

Chapter 7

DNA Damage, Response, and Repair in Plants Under Genotoxic Stress

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Abstract Several environmental and endogenous factors create a variety of lesions in the genome of an organism. These lesions could potentially be genotoxic and might lead to mutations, which could be lethal. All organisms exhibit a prompt response against DNA damage, which is referred to as the DNA damage response. The existence of elaborate, evolutionarily conserved systems to repair the damage, mostly at the expense of huge amount of energy, points to the importance of safeguarding the integrity of DNA. Most of the current understanding about DNA damage response and repair pathways has been distilled through decades of research on prokaryotes, yeast, and mammalian systems. The response to genotoxic stresses and the repair mechanisms involved in plants has only recently begun to be investigated. Herein, we present a comprehensive account of the types of DNA damage, the DNA damage response, and the repair pathways with reference to the recent insights gained from the plants. Although, the underlying common theme runs through to the plants, the mechanisms in plants demonstrate some unique features. Also, there are several missing links that need to be unravelled for inferring a complete picture. A thorough understanding of the mechanisms involved would aid in devising strategies to help plants avoid irreparable damages to their genome upon exposure to genotoxic agents.

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Introduction

The genome contains the blueprint of life. Protecting the sanctity of the information in DNA is a cellular imperative for maintaining and perpetuating life. Though intrinsically more stable than other macromolecules, DNA is nonetheless prone to damages that can compromise its function and the eventual fate of an organism. A variety of processes, some endogenous and other catalyzed by environmental agents, can undermine the integrity of DNA. The endogenous sources of damage include metabolic by-products and stalled replication forks while environmental agents like ultraviolet (UV) and ionizing (IR) radiation and chemical mutagens are the exogenous ones. To combat the DNA damage, organisms contain elaborate cellular networks termed the DNA damage response (DDR) that are critical to maintain the integrity of the genome (Ciccia and Elledge 2010). The components of this response include processes to detect the damage, to relay the signal to specific regulators in the cell, and to elicit the production of effector molecules (Vespa et al. 2005; Culligan et al. 2006). The orchestration of these processes ultimately leads to the repair of DNA lesions or the induction of cellular death, if the damages are beyond repair. The DDR pathways are fundamental to life and most of them have been conserved through the evolution. Much of the current understanding of these mechanisms derives from the research done in prokaryotic, yeast, and mammalian systems. The delineation of DDR in plants has only been attempted lately.

Plants are continuously exposed to environmental, edaphic, and other anthropogenic stresses. These include extremes of temperature, drought, UV-B, IR, as well as air and soil pollutants. In addition to severely impacting the structural, enzymatic, and nonenzymatic components of plants, these stresses potentially threaten the plant genomes (Wasi et al. 2013). Unrepaired DNA damages can lead to mutations, which can impact the stability of a plant's genomes, its growth and productivity, and might threaten the survival of the plant (Singh et al. 2010; Biedermann et al. 2011). Being sessile, plants cannot evade these stresses and combat is the only alternative for their survival and growth. It is, thus, imperative that an efficient and specific DDR system be in place in plants to cope with DNA damage (Yoshiyama et al. 2013). The sequencing of several plant genomes has given huge impetus to the study of DDR in plants, with many components of the system having been discovered in plant genomes (Mannuss et al. 2012). Although the basic mechanism remains conserved in eukaryotes, several plant-specific regulators have been reported, alluding to the existence of some unique DDR systems in plants.

In this chapter, we have endeavored to summarize the current understanding of DDR in plants. The following sections in the chapter provide details on the DNA damaging agents, types of DNA damage, mechanism of genotoxic stress perception, transduction of the signal, and repair of the damage.

DNA Damaging Agents

Different intrinsic and extrinsic factors, classified either as physical or chemical, are capable of damaging the genetic material.

Physical Agents

Ultraviolet and ionizing radiation are the most common physical factors contributing to DNA damage. The innocuous sunlight, essential for photosynthesis in plants, also contains radiations that are potentially genotoxic. These energy-rich radiations include UV-C (100–280 nm), UV-B (290–320 nm), and UV-A (320–400 nm). UV-C is filtered out by absorption in the upper stratospheric and ozone layers but UV-B and UV-A enter the atmosphere, their amount reaching the earth's surface dependent on latitude and elevation, as well as the cloud cover and canopy density. UV-B, on account of its higher energy, is potentially more deleterious to living organisms. Besides damaging proteins and biomembranes, UV-B is strongly mutagenic (Pang and Hays 1991; Britt 1999). It is absorbed by the DNA and may lead to the generation of cyclobutane pyrimidine dimers (CPDs) and to a lesser extent pyrimidine (6-4) pyrimidone dimers (6-4 PPs; Friedberg et al. 2006). These lesions affect the transcription and also result in error-prone replication. Besides, UV light is strongly absorbed by many environmental organic contaminants, thereby, increasing their toxicity (Huang et al. 1993). The UV-A and visible spectrum of light cannot be absorbed by the native DNA. However, they can still damage DNA through reactive oxygen species (ROS) generation via photosensitizing reactions (Alscher et al. 1997; Iovine et al. 2009). ROS can induce a variety of DNA damages like base and nucleotide modifications, especially in guanine-rich sequences and may even cause strand breaks (Wiseman and Halliwell 1996; Tuteja et al. 2001; Tuteja and Tuteja 2001).

