Chapter 13

Plant Genome Stability: General Mechanisms

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Chapter Outline

1. INTRODUCTION

The maintenance of genome stability in every organism encompasses a complex of measures aimed at a precise replication of a native sequence or the repair of damaged DNA in order to avoid any alterations in the genetic material in somatic cells and in the progeny. Due to a wide number of intra- and extracellular genotoxic agents, different components of DNA can be damaged including the sugar residues, phosphodiester linkages, and purine and pyrimidine bases [1]. Hence, depending on DNA damage, different pathways are involved in the sensing of distinct lesions, their recognition and repair. It is believed that an efficient DNA repair is of a particular importance for plants because unlike most of the higher eukaryotes, they do not set aside gametes during early sporophytic development [1]. Thus, any stress factor encountered during plant sporophytic development can potentially affect the genome of the predecessors of gametes and be passed on to the progeny. Taking into account that plants are sessile organisms with an associated inability to initiate avoidance response during stress exposure, it is safe to assume that the genome maintenance mechanism has to be robust enough to cope with genotoxic factors for an intact passage of genetic information.

The typical external sources of DNA damage are UV-B, ozone, high temperatures, drought, air, and soil pollutants including heavy metals [2]. Reactive oxygen species (ROS) belong to the internal agents which may cause single-strand breaks (SSBs) either through the damage of deoxyribose units or covalent alterations of bases [2]. ROS are continuously generated in plant cells during normal oxidative cellular processes and possess a potential danger to the integrity of the plant genome even in the absence of environmental stressors. DNA damage can also occur spontaneously during DNA replication—the collapsed replication forks or replication through SSB [3]. In most of the cases, to mitigate the cytotoxic effects of DNA damage, the early detection, cell-cycle arrest, and the rapid repair of damaged regions have to take place. In this chapter, we provide a short review of the major DNA-repair pathways in plants that play a key role in the maintenance of genome stability.

2. DNA-DAMAGING AGENTS

Exogenous genotoxins as well as metabolic derivatives can react with DNA and cause a number of different base modifications and even SSBs. Extracellular DNA-damaging agents, such as salt, heavy metals, extreme alterations in the ambient

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temperature, water supply, pathogens, and elicitors can increase the level of DNA lesions and activate responsive pathways [4]. A common factor that links all of the stressors together is the generation of ROS that are also constantly produced in mitochondria and chloroplasts during respiration and photosynthesis, respectively [5]. The hydroxyl radical (·OH) is one of the most active ROS which effectively interacts with biomolecules at the diffusion-controlled rates [6]. Due to a high reactivity of ·OH, it essentially reacts with biomolecules in the place of its generation in a reaction volume of less than 2 nm. ·OH is mostly generated in the Fenton reaction as a product of the interaction of reduced redox-active metal ions (eg, Fe^{2+} and Cu^{+}) with intracellular hydrogen peroxide. The main targets of ·OH are thymine nucleobases that eventually can give rise to *cis*- and *trans*-diastereomers of 5,6-dihydroxy-5,6-dihydrothymine. The oxidation of cytosine leads generates intermediate products which are highly unstable and give rise to analogues of uracil. In turn, the degradation products of guanine include 8-oxo-7,8-dihydroguanine (8-oxoGua) and 2,6-diamino-4-hydroxy-5-formamidopyrimidine (Fapy-Gua) with a higher efficiency of the intracellular generation of Fapy-Gua as compared to 8-oxoGua [7]. Similarly, the oxidation of adenine also leads to the formation of 8-oxoAde and Fapy-Ade. Singlet oxygen ($^1O_2$) is another ROS that is the main contributor of UV-A irradiation-induced oxidative damage and may cause DNA lesions by reacting selectively with guanine components [8]. As a result, 8-oxo-7,8-dihydro-20-deoxyguanosine (8-oxodGuo) is exclusively formed in the oxidation reaction. The oxidized guanine adducts are mainly associated with G-A and G-T mutations. In addition, the alkylation of guanine can lead to G-C mutation [9].

The artificial mutagenesis is also widely exploited in research by the utilization of ethyl methane sulfonate (EMS) to generate alkylation products, including O6-ethylguanine. Eventually, the DNA-replication machinery recognizes the modified base as adenine leading to G-A mutations, thus permanently altering the sequence of the genome [10,11].

3. SENSING DNA DAMAGE

The detection of DNA damage is the first step in the DNA-repair pathway. It is believed that there are few mechanisms for DNA lesions detection depending on the nature of damage. The two main proteins involved in the DNA-damage sensing in *Arabidopsis* are the phosphoinositide-3-kinase-related protein kinases ATM (ataxia telangiectasia mutated) and ATR (ataxia telangiectasia and Rad3-related) (Fig. 13.1). While the ATM protein is activated by double-strand breaks (DSBs), ATR responds to replication defects [12,13] (Table 13.1). ATR-deficient plants also demonstrate a hypersensitivity to UV-B radiation and show changes in G2-phase cell-cycle checkpoints [14,15]. The kinase activity of both proteins results in the phosphorylation of several hundred target proteins in animals, including H2AX, a histone 2A isoform, Nbs1, and the checkpoint-related protein kinases Chk1 and Chk2 [16]. The phosphorylation of H2AX in dividing root cells occurs very rapidly and demonstrates a peak of accumulation at10-min post-irradiation [17]. As a result of a wide variety of protein phosphorylation by ATM/ATR proteins, a rapid relocation of the DSB-repair

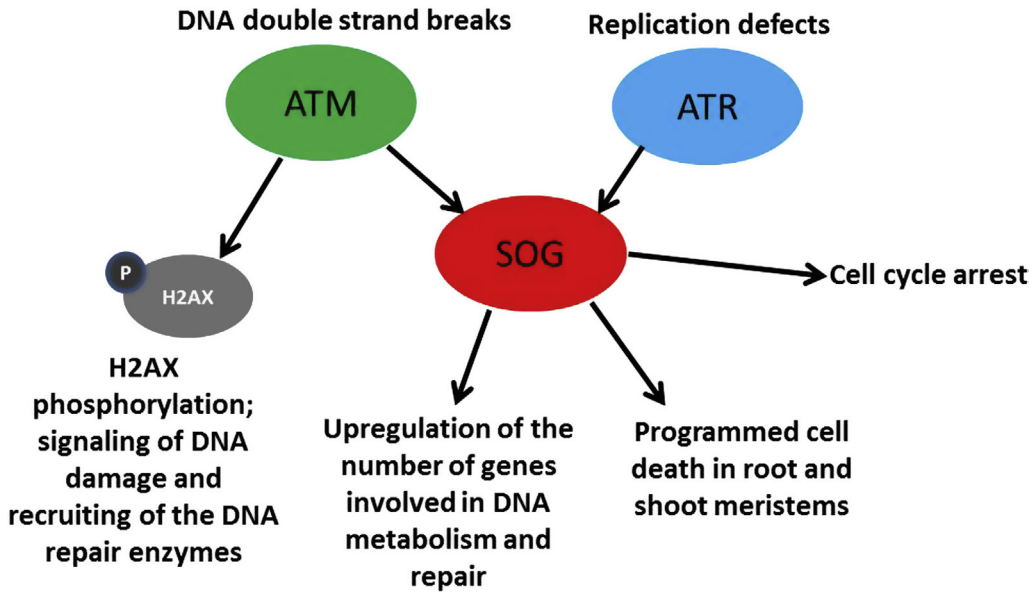

FIGURE 13.1 **DNA-damage signaling in plants.** The phosphoinositide 3-kinase-like protein kinases ATAXIA TELANGIECTASIA MUTATED (ATM), ATAXIA TELANGIECTASIA MUTATED, and RAD3-RELATED (ATR) are involved in the DNA-damage response in plants.

proteins occurs along with the activation of the cell-cycle checkpoint and initiation of DNA repair [18]. In addition, transcriptional changes in response to DNA damage play a vital role in lesion repair. While the ATM protein is required for the upregulation of genes involved in DNA metabolism, cell cycle, and homologous recombination (HR) repair, the ATR protein plays a minor role in the regulation of gene expression [19]. At the same time, NHEJ genes demonstrate a negligible transcriptional upregulation in response to DNA damage that is apparently due to a constitutive expression of genes in somatic tissues. In addition to the ATM/ATR proteins, a unique plant-specific transcription factor SUPPRESSOR OF GAMMA RESPONSE 1 (SOG1) has been demonstrated to play a role of a central regulator in the DNA-damage response [20]. It has been suggested to perform similar functions to the mammalian p53 protein involved in the cell-cycle checkpoint.

The UV-induced photoproducts are recognized by the DNA damage-binding (DDB) complex that consists of DDB1, DDB2 (XPE), Cullin4, and Rbx1 proteins [21]. In addition, in the case of DSBs, free DNA ends are detected by the KU70 and KU80 complex as well as by the MRN complex [12,22].

4. CHROMATIN ARCHITECTURE AND DNA REPAIR

Following the recognition of DNA lesions, the chromatin-remodeling enzymes provide an access of repair proteins to the damaged DNA. The evolutionarily conserved Chromatin assembly factor 1 (CAF-1) complex plays a key role in the deposition of H3 and H4 histones on to a newly synthesized DNA molecule [23] (Table 13.1). CAF1 is a chaperone complex consisting of FASCIATA 1 (FAS1), FAS2, and Multicopy suppressor of IRA1 (MSI1) subunits in *Arabidopsis* [24]. Mutations of CAF-1 components cause a hypersensitivity to genotoxins, the elevated level of DSBs, a constitutive activation of the DNA-damage response, including H2AX phosphorylation and RAD51 induction concomitant with a 40-fold increase in the rate of HR [25,26]. Similarly, in *Arabidopsis*, the overexpression of the RAD54a protein which is a member of the SWItch/Sucrose nonfermentable (SWI2/SNF2) superfamily and a chromatin-remodeling factor promotes HR and increases the rate of gene targeting by almost 30-fold [27]. In addition, screening of RNAi plants deficient in representatives of the SWI2/SNF2 subfamilies revealed their hypersensitivity to genotoxins, further highlighting the importance of chromatin-remodeling factors in the DNA repair [28].

