathway (Verhage et al., 1996). In 1997 a structural homolog of *Rad23*, *OSRad23*, was reported from rice but its role in NER has not been determined (Shultz and Quatrano, 1997). Several putative *Rad23* homolog in *Arabidopsis* EST databases have been observed.

Until recently, the only evidence of the existence of light-independent DNA repair pathways in plants came from physiological studies based on dark repair of UV-induced pyrimidine dimers (Eastwood and McLennan, 1985; Quaite et al., 1994; Taylor et al., 1996). In ad-

dition, UV-sensitive *Arabidopsis* mutants defective in dark repair have been described (Britt et al., 1993; Harlow et al., 1994; Jiang et al., 1997). Recently, a few plant homologs of some of the components involved in mammalian NER pathway have been reported. The molecular evidences for the conservation of the NER pathway came from the cloning of *Arabidopsis XPB* (*araXPB*) (Ribeiro et al., 1998) and the lily homolog of human *ERCC1* (Xu et al., 1998). The araXPB protein shared 50% identity and 70% conserved amino acids with yeast and human homologs. The plant XPB contained all the functional domains found in the other proteins, including nuclear localization signal, DNA-binding domain, and helicase motifs, suggesting that it might be playing a role in NER in plant cells (Ribeiro et al., 1998). However, the DNA unwinding activity of plant XPB has not been determined yet.

The plant *ERCC1* gene was isolated from male germline cells of lily, and the deduced amino acid sequence contained striking homology with human ERCC1 and yeast Rad10 (Xu et al., 1998). The lily *ERCC1* homologs were present in the genomes of taxonomically diverse plant species, including *Arabidopsis thaliana, Brassica napus, Zea mays, Oriza sativa, Nicotiana tobacum,* and *Lycopersicon esculentum,* as shown by Southern blot analysis of genomic DNAs (Xu et al., 1998). These findings suggested that the NER role of the *ERCC1* gene might be conserved in higher plants. The lily ERCC1 expression was also reported to be upregulated in the male germline cells of plants (Xu et al., 1998). Pollen, as part of its developmental process, is exposed to solar UV radiation and other environmental mutagens after being released from the anther. This exposure could inevitably lead to DNA damage in both vegetative and generative cell nuclei of pollen. The up-regulation of ERCC1 homolog in lily generative cells suggested that a highly active DNA repair mechanism existed in male germline cells in order to protect germline DNA from heritable mutations resulting from damaged DNA (Xu et al., 1998). The protein encoded by the lily *ERCC1* gene was also able to correct the sensitivity to the cross-linking agent mitomycin C in *ERCC1*-deficient Chinese hamster over cells that further suggested that the NER mechanism is conserved in yeast, animals, and higher plants (Xu et al., 1998).

Recently, two different groups have reported the cloning of plant (*Arabidopsis*) homolog of human *XPF* and *S. cerevisiae RAD1* and they gave the name of the gene as *UVH1*, *Rad1* (Liu et al., 2000), and *AtRAD1* (Gallego et al., 2000). The *UVH1/Rad1* gene was cloned from *Arabidopsis* by positional cloning methods and has been mapped to chromosome 5 and also shown to be the product of a repair endonuclease (Liu et al., 2000). The *UVH1/Rad1* gene was shown to be strongly expressed in flower tissue as well as in other tissues (Liu et al., 2000). The *AtRAD1* gene from the other group is also shown to be involved in NER in the dark and is also shown to be ubiquitously expressed, which suggested that plant NER has some other roles (Gallego et al., 2000). Recently, the *XPF* gene was knocked out in tobacco by introducing *AtXPF* antisense in tobacco, which resulted in the increased UV sensitivity (Crockett et al., unpublished data). The transgenic tobacco plant carrying on *AtXPF* promoter *GUS* gene fusion showed relatively higher levels of GUS expression in generative cells of pollen and seeds. These findings suggested a key role of NER pathway in maintaining germline

DNA integrity and also in the removal of endogenous DNA lesions in seed, which accumulated during the seed aging process (Crockett et al., unpublished data).

D. Photoreactivation (PR) and Photolyases

It is clear now that UV radiation can damage plants, decreasing growth, and productivity (Teramura, 1983). The UV-induced DNA damages (CPDs and 6-4 pps) are removed or repaired by two different repair pathways. One is NER, which is described above, and other is PR which is a direct reversal phenomenon. PR is performed by the combined action of one or more proteins termed 'photolyases' and visible light in an error-free fashion (Sancar, 1994; Britt, 1999; Thoma, 1999). The photolyases specifically recognize and bind to the pyrimidine dimers form a complex that is stable in the absence of light. After absorbing a blue light photon, the pyrimidine dimers are reversed to pyrimidine monomers without excision of the damaged bases (Sancar, 1996; Thoma, 1999) as shown in Figure 6. Photorepair of CPDs have been reported in several plant species, including gingko (Trosko and Mansour, 1969), *Arabidopsis* (Pang and Hays, 1991; Britt et al., 1993), alfalfa (Quaite et al., 1994), soybean (Sutherland et al., 1996), cucumber (Takeuchi et al., 1996), rice (Hidema et al., 1997, Hidema et al., 2000), maize (Stapleton, 1992; Stapleton et al.,1997), and wheat (Taylor et al., 1996a).

Photolyases are known to be very specialized in terms of their substrate specificity (Britt, 1999). CPD photolyases were reported in bacteria, fungi, plants, invertebrates, and many vertebrates, while 6-4 photolyases were identified in silkworm, frog, fly, and rattlesnakes (Yasui et al., 1994, Sancar, 1996; Todo, 1999). The first higher eukaryotic photolyase was CPD specific and cloned from goldfish (Britt, 1999). Two distinct photolyases, one specific for CPDs and other specific for 6-4 products, were reported from *Arabidopsis* seedling (Chen et al., 1994). The two classes of CPD photolyases share only 10 to 15% sequence identity (Yasui et al., 1994). Class I is a microbial CPD photolyase and Class II is metazoan CPD photolyase. The *Arabidopsis* homolog of class II photolyase sequence (*PHR1*) corresponds to a gene (*UVR2*), which was identified via classic genetic analysis (Ahmad et al., 1997; Jiang et al., 1997). The phylogenetic analysis of photolyase homologs are described in Nakajima et al. (1998) and Britt (1999). Photolyases contain two prosthetic chromophores: $FADH_2$ and either methenyl tetrahydrofolate or 8-hydroxy-5-deazaflavin (Sancar, 1994). The second chromophore works as a light-harvesting antenna. The wavelength of light for photoreactivation ranges from the visible (500 nm) to the UV-B (300 nm). The existence of PR in plant was first reported by Ikenaga and Mabuchi (1966), who demonstrated that the frequency of endosperm mutations generated by UV radiation of maize pollen dropped substantially if the pollen was exposed to visible light after UV irradiation. A photolyase cDNA was reported earlier from white mustard (Batschauer, 1993). The crystal structures of CPD photolyase of *E. coli* and *Anacystis nidulans* have been resolved. This suggested that the photolyases flip the pyrimidine dimer out of the duplex into the hole that contains the catalytic cofactor (Park et al., 1995; Tamada et al., 1997) followed by splitting the cyclobutane ring after light absorption.