IR is another major mutagen that damages DNA, either directly or indirectly through the production of free radicals (Ward 1975). Chromosomal breaks, inversions, duplications, and translocations can result from double-strand (DSBs) and single-strand breaks (SSBs) caused by the absorption of IR by the sugar–phosphate backbone in the DNA. The indirect effect of IR is mediated through the radiolysis of water which generates OH radicals, the most damaging of all the ROS that attack the DNA along with proteins, lipids, and other cellular constituents.

Chemical Agents

Chemically, genotoxic agents could either be inorganic or organic. Heavy metals like Cd^{2+} , Cu^{2+} , Ni^{2+} , and Pb^{2+} are the main inorganic genotoxic compounds which cause the generation of free radicals that damage the DNA (Imlay and Linn 1988).

Metal ions can also influence the efficiency and fidelity of DNA replication. Ions like Ni^{2+} and As^{3+} change the stability of DNA by altering their methylation pattern (Davis et al. 2000).

Genotoxic organic compounds can affect the DNA in different ways. Alkylating agents like ethylmethane sulfonate (EMS) and methylmethane sulfonate (MMS) cause DSBs while aromatic compounds such as polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) are mutagenic because they intercalate between the DNA strands.

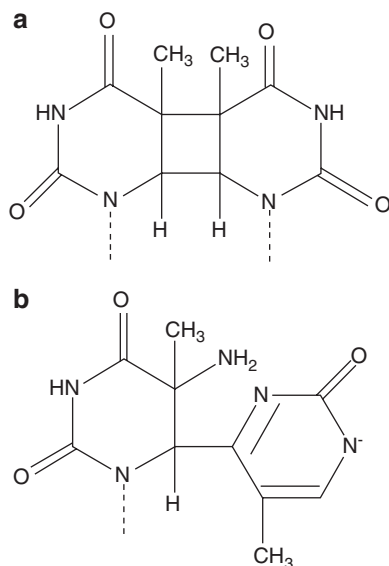
Types of DNA Damage

DNA damage caused by physical or chemical agents can be broadly classified into three types of lesions: mismatched bases, double-strand breaks, and chemically modified bases. The damages induced by the different agents are discussed below.

UV-Induced DNA Damage

A major type of DNA damage induced by UV-B is the formation of dimers between adjacent pyrimidines—the CPDs and 6-4 PPs (Mitchell and Nairn 1989; McGregor 1999; Fig. 7.1). CPDs can occur in any of the diastereoisomeric forms, i.e., cis/trans

Fig. 7.1 Pyrimidine dimers formed upon UV-B irradiation. Structure of a cyclobutane pyrimidine dimer (**a**) and a pyrimidine 6-4 pyrimidone dimer (**b**)



(with respect to the relative position of pyrimidine rings) and syn/anti (with reference to relative orientation of C5–C6 bonds). Whereas cis-syn is the predominant CPD form in DNA, the trans-syn occurs exclusively in single-stranded DNA. The 6-4 PPs are formed between adjacent TT, TC, and CC nucleotides depending upon the UV wavelength and adjacent sequence (Mouret et al. 2006). CPDs and 6-4 PPs make up approximately 70–80 % and 20–30 %, respectively, of the total UV photo-products (Mitchell and Nairn 1989). The pyrimidine dimers inhibit DNA replication and transcription by inducing structural distortions within the DNA, produced as a consequence of bending and unwinding of the DNA helix (Demple and Harrison 1994).

UV-induced ROS can damage DNA mostly by the formation of 8-hydroxydeoxyguanosine (8-OHdG) at the 5'-site of –GG– sequence in the double-stranded DNA. This modified guanine pairs with adenine at 50 % probability causing G:C to T:A transversions (Ito et al. 1993).

IR-Induced DNA Damage

Like UV, IR, too, can induce DNA damage both by being directly absorbed and by production of ROS as a consequence of radiolysis of water. The most common DNA lesions generated by IR include sugar and base damage, single- and double-strand breaks, and DNA–DNA and DNA–protein cross-links (Belli et al. 2002). Direct absorption of IR by the sugar–phosphate backbone of DNA generates nicks and DSBs. IR-induced SSBs are characterized by the presence of a one nucleotide gap containing a 5'-phosphate and either a 3'-phosphoglycolate or a 3'-phosphate (Henner et al. 1983).

Hydrolysis-Induced DNA Damage

Hydrolysis of glycosidic bonds between bases and sugar–phosphate backbone in DNA results in the formation of apurinic/apyrimidinic (AP) sites. These sites prevent normal DNA replication and transcription and are the most common locations for the creation of DSBs because of the collision of replication forks. These lesions are generally recognized and repaired under normal conditions, but their occasional bypass during replication can be mutagenic (Gentil et al. 1984).

Cytosine and methyl-cytosine can undergo hydrolytic deamination to uracil and thymine, respectively. During replication, this can lead to C:G to T:A transition because of mispairing with adenine. Though, the unusual presence of uracil in the DNA is promptly recognized and the lesion is repaired, the transition to thymine is difficult to detect and frequently leads to point mutation.