In the process of DSB repair, especially during HR, the cohesion of sister chromatids plays a vital role in promoting the recombination. DNA damage–induced cohesion is stimulated by the large ATPases—structural maintenance of chromosomes (SMC) proteins [29,30]. *Arabidopsis* mutants deficient in the SMC6 homologue MIM (hypersensitive to MMS, Irradiation and MMC) demonstrate the reduced levels of DSB repair, including low rates of intrachromosomal HR and an increased sensitivity to a broad range of genotoxins [31,32].

In addition to DSB repair, the histone acetyltransferases HAM1 and HAM2 have been implemented in the repair of UV-B-induced DNA damage [33]. Similarly, the histone H3/H4 chaperone ANTI-SILENCING *FUNCTION1* (ASF1) is also involved in the repair of UV-B-induced DNA damage, thus further reinforcing the importance of chromatin modifiers in DNA-damage repair [34].

TABLE 13.1 Proteins Involved in the DNA Damage Sensing and Chromatin Composition in Plants

Pathway	Protein Name	Function
DNA-damage sensing and response	Ataxia telangiectasia mutated (ATM)	Activated by double-strand breaks and is required for the upregulation of genes involved in the DNA metabolism, cell cycle, and HR repair
	Ataxia telangiectasia and Rad3-related (ATR)	Responds to replication defects; plays a minor role in the regulation of gene expression
	Suppressor of gamma response 1 (SOG1)	Plays a role of the central regulator in the DNA-damage response
Chromatin composition	Chromatin assembly factor 1chaperone complex (CAF-1)	Involved in the deposition of H3 and H4 histones on to newly synthesized DNA
	Structural maintenance of chromosomes (SMC) proteins	The large ATPases that stimulate the DNA damage–induced cohesion
	Histone acetyltransferases HAM1 and HAM2	Involved in the repair of UV-B-induced DNA damage

5. PHOTOREACTIVATION

During their life cycle, plants are constantly exposed to the ultraviolet radiation (UVR). Solar UVR that reaches the Earth's surface is divided into three wavelength ranges: the UV-A (315–400 nm), UV-B (280–315 nm), and UV-C (200–280 nm) spectrum. While the first two types of UVR can reach the surface of the planet, the third type is strongly absorbed by oxygen and ozone in the atmosphere [1]. The UV-B radiation is also partially filtered through the stratospheric ozone layer and represents 1.5% of the total radiation that reaches the Earth's surface. At the same time, it is one of the most damaging types of solar radiation which reaches the surface [35]. More importantly, during the end of the 20th century, a decreasing layer of stratospheric ozone caused an increase in the amount of UV-B irradiation reaching the surface of our planet [36]. UVR can potentially damage almost all biomolecules; however, DNA damage is considered to be the most pronounced as compared to the damage of proteins and lipids [37]. UVR-induced alterations to the DNA structure include photosensitization reactions and dimer production between adjacent pyrimidine residues in the DNA strand caused either by UV-A/visible light or UV-B, respectively. UV-A/visible light can induce the generation of ROS, including $^1O_2$ which is very potent in damaging biomolecules [38]. In addition to pyrimidine dimers, UV-B light can produce the oxidized and hydrated bases, SSBs and cross-links (both DNA–protein and DNA–DNA) that can lead to the growth and development retardation in plants [39,40].

The two major types of pyrimidine dimers include the cyclobutane pyrimidine dimer (CPD) and the pyrimidine-pyrimidone (6-4) photoproduct (6-4PP). When induced by wavelengths longer than 290 nm, the last class of pyrimidine dimers may occasionally be converted to a Dewar isomer. Structurally, both CPD and 6-4PP can introduce distortions into the DNA double helix leading to either slight bending or even unwinding of the strands [40]. This in turn can impede the transcription and result in error-prone replication [41,42]. In plants, the occurrence of CPDs following UV-B exposure prevails as compared to 6-4PP. Although plants may tolerate a low level of CPDs in their genome [43,44], the pyrimidine dimers can jeopardize plant development due to the mutagenic and cytotoxic effects. Therefore, an efficient removal of pyrimidine dimers from the DNA structure is of a paramount importance for plant survival. A number of DNA-repair pathways can be engaged to restore the genome integrity following UV exposure, including photorepair (photoreactivation), base excision repair (BER), nucleotide excision repair (NER), or mismatch repair (MMR) [45]. Overall, depending on the light requirement, the pathways are divided into "dark" and "light" repair pathways. Dark repair pathways including BER, NER, MMR, and others are relatively inefficient in removal of UV-induced DNA lesions. At the same time, the photoreactivation process is considered more effective and depends on the wavelength of 350–450 nm [46]. The dimer splitting reaction is catalyzed by 450–550 amino acids-long monomeric enzymes called photolyases that upon binding to the UV-damaged DNA absorb UV-A light (350–450 nm) to induce cyclic electron transfer, split the CPD ring, and restore the bases to their normal state [47] (Table 13.2). The absorbance of photons is performed by cofactors, one of which is always a two-electron reduced form of flavin adenine dinucleotide ($FADH^-$), while another one is either a reduced pterin methenyltetrahydrofolate (MTHF) or 8-hydroxy-7,8-didemethyl-5-deazariboflavin (8-HDF). While the FAD cofactor is necessary for both a specific binding to the damaged DNA and for the reaction to occur, other chromophores are not essential for catalysis under normal light conditions and have no effect on enzyme-binding specificity [47]. At the same time, under the limiting light conditions, the second chromophore may increase the rate of DNA lesions repair by 10- to100-fold depending on the wavelength during catalysis. The dimer-splitting reaction includes two steps: in the "dark reaction," the photolyase binds to a pyrimidine dimer lesion, flips the dimers out of the double helix into the enzyme's catalytic site and forms a stable enzyme–substrate complex; in the light-dependent step, the chromophore absorbs a photon and transfers the excitation energy to $FADH^-$ followed by one-electron transfer to the enzyme-bound pyrimidine complex [1] (Fig. 13.2). Eventually, the covalent bond between two pyrimidine dimers is split, and the electron is transferred back to FADH. Similarly, 6-4PP is repaired by 6-4 photolyases, but with the inclusion of the thermal conversion step of 6-4PP to an unstable oxetane intermediate before the photochemical reversion steps. Distinct photolyases are responsible for the repair of different dimers and the photolyase that repairs one type of dimer cannot repair another. For instance, in *Arabidopsis*, whereas the *UVR2* gene encodes a photolyase (PHR1) that recognizes only CPDs, the *UVR3* gene encodes an enzyme that acts on 6-4PP [35]. Although the efficiency of CPD photorepair is lower as compared to 6-4PP, the quantum yield of photolyase repair of the first DNA lesion is higher as compared to that of the second one [47,48]. Similarly, the ATP-dependent NER pathway is 9.5–10.7 times more efficient in the repair of 6-4PP as compared to CDPs [47,49].

Both the induction and rate of photoproduct repair in plants are temperature dependent: they are the lowest at 0°C and the highest at around 30°C followed by either a further stabilization or decline of repair rate at higher temperatures [50,51]. In addition, the choice of the pathway for the repair of DNA lesions caused by UV-B irradiation depends on the plant's developmental stage as well as the severity of the damage. For instance, in alfalfa seedlings, whereas at the relatively low levels of pyrimidine dimers, the error-free photoreactivation mechanism is involved, at the higher DNA-damage levels, both the photoreactivation and excision-repair pathways repair DNA lesions [52]. Thus, when the level of pyrimidine dimers is

TABLE 13.2 Proteins Involved in the Photoreactivation and Base Excision Repair Pathways

Pathway	Protein Name	Function
Photoreactivation DNA-repair pathway	Photolyases	Catalyze the splitting of UV-induced pyrimidine dimers; photolyases absorb UV-A light to induce cyclic electron transfer to split cyclobutane pyrimidine dimer ring and restore the bases to normal state
Base excision repair (BER)	Glycosylases	Catalyze the recognition and excision of the damaged or incorrect base by hydrolysis of the *N*-glycosidic bond between the damaged base and the sugar
	Apurinic/apyrimidinic (AP) endonuclease	Cleaves the sugar–phosphate backbone at the 5′-region of the AP site leaving 3′-OH and blocking 5′-deoxyribose-5-phosphate (5′-dRP) termini
	DNA Pol λ	Possibly performs the gap filling in the short-patch repair
	Replicative DNA polymerase complex Pol δ/ε	Catalyzes DNA synthesis in the long-patch repair
	Flap endonuclease FEN1	Endonuclease which removes a "flap" structure generated by DNA polymerase complex Pol δ/ε in the long-patch repair
	Ligase I	Possibly involved in the repair of both single- and double-strand breaks in planta

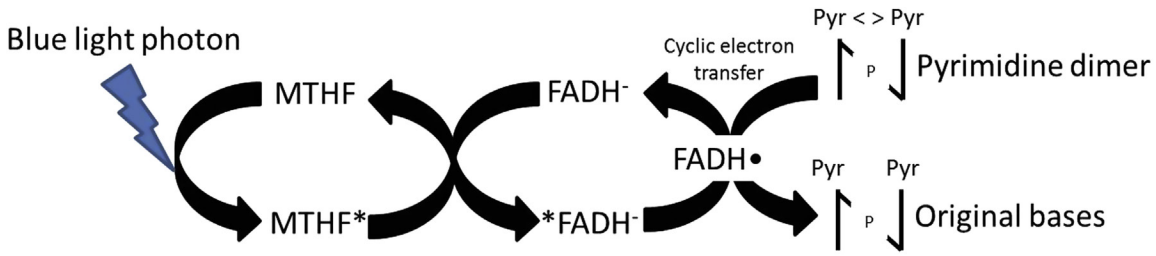

FIGURE 13.2 A simplified scheme of the reaction catalyzed by the CPD photolyase enzyme. The pterin methenyltetrahydrofolate (MTHF) chromophore absorbs a blue light, and the excited (MTHF*) transfers energy to the fully reduced flavin $FADH^-$. The last one, in its excited form ($*FADH^-$), induces a cyclic electron transfer step that leads to the splitting of the pyrimidine dimer into two pyrimidine monomers. The flavin coenzyme eventually is converted into the active two-electron fully reduced state. *Adapted from Bray CM, West CE. DNA repair mechanisms in plants: crucial sensors and effectors for the maintenance of genome integrity. New Phytol 2005;168(3):511–28.*

under 30 dimers per 10^6 bases, the external light energy is used for DNA repair, whereas when the DNA damage is more severe, the ATP-dependent pathway is involved.