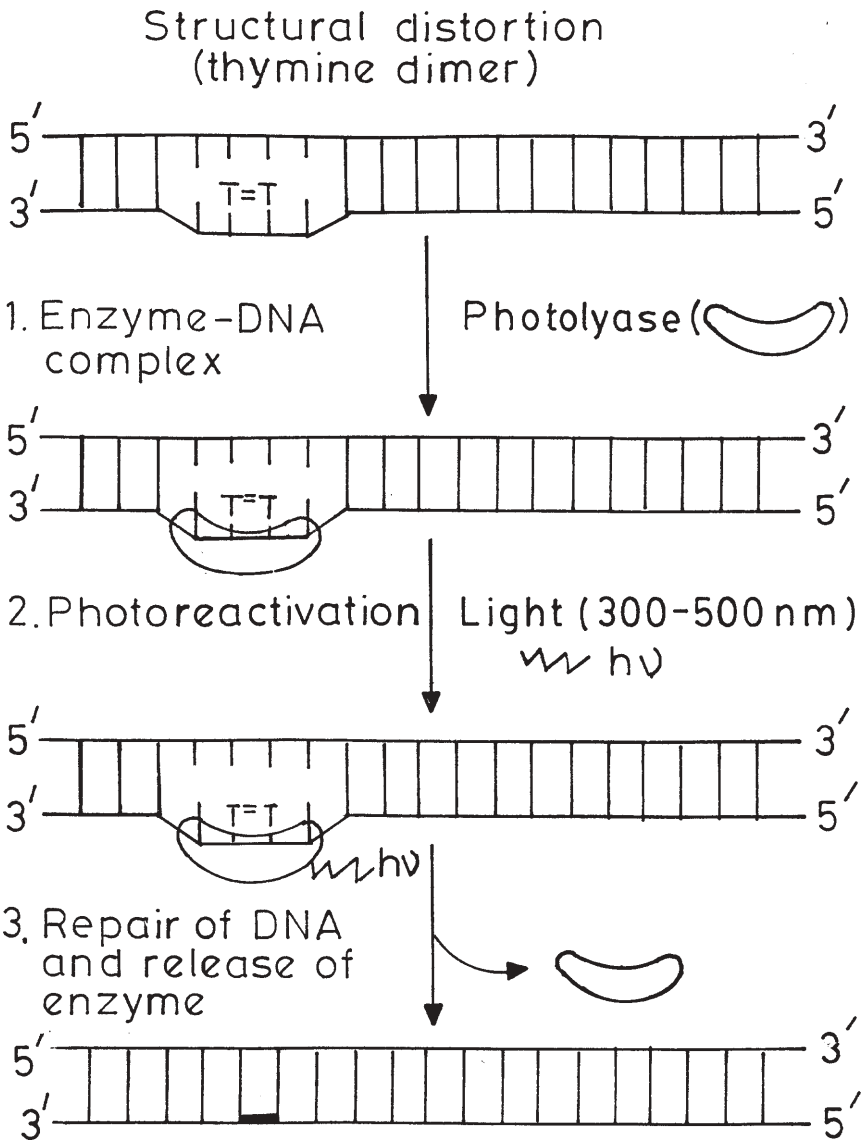

FIGURE 6. Schematic illustration of photoreactivation. Photolyase binds to pyrimidine dimer (T = T), split the dimer and restore it to native pyrimidine monomer in a light-dependent reaction.

The plant blue light photoreceptor genes were discovered during the photolyase research and named *CRY* after cryptochrome. The CRY of plant and animal have some sequence homology with DNA photolyase (Todo, 1999), but they do not contain any repair activity. In yeast it is known that repair by photolyase is faster than NER especially in nucleosome-free regions, for example, promoters and origin of replication regions (Thoma, 1999). The photolyase and NER can also play complementary roles in the genes that are transcribed by RNA polymerase II; they remove the damage in the nontranscribed and transcribed strands, respectively (Livingstone-Zatchej et al., 1997; Suter et al., 1997; Thoma, 1999). Aboussekhra and Thoma (1999) have shown that NER can remove CPDs, which are not accessible to photolayase, such as CPDs gen-

erated in the TATA-box of the yeast *SNR6* gene.

Increased sensitivity to UV radiation may result from failure to repair photodamage in DNA. The UV radiation-sensitive *UVR1* mutant of *Arabidopsis* cannot photorepair 6-4 photoproducts (Britt et al., 1993). Landry et al. (1997) showed that another *Arabidopsis* UV radiation-sensitive mutant, *UVR2-1*, was deficient in photorepair of CPDs and cloning and sequencing of the photolyase genes from UV radiation-sensitive and wild-type strains indicated alterations of DNA sequence that led to defective photolyase protein (Ahmad et al., 1997). These data suggested that such deficiencies might be ameliorated by restoration of photolyase function in photorepair-deficient strains. However, for such restoration exact knowledge of the molecular origin of their photorepair deficiency is essential, as suggested by Hidema et al. (2000). It is important to know whether the deficiency is in the photolyase protein itself, or it has resulted from other cause, such as a regulatory mutation that causes the production of fewer normal photolyase molecules per cell. The UV-B radiation-sensitive rice cultivar Norin 1, a progenitor of many Japanese commercial rice strain, has been reported to be deficient in photorepair of CPDS (Hidema et al., 1997). Recently, the same group, Hidema et al. (2000), reported that the photorepair deficiency in Norin 1 is due to the alteration in the structure of the photolyase enzyme rather than a regulatory mutation. Their findings indicated that a strategy of increasing the activity of photolyase through selective breeding or engineering could increase resistance to UV radiation and perhaps the productivity of such cultivars (Hidema et al., 2000).

E. Bypass Damage Repair and DNA Polymerases

It is known that the pyrimidine dimers can act as blocks to DNA replication, so cells have to possess specialized low fidelity and often error-prone DNA polymerases that can bypass such lesions and promote replication of damaged DNA (Johnson et al., 1999c). Usually at cell-cycle checkpoints, these damages are repaired before replication or cell division proceeds. If the damage is not repaired properly, then the cell cycle checkpoints can lead to the inhibition of cell cycle progression (Costanzo et al., 2000). In yeast *S. cerevisiae* the catalytic subunit (Rev3) of DNA polymerase ζ together with an accessory factor Rev7 can bypass pyrimidine dimers and other adducts in DNA (Lawrence and Hinkle, 1996). Some other polymerases, for example, yeast, Rev1 (hREV1 in human), *E. coli* UmuD'2C (*E. coli* Pol V), *E. coli* DinB (Pol IV), are also known for bypassing the damage before the action of replicative polymerases (Nelson et al., 1996; Gerlach et al., 1999; Lin et al., 1999; McDonald et al., 1999; Tang et al., 1999; Wagner et al., 1999). The DNA polymerase encoded by yeast *RAD30* and human *hRAD30A* also bypasses a cis-syn thymine-thymine dimer efficiently and accurately (Johnson et al., 1999 a,b; Masutani et al., 1999; Washington et al., 1999; Johnson et al., 2000b, Washington et al., 2000). The damage bypassed by polymerases that insert wrong residues result in mutations, which can create more problems.

The human gene *hRAD30B* (McDonald et al., 1999), which is a analog of yeast *RAD30B*, encodes the ninth eukaryotic DNA polymerase named Pol ι, which misincorporates deoxynucleotides at a high

rate (Johnson et al., 2000 a). In general, DNA polymerases extend from mispaired bases at about the same frequency at which they insert the respective mispair (Goodman et al., 1993). A new model for mutagenic bypass of DNA lesions in eukaryotes showed that two DNA polymerases (ι and ζ) act sequentially (Johnson et al., 2000 a). Pol ι functions in damage bypass by inserting deoxynucleotides opposite DNA lesions, whereas Pol ζ acts at the subsequent step of extending from them (Gibbs et al., 1998; Johnson et al., 2000a).