Alkylation-Induced DNA Damage

7-Methylguanine, 3-methyladenine, and O⁶-methylguanine are the most common lesions produced by alkylating agents like methyl methane sulfonate (MMS) and ethyl methane sulfonate. While 7-methylguanine is a neutral mutation, 3-methyladenine blocks DNA synthesis creating DSBs and O⁶-methylguanine pairs with thymine to produce G:C to A:T transition.

Oxidation-Induced DNA Damage

Increased ROS production is a common manifestation of many abiotic stresses in plants with chloroplasts and mitochondria being the major sites of production. ROS-induced oxidative damage to DNA includes modified bases and sugar, abasic sites, strand breaks, DNA–protein cross-links. The most common oxidation product of purines is 7,8-dihydro-8-oxoguanine, also known as 8-oxoguanine, which can base pair equally well with adenine and cytosine (Maki and Sekiguchi 1992). Thymine glycol is the main oxidatively modified form of pyrimidines and can block DNA replication. Furthermore, ROS can react with sugar of the sugar–phosphate backbone leading to SSBs and DSBs.

DDR Pathway: Perspectives from Animals and Plants

The DDR pathway has mostly been investigated in animals and yeast. At the molecular level, it consists of a number of components including DNA damage sensors, signal transducers, mediators, and effectors. In mammals, ataxia telangiectasia mutated (ATM), ATM, and Rad3-related (ATR), and DNA-dependent protein kinase subunit (DNA-PKcs) are the major regulators of DDR and are rapidly activated in response to DNA damage (Sirbu and Cortez 2013). DSBs are mostly sensed by ATM which initiates a cascade of steps that comprise the ATM pathway. ATR, on the other hand, can respond to a variety of DNA lesions, especially those associated with DNA replication to commence the ATR pathway (Cimprich and Cortez 2008).

Sensing the Damage

The different types of DNA damages are sensed by either the ATM or ATR pathway and culminate into a suitable response depending upon the severity of the damage. In mammals, the DSBs are recognized in the ATM pathway by a ternary MRN complex that comprises the proteins MRE11, RAD50, and NBS1 (Rupnik et al. 2010). This

complex assembles at the site of DSBs and recruits ATM whereby the interaction with NBS1 activates the kinase activity of ATM resulting in the phosphorylation of target proteins, especially the histone variant H2AX. γ H2AX, the phosphorylated form of H2AX, further recruits other DDR proteins (Dickey et al. 2009). Homologs of MRE11, RAD50, and NBS1 have been reported in *Arabidopsis thaliana* (Hartung 1999; Gallego and White 2001; Akutsu et al. 2007). Phosphorylation of H2AX by AtATM and AtATR, the *A. thaliana* homologs of ATM and ATR, has also been reported in response to DSBs (Friesner et al. 2005). That the induction of γ H2AX was observed to be impaired in the *rad50* and *mre11* mutant plants suggested the involvement of the MRN complex in the phosphorylation of H2AX via the ATM and ATR homologs in plants (Amiard et al. 2010).

In mammals, replication protein A (RPA), a single-strand DNA (ssDNA)-binding protein acts as a sensor in the ATR pathway. The RPA-ssDNA complex formed at the site of stalled replication fork (near a DNA lesion) is bound by an ATR-interacting protein (ATRIP) which recruits ATR, eventually activating the check point kinase 1 (CHK1; Zou and Elledge 2003). Alternatively, the ATR pathway can be initiated by recognition of the damage by the RAD9/RAD1/HUS1 (9-1-1) complex. This complex is a ring-like clamp that is loaded onto ssDNA at the damage site with the help of a clamp loader, RAD17 (Bermudez et al. 2003). The homologs of RPA, 9-1-1 complex, and RAD17 have been identified in the *A. thaliana* genome (Takashi et al. 2009; Heitzeberg et al. 2004). Functional characterization of mutants of these homologs suggests that the DNA sensing mechanisms via the ATM and ATR pathway are conserved between animals and plants.

Transducing the Signal

For proper response, information about the damage detected by the sensors is amplified and transduced to the effector proteins by the mediation of transducers. These transducers are mostly serine/threonine kinases which initiate a sequence of phosphorylation steps. ATM/ATR and CHK1/CHK2 are the main transducers in animals. Activation of ATM involves its autophosphorylation and subsequent monomerization of the ATM dimer (Bakkenist and Kastan 2003), whereas ATR is brought into action by its recruitment to RPA-ssDNA complex by ATRIP (Ball and Cortez 2005; Warmerdam et al. 2010). The downstream substrates for phosphorylation by ATM and ATR include CHK1, CHK2, p53, NBS1, and BRCA1 (Matsuoka et al. 2000; Gatei et al. 2003; Zhao and Piwnica-Worms 2001). Of these, CHK1 and CHK2 are the main transducers which have common phosphorylation substrates like p53, BRCA1, E2F1, and CDC25A (Kim et al. 2007).