The expression profile of genes involved in the photoreactivation and excision repair during the development has a clear tissue-dependent distribution in plants. For instance, the expression of genes involved in the excision-repair pathway is more profound in proliferating tissues. At the same time, the CPD photolyase is expressed in nonproliferating tissues, such as mature leaves and elongation zone of roots [53]. Furthermore, the DNA damage is not repaired efficiently in mature leaves in the dark, suggesting that the photoreaction is the major DNA-repair pathway for UV-induced DNA lesions in nonproliferating tissues. As expected, the examination of the photolyase protein distribution in *Arabidopsis* tissues using Western blot analysis revealed the highest level of both CPD and 6-4PP photolyases in aerial tissues including florets and leaves, and the level was very low in roots [51]. In addition, the highest level of 6-4PP photolyase was detected in siliques, and the protein is constantly expressed throughout the plant development. In contrast, the level of CPD photolyase was low in leaves of young *Arabidopsis* plants (7-day seedlings) with a further increase as plants get older (7–14 days), followed by a decrease in leaves of mature plants (6 weeks old). The low transcript level of CPD photolyase found in young leaves can apparently be compensated by a higher expression of excision-repair genes as compared to mature tissues found in *Arabidopsis* and wheat [46,53].

A more severe damage of bases and nucleotides is usually repaired through the excision repair pathways. Since many types of genotoxins introduce lesions only to a single strand of the double helix, the second strand can be used as a template for the repair. Depending on the DNA damage, the distinct but evolutionary conserved excision-repair pathways are

involved that have a few common steps including: the recognition of a lesion and the excision of the damaged region, repair synthesis, and ligation [1].

6. BASE EXCISION REPAIR

BER is the major pathway for protecting DNA from genotoxic agents [54]. It is involved in the repair of DNA damages caused by ROS, alkylation, deamination, abasic (apurinic and/or apyrimidinic, AP) sites, and SSBs. The initial step of the classic BER pathway involves the recognition and excision of the damaged or incorrect base by hydrolysis of the *N*-glycosidic bond between the damaged base and the sugar catalyzed by glycosylases. The resulting site can become AP and acquire a single-stranded DNA break or a 1-nt gap flanked with a 5′-phosphate [55]. Different glycosylases are specific for every base adduct that they remove (Table 13.2). For instance, there are distinct glycosylases for the excision of either of 3-methyladenine or 8-oxoG adducts in plants [56,57]. The 8-oxoG residue is recognized as thymidine during replication, thus leading to G-T mutations. DNA glycosylases scan the DNA in search for damaged bases. During this process, the enzymes kink or bend the DNA double helix and flip the base into the catalytic site. The damaged bases usually destabilize the structure of DNA and are therefore more easily flipped out. In addition, the catalytic site of glycosylases is complementary to a specific structure and charge distribution on the damaged base, thus once in the catalytic pocket, the *N*-glycosidic bond is hydrolyzed [58]. The AP sites may also arise randomly by a spontaneous hydrolysis of the *N*-glycosylic bond [59]. Regardless of the cause of the appearance of AP sites, the following steps are parts of the SSB-repair pathway including an incision at the AP site, the formation of a gap, DNA-repair synthesis, and ligation. In the case of the monofunctional glycosylase, the AP site is further processed by an AP endonuclease which cleaves the sugar phosphate backbone at the 5′-region of the AP site leaving 3′-OH and blocking 5′-deoxyribose-5-phosphate (5′-dRP) termini [42,60]. The *Arabidopsis* genome encodes three homologues to human AP endonucleases, such as Arp, Ape1L, and Ape2. The enzymes have been suggested to act during embryo development and even programmed deletion of certain bases in gene promoters activated during the development process [61,62].

The bifunctional glycosylases, for instance, an 8-oxoG DNA glycosylase/AP lyase (OGG1), can perform both functions: the hydrolysis of *N*-glycosidic bond and the cleavage of the sugar–phosphate backbone. Other vital glycosylases which are responsible for the excision of oxidized purines and uracils are formamidopyrimidine DNA glycosylase (FPG) and uracil DNA glycosylase (UDG), respectively [61]. Curiously, while in bacteria, FPG is able to recognize and remove the 8-oxoG lesion, in plants, its homologue has structural differences that result in the minimal or no activity in DNA containing this oxidized base [63]. The appearance of uracil in DNA can arise due to a wrong incorporation of dUMP during the replication process as well as a hydrolytic deamination of cytosine [61].

The *Arabidopsis* genome encodes at least nine bifunctional glycosylases, the seven of which have been confirmed to have the AP lyase activity in vitro [54]. In addition, some of the glycosylases are actively involved in the DNA demethylation process by removing 5-methylcytosine [64].

A gap in the damaged strain may be filled in either by the insertion of a single nucleotide (short-patch repair, SP) or by DNA synthesis including a few nucleotides (long-patch repair, LP) [54,65] (Fig. 13.3). While in the former scenario, the gap filling is performed by DNA polymerase β (Pol β), in the latter case, DNA synthesis is done by the replicative DNA polymerase complex Pol δ/ε. The Pol β complex has an intrinsic deoxyribose lyase activity and is also capable of removing the sugar left by the monofunctional DNA glycosylase. Therefore, the polymerase releases the blocking 5′-dRP terminus, thus allowing for strand ligation by DNA ligase III. In the case of the LP pathway, the polymerases carry out the displacement of the strand containing the 5′-dRP terminus by 2–10 nucleotides at the 3′-region to the abasic site [1]. The generated flap structure is removed by the 5′-flap endonuclease FEN1 assisted by a proliferating cell nuclear antigen (PCNA) followed by ligation step by DNA ligase I. It seems that the choice of the BER-repair pathway depends on the nature of lesion as well as the type of glycosylase that initiates the repair [65]. The SP-repair pathway in plants seems to be missing distinct homologues of mammalian Pol β and DNA ligase III. At the same time, it has been shown that both the SP and LP pathways are active in *Arabidopsis* protein extracts and plants encode DNA ligase I that might be involved in the repair of both SSBs and DSBs in planta [54,66]. In addition, the *Arabidopsis* genome contains a gene which codes for DNA polymerase λ that contains N-terminal region that is similar to a human Pol λ. This polymerase has been shown to have the dRP-lyase activity in vitro, thus suggesting for its possible involvement in the BER pathway instead of Pol β [67].

In mammalian cells, in addition to Pol β, an X-ray repair cross-complementing protein 1 (XRCC1) is involved in the SP pathway. Although the *Arabidopsis* homologue of XRCC1 does not contain domains required for the interaction with Pol β and ligase III, it still contains the conserved BRCT domain responsible for the interaction with poly(ADP-ribose) polymerase (PARP). However, the XRCC1 homologue of rice has been hypothesized to interact with the plant Pol λ in the presence of the PCNA protein [68]. In *Arabidopsis*, the *PARP* gene has been shown to be responsive to DNA-damaging agents [69].

