REVIEW

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Protecting DNA from errors and damage: an overview of DNA repair mechanisms in plants compared to mammals

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Abstract The genome integrity of all organisms is constantly threatened by replication errors and DNA damage arising from endogenous and exogenous sources. Such base pair anomalies must be accurately repaired to prevent mutagenesis and/or lethality. Thus, it is not surprising that cells have evolved multiple and partially overlapping DNA repair pathways to correct specific types of DNA errors and lesions. Great progress in unraveling these repair mechanisms at the molecular level has been made by several talented researchers, among them Tomas Lindahl, Aziz Sancar, and Paul Modrich, all three Nobel laureates in Chemistry for 2015. Much of this knowledge comes from studies performed in bacteria, yeast, and mammals and has impacted research in plant systems. Two plant features should be mentioned. Plants differ from higher eukaryotes in that they lack a reserve germline and cannot avoid environmental stresses. Therefore, plants have evolved different strategies to sustain genome fidelity through generations and continuous exposure to genotoxic stresses. These strategies include the presence of unique or multiple paralogous genes with partially overlapping DNA repair activities. Yet, in spite (or because) of these differences, plants, especially *Arabidopsis thaliana*, can be used as a model organism for functional studies. Some advantages of this model system are worth mentioning: short life cycle, availability of both homozygous and heterozygous lines for many genes, plant transformation techniques, tissue culture methods and reporter systems for gene expression and function studies. Here, I provide a current understanding of DNA repair genes in plants, with a special focus on *A. thaliana*. It is expected that this review will be a valuable resource for future functional studies in the DNA repair field, both in plants and animals.

Introduction

All organisms are constantly exposed to environmental stresses as well as genotoxic products from endogenous metabolic processes that induce, either directly or indirectly, DNA damage [1]. Another source of DNA damage includes base–base mismatches produced by the incorporation of a non-complementary Watson–Crick base and unpaired nucleotides caused by misalignment of the two DNA strands, both generated during DNA synthesis. These DNA biosynthetic errors differ from typical DNA damage in that they involve undamaged bases and exist only transiently provided that the DNA remains double-stranded [2]. To counteract these genome integrity threats, all organisms have evolved the DNA damage response (DDR) [3, 4]. The DDR is a pathway that transduces the DNA damage signal into activation of various pathways that leads to DNA repair, cell cycle checkpoint and programmed cell death. Plant DDR also includes endoreduplication. Endoreduplication involves replication of the nuclear DNA without cytokinesis [5]. Here, I only focus on DNA repair mechanisms in mammals and plants. Plants are distinctly different from mammals in that they lack a reserved germ line and are sessile. Gametes arise from meristem cells that

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have been exposed to the continuous effect of environmental and endogenous mutagens and have divided many times. Thus, plants have evolved particularly efficient DNA repair mechanisms. Advancements in this field have been provided by the Arabidopsis genome sequence [6]. Plants have orthologs of most of the genes involved in mammalian DNA repair pathways [7–9]. However, several interesting features may be noted: the presence of unique genes, the presence of multiple gene copies and the absence of well-characterized genes in plant genomes [9, 10]. Such differences suggest plant-specific DNA repair pathways. Provided here is a review of major findings over the last decade related to mammal and plant DNA repair mechanisms, with a special focus on DNA repair genes from *A. thaliana*.