Whether the bypass repair is responsible for mutagenesis in plants remains to be studied in detail. However, in maize pollen the UV-induced mutations were found to be nontransmissible, which suggested that the mutations are of deletion type rather then point mutation (Nuffer, 1957).

F. Double-Strand Break (DSB) Repair

As early as in 1931, by using cytological tools, Barbara McClintock showed that the outcome of radiation-induced chromosome breaks is translocations, deficiencies, ring chromosomes, and end fusions (McClintock, 1931). She suggested that the broken ends can fuse with the same or other broken ends or chromosomes, we now call this DSB repair. DSBs are usually created by ionizing radiation (Dizdaroglu, 1992), chemical agents, during meiosis (Friedberg et al., 1995) or transposition reaction (Lieber, 1991) and also as a result of a nick in the single-stranded region due to mechanical stress of a chromosomes (Britt, 1999). The unprotected DSB is a dangerously cytotoxic lesion. If not repaired immediately, DSBs can be attached to by unwelcome recombination events, which can lead to loss of portions of chromosomes or to rearrangements (error prone). Usually DSBs are repaired by either homologous recombination or by nonhomologous end-joining (NHEJ), which involves the joining of two broken ends (Britt, 1999). Homologous recombination is particularly effective in S phase when the break can be repaired using genetic information from a sister chromatid, whereas NHEJ is thought to be effective at all times in the cell cycle (Takata et al., 1998; Essers et al., 2000). NHEJ also plays an important role in DSB repair during V(D)J recombination in antibody coding fragments (Blunt et al., 1995). The model of DSB is shown in Figure 7. In general, the organisms with relatively compact genomes (yeast, bacteria, etc.) repair the DSB via homologous recombination, provided some homology is available and organisms with larger genomes follow NHEJ (Britt, 1999). In plants the DSB repair products have been characterized as excision products of transposable elements, or insertion products of *Agrobacterium* T-DNA (Ohba et al., 1995; Scott et al., 1996).

Recombination is initiated by a 5' to 3′ exonuclease (Figure 7) that generates long single-stranded 3′ tails (White and Haber, 1990; Sugawara and Haber, 1992). In homologous recombination, one of these tails invades a homologous DNA duplex. Overall, this resulted in a formation of Holliday Junction, which resolved and forms two recombinant DNA molecules with or without crossing over. The DSB repair by homologous recombination with another allele can be achieved with high fidelity, whereas repair by NHEJ may result in lost or changed genetic information.

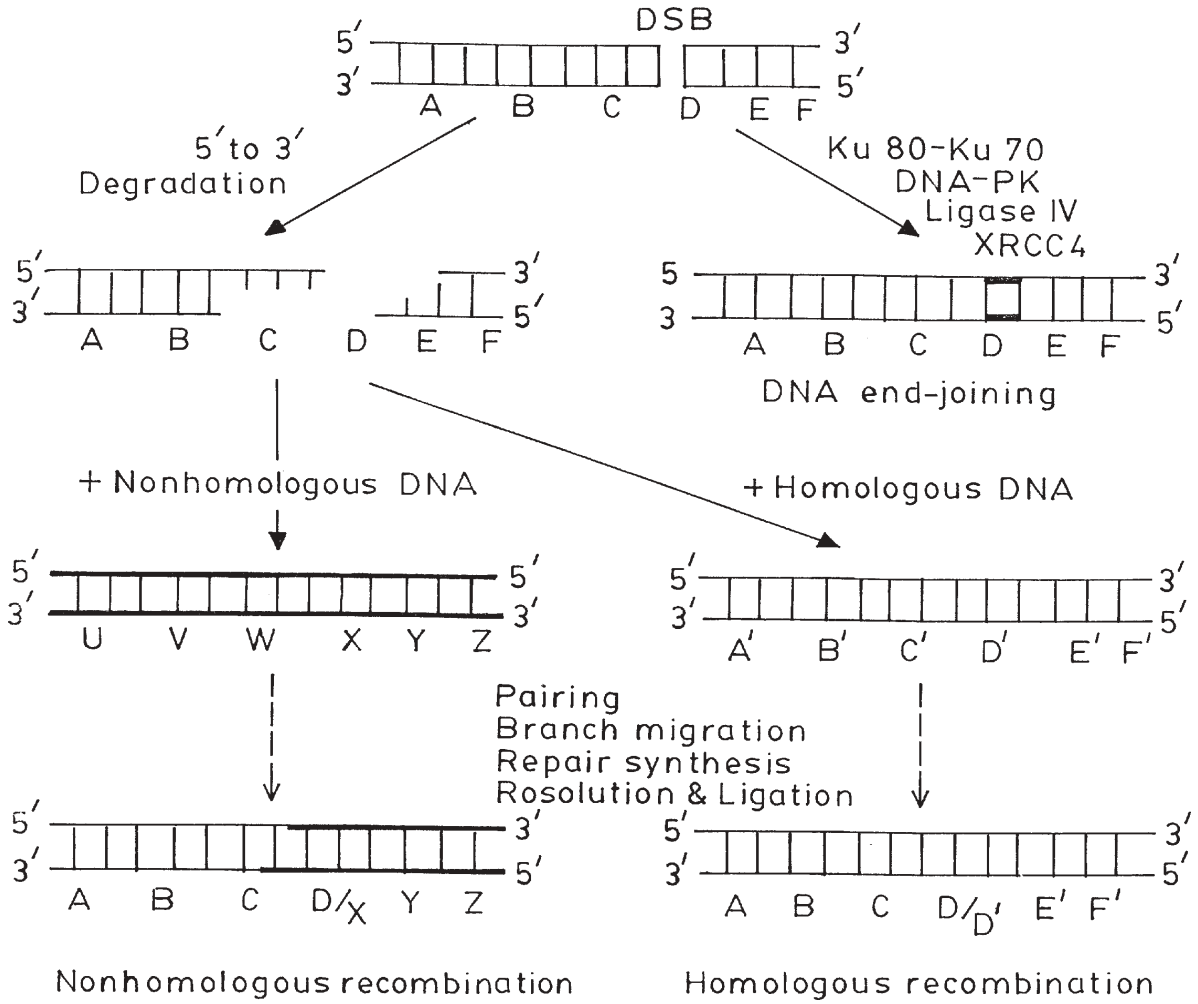

FIGURE 7. Models for double-strand break repair. In these schematic models, double-strand breaks caused by either irradiation or chemical assault or during meiosis and transposition reactions or due to mechanical stress can be repaired by nonhomologous end joining or nonhomologous recombination or homologous recombination.

In plants, the DSB repair is still poorly understood. Evidence from transformed plants suggests that the majority of transformed DNAs are integrated via NHEJ. However, plants are capable of repairing DSB via either pathway. **I**n plants a few genes that are involved in DSB repair have been isolated. The homologs of RecA genes (*DMC/LIM15/RAD51*) have been reported from plants (Kobayashi et al., 1993; Klimyuk and Jones, 1996; Doutriauk et al., 1998). The RecA-like recombination protein was shown to be localized on chromosomes of the lily at various meiotic stages (Terasawa et al., 1995). The DSBs in plant somatic tissues can be repaired by homologous recombination. Greater induction of homologous recombination was observed when site-specific DSBs were created using transposon or rare-cutting endonucleases. The first molecular data on end-joining in plants came from the analysis of footprints left behind after transposable element excision (Rinehart et al., 1997). These footprints usually consist of minor changes (i.e., a few base pair deletions, or inversion), and the exact configuration of DNA ends produced by transposon excision remains unknown. Recently, a Rad50 homologue gene and the encoded protein have been isolated from *Arabidopsis* and shown to be involved in meiosis and DSB repair in plants (Gallego et al., 2001).