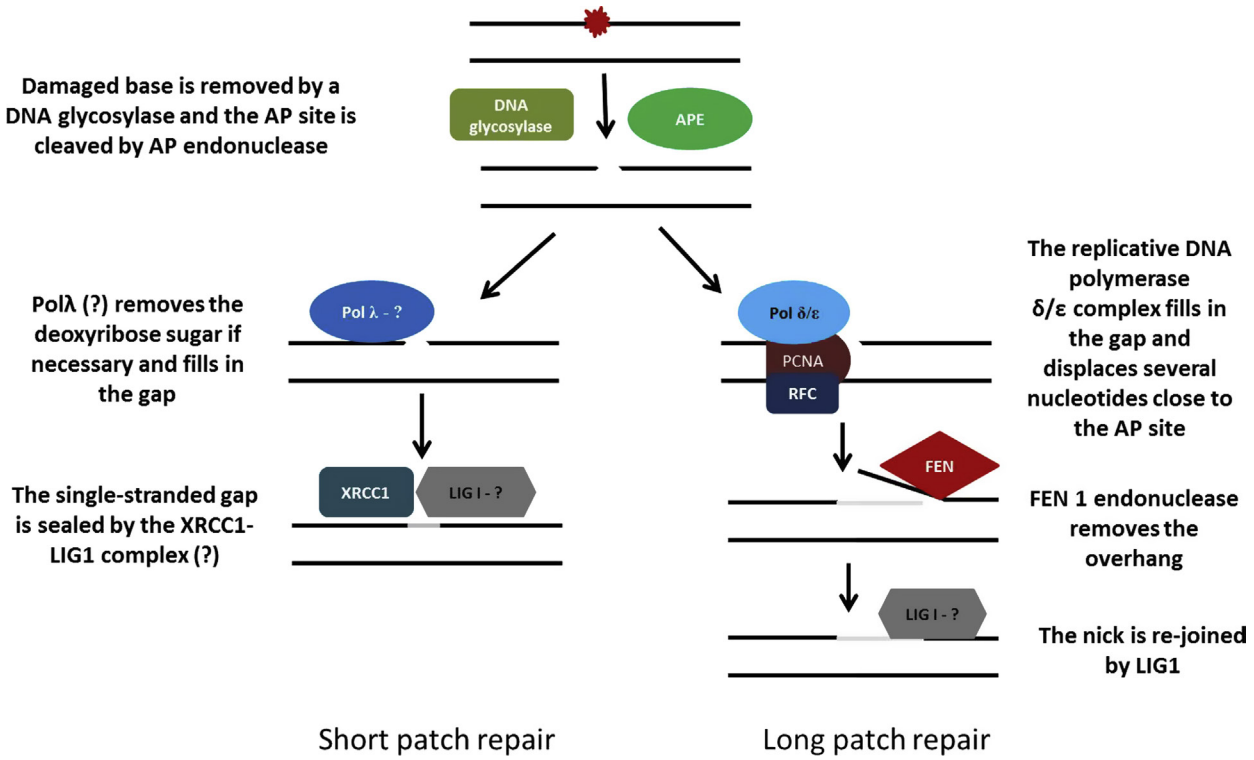

FIGURE 13.3 **Short- and long-patch repair of the base excision-repair (BER) pathway.** In the short-patch repair, the damaged base is cleaved off by either a DNA glycosylase or a bifunctional DNA glycosylase-endonuclease. Then an Apurinic/apyrimidinic endonuclease (APE) cleaves the sugar–phosphate backbone at the 5′-region of the AP site. Pol β removes the deoxyribose sugar if necessary and fills in the gap. In animals, the single-stranded gap is ligated by the XRCC1–LIG3 complex. Due to the absence of the definitive LIG3 homologue, this function is possibly performed by LIG1. In the long-patch repair, after base removal and APE nicking, the replicative DNA Pol δ/ε complex fills in the gap. During this process, a couple of nucleotides close to the AP site are displaced. The generated flap structure is removed by the 5′-flap endonuclease FEN1, and the nick is re-joined by LIG1. *Adapted from Bray CM, West CE. DNA repair mechanisms in plants: crucial sensors and effectors for the maintenance of genome integrity. New Phytol 2005;168(3):511–28.*

PARP1 has a high affinity for SSB intermediates produced during BER, but at the same time, it has been reported to have a negative effect on the rate of BER in animal cells and is not essential for an efficient completion of BER [70].

7. NUCLEOTIDE EXCISION REPAIR

The NER pathway is assumed to be less specific for a variety of DNA lesions because the enzymes involved in this pathway detect modifications broadly by conformational changes to the DNA double helix. NER is involved in the repair of DNA damage that causes significant distortions in the structure of the double helix, such as UV-photoproducts and bulky covalent lesions. Following the recognition, NER proteins remove a 24–32 oligonucleotide stretch with the altered nucleotide from the damaged strand, and the repair is completed by DNA synthesis and ligation [61]. The pathway includes two different modes, such as global genome repair (GGR) and transcription-coupled repair (TCR), with the difference between them being in the way of lesion recognition (Fig. 13.4). In plants, the pathway was discovered in the experiments involving EMS mutagenesis that resulted in a group of mutants which demonstrated a hypersensitivity to UV-C and γ-irradiation [71,72]. Thus, in addition to having an active photoreactivation pathway, plants rely on the NER pathway for the repair of UV adducts in DNA.

The recognition of DNA lesions in plants in the GGR pathway is performed by the multiprotein complex involving the xeroderma pigmentosum group C (XPC), RAD23, and centrin 2 (CEN2) [58]. The *Arabidopsis* homologue of the human XPC protein is *AtRAD4* [73]. Since the XPC protein is not capable of recognizing the UV-induced CPDs with a high efficiency, the DNA damage–binding (DDB) complex enhances its binding to the damaged DNA. The DDB complex consists of DDB1, DDB2 (XPE), Cullin4, Rbx1 proteins and is also complexed with an E3-ligase that targets specific E2-ubiquitin conjugating enzymes to other proteins including XPC. Upon the ubiquitination, the XPC protein acquires a higher affinity for the UV-damaged DNA. In addition, for the NER pathway to progress, the removal of nucleosomes around the damaged DNA region has to occur. In animal cells, it has been shown to be strictly related to the ubiquitination of H3 and H4

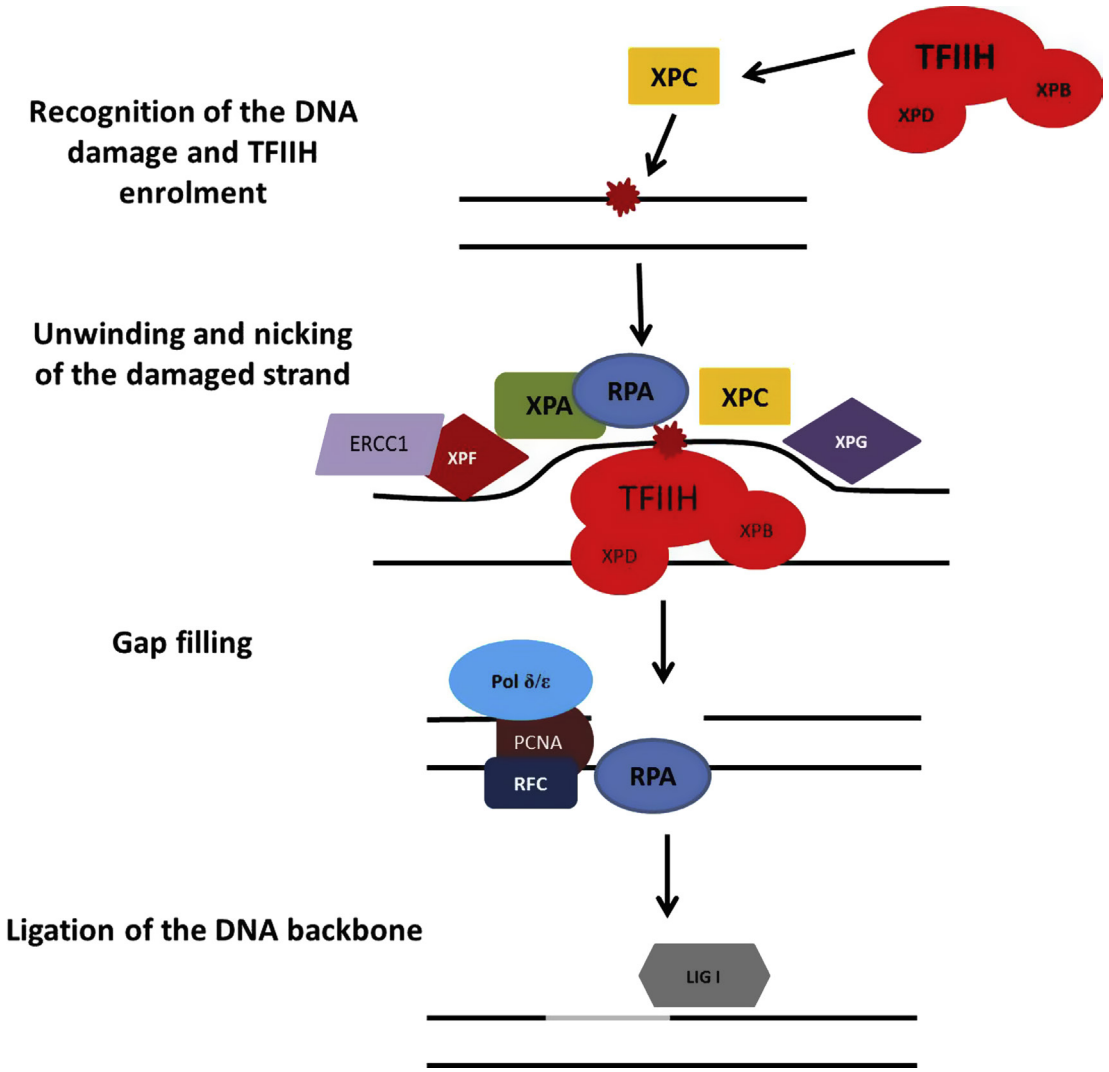

FIGURE 13.4 Global genomic repair of the nucleotide excision-repair (NER) pathway. The damaged DNA is recognized through binding by the XPC complex followed by recruiting the TFIIH complex. The complex contains nine subunits, including two helicases (XPB and XPD) which unwind the damaged region. The endonucleases XPG and XPF-ERCC1 allow a release of the single-stranded oligonucleotide. Following incision, a replication factor C (RFC) clamp loader adds PCNA at the 5′-site, and the replicative Pol δ/ε fill in the gap by rejoining the phosphodiester backbone by the DNA ligase I. *Adapted from Bray CM, West CE. DNA repair mechanisms in plants: crucial sensors and effectors for the maintenance of genome integrity. New Phytol 2005;168(3):511–28.*

histones that is performed by the components of an E3 ubiquitin ligase in response to UV exposure [74]. Mutation of the components involved in the DDB complex renders *Arabidopsis* plants hypersensitive to bulky DNA adducts caused by UV or cisplatin [21]. In line with these results, the overexpression of *DDB1A* and *DDB2* genes in *Arabidopsis* enhances UV-C tolerance in plants.

The recruitment of NER components following the stalling of the RNA polymerase II at the lesion site during transcription involves a Cockayne syndrome (CS) CSA and CSB proteins that may help remove the RNA polymerase complex and facilitate DNA repair. Although plants have two orthologues of CSA and one of CSB, their exact role remains uncharacterized [58].

Following the recognition of the DNA lesion, the XPC protein recruits a TFIIH complex that acts both as an RNA polymerase II transcription factor and a vital component involved in DNA repair. The complex contains nine subunits, including two helicases XPB and XPD which act in the 3′→5′- and 5′→3′-directions, respectively, and open the DNA helix around the lesions. Curiously, another transcription elongation factor II-S (TFIIS) stimulates RNA Pol II to bypass DNA regions containing specifically 8-oxoG in animal cells, thus preventing cell death due to the oxidative damage of DNA [75]. The TFIIS homologue was identified in plants, and the gene was shown to be responsive to stress in both the aerial parts and roots [76,77].