AtATM and AtATR, the *Arabidopsis* homologs of mammalian ATM and ATR have been identified. Analysis of the *atm* and *atr* mutants revealed their sensitivity to DNA damage inducing agents signifying the conservation of the role of these proteins as signal transducers in both animals and plants (Garcia et al. 2003; Culligan et al. 2004). Orthologs of ATRIP have also been reported in *A. thaliana*

(Sweeney et al. 2009; Sakamoto et al. 2009). Although the orthologs of CHK1 and CHK2 have not been identified in plants the presence of other kinases having functions similar to these two proteins has been alluded to, in view of the presence of mediator proteins like BRCA1 and E2F in plants (Lafarge 2003; Inze and de Veylder 2006).

Between the Signal and Response: The Mediators

Different mediator proteins that regulate DDR have been identified and their roles have been described. The two most common mediators involved in the ATM pathway are BRCA1, mediator of DNA-damage checkpoint protein 1 (MDC1), and p53-binding protein (53BP1; Stewart et al. 2003; Stucki and Jackson 2004). DNA topoisomerase-2-binding protein 1 (TOPBP1) and CLASPIN have been demonstrated to regulate the ATR pathway (Garcia et al. 2005; Kumagai et al. 2004). Many of these proteins interact with other phosphorylated proteins through a conserved BRCA1 associated C-terminal (BRCT) domain (Manke et al. 2003). These mediator proteins function as scaffolds for many DNA damage repair proteins. AtBRCA1, a homolog of BRCA1 with two BRCT domains, which is strongly induced by IR, has been reported in *A. thaliana* (Culligan et al. 2006; Lafarge 2003). Also, an *Arabidopsis* MEIOSIS DEFECTIVE 1 (ME1) protein with five BRCT domains having 40% similarity to the human TOPBP1 has been reported although its role as a mediator in DRR remains to be confirmed (Mathilde et al. 2003). Other mediators are yet to be identified in plants.

Responding to the Damage: The Effectors

Cellular response to DNA damage depends on the severity of the damage. A cell tries to repair the damage by halting its progress in the cell cycle and stopping DNA replication followed by the activation of DNA repair pathways. If the damage is extensive and beyond repair, the cell prefers to initiate apoptosis. The ultimate result of DNA damage is decided by the action of effector proteins. In animals, the tumor suppressor protein p53 is the most important effector (Helton and Chen 2007). The amount and activity of this transcription factor is regulated very precisely by post-translational modifications, of which phosphorylation is the most crucial (Taira and Yoshida 2012). Under normal conditions, p53 levels are maintained at low levels by MDM2-mediated ubiquitination and proteasomal degradation (Xirodimas et al. 2004). DNA damage leads to phosphorylation of p53 by ATM, ATR, CHK1, and CHK2; phosphorylation inhibits the interaction of p53 with MDM2, thereby, resulting in its stabilization and activation (Shieh et al. 1997; Appella and Anderson 2001). Despite the conservation of many DRR proteins between animals and plants,

a plant p53 homolog has long eluded plant scientists. Presence of factors with functions similar to that of p53 has been envisaged; SOG1 has been described as one such factor.

An essential prerequisite for initiating DNA repair is the halt or arrest of cell cycle to prevent the replication of damaged DNA or allow the division of cells carrying such DNA. Cyclin-dependent kinase (CDK) inhibitors, p21 and WEE1 kinase, are the major effectors that halt the cell cycle in response to DNA damage in animals (Abbas and Dutta 2009; Sorensen and Syljuasen 2012). While p21 arrests the cells at G1 in a p53-mediated manner (Reinhardt and Schumacher 2012), WEE1 prevents mitotic entry (McGowan and Russell 1995). In addition, several CDC25 phosphatases are also cell-cycle effectors. These phosphatases remove the inhibitory phosphate groups on CDKs to control the progression of cell cycle. Upon DNA damage, CDC25 is itself inactivated by phosphorylation mediated by CHK1 and CHK2 leading to the cell-cycle arrest (Karlsson-Rosenthal and Millar 2006). Although, homologs of p21, like its immediate regulator p53, have not been identified in plants several other CDK inhibitors (CDKIs) have been reported from *A. thaliana*. These CDKIs belong to two families, namely Kip-related protein (KRP) family and SIAMESE/SIAMESE-RELATED (SIM/SMR) family. DSBs strongly induce the expression of SMR4 and SMR5, indicating their involvement in cell-cycle arrest (Yoshiyama et al. 2009). AtWEE1 is the WEE1 homolog in *A. thaliana* that is activated by DNA damage or DNA replication arrest in an AtATM- or AtATR-dependent manner, respectively (de Schutter et al. 2007). It has been demonstrated to control cell-cycle arrest in the DDR pathway. Although a CDC25-like protein has been identified in *A. thaliana*, its role in cell-cycle regulation is not confirmed (Spadafora et al. 2011).

By applying brakes on the cell cycle, cell buys time to make amends to the damaged DNA. A number of conserved DNA repair pathways viz., nucleotide excision repair (NER), base excision repair (BER), homologous recombination (HR), non-homologous end-joining (NHEJ), and direct repair (DR) have been identified in yeast, animals, and plants. If the damage is beyond repair, animal cells activate apoptosis, the exquisitely regulated pathway to kill cells with compromised genomes. The diversion of cells from DNA repair pathways to apoptosis is mediated by the transcriptional activation of genes such as *BAX*, *PUMA*, and *NOXA* by p53 (Vousden and Lu 2002). Plants, too, have programmed cell death (PCD), but it is distinct from the apoptotic cell death in animals because plants lack the core apoptotic machinery. PCD involving AtATM and AtATR has been observed in shoot and root apical meristem in plants in response to DNA damage (Fulcher and Sablowski 2009). Metacaspases (MCs) that are structurally similar to animal caspases, the initiator proteins for apoptosis have also been reported in plants (Uren et al. 2000; Lam and Zhang 2012). Overexpression of some of these MCs increased the cell death upon treatment with ROS-inducing agents while their loss resulted in delay or decrease of cell death (Lam and Zhang 2012). It has been suggested that some of these pathways might be regulated by SOG1 in plants.