The repair of DSBs by NHEJ requires the products of the *XRCC4*, *XRCC5*, *XRCC6*, and *XRCC7* genes in mammalian system (reviewed by Chu, 1997; Critochlow and Jackson, 1998). *XRCC4* encodes a protein (XRCC4) that forms a heterodimer with ligase IV that rejoins two DNA ends in the last step of V(D)J recombination and NHEJ to repair DSBs. XRCC4-defective cells are extrememly sensitive to ionizing radiation and disruption of the XRCC4 gene or ligase IV results in embryonic lethality in mice (Barnes et al., 1998). Recently, the crystal strucutre of XRCC4 has been resolved at 2.7 A, which suggests a dumbbell-like tetramer structure (Junop et al., 2000). *In vitro*, XRCC4 stimulates adenylation of ligase IV, the first chemical step in DNA ligation (Modesti et al., 1999). XRCC4 may also bridge two ends for ligation, perhaps by interacting with Ku protein already present at the ends (Chen et al., 2000). *XRCC5* and *XRCC6* encode the 70- and 80-kDa subunit of Ku, and *XRCC7* encodes the catalytic subunit of DNA-dependent protein kinase (DNA-PKcs). In mammalian system, it has been shown that DNA-PK is involved in the major pathway for repairing DSB (Jeggo et al., 1995). DNA-PK is a heterotrimer consisting of a 450-kDa catalytic subunit (DNA-PKcs) and Ku80 and Ku70 regulatory subunits (Anderson, 1993; Tuteja et al., 1994, Tuteja and Tuteja, 2000). The Ku80 and Ku70 of DNA-PK bind to DNA ends (broken ends), which activate the kinase activity of DNA-PKcs. The DNA-PKcs is a serine/threonine protein kinase and can phosphorylate a variety of substrates *in vitro* (Tuteja and Tuteja, 2000). Finally, the Ku80 and Ku70 bring the DNA broken ends together and then DNA ligase joined the ends (Figure 7). The end-binding activity of Ku serves as an entry site for other proteins and the phosphorylation activity of DNA-PKcs activate other repair factors (Tuteja and Tuteja, 2000). DNA-PK is the only factor known to participate in NHEJ. DNA-PKcs is a member of the phosphatidylinositol 3-kinase (PI3K)-related kinase family, as are ATM and ATR. All three proteins exhibit a strong sequence homol-

ogy to the PI3Ks, especially in the catalytic core domain that binds and phosphorylates the phosphoinositol head group of phosphtidylinositol. However, no phosphorylation of lipid substrates has been observed by DNA-PKcs or by other members of this PI3K-related family of kinases (Carpenter and Cantley, 1996; Wymann and Pirola, 1998).

The mutants deficient in Ku or DNA-PKcs are deficient in the repair of DSBs and also sensitive to lethal effects of ionizing radiation. A new activity of Ku as an unwinding enzyme was also reported (Tuteja et al., 1994; Tuteja et al., 1997). The analog of Ku in plant has not been reported yet. However, we have shown through Western blotting that the Ku or Ku-like protein are also present in plant (Tuteja and Tuteja, 2000). However, by using human Ku antibodies and pea cDNA library, we got the positive clone that later turned out to be nonspecific and actually was the clone of calnexin, a Ca^{2+}-binding molecular chapareon protein (Ehtesham et al., 1999).

In plants NHEJ has been analyzed in a more direct and thorough manner. Linearized plasmid DNA has been introduced into tobacco cells, and novel joints that formed between the plasmid ends have been sequenced (Gorbunova and Levy, 1997). In another study, DSBs were induced at an I-SceI recognition site, within a negative selectable marker genome on a chromosome, and new junctions were sequenced (Salomon and Puchta, 1998). Gorbunova and Levy (1997) summarized the results of these studies as follows:

- End joining by simple ligation with no sequence alteration is rare.
- End-joining is usually associated with deletions ranging from 1 bp up to > 1 kb.
- Rejoining frequently occurs at short repeats.
- A distinct feature of the junctions is the presence of relatively large insertions, up to 1.2 kb long.

The filler DNA could consist of a simple insertion corresponding to a plasmid or genomic sequence. Insertions might also have a complex structure consisting of a patchwork of different plasmid and tobacco genomic sequences.

In response to DSB, different repair routes can be taken. The break can also be simply ligated, without any modification of the ends or different events can happen at the broken site, such as exonuclear degradation or exposure of 3′-ss end, which was proposed to be the first step of DSB repair pathway in plants. When annealing of the exposed ends occurs at sites of microhomology, as often found in plants (Gorbunova and Levy, 1997; Salomon and Puchta, 1998), this results in a deletion of the region between the microrepeats. This process is reminiscent of the single strand annealing (SSA) mechanism, which was also proposed for extrachromosomal homologous recombination events in plants (Puchta and Hohn, 1991). The outcome of DSB repair is also the presence of templated filler DNA at the repair site (Gorbunova and Levy, 1997). Formation of such filler DNA can be best explained by the synthesis-dependent strand annealing (SDSA) mechanism that involves template invasion and DNA synthesis (Gorbunova and Levy, 1999). The SSA and SDSA appear to be the two prominent mechanisms for DSBs repair in plants. They can be used either for homologous or nonhomologous recombination.

G. Mismatch Repair (MMR)

Because of the errors of replication or homologous recombination, mismatched bases can be produced (Friedberg et al., 1995; Friedberg and Wood, 1996). The MMR basically discriminates between the correct and incorrect bases and finally corrects the error after DNA synthesis. Most of the present understanding of eukaryotic MMR has come from studies of the eukaryotic homologs of the bacterial MutS and MutL proteins (Kolodner, 1996; Kolodner and Marsischky, 1999). The model of MMR in *E. coli* is shown in Figure 8. In *E. coli* the MutS dimer recognize the mispairs and then bind on it (Grilley et al., 1989) followed by MutL binding, which activates MutH (endonuclease) that makes a single-strand incision. This incision by MutH can be on either side of the mismatch (Au et al., 1992). Subsequently, the excision initiates at the incision (nick) and proceeds toward the mismatch (Grilly et al., 1993). To fill the gap (100 to 1000 nucleotide gap) the original template strand can then be replicated and finally sealed by ligation (Figure 8). The proteins involved in the last step of eukaryotic MMR are DNA pol δ, RP-A, PCNA, and RFC (for a review see Kolodner, 1996; Kolodner and Marsischky, 1999).

The homologs of MutS and MutL have been reported in yeast and human (Modrich, 1994; Drummond et al., 1995; Palombo et al., 1995; Hunter et al., 1996; Johnson et al., 1996; Palombo et al., 1996). In plant, the homolog of MutS was reported from *Arabidopsis* (Culligan and Hays, 1997), but the function has not been determined yet.