Additionally, the gene was up-regulated during seed imbibition that also requires the active DNA damage–repair process. Nevertheless, it still remains to be shown whether functions of the DNA lesion bypass pertain to the described homologue.

The *Arabidopsis* genome encodes two copies of XPB (*AtXPB1* and *AtXPB2*) which contain the conserved ATPase and helicase domains and also a homologue of XPD (*AtXPD*) [78]. While a complete knockout of the *AtXPB1*gene in *Arabidopsis* does not affect plant sensitivity to UV exposure, a mutation of the *AtXPD* gene is lethal. This apparently is due to a functional redundancy of *AtXPB1* and *AtXPB2* in the NER pathway. At the same time, both genes have been shown to be important during early stages of plant development because the *atxpb1* plants demonstrate a developmental delay, low seed viability, and a loss of germination synchrony.

The unwinding of the DNA helix around the lesion site exposes ssDNA and allows a replication protein A (RPA) to coat the strand [58]. In addition to the RPA protein, XPA is vital for the opening of the preincision complex followed by the recruitment of the XPF-ERCC1 complex by XPA. Although, plants appear to lack the orthologue of XPA, mutations of plant orthologues of XPF and ERCC1 render them hypersensitive to DNA-damaging agents. The cleavage at the 5′-end catalyzed by the XPF-ERCC1 nuclease complex releases the damaged DNA strand as a 24–32 oligonucleotide. The *Arabidopsis* genome encodes *AtRAD1*, a homologue of the XPF gene, the mutation of which causes a small reduction in the rate of CPD repair and hypersensitivity to DNA-damaging agents [79,80]. In addition, the RAD1/ERCC1 complex has been implemented in the removal of DNA flap structures generated during recombination. Following the incision, a replication factor C (RFC) clamp loader adds PCNA at the 5′-site, and replicative Pol δ/ε fill in the gap by the rejoining of the phosphodiester backbone by the DNA ligase (Table 13.3). The recognition of DNA adducts is the rate-limiting step in the NER pathway. This is due to the fact that the TCR pathway repairs different lesions at a constant rate, whereas the efficiency of GGR depends on the type of a lesion [81].

8. MISMATCH REPAIR

A mismatch is the mutagenic incorporation of an incorrect nucleotide that can occur during replication of both native and damaged DNA. An MMR pathway is involved in the efficient removal of erroneous nucleotides incorporated by the replicative DNA polymerase (Fig. 13.5). Although the proofreading function of the replicative polymerase limits the misincorporation of one nucleotide per 10^6–10^7 bp, this value is still high for the effective maintenance of genome integrity. Therefore, the error rate is further reduced by the MMR mechanism to one misincorporated base per 10^9–10^{10} nucleotides in the nascent DNA chain [61]. In addition, the MMR pathway may be also involved in eliminating mismatches at recombination sites. This allows to prevent recombination events which can cause inappropriate chromosome rearrangements [82]. Eukaryotic MMR involves the MutS HOMOLOGUE (MSH) proteins that are the evolutionarily conserved homologues of prokaryotic MutS. The *Arabidopsis* genome encodes seven MSH proteins, with MSH7 being unique to plants [83]. The heterodimeric protein complexes that include MSH2, 3, 6 and 7 are involved in the recognition of mismatches. Every dimer seems to be responsible for the recognition of specific lesions in the DNA sequence. For instance, the analysis of the in vitro produced MSH proteins revealed that an MSH2:MSH6 pair showed preference for a (T/G) base/base mispair and a one-nucleotide (+T) loop out [84]. At the same time, the heterodimer showed a minimal recognition of homoduplex (T/A) DNA, (C/C) heteroduplex, or to the three-nucleotide (+AAG) loop out. The recognition of the last DNA lesion, however, was best achieved by the MSH2:MSH3 heterodimer, although the binding to (C/C) and (T/G) mismatches was weak. Similarly to the MSH2:MSH6 pair, the MSH2:MSH7 heterodimer demonstrated an affinity for the (T/G) mispair and almost no binding to other lesions. While mutation of the *AtMSH2* gene in *Arabidopsis* is critical for the repair of mismatches in germline cells, it seems to be dispensable for MMR in somatic cells [83]. Similarly, both the *AtMSH4* and *AtMSH5* genes have been implemented in the DNA repair in gametes [85,86]. The expression of the genes is critical for floral organs, and their mutations cause a severe reduction in fertility due to meiotic defects. Moreover, the localization of AtMSH5 to the chromatin was compromised in the absence of AtMSH4.

The recognition of mismatches is linked to strand incision in eukaryotes by few orthologues of the prokaryotic MutL protein: MLH1, MLH2, MLH3, and PMS2. The *Arabidopsis* genome encodes orthologues of MLH1, PMS2, and a relatively distinct orthologue of MLH3 [83]. In animal cells, the MLH1:PMS2 pair plays a key role in the differentiation between the template and nascent DNA strands. Following the recognition step, the heterodimer catalyzes the excision of a stretch of the nascent strand DNA containing the erroneous nucleotide. The excision is terminated right after the mismatched base on the strand, and the DNA polymerase fills in the stretch of cleaved DNA followed by ligation of the strand by DNA ligase I [61] (Table 13.3).

A curious connection between MMR and an epigenetic pathway of the regulation of gene expression comes from 2015 studies on the *MSH1* gene [87,88]. MSH1 is a homologue of the yeast MSH1 protein which is absent in mammalian cells [89]. The protein is encoded in the nucleus but is localized to mitochondrial and chloroplast nucleoids and is involved in organelle genome stability [90]. Mutation of the *MSH1* gene increases the recombination rate of repeated sequences in the

TABLE 13.3 The Major Components of the Nucleotide Excision Repair and Mismatch Repair Pathways

Pathway	Protein Name	Function
Nucleotide excision repair (NER)	Multiprotein complex involving a Xeroderma pigmentosum group C (XPC), Rad23 and Centrin 2 (CEN2) proteins	Responsible for recognition of DNA lesions in plants during the global genome-repair pathway
	DDB complex consisting of DDB1, DDB2 (XPE), Cullin4, RBX1 proteins	Enhances binding of XPC protein to UV-induced cyclobutane pyrimidine dimers in the damaged DNA
	E3-ligase	Targets specific E2-ubiquitin conjugating enzymes to the XPC protein and others
	Cockayne syndrome (CS) CSA and CSB proteins	In plants, the exact role of orthologues remains uncharacterized. In animals, the proteins help to remove stalled RNA polymerase complex to facilitate the DNA repair
	TFIIH complex which contains nine subunits, including two helicases—XPB and XPD	Acts both as an RNA Pol II transcription factor and the vital component involved in the DNA repair
	Transcription elongation factor II-S (TFIIS)	Stimulates RNA Pol II to bypass DNA regions containing specifically 8-oxoG in animal cells; in plants, the homologue has been identified, but the function remains unknown
	Replication protein A (RPA)	Binds to ssDNA to stabilize it after unwinding of the DNA helix
	XPF-ERCC1 nuclease complex	Catalyzes the cleavage at the 5′-end that releases the damaged DNA strand as a 24–32 oligonucleotide; the *Arabidopsis* genome encodes a homologue of the XPF gene—*AtRAD1*
	Replicative Pol δ/ε	Fill in the gap
	LIGASE I	Possibly involved in the rejoining of DNA strands
Mismatch repair (MMR)	MutS homologue (MSH) proteins, such as heterodimeric protein complexes MSH2, 3, 6, and 7	Involved in the recognition of mismatches
	MSH1	The protein is encoded in nucleus but is localized to the mitochondrial and chloroplast nucleoids and is involved in organelle genome stability

mitochondrial genome, causes cytoplasmic male sterility, and a number of phenotypic abnormalities in plants [89]. This effect seems to be universal across plant kingdom and is referred to as developmental reprogramming [91]. Curiously, the *msh1*-associated phenotype can persist for multiple generations even when gene expression is restored to a wild-type level [92]. Furthermore, reciprocal crosses between the dwarf sorghum line with the *msh1*-associated phenotype (but with the WT genetic background) and the WT line resulted in the F1 progeny which demonstrated an enhanced vigor as compared to genuine WT plants. Similar results were obtained in other plant species including tomatoes, soybean, tobacco, and *Arabidopsis* [88,89]. A global analysis of a DNA methylation profile in *msh1* plants revealed alterations in the methylation level at CG and non-CG positions as compared to WT plants [87]. At the same time, no differences were detected in the DNA sequence itself that would argue against the developmental reprogramming phenotype caused by mutations in the genome. Since the gene is responsive to stress conditions in somatic tissues [91,93], it can be speculated that MSH1 is a novel component of the environmental sensing apparatus of plants which links the detection of alterations in ambient conditions through plastids to epigenetic responses of the whole plant [87].