DNA Repair Pathways in Plants

Maintenance of genomic integrity is crucial to life. This comes at a huge premium in terms of energy expenditure and elaborate repair framework that organisms need to maintain. Although damages that do not interfere with the replication or transcription can sometimes be innocuous, those that hinder the activity of DNA and RNA polymerases are very harmful for the organism and need to be compulsorily repaired. Most of the repair mechanisms have been conserved through evolution and homologs of different elements have been identified across kingdoms. The whole genome sequence of *A. thaliana* and rice has revealed the presence of many repair proteins that are similar to those in humans. Characterization of mutants and overexpressing lines has provided functional validation for these proteins. A comprehensive list of DNA repair genes in the rice genome was provided by Kimura and Sakaguchi (2006). Like animals, plants are also equipped with a variety of repair pathways catering to the different types of DNA damages. These damages can be rectified through a number of pathways. Herein, we have described the main pathways, namely photoreactivation/photorepair (PR), base excision repair (BER), and nucleotide excision repair (NER).

Photoreactivation/Photorepair (PR) Pathway

As inherent in the name, photoreactivation is a light-dependent pathway majorly responsible for the repair of UV-B-induced DNA damage, especially CPDs. This pathway utilizes light-mediated reversion of the damage by photolyases (Yasui and Eker 1998). Photolyases generally contain two chromophoric co-factors, one of which is always the two electron reduced form of FAD (FADH⁻; Sancar 2003). FADH⁻ acts as a transient electron donor to reverse the cross-link between the bases. The second chromophore acts as an antenna pigment to excite the electron donor. The reaction mechanism proceeds via a free radical mechanism. Photolyases bind specifically to DNA lesions absorbing blue/UV-A (320–400 nm) light and reducing pyrimidine dimers to monomers (Kimura et al. 2004; Fig. 7.2). A number of factors including quality, timing, and quantity of photoreactivating light as well as the severity of the damage affect this repair (Sutherland et al. 1996; Takeuchi et al. 1996; Stapleton et al. 1997). Although, PR is present in prokaryotes and eukaryotes including some species of plants and animals, it is not universal, with many species, including humans, lacking it (Todo 1999). The genes for photolyases have been cloned from higher plants such as *A. thaliana*, *Cucumis sativus*, *Oryza sativa*, and *Spinacia oleracea*. Six genes with photolyase activity have been identified in the *Arabidopsis* genome. The common UV-induced lesions, CPD and 6-4 PP, are specifically recognized by photolyases PHR1/UVR2 and UVR3, respectively. *Arabidopsis* mutants for these photolyases were defective in PR (Jiang et al. 1997). Similarly, rice cultivars with deficient PR of CPDs were reported to carry a mutation