IV. ROLE OF DNA HELICASES IN DAMAGE AND REPAIR

The DNA helicases are ATP-dependent DNA unwinding enzymes and thus play an important role in repair as well as in replication, recombination, and transcription (Tuteja and Tuteja, 1996; Tuteja, 1997). The unrepaired DNA lesions can affect the outcome of all these processes. The helicases are among the first proteins that would encounter DNA damage during all the processes above. The interstrand or intrastrand DNA lesions can block or impair the DNA helicase action. The XPB and XPD are repair helicases that are required for local unwinding of the damage area of duplex DNA in NER in the mammalian system (Schaeffer et al., 1993; Schaeffer et al., 1994). During DNA repair, especially in NER, the damaged area on DNA has to unwind first in order to get proper repair, because most of the DNA repair machinery enters through single-stranded DNA. This single-stranded DNA is provided transiently by the DNA repair helicase(s). This unwinding process by DNA helicases is an energy-requiring process that comes through the hydrolysis of ATP by the intrinsic DNA-dependent ATPase activity of the helicase protein itself (Tuteja, 2000).

DNA helicases generally bind to ssDNA or ss-/ds-DNA junctions and translocate unidirectionally along the bound DNA into the duplex region, disrupting the hydrogen bonds linking the two strands (Tuteja and Tuteja, 1996). The XPB can unwind DNA in the 3′ to 5′ direction (Schaeffer et al., 1993; Roy et al., 1994) similar to previously reported human DNA helicases (HDH) I, II, III, V, and VI (Tuteja et al., 1990; Tuteja et al., 1992 to 1995), pea chloro-

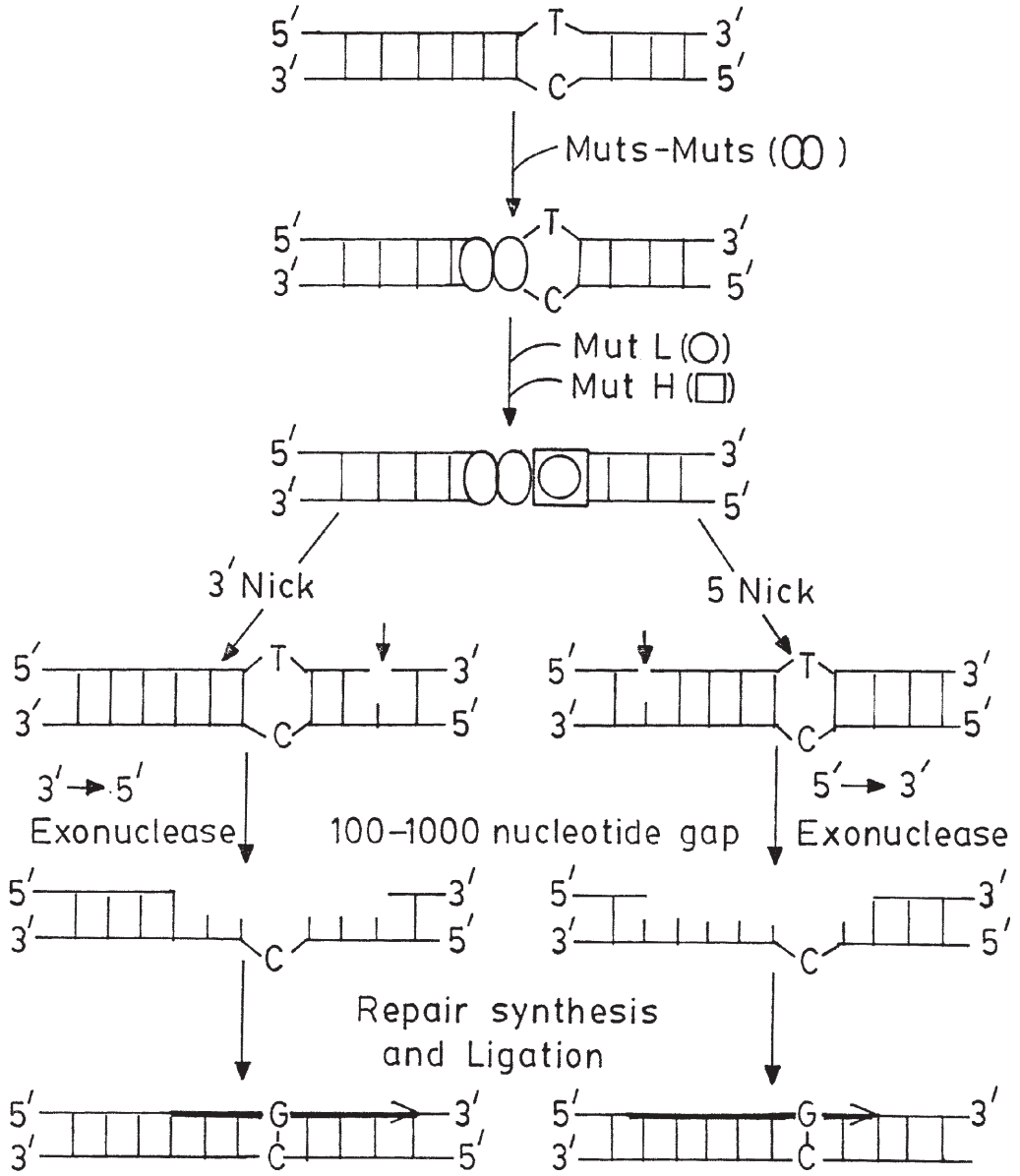

FIGURE 8. A model for mismatch repair in *E. coli*. The eukaryotic process is believed to share many of the features shown here.

plast DNA helicases I and II (Tuteja et al., 1996; Tuteja and Phan, 1998), pea nuclear DNA helicase 45 (Pham et al., 2000), and pea nuclear DNA helicase 65 (Tuteja et al., 2001). However, XPD unwinds the DNA opposite to XPB polarity, that is, in 5′ to 3′ direction (Sung et al., 1993), which is similar to HDH IV (Tuteja et al., 1991). In order to better understand NER in plant, it is important to isolate the DNA repair helicases along with other components. These repair helicases could be analogs of XPB and XPD that need to be cloned in an expression vector for purification of the encoded proteins. Furthermore, these proteins need to be characterized in detail. XPC-hHR23B may assist this strand separation by interacting with TFIIH (Drapkin et al., 1994). The XPB and XPD both contain the seven conserved domains (helicase motifs) of the super-

family of DNA and RNA helicases (Gorbalenya and Koonin, 1993; Tuteja, 2000). However, the sole presence of helicase motifs in the protein may not prove that it is a helicase. For example, an *E. coli* Mfd protein (a strand-specific repair protein) (Selby and Sancr, 1993) and a human Rad54 protein (Swagemakers et al., 1998) both contain conserved helicase motifs but lack the unwinding activity. So a direct biochemical demonstration of ATP-dependent DNA or RNA unwinding activity is essential before a protein is classified as helicase (Tuteja, 2000).

In *E. coli* the Uvr_2B and UvrD are the repair DNA helicases involved in NER. The DNA unwinding activity of UvrD helicase was shown to be exquisitely sensitive to major groove DNA intercalators (George et al., 1992), while it was moderately sensitive to UV radiation. In general, the UV-induced lesions or other helix-distorting intrastrand adducts are better tolerated by DNA helicases than those produced by intercalating agents. However, in yeast *S. cerevisiae* the Rad3 protein, a repair helicase, is absolutely required in NER. It is found to be inhibited by UV damage and intrastrand cisplatin adducts located on the strand along which the enzyme translocates, whereas lesions on the opposite strand had no effect (Villani and Tanguy Le Gac, 2000). Calf thymus DNA helicase E is a 3′ to 5′ helicase that can unwind DNA duplex from nicks and has been suggested to play a role in DNA repair (Turchi et al., 1992).