9. DNA DOUBLE-STRAND BREAK REPAIR

An efficient repair of DSBs is of a particular importance for plant growth and development. The progression of DNA replication in the presence of DSBs can lead to the loss of chromosome fragments that can be detrimental in actively

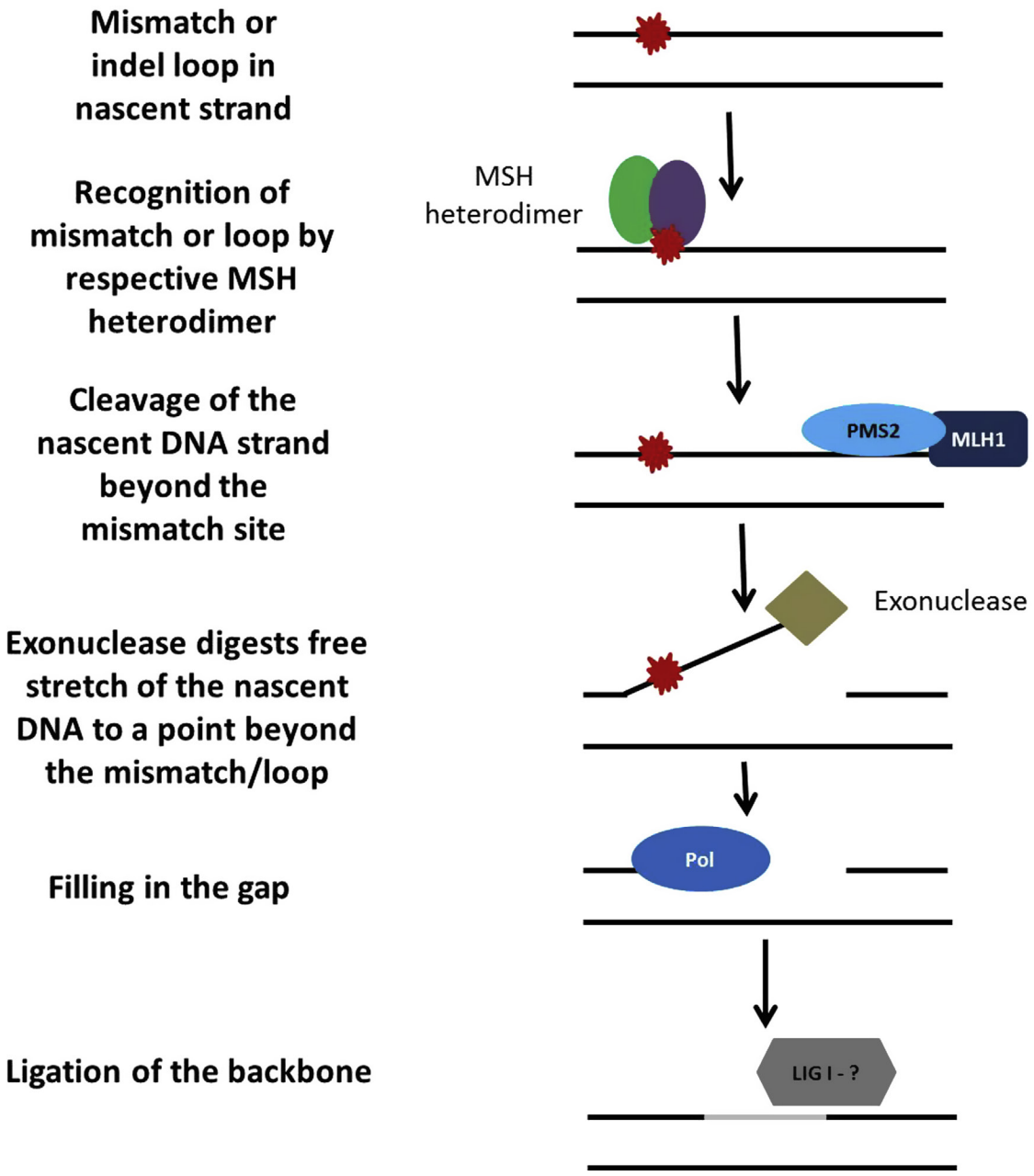

FIGURE 13.5 A simplified scheme of the mismatch repair (MMR) pathway in plants. A mismatch or loop is recognized by an appropriate MSH heterodimer (s) followed by cleavage of the nascent strand catalyzed by MLH1/PMS2. This promotes the unwinding of the DNA helix and digestion of the nascent strand to a point beyond the mismatch lesion/loop. The gap is filled in by DNA polymerase followed by backbone ligation. *Adapted from Bray CM, West CE. DNA repair mechanisms in plants: crucial sensors and effectors for the maintenance of genome integrity. New Phytol 2005;168(3):511–28.*

dividing cells [94]. In addition, an incorrect repair can cause chromosome fusions leading to dicentric chromosomes and anaphase bridges.

There are two major pathways involved in the repair of DNA DSBs (DSB) in living cells: homologous recombination (HR) and nonhomologous end-joining (NHEJ) or illegitimate recombination. The two pathways are considered to be responsible for maintaining a balance of genome stability versus genetic diversity, and the NHEJ pathway significantly prevails over HR in vascular plants [1]. The rate of the HR-mediated DSB repair in somatic cells is quite low and is about 1 in 10^3 repair events [95]. At the same time, when DSB occurs between tandem repeats, 30% of DSBs can be repaired by a single-strand annealing (SSA) and approximately 7%—by a synthesis-dependent strand annealing (SDSA) [96,97]. Similarly, the induced DSBs significantly increase the rate of HR that is probably concomitant with an increase in the frequency

of repair through the NHEJ pathway. In addition, DNA-repair mutants, such as *uvr2–1* (CPD-photolyase), *atrad50*, and *atcen2* also demonstrate an increase in the rate of HR repair (Table 13.4).

9.1 Homologous Recombination

The HR pathway plays a vital role in both DSB repair in somatic cells and meiotic recombination during gametogenesis. The pathway includes RPA proteins and proteins in the RAD52 epistasis group, such as RAD51, RAD52, RAD54, and the MRN complex of RAD50, MRE11 and yeast XRS2/human NBS1. In addition, HR also requires the RAD51-like proteins. The *Arabidopsis* genome contains seven RAD51 homologues that are divided into two ancient groups: RADα and RADβ subfamilies [98]. While the former subfamily includes both RAD51 and DMC1 proteins, the latter one contains the RAD51B, RAD51C, RAD51D, XRCC2, and XRCC3 proteins. The RAD51, DMC1, RAD51C, and XRCC3 proteins have a unique role in meiotic HR and are necessary for a normal fertility [99]. In addition, the RAD51, RAD51C, and XRCC3 proteins are involved in DNA repair in somatic cells [1].

In yeast cells, Rad52 is a key protein involved in DBS repair and HR. It promotes DNA annealing and is involved in Rad51-mediated strand invasion. Eventually, Rad52 may participate in capturing the second DNA end followed by strand annealing to the D-loop and the formation of a Holliday junction [100]. The *Arabidopsis* genome also contains two homologues of the *RAD52* gene, the mutation of which causes a reduced fertility, a sensitivity to cross-linking drug mitomycin C, and the reduced level of intrachromosomal recombination compared to wild-type plants [101]. Similarly, null mutations of other components of the HR pathway, such as *AtRAD51*, *AtRAD50*, and *AtMRE11* result in a sterility due to severe meiotic defects [102–104].

Overall, three distinct models of HR are recognized: DSB repair (DSBR), SDSA, and SSA, with all three pathways being active in plants [105] (Fig. 13.6). The DSBR model is best described as a part of meiotic recombination [1]. The meiotic recombination occurs between homologous chromosomes rather than sister chromatids to stimulate the mixing of parental

TABLE 13.4 Proteins Involved in the DNA Double-Strand Break Repair

Pathway	Protein Name	Function
Homologous recombination	RAD51	Required for homology search
	The RAD51-like proteins: DMC1, RAD51B, RAD51C, RAD51D, XRCC2, and XRCC3	Involved in the steps of strand invasion and later on during the recombination
	RAD52	Promotes DNA annealing and is involved on RAD51-mediated strand invasion
	RAD54	Promotes chromatin remodeling and protein displacement from dsDNA and interacts with RAD51 protein that stimulates DNA strand exchange activity
	RECQ4A helicase	Plays a major role in replication fork regression
	FANCM helicase	Inhibits the formation of crossover recombinants during meiosis, thus favoring non-crossover resolutions
Nonhomologous end-joining	KU70 and KU80 heterodimer	Protects the DNA ends from exonuclease activity
	MRE11–RAD50–NBS1 (MRN)	Involved in the DNA-damage repair, DNA replication, meiosis, and telomere maintenance
	Poly(ADP-ribose) polymerase(PARP)	Involved in DNA-damage response in *Arabidopsis*
	XRCC1	A key protein in BER and single-strand break repair; acts as a scaffold for other DNA-repair proteins; involved in KU-independent alternative end-joining pathway that results in the large deletions at the joints
	DNA ligase IV	Is a specialized ligase that catalyzes a final step in the NHEJ pathway and together with its cofactor XRCC4 interacts with KU to seal a joint

genomes. The process starts by the creation of DSB and the formation of a Holliday junction followed by processing of ends to produce long 3′-tails. The single-stranded nucleoprotein filament mediates homology search and the invasion of the homologous chromosome. An in vitro study has shown that homology search requires a single RAD51 protein. The protein is also vital for plant reproduction because the *Arabidopsis atrad51* mutants are sterile [102]. At the same time, the mutant does not display any phenotypic abnormalities and is not hypersensitive to DNA-damaging agents. Additional RAD51-like proteins are