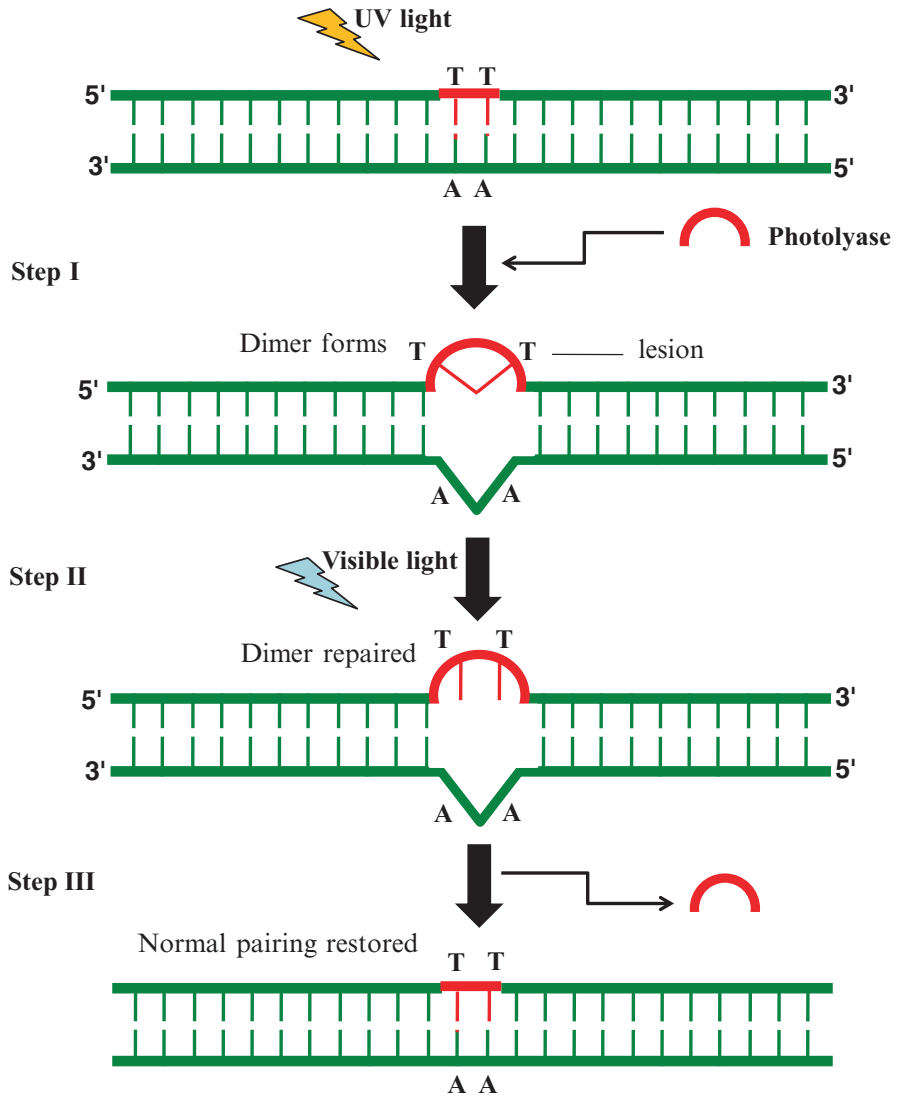


Fig. 7.2 Schematic representation of photoreactivation mechanism. The enzyme photolyase binds to the site of structural distortion in the DNA caused because of pyrimidine (thymine in this case) dimer (Step 1) and catalyzes the light (blue) activated reversion of the dimer to the monomeric form (Step 2). The enzyme is released from the DNA after the repair (Step 3)

in the CPD photolyase (Hidema et al. 2000; Teranishi et al. 2004). In contrast, transgenic rice overexpressing CPD photolyase were demonstrated to be tolerant to growth inhibition caused by UV-B-induced damage and accumulated lower CPD lesions in leaves during growth under UV-B irradiation (Hidema et al. 2007). Further, diurnal changes in CPD photolyase expression have been observed in

cucumber, suggesting regulation of the gene to prevent growth suppression by UV (Takahashi et al. 2002). In *Arabidopsis*, the overexpression of CPD photolyase resulted in a modest increase in biomass under UV-irradiated conditions (Kaiser et al. 2009). In addition, different ecotypes of *A. thaliana* have been demonstrated to possess variable UV-B response (Kalbina and Strid 2006).

Base Excision Repair

Only a minority of damaged bases in DNA are repaired by direct damage reversal; most of these are removed by excision repair (Lindahl and Wood 1999). Damaged bases that do not distort the helical structure of DNA are repaired by BER. Such damaged bases could be the result of spontaneous deamination or base loss or could be induced by IR, oxidative or methylating agents. In this multi-step repair pathway, specific DNA glycosylases excise the damaged base thereby creating an abasic (AP) site that is subsequently filled in by the correct DNA sequence (Lindahl and Wood 1999; Mol et al. 1995; Tuteja and Tuteja 2001). The repair mechanism has been classified into two sub-pathways: short-patch BER and long-patch BER. In short-patch BER, a glycosylase with specificity for a particular base-adduct slides along the minor groove of DNA helix and localizes to the site of the lesion where it removes the damaged base creating an AP site (Fromme et al. 2004). The abasic site is then recognized by an AP-endonuclease that nicks the DNA backbone on 5'-end of the AP site. Thereafter, the 5'-terminal deoxyribose-phosphate residue is excised by DNA polymerase β using its AP lyase activity (Matsumoto and Kim 1995). The missing base is then put in place by DNA polymerase β , and the nick is sealed by DNA ligase I or III with the help of XRCC1 (Fig. 7.3). In the case of long-patch BER, instead of the replacement of a single damaged base, a longer patch (2–10 nucleotides) is resynthesized by nick translation in conjunction with strand displacement in the 5'–3' direction, generating a flap-like structure. The flap is removed by a flap endonuclease, FEN-1, with the aid of PCNA (Wu et al 1996; Klungland and Lindahl 1997; Fig. 7.3). The nick translation is catalyzed by DNA polymerase β or δ and the backbone is sealed by DNA ligase I (Fortini et al. 1998).

The study of BER in plants has revealed the presence of several homologs of different component proteins in *A. thaliana* and *O. sativa* (Kimura and Sakaguchi 2006). For example, glycosylases with specificity for particular modified bases have been reported (Santerre and Britt 1994; Garcia-Ortiz et al. 2001; Dany and Tissier 2001; Murphy and Gao 2001). Similarly, *Arabidopsis* XRCC1-like protein and rice FEN1 and DNA polymerase δ have been isolated (Martínez-Macías et al. 2013; Kimura et al. 2003; Uchiyama et al. 2002). However, plant homologs of DNA polymerase β and DNA ligase III have not yet been reported.