Ku protein is another DNA helicase (human DNA helicase II) that plays an important role in double-strand break repairs (Tuteja et al., 1994; Tuteja and Tuteja 2000). Ku is a heterodimeric protein composed of ~70 and ~80 kDa subunits (Ku 70 and Ku 80) originally identified as an autoantigen recognized by the sera of patients with autoimmune diseases. It is an abundant nuclear protein found in vertebrates, insects, yeast worms, and also in plant (Tuteja and Tuteja, 2000). Ku contains ssDNA-dependent ATPase, DNA end binding, and ATP-dependent DNA unwinding activities (Tuteja et al., 1994). It prefers to unwind the partially, unwound DNA substrates and moves in the 3′ to 5′ direction on the bound strand (Tuteja et al., 1994). Ku also functions as a regulatory subunit of DNA-PK that phosphorylates many proteins, such as p53, SV-40 large T antigen, RP-A, hsp90, c-jun, C-Fos, Sp-1, C-Myc, TFIID, etc. (Tuteja and Tuteja, 2000). The DNA unwinding activity of Ku was found to be severely inhibited by DNA intercalating agents such as nogalamycin, daunorubicin, actinomycin, and ethidium bromide (Tuteja et al., 1997).

In general, intrastrand DNA damage seems to affect DNA unwinding activity of the helicase *in vitro* when placed on the strand to which the protein translocates with exception to the SV-40 T antigen and RecA helicases (Villani and Tanguy Le Gac, 2000). The DNA helicases involved in initial stages of NER are more sensitive to DNA damage (e.g., UvrAB, Rad3, or XPD) when compared with the helicases that are involved in the subsequent removal of the damaged DNA (e.g., UvrD).

V. DESSICATION-RELATED DAMAGE, TOLERANCE, AND DNA REPAIR

Plant seed, the product of fertilization, during its final stages of development and maturation undergoes desic-

cation, resulting in a significant reduction in its water content. In addition, metabolic activities are also reduced to undetectable levels, leading seed embryo in quiescent state. Under low temperature and moisture, seed can now be stored for long periods until it is reactivated by favorable seed germination conditions. Seed viability loss has been shown to result in a loss of membrane integrity, impaired RNA, and protein synthesis and ATP production (Priestley et al., 1985; Bailly et al., 1996). Ultrastructural, biochemical, and biophysical studies have shown that the loss of desiccation tolerance is correlated with an irreversible alteration to the physical properties of membrane lipid bilayers, increased liquid crystalline-gel phase transition temperature, deesterification of phospholipids, and an accumulation of several products of peroxidized lipids. Observations that lipid-rich seeds tend to have a limited longevity tend to suggest that seed aging based on lipid peroxidation is important (Priestley et al., 1985).

Free radicals and lipid peroxidation are considered to be major cause of seed deterioration (Leprince et al., 1996). Ungerminated seeds and pollen are known to contain and accumulate free radicals during artificial aging (Priestley et al., 1985) and germinating seeds after dehydration (Senaratna and McKersie, 1986). Changes in lipid peroxidation and in the molecular defenses against the activated forms of oxygen have also been observed in dry stored seeds and seed after accelerated aging. The loss of seed viability has been correlated with the MDA (malondialdehyde) content, a product of lipid oxidation. Reuzeau and Cavalie (1995) found a significant difference in the MDA content of seeds able or unable to germinate. A higher lipid peroxidation was found in seeds unable to germinate. It has been suggested that secondary toxic products produced as a result of oxygen-dependent reactions in imbibed seeds by the accumulation of oxygenated fatty acids could participate in altered cellular components leading to a decrease in seed viability (Gidrol et al., 1989; Reuzeau and Cavalie, 1995). When such seeds are hydrated, toxic oxygen products, if they are not efficiently detoxified, can allow damaging reactions and prevent seed germination. Because dry seeds are affected in their ability to detoxify O_2-related toxic products, it seems reasonable to suggest that important changes in soluble and membrane entities might interfere in the signal transduction necessary for the initiation of germination process (Reuzeau and Cavalie, 1995). Similar conclusions on the role of lipid peroxidation were drawn when accelerated aging of seed was studied (Bailly et al., 1996). Nonviable seeds with accelerated aging induced the accumulation of malondialdehyde, indicating that seed deterioration was associated with lipid peroxidation. No direct correlation between lipid peroxidation and deterioration in membrane integrity was found. However, loss of seed viability was associated with a decrease in superoxidase dismutase, catalase and glutathione reductase (GR) activities. This study established a clear relationship between the loss of detoxifying enzymes such as catalase, and GR, lipid peroxidation, and seed deterioration during accelerated aging. Whether oxidative damage and decrease in SOD, catalase, and GR activities are the cause or consequence of the loss of seed viability was not established (Bailly et al., 1996).

The desiccation tolerance mechanisms allow cells to withstand the loss

of the major percentage of its water content and to survive in a dry state for prolonged periods. The seeds, spores, wind-dispersed pollen, and specialized 'resurrection' species can withstand desiccation without incurring cellular damage, and they naturally dehydrate to moisture contents that will result in cell death in other plant tissues.

The role of free radicals in loss of desiccation tolerance in germinating seed has also been studied (Leprince et al., 1990). Desiccation of 24 h during germination resulted in a significant increase in lipid peroxidation in the dehydrated tissues, and rehydration not only fails to reduce lipid peroxidation but resulted in a 15-fold increase in MDA accumulation, indicating that desiccation treatment results in irreversible peroxidative damage to lipids. It is not clear whether oxygen radicals are the cause of or a result of the damage following desiccation, but it is well established that desiccation alters the properties of membranes (Senaratna and McKersie, 1986). A desiccation-induced rise in lipid peroxid-ation originates from oxygen radicals and a loss of desiccation tolerance is a consequence of increased formation of one or more forms of activated oxygen coupled with decreased protection from SOD and peroxidases. It is now clear that damages to DNA are harmful, which is why the repair of DNA damage is of vital importance to the cell in order to keep its normal function.

A. In Bacterial Spores

Desiccation tolerance of bacterial spores depends on the conversion of B-form DNA to a more condensed A-form (Setlow, 1992a,b). The DNA in dormant spores of *Bacillus* species is associated with alpha/beta-type, small acid-soluble proteins (SASP), which are double-stranded DNA-binding proteins whose amino acid sequence has been highly conserved in evolution. The expression of these proteins occurs at a specific stage of spore maturation when the B-form DNA duplexes lose water and are converted to A-form conformation (Setlow, 1992a,b). These proteins bind to A-form, conferring stability on the DNA during the final stages of dehydration and through the dehydrated stage. These proteins are released by the action of the sequence-specific protease (termed GPR) that degrades SASP during germination. These spore protease of *Bacillus* are synthesized during sporulation as an inactive precursor termed P46. The activation of this protease during rehydration leads to the removal of SASPs and the return of the DNA to the B-form (Illadas-Aguiar and Setlow, 1994). The saturation of spore DNA with SASP provides protection against DNA base loss due to wet heat (i.e., de-purination) and breakage of DNA single-strands by desiccation and hydrogen peroxide. The spores lacking a/b SASP are much more sensitive to desiccation, dry and wet heat, and hydrogen peroxide than are wild-type spores. All these treatments have been shown to kill spores in large part by damaging DNA (Setlow and Setlow, 1996). Binding of a/b type SASP also helps in conferring resistance to UV damage (Setlow and Setlow, 1996).