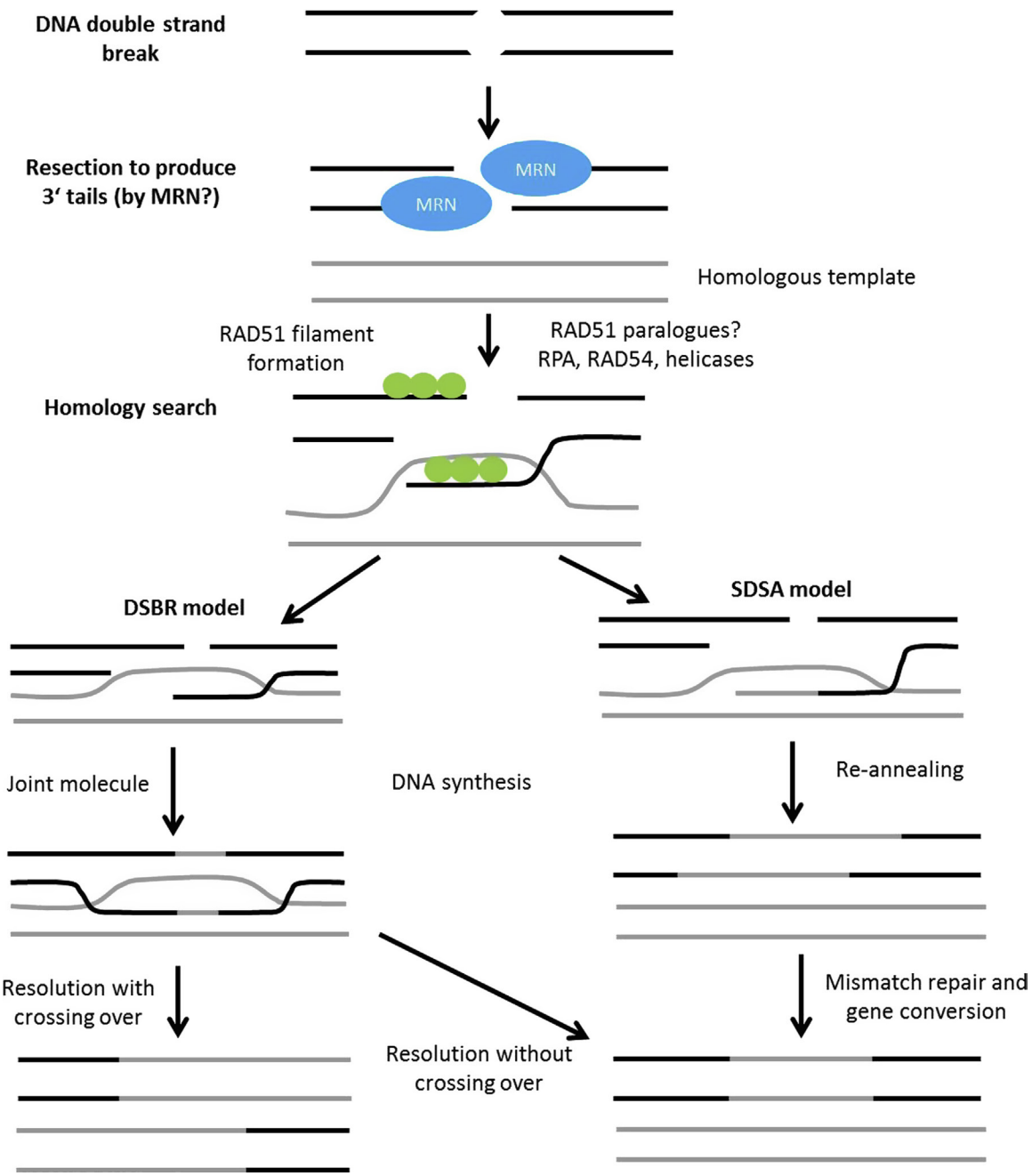

FIGURE 13.6 **The homologous recombination pathways of DNA double-strand break repair.** Three different models of homologous recombination (HR) pathways are recognized in plants, including double-strand break repair (DSBR), synthesis-dependent strand annealing (SDSA), and single-strand annealing (SSA). The former one is considered to be the most active pathway (not shown); it functions when a break occurs between the repeated sequences and results in the deletion of the intervening sequence. The SDSA model explains most of the recombination products observed in plants in the nonrepeat regions. The region copied during the recombination in this pathway remains unaltered. The DSBR model describes crossing over of chromosomes during meiosis. MRN is a complex of MRE11, RAD50, and NBS1. *Adapted from Waterworth WM, Drury GE, Bray CM, West CE. Repairing breaks in the plant genome: the importance of keeping it together. New Phytol 2011;192(4):805–22.*

also required in strand invasion steps and later on during the recombination [104]. In *Arabidopsis*, it has been speculated that the RAD51B, RAD51D, and XRCC2 proteins might interact with each other to form a protein complex that also cooperates with RAD51C in the HR pathway. Moreover, the RAD51B, RAD51D, and XRCC2 proteins are partially redundant because a triple mutant of the three genes demonstrates a higher sensitivity to bleomycin than single and double mutants [106].

The search for the homologous sequence will not be possible without alterations in the chromatin structure; therefore, the chromatin-remodeling enzymes and helicases assist in homology search. The invading strand may have differences in the sequence resulting in the occurrence of the heteroduplex DNA leading to the repair of mismatches by MMR [1]. The DNA synthesis is then initiated on the invaded strain by using a homologous DNA as a template. Eventually, the invading strand is ligated to the other side of DSB leading to the formation of two Holliday junctions that may undergo either a crossover or gene conversion to be resolved.

In the process of SDSA, a sister chromatid, the homologous chromosome, or an ectopic region of homology in the genome can be used as a recombination substrate. The repair mechanism is used both in meiotic and somatic cells, and similarly to DSBR, it is initiated by the generation of long single-stranded 3′-ends that invade a homologous stretch followed by the DNA synthesis. Unlike the DSBR pathway, SDSA rarely involves the formation of Holliday junctions and crossovers. This is achieved by annealing of the newly synthesized DNA to the other side of DSB to promote break repair, thus avoiding the formation of joint molecules [107]. The avoidance of crossovers during SDSA eliminates the possible mutagenic effect of recombination that can occur between ectopic regions of homology. At the same time, the Holliday junction can take place in the process when the recombination occurs between sister chromatids. SDSA is the main pathway of conservative HR repair in somatic cells in plants [108]. The involvement of SDSA over DSBR also plays a key role in the maintenance of genome stability. This is due to the contribution of crossovers to the DSBR process that if occurred in somatic cells at ectopic positions in chromosomes could potentially result in dicentric and acentric chromosomes, thus causing genome instability.

The SSA mechanism, in turn, can utilize tandem repeats that are arranged in a close proximity. In the genome, local duplication events as well as clustered ribosomal genes can become the preferred substrates for SSA. The DNA sequence between tandem repeats is removed during the recombination process, thus suggesting for an additional mechanism (NHEJ is the other one) that can cause DNA loss during the evolution and is mutagenic [1]. In the process of SSA following the DSB induction, the homologous regions are getting exposed during the resection. The partially complementary strands can directly anneal to each other, and the chimeric DNA-double helix can be formed. The extra 3′-overhangs can be trimmed or the single-stranded gaps can be filled in through the DNA synthesis. Eventually, the DNA backbone is joined together by the DNA ligase. The main difference between SSA and SDSA is that the latter pathway requires the strand exchange, whereas the former one does not. Unfortunately, no proteins have been characterized yet that are exclusively necessary for the SSA pathway. At the same time, the recombination process in the SDSA pathway requires homologues of the recombinase RecA, AtRAD51, AtXRCC3, AtRAD54, DNA helicases AtRECQ4A, and AtFANCM, as well as nucleases like AtMUS81 [108].

9.1.1 Replication-Associated HR

The HR pathway may be also involved in processing the stalled replication forks. A number of factors can cause stalling of the replication forks, including cross-linked DNA strands, the presence of the inhibitory modified base in the strand, and an increased tension of dsDNA due to supercoiling caused by an improper function or inactivation of topoisomerases [108]. Because the free ends of the double-stranded DNA are not available in the process, the repair cannot be done by the NHEJ and SSA pathways. Therefore, it is assumed that in animal cells, the pathway is more important during the S phase as compared to gap phases where the NHEJ and SSA pathways prevail. The repair can progress according to the two main scenarios: the replication bypass and the formation of one-sided DSB. In the case of the replication bypass, translational polymerases can synthesize the complementary strand besides the altered DNA bases. At the same time, if the DNA strand cannot be elongated due to a severe damage of the template strand, the second daughter strand can still be synthesized in the process known as "overshoot synthesis." The process resembles the steps involved in the SDSA mechanism and relies on the sister duplex strand for copying the information onto the shorter daughter strand, thus avoiding the damaged part at the template strand [108]. In the second scenario, DSB can form when the polymerase encounters a nick on one of the template strands or when the replication fork stalls due to the endonuclease activity. Since there is no second end present on another side of the break, the NHEJ pathway cannot be engaged in the repair. Thus, the one-sided DSB is repaired using the homologous sequence present on the sister chromatid.

Curiously, in the studies on *Arabidopsis* mutants, two inhibitors of the HR pathway were revealed, RECQA and FANCM. The genes seem to be involved in different mechanisms of HR suppression because the double mutant demonstrated higher spontaneous HR frequencies compared to the single mutants [108].

9.2 Nonhomologous End-Joining

Experiments involving a stable transformation of either somatic cells or gametes revealed that in most of the cases, the foreign DNA integrates randomly into the genome of higher plants without utilizing the regions of homology to the endogenous DNA. This and other observations have led to the conclusion that the majority of DSBs in plant cells are repaired through NHEJ in higher eukaryotes [1]. It is believed that there are at least two different mechanisms of NHEJ repair, such as classical and alternative NHEJ pathways (cNHEJ and aNHEJ, respectively) that differ in both the key players involved in the pathways and the final outcome of the repair process [109]. While in the cNHEJ pathway, DSB repair does not require the microhomology at the joints, the aNHEJ mechanism requires small homology regions. The ends in the first pathway are protected against the exonuclease activity by binding the KU70/80 heterodimer followed by ligation mediated by ligase IV. In addition, in yeast, if the ends of the damaged DNA do not carry the 5′-phosphate and 3′-hydroxyl group required for ligation, they can be processed by the MRE11–RAD50–XRS2 (MRX) complex [110] (Fig. 13.7). In plants, a similar complex—MRE11–RAD50–NBS1 (MRN)—is involved in DNA-damage repair, DNA replication, meiosis, and telomere maintenance. The complex recognizes DSBs by its ability to bind to the ends of DNA. Following binding, the complex unwinds and initiates the processing of the ends. The *mrnatr* double mutants demonstrate the growth retardation effect caused by the accumulation of DNA lesions and cell death [17]. Similarly, KU-deficient mutants are hypersensitive to DNA-damaging and alkylating agents, further suggesting the involvement of these genes in the DNA-repair process [111,112]. Additionally, mutants compromised in the KU70 protein demonstrate drastic telomere deregulation leading to an increase in the telomere length as compared to WT plants.