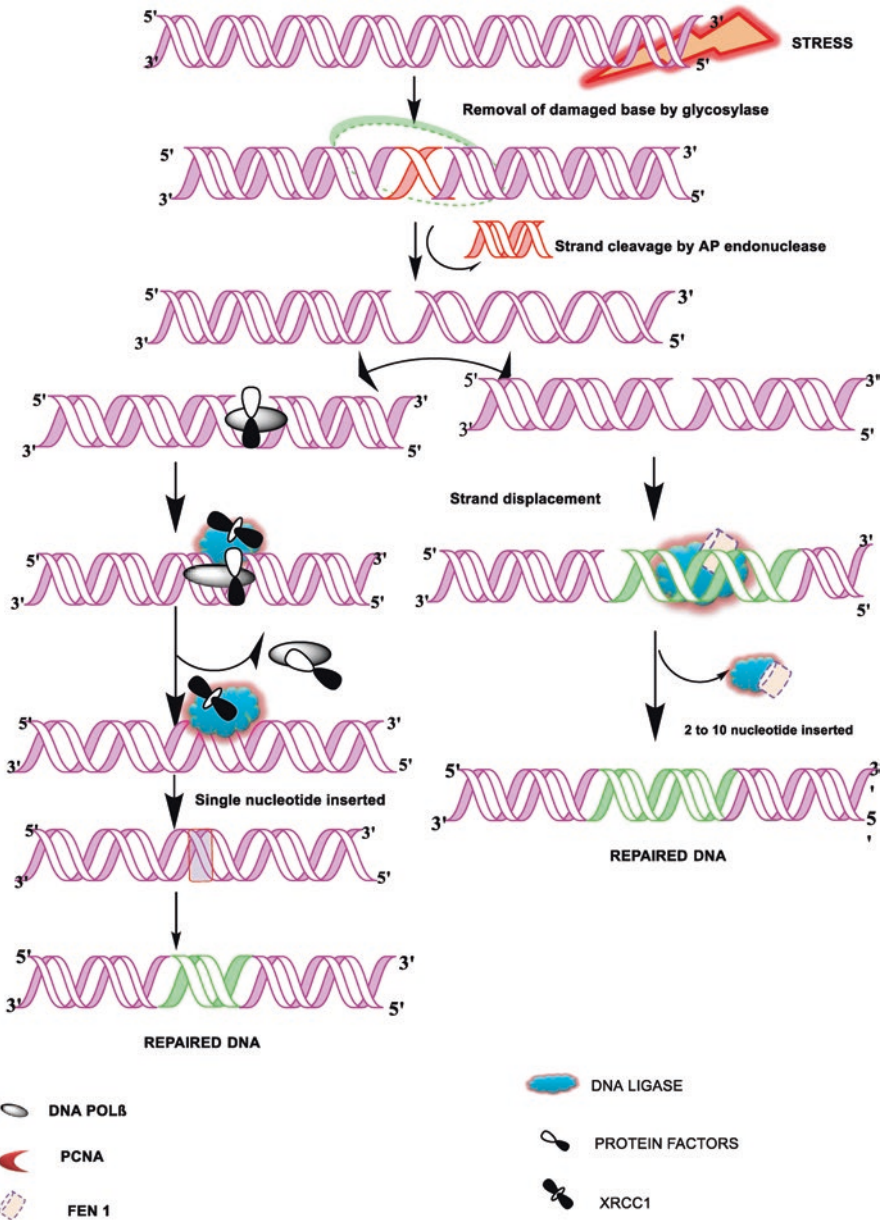


Fig. 7.3 Schematic representation of base excision repair pathway. Please refer to the text for explanation of the steps in the pathway