The major deleterious DNA photoproduct generated by UV irradiation of growing cells is a cyclobutane-type dimer between adjacent thymine residues (termed TT), but TT is not formed

in spores. The major UV photoproduct formed in spore DNA is a thymine-thymine adduct termed the spore photoproduct (SP), which is also formed between adjacent thymine residues. SASP deficient spores are ~30 times more UV sensitive than the wild-type spores, and UV generates a large amount of TT in deficient spores. According to Setlow and Setlow (1996), the generation of TT is the cause for the increased UV sensitivity of mutant spores. These authors examined the expression of genes involved in DNA repair during germination and outgrowth of both wild type and a-b-spore, which were exposed to a variety of treatments. This study has shown that the repair of DNA damage during spore outgrowth is an important component of spore resistance to a number of DNA-damaging treatments. The SASP-type proteins have not been identified in plants as such. However, other proteins may also play an important role in survival of dehydrated seeds and pollen. Dehydrinds and LEA-type proteins may have some role in desiccation tolerance.

The *Deinococcus radiodurans* is a non-spore-forming bacterium that exhibit a remarkable capacity to resist the lethal effects of ionizing radiation. It has been reported that *D-radiodurans* ionizing radiation resistance is incidental, a consequence of this bacterium's adaptation to dehydration (Mattimore and Battista, 1996). The authors showed that *D. radiodurans's* cellular responses to ionizing radiation and dehydration significantly overlap by showing that 41 ionizing radiation-sensitive strains of *D. radiodurans* were also sensitive to desiccation. It was also inferred that loss of DNA repair ability in the ionizing radiation-sensitive strains was responsible for the desiccation sensitivity of these strains.

B. In Seeds

It has been known for more than 2 decades that a feature of loss of viability in stored dry seed is the increasing loss of integrity of nuclear DNA with increasing levels of low-molecular-weight oligonucleotide fragments (Cheah and Osborne, 1978, Elder et al., 1987; Elder and Osborne 1993; Leprince et al., 1996). When rye embryos that are nonviable are imbibed for 2.5 h the extent of fragmentation of DNA shows a significant increase pointing toward the continuous activity of endonuclease cleavage in the absence of DNA repair (Cheah and Osborne, 1978; Elder et al., 1987). Embryos of imbibed dormant *A. fatua* also showed an efficient repair of radiation-induced lesions in the DNA of dry seeds, and this DNA repair also remained stable when embryos were dried back. On the basis of results from various experiments, Boubriak et al. (1997) have suggested that the failure to restore genetic integrity to the genome on rehydration of an embryo whose DNA is desiccation damaged could be a major factor in determining desiccation tolerance. The ability to repair DNA when embryos are rehydrated is critical to maintaining a transcriptionally functional genome.

Elder et al. (1987) have shown accumulation of single- and double-strand breaks in the embryos of seeds during the loss of viability in the dry state. In contrast to hydrated cell, which can effectively repair DNA damage, the nuclei of dry cells have to accumulate DNA damage until rehydration activates DNA repair machinery. The stability of the DNA on desiccation and the capacity for repair of DNA on rehydration have been suggested as integral components

of a total desiccation tolerance mechanism.

C. DNA Integrity in Pollens

Osborne and Boubriak (1994) have shown that dry birch pollen (14% moisture content) can be fully hydrated in moist air and dehydrated back to the original dry weight without the loss of germinability and without the fragmentation damage detectable in the extracted DNA. At the time of anther dehiscence, birch pollen is haploid and bi-nucleate and arrested at the G2 stage of the cell cycle. The authors addressed the question whether there is evidence for DNA repair during the air hydration of birch pollen. Pollen that has been exposed to increasing durations of hydration in moist air prior to the transfer to the liquid germination medium showed similar levels of H3-methyl thymidine. It was suggested that an excision repair measured as unscheduled DNA synthesis does not occur during the relatively low level of hydration possible in moist air. As pollen gets exposed naturally to UV in solar radiation following release from the anther, it is the likely candidate for UV-induced heritable mutations.

The role of DNA repair in the desiccation tolerance of pollen and spores is not unlike that ascribed to DNA repairs in bacterial spores (Setlow, 1992a,b). In their dehydrated state, pollen and seed continue to accumulate DNA damage that cannot be repaired until the rehydration occurs. The low water content in the desiccated stage prevents enzymatic activity and therefore DNA repair. No evidence of DNA repair has been observed in dry pollen or seed. Thus DNA damage cannot be repaired until pollen and seed are rehydrated. Given the DNA damage that may accumulate in pollen and spores during the dormant period, they may contain high levels of enzymes needed for DNA repair.

Jackson and Linskens (1978) made comprehensive analysis of DNA repair system in Petunia pollen. The mature pollen of Petunia carries generative nucleus blocked at G2 stage of cell cycle. The addition of 4-nitroquinolide 1-oxide to the liquid germination medium led to unscheduled DNA synthesis in pollen. Mature Petunia pollen is bicellular and thus has two nuclei, that is, generative and vegetative nuclei. In these studies on Petunia pollen it was not feasible to discern whether the repair synthesis takes place in either or both nuclei of pollen. It was shown that unscheduled DNA synthesis occurs after ultraviolet radiation. As this unscheduled DNA synthesis was not affected by hydroxyurea, an agent used to discriminate between replicative and repair synthesis, the authors suggested that dark repair of DNA takes place in germinating pollen of *Petunia* (Jackson, 1987).

VI. DNA REPAIR GENES IDENTIFIED BY SEQUENCE HOMOLOGY

Recently, Wood et al. (2001) have described a catalog of 130 known human repair genes on the basis of function or sequence homology to known repair genes. Here we are mainly describing the plant repair genes found on the basis of sequence homology. The completion of the first plant genome sequence of *Arabidopsis thaliana* is a landmark of twentieth century in plant research (The *Arabidopsis* Genome Ini-

tiative, 2000). The information it contains will certainly give a boost to every area of plant science, including DNA repair in near future. *A. thaliana* is a modest little flowering brassica plant choosen first for plant genome sequence because it has many advantages for genome analysis, including a small size, large number of offspring, a short generation time, and a relatively small nuclear genome. In 1977 William Curtis, a British botanist and apothecary, described *A. thaliana* in his flora landinensis as a plant of "no particular virtues or uses", but now with the report of its genome sequence it becomes the most significant plant in the kingdom (Dennis and Surridge, 2000). The sizes of the *Arabidopsis* genome of nuclear, plastid and mitochondrial are 125 Mb, 560 kb, and 367 kb, respectivley. It has five chromosomes (chromosomes 1 to 5) with respective sizes of 29.1, 19.6, 23.2, 17.5, and 26.0 Mb. The evolution of *Arabidopsis* involved a whole-genome duplication, followed by subsequent gene loss and extensive local gene duplications. The nuclear genome contains 25,498 gene encoding proteins from 11,000 families, which is the largest gene set published to date (The *Arabidopsis* Genome Initiative, 2000).