The aNHEJ mechanism resembles the SSA-repair pathway because similarly to the last mechanism, the 3′-resection of the broken ends occurs by a specific exonuclease enzyme complex followed by microhomology search, trimming and ligation of the broken ends by DNA ligase IV. In *Arabidopsis*, both PARP1 and XRCC1 are involved in the aNHEJ pathway, and it seems that the PARP1 protein competes with KU80 for DSBs [113]. The result of NHEJ repair is almost always a genomic change caused by either deletions of different sizes or insertions. In addition, if more than one break is induced simultaneously, the potential outcome can be a rearrangement, which leads to the generation of a new sequence combination. In most cases, the reshuffling of the genome would be detrimental to the viability of the progeny; therefore, it will not be propagated. In rare occasions, however, small rearrangements of chromosomes can be inherited, thus possibly affecting the speciation [108].

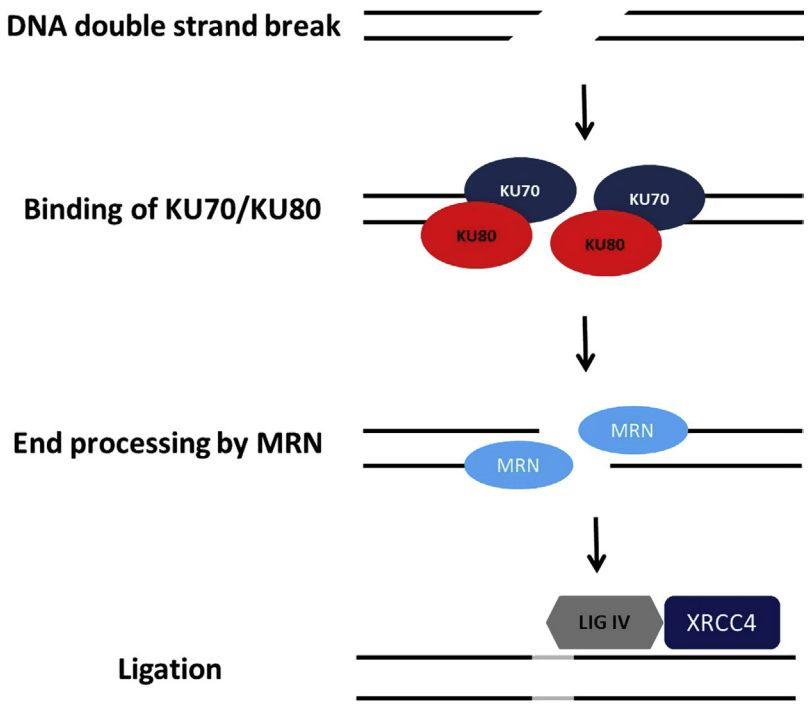

FIGURE 13.7 The nonhomologous end-joining pathway of DNA double-strand break repair. In the classical nonhomologous end-joining (NHEJ) pathway, the recognition of free DNA ends is performed by the KU70–KU80 complex. Later, the MRN complex (contains MRE11, RAD50, and NBS1 proteins) binds to DSB, and if the damaged ends do not carry the 5′-phosphate and 3′-hydroxyl group required for ligation, it unwinds the strands and initiates processing the ends. The DNA LIGASE 4–XRCC4 complex seals the phosphodiester backbone. In the absence of KU proteins, the repair can occur followed by microhomology search and requires the presence of either MRE11 or LIG4. *Adapted from Waterworth WM, Drury GE, Bray CM, West CE. Repairing breaks in the plant genome: the importance of keeping it together. New Phytol 2011;192(4):805–22.*

10. DNA REPAIR IN ORGANELLES

The maintenance of genome stability in plants also includes an efficient DNA-damage repair in chloroplasts and mitochondria which are the main factories of ROS in the cell. Unfortunately, the DNA-repair mechanisms in these organelles remain largely unexplored, although the 2014 studies provided evidence of resemblance of the HR pathway in chloroplasts to that observed in bacteria [114,115]. The HR repair in bacterial cells is performed by the RecA/RecBCD pathway [116]. RecA proteins that are targeted to mitochondria and chloroplasts were described in *Arabidopsis*, suggesting that the HR pathway is also active in organelles [115,117]. In addition, such components of the BER pathway as the endonuclease III homologues and the AP endonuclease were also identified in *Arabidopsis* chloroplasts [118]. Similarly, the BER pathway is also active in mitochondria because the uracil DNA glycosylase associated with mitochondrial membranes has been characterized in the previous study [119]. At the same time, substrates for the NER pathway are likely to be repaired by alternative pathways since there is little evidence for the presence of this pathway's components in organelles [61,119].

11. FUTURE PERSPECTIVE

The ability of plants to maintain the genome integrity in response to external and internal cues is detrimental for both the survival of an individual plant and the transmission of intact genetic information to future generations. At the same time, due to errors in DNA-repair pathways, genetic variation can lead to the genetic diversity, the appearance of altered traits, which occasionally can be beneficial in the new environment. Although our understanding of DNA-repair pathways in plants is far from complete, it is now clear that the utilization of DNA-repair components can benefit applied studies including those in genome editing and breeding for crop varieties with improved DNA-repair functions. In the case of genome editing, benefits of either the NHEJ or HR pathway are harnessed for the targeted gene disruption or insertion, respectively, by using designed endonucleases. Similarly, crop varieties with improved DNA-repair pathways may be more stress tolerant, whereas unrepaired DNA damage directly correlates with yield loss [61]. A deeper understanding of DNA-damage repair in organelles will also be essential for biotechnology applications since stable modifications of the organelle genome can be beneficial for both the improvement of photosynthesis and the avoidance of the spread of the modified genome through cross-pollination. Therefore, further elucidation of DNA-repair pathways in plants will be valuable for the generation of crops with improved traits.

GLOSSARY

Base excision repair DNA damage–repair process which is primarily responsible for the removal of small base lesions from the genome.

Homologous recombination repair Type of double-strand break repair in which nucleotide sequences are exchanged between two identical or very similar molecules of DNA during genetic recombination.

Mismatch The mutagenic incorporation of an incorrect nucleotide that can occur during replication and recombination of both native and damaged DNA.

Mismatch repair DNA-repair process involved in removing mismatches from the DNA structure.

Nonhomologous end-joining repair Double-strand break-repair pathway that involves simple rejoining of the broken ends of the DNA molecule either in the presence or absence of microhomology regions between the broken ends.

Nucleotide excision repair DNA damage–repair process which is responsible for removing bulky lesions in DNA that cause the disruption of the DNA helix.

Photoreactivation Repair process of UV-damaged DNA by photolyase enzymes with the utilization of the light source with the longer wavelengths.

LIST OF ACRONYMS AND ABBREVIATIONS

5′-dRP 5′-deoxyRibose-5-phosphate termini
6-4PP Pyrimidine-pyrimidone (6-4) photoproduct
8-HDF 8-hydroxy-7,8-didemethyl-5-deazariboflavin
8-oxoAde 8-oxo-7,8-dihydroadenine
8-oxoGua 8-oxo-7,8-dihydroguanine
8-oxodGuo 8-oxo-7,8-dihydro-20-deoxyadenosine
AP Apurinic/apyrimidinic
ASF1 *Anti-silencing function1* gene
ATM Ataxia telangiectasia-mutated protein
ATR Ataxia telangiectasia and Rad3-related protein
BER Base excision repair

CAF1 Chromatin assembly factor 1
CEN2 Centrin 2
Chk1 and Chk2 Checkpoint-related protein kinases
cNHEJ and aNHEJ Classical and alternative NHEJ, respectively
CPD Cyclobutane pyrimidine dimer
CSA Cockayne syndrome protein A
DDB DNA damage–binding complex
DSBR DSB repair by the HR pathway
EMS Ethyl methane sulfonate
FADH Flavin adenine dinucleotide
Fapy-Gua 2,6-diamino-4-hydroxy-5-formamidopyrimidine
FAS1 FASCIATA 1 protein
FEN1 5′-flap endonuclease
FPG Formamidopyrimidine-DNA glycosylase
GGR Global genome repair
H2AX Histone 2A isoform
HR Homologous recombination
LP Long-patch repair
MIM Hypersensitive to MMS, irradiation and MMC
MMR Mismatch repair
The MRX and MRN complexes MRE11–RAD50–XRS2 and MRE11–RAD50–NBS1 complexes, respectively.
MSH MutS Homologue
MSI1 Multicopy suppressor of IRA1
MTHF Pterin methenyltetrahydrofolate
Nbs1 Nijmegen breakage syndrome gene
NER Nucleotide excision repair
$^1O_2$ Singlet oxygen
OGG1 8-oxoG DNA glycosylase/AP lyase
·OH Hydroxyl radical
PARP Poly(ADP-ribose)polymerase
PCNA Proliferating cell nuclear antigen
Pol β DNA polymerase β
RFC Replication factor C clamp loader
ROS Reactive oxygen species
RPA Replication protein A
SDSA Synthesis-dependent strand annealing
SMC Structural maintenance of chromosome proteins
SOG1 Suppressor of gamma response 1
SP Short-patch repair
SSA Single-strand annealing
SSB Single-strand break
SWI2/SNF2 SWItch/Sucrose nonfermentable
TCR Transcription-coupled repair
TFIIH Transcription factor II H
TFIIS Transcription elongation factor II-S
UDG Uracil DNA glycosylase
UV Ultraviolet radiation
XPC Xeroderma pigmentosum group C
XRCC1 X-ray repair cross complementing protein 1

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