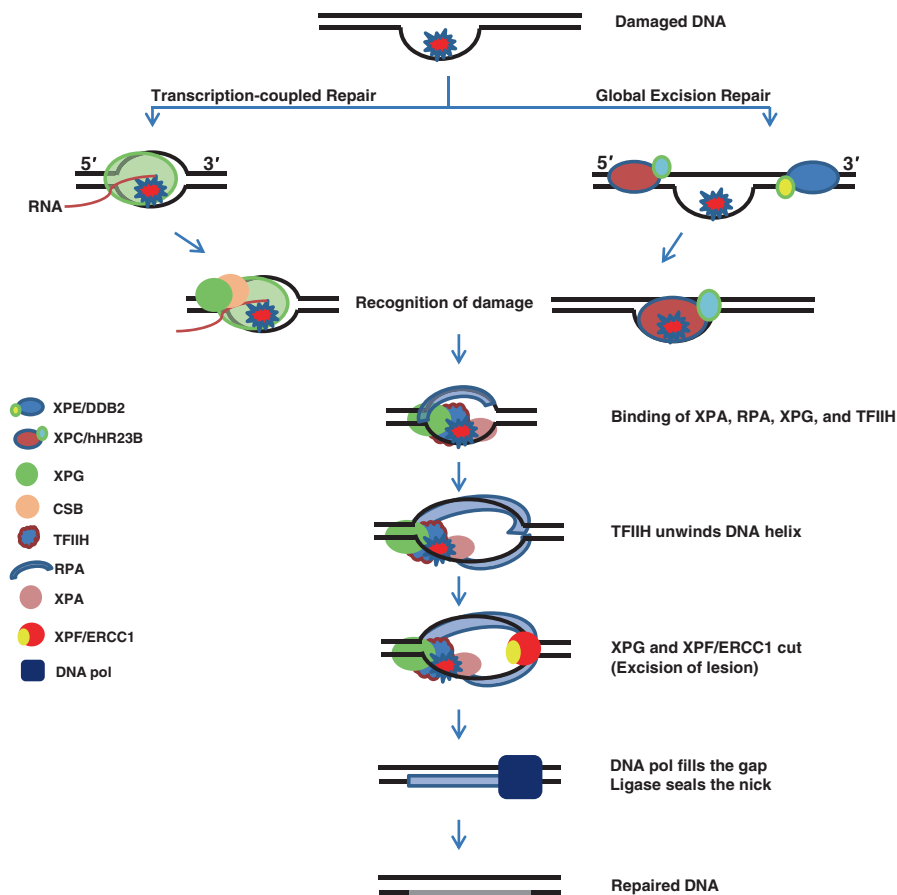


Fig. 7.4 Schematic representation of nucleotide excision repair. Please refer to the text for explanation of the steps in the pathway. (Adapted from Fuss and Cooper 2006)

Nucleotide Excision Repair

Lesions that cause large distortions in the helical structure of DNA are generally restored by nucleotide excision repair. The steps involved in this mechanism include recognition of the damaged site, opening of the double helix by the action of helicases and endonucleases, excision of a DNA segment carrying the lesion, and replacement of the segment using the template provided by the intact complementary strand followed by ligation of the nick (Costa et al. 2003; Fig. 7.4). The proteins involved in NER are sequentially assembled at the site of the lesion (Volker et al 2001). NER is classified into two distinct sub-pathways that differ in the DNA damage recognition: transcription coupled repair (TCR) and global genome repair (GGR). RNA polymerase II detects the lesion in TCR with the help of CSB and CSA. CSB has been demonstrated to alter DNA conformation and remodel

chromatin in an ATP dependent manner (Citterio et al. 2000). In GGR, XPC-hHR23B is the first factor to be involved in the lesion detection (Volker et al. 2001). In general, XPC-hHR23B complex is stabilized by hCEN2. Some other factors like XPE may aid in this recognition (Kusumoto et al. 2001). Subsequent to damage recognition other NER factors are recruited, which include TFIIH, XPD, XPA, XPB, RPA, XPF, and XPG (Evans et al. 1997). TFIIH is the basal transcription initiation factor that comprises nine different proteins (Winkler et al. 1998; Coin et al. 1999). XPD and XPB are ATP-dependent helicases that unwind the DNA helix in 5'–3' and 3'–5' direction, respectively (Schaeffer et al. 1994; Roy et al. 1994). The replication protein A (RPA) binds to DNA to provide complete opening of the helix around the lesion (Christians and Hanawalt 1993). XPA homodimer binds to RPA to form XPA2-RPA complex that provides proper three-dimensional orientation of the NER components required by excision of damage carrying DNA segment (Missura et al. 2001; Yang et al. 2002). With the DNA lesion recognized and helix around it unwound, XPG carries out structure-specific 3'-endonucleolytic activity (2–8 nucleotide away from the lesion) in conjunction with the 5'-endonucleolytic activity (15–24 nucleotide away from the lesion) of an XPF-ERCC1 complex. Thus, the lesion carrying segment of DNA is excised and is dissociated. Thereafter, the resultant gap is filled by DNA polymerase ϵ and δ using the 3'-end left by the XPF-ERCC1 incision as the primer (Hunting et al. 1991; Coverley et al. 1992). These polymerases require the presence of PCNA and RFC (Wood and Shivij 1997). Finally, the 5'-end of the newly synthesized DNA segment is sealed with the parent strand by DNA ligase I (Tomkinson and Levin 1997).

Most of the genes of NER pathway have been reported from *A. thaliana* (The *Arabidopsis* Genome Initiative 2000). Many genes with sequence similar to those of yeast and mammals have been cloned (Schultz and Quatrano 1997; Sturm and Lienhard 1998; Taylor et al. 1998; Costa et al. 2001; Liu et al. 2003; Dubest et al. 2004; Kimura et al. 2004). In addition, NER-related genes like PCNA, DNA polymerase δ , RPA, and CSB have been cloned from rice (Kimura and Sakaguchi 2006). The functional analysis of these genes and characterization of many mutants defective in some of them confirms the importance of NER in DNA damage repair in plants and the conservation of this pathway across the living kingdoms.

Conclusions

DNA damage response and repair pathways play a crucial role in maintenance of the integrity of genome and have been conserved to a great extent in various organisms. Many of these pathways have been thoroughly examined in mammals and yeast. Studies on plants have lagged behind in this regard. However, with the increasing genomic data available as a consequence of high-throughput sequencing efforts, homologs of different proteins involved in these pathways have been identified and functionally evaluated. Although most of the components are present, some crucial factors like p53, XRCC, DNA polymerase γ , DNA ligase III, etc. have been

conspicuously absent. Furthermore, some genes like CSB, RPA, PCNA, and FEN1 are present in multiple copies in plants. Some repair defects, for example, RAD50 knockout, which are lethal in animals, have been reported to have no physiological abnormalities in plants. Existence of novel plant-specific DDR components in plants has been alluded to in plants. The ongoing genetic, proteomic, and knock-out based strategies are expected to unveil novel DNA damage sensing and repair components in plants.

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