Comparing the *Arabidopsis* genome with other species (Eisen and Hanawalt, 1999) indicated that *Arabidopsis* has a set of DNA repair and recombination (RAR) genes similar to most other eukaryotes. The pathways represented include PR, DNA ligation, NHEJ, BER, MR, NER, and many aspects of DNA recombination. The *Arabidopsis* RAR genes include homologues of many DNA repair genes that are defective in different human diseases. For example, hereditary breast cancer and nonpolyposis colon cancer, *Xeroderma pigmentosum* (*D-XPD, F-XPF, B-ERCC3*), *Ataxia telangiectasia* (*ATM*), and Cockayne's syndrome (*CSA, CSB*) (The *Arabidopsis* Genome Initiative, 2000). The presence of additional homologues of many RAR genes was found in *Arabidopsis* genome. This is seen for almost every major class of DNA repair, including recombination (four *RecA*), DNA ligation (four *DNA ligase I*), PR (five class I photolyase and one class II photolyase), and NER (six *RPA1*, two *RPA2*, two *Rad25*, three *TFB1*, and four *Rad23*). Regarding BER, *Arabidopsis* contianed 16 homologues of DNA base glycosylates, which is the maximum number known for any species. It also contains three homologues of AP endonuclease. Some of the extra copies of RAR genes originated through relatively recent gene duplication because many of the sets of genes were more closely related to each other than to their homologues in any other species. The presence of duplicate (paralogous) genes might be responsible for greater repair capacity.

The multiplicity of RAR genes could also be partly due to the transfer of genes from the organellar genomes to the nucleus. Repair gene homologues that appear to be of plastid origin include the recombination protein RecA, RecG, and SMS, two class I photolyase homologues, Fpg, two MutS2 proteins, and the transcription repair coupling factor Mfd. The homologues of *E. coli* repair gene *UvrABC* was found to be absent in *Arabidopsis*. As *Arabidopsis* showed many similarities to the set of DNA repair genes found in other eukaryotes, it will not be difficult to determine the functions of these gene-encoded proteins in the plant system (The *Arabidopsis* Genome Inititative, 2000). Recently, the *A. thaliana Rad50* homologue (*AtRad50*) has been isolated and shown to be lo-

cated on chromosome 2 (Gallego et al., 2001). The Rad50 protein is involved in the cellular response to DNA double-strand break (DSBs), including the detection of damage, activation of cell-cylce checkpoints, and DSB repair via recombination. Gallego et al. (2001) have shown that Rad50 homologue protein is involved in meiosis and DNA repair in plants.

VII. CONCLUSIONS AND FUTURE PROSPECTS

The survival of organisms depends on the accurate transmission of genetic information from one cell to its daughters. Such faithful transmission requires not only extreme accuracy in the replication of DNA and the precision in chromosome distribution, but also the ability to survive spontaneous and induced DNA damage while minimizing the number of heritable mutations. To achieve this fidelity, cells have evolved several DNA repair mechanisms that can monitor the structure of chromosome and coordinate repair and cell-cycle progression. These repair mechanisms also help in maintaining the genome integrity and in regulating mutation rates. If the DNA damage is not repaired properly, it can lead to various complications, including cell death or mutations. The mutagenicity may be caused by persistent, unrepaired adducts that are subsequently converted to mutations through DNA replication or to mutation fixation through misrepair.

In general, the damaged proteins and RNA molecules can be replaced using information encoded in the DNA, but the DNA molecules themselves are irreplaceable. Therefore, the damaged DNA has to be repaired. DNA repair is possible largely because the DNA molecule consists of two complementary strands. DNA damage in one strand can be removed and accurately replaced by using the undamaged complementary strand as a template. DNA can become damaged by a variety of means, some spontaneous, others catalyzed by environmental agents or defect in replication, which can leave mispaired bases. The chemistry of DNA damage is diverse and complicated. Consequently, the cellular response to this damage includes a wide range of enzymatic systems that catalyze some of the most interesting chemical transformations in DNA metabolism.

During the last several years, considerable progress has been made in elucidating the components of the eukaryotic DNA damage response and DNA repair pathways. How damaged DNA activated DNA damage response proteins is a question of central importance to be addressed in near future. In principle, a sensor protein should have the ability to interact with damaged DNA. As there are many ways to damage DNA, it is important to determine whether there is a sensor for each type of damage. If the damage itself is sensed through the interaction with a sensor protein, this sensor protein may potentially interfere with repair proteins that also need access to damaged DNA. The cell must be aware not only of damage, but also of when the damage is repaired, because the completion of repair should be the signal for termination of the damage response. Further work is needed to define the activation of damage-signaling kinases. Another area ripe for future exploration is the relationship between the DNA damage signaling pathway and telomeric DNA structures. Any DNA

lesions that block the movement of DNA and RNA polymerases may kill cells by interfering with replication in actively growing and dividing cells or by blocking transcription and thus depriving nondividing cells of an essential protein. Current models dealing with replication of damaged DNA postulate the initial dissociation of replicative DNA polymerases when encountering sites of DNA damage. Replication can then restart beyond leisons, or DNA polymerases specialized in translesion synthesis can temporarily replace replicative polymerases to synthesize across the lesion; replicative enzymes then take over again. The DNA helicase can help in this bypass reaction; however, this helicase-mediated bypass hypothesis is still speculation.

In modern biology the DNA repair study occupies a central place. The DNA repair field in eukaryotes has a strong connection with replication, transcription, recombination, and cell cycle control and checkpoints (G1/S and G2/M). Checkpoints are mechanisms that allow cells to deal with DNA damage or other insults. A major role of checkpoints is to delay cell cycle transitions in order to allow time for the damage to be repaired. Therefore, it is important to understand how checkpoints regulate the basic cell cycle machinery. The overall progress of research on DNA repair in plant cell has been very slow when compared with other systems. However, now this field has started progressing fast in plants especially after the isolation of several repair protein and genes. However, the twentieth century began with the rediscovery of Mendel's rules of inheritance in pea (De Vries, 1900), and it ends with the elucidation of the complete sequence of *Arabidopsis*. The analysis of completed sequence provides insights into the genetic basis of the similarities and differences of diverse multicellular organisms. Regarding DNA repair genes, it appears that they are highly conserved between plants and mammals than within the animal kingdom, perhaps reflecting common factors such as DNA methylation.

DSB repair in plants is error prone, with the main pathway of repair being illegitimate recombination. Such an error-prone DNA repair mechanism might have an important impact on plant evolution. In plant the DSB repair products have largley been characterized as excision products of transposable elements or insertion products of T-DNAs. These products are similar to mammalian DSB repair products, which also suggests that plant DSB repair is similar to mammals. There is an urgent need to study the Ku and DNA-PK in plants in order to understand the mechanism of DSB repair. Still there is a need to identify all the repair genes in plants as reported in other systems. One way of identifying the genes involved in DNA repair is to study mutants that are sensitive (or resistant) to DNA-damaging agents. The identification of mutants that are selectivley altered in only one type of DSB repair will shed light on the interrelations between the different DSB repair pathways. It might also allow the selective activation of a homologous pathway to achieve gene targeting.

There is still much to be done. Various models proposed for repairs are largely hypothetical and require further experimental support. Cloning of all the repair genes and studying knockouts or overexpression of these genes could provide a solution for a longstanding problem of gene targeting in plants. Further, the crystallographic studies of DNA repair enzymes will reveal a wide range of

structural motifs used to recognize specific lesions in DNA. These studies will also help to unravel the catalytic mechanism by which these lesions are repaired. We hope that in future new technologies, such as gene machines for reverse genetics, sequence analysis of whole plant genomes, and utilization of DNA chips, will give a boost to unravel the mystery behind DNA repair mechanisms. Also in the near future proteomics will provide a wealth of protein-protein interaction data, which will add to the knowledge of repair proteins function. The challenge for future research is to uncover the exact molecular mechanisms in plant DNA repair system through which these repair proteins repair the DNA damage in cooperation with other proteins. More research is needed in plants to answer the many questions that still surround the repair proteins and their many targets.

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