Photoreactivation

Photoreactivation is the direct reversal of major lesions induced by ultraviolet (UV) light on two adjacent pyrimidine bases, such as cyclobutane pyrimidine dimers (CPDs) and pyrimidine (6-4) pyrimidones (6-4 photoproducts or 6-4 PPs). The history of the discovery of this repair system has been recently reviewed [11]. Photoreactivation relies on single light-activated flavoenzymes called photolyases. Photolyases belong to the photolyase/cryptochrome family and have been classified into three related subfamilies known as class I CPD photolyase, class II CPD photolyase, and 6-4 photolyase, based on their sequence homology and substrate specificity [12–14]. Multiple sequence alignment analysis of members from the three subfamilies indicates a highly functional conservation of a photolyase-homologous region (PHR) domain that binds the chromophore FAD (flavin adenine dinucleotide) [15, 16]. Class I CPD photolyases have been reported in diverse microbial organisms such as archaea, eubacteria, and fungi; class II CPD photolyases have been found not only in archaea, eubacteria, some viruses, but also in higher organisms such as animals and plants; and 6-4 photolyases have been identified in cyanobacteria and certain eukarya [15, 17]. Humans and other placental animals have lost photolyase activities. All photolyases use the energy of visible or blue light ($\lambda = 320–500$ nm) for the catalytic cleavage of the photoproducts and contain the fully reduced FADH$^-$ as the catalytically active cofactor [13, 14]. All photolyases characterized to date also contain a second chromophore, such as methenyltetrahydrofolate found in the majority of the enzymes or 7,8-didemethyl-8-hydroxy-5-deazariboflavin, FAD or FMN (flavin mononucleotide) in others that functions as a photoantenna to enhance light absorption [14, 18–20]. Upon blue light absorption, the excitation energy is efficiently transferred from the photoantenna to

FADH$^-$ by Förster resonance energy. The resulting FADH^{-*} donates an electron to the DNA lesion with the generation of two canonical pyrimidines [12, 14, 19–21].
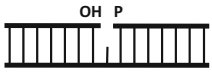
Photoreactivation in plants

Arabidopsis thaliana contains two specific class II CPD photolyases [PHR1, also named UVR2 (At1g12370) and PHR2 (At2g47590)] and one 6-4 photolyase [UVR3 (At3g15620)]. The FAD-binding domains of plant PHR2 proteins are smaller and less conserved than in the other subfamilies [15]. Several articles provide a summary of the molecular cloning, the tissue-specific and light-dependent regulation and the characterization of mutants of CPD photolyase and 6-4 photolyase genes from various plant species (e.g., *A. thaliana*, *Oriza sativa*, *Sinapis alba*) [22–24]. Recent evidence suggests that At*PHR1* expression induced by UV-B is primarily regulated by the UVR8 (UV RESISTANCE LOCUS8)-dependent pathway but is also mediated by a UVR8-independent pathway [25]. The latter one was found to be correlated with UV-B-induced CPD levels [25].

The crystal structure of a UVR2 homolog has recently been determined in rice and compared with prokaryotic class I CPD photolyases [26]. Results indicate that both enzymes recognize CPD damage in similar active sites but create a different conformational distortion in the DNA duplex [26]. The crystallographic structure and molecular mechanism of *A. thaliana* 6-4 photolyase have also been determined [27, 28]. After light excitation, there is initially a forward electron transfer followed by a cyclic proton transfer involving an active-site histidine residue [28]. This proton transfer is the determinant step in the repair and determines the overall quantum yield (Φ = number of dimers repaired per number of photons absorbed) [28]. For comparison, the reported Φ for CPD and 6-4PP repair are 0.82 and 0.1, respectively, and the whole catalysis reaction takes less than a nanosecond for CPD repair or tens of nanoseconds for 6-4PP repair [29]. The complete photocycle in real-time from the initial 6-4 PP to several intermediates and subsequent conversion to two thymine bases catalyzed by the *A. thaliana* (6-4) photolyase has been recently well reviewed [21, 29].

Base excision repair

Base excision repair (BER) recognizes and repairs lesions such as deaminated, oxidized and alkylated bases, abasic (apurinic and/or apyrimidinic, AP) sites and single-strand breaks (SSBs). BER occurs in several steps (Table 1A; see references [30–32] for recent reviews in mammalian BER). (1) Lesion recognition and removal by DNA glycosylases.

Table 1 Overview of steps (A) and *A. thaliana* required factors (B) involved in BER

A		**B**	
Step	**Schematic representation**	**Gene** [a]	**AGI locus code** [b,c]
i) Lesion recognition and removal		OGG1	At1g21710
		FPG	At1g52500
		NTH1	At2g31450
		NTH2	At1g05900
		DME	At5g04560
		ROS1	At2g36490
		UNG	At3g18630
		DML3	At4g34060
		MBD4L	At3g07930
ii) Cleavage of the sugar–phosphate backbone at the AP site [d]		ARP	At2g41460
		APE1L	At3g48425
		APE2	At4g36050
iii) Processing of unconventional ends to 3′ OH and 5′ phosphate		ZDP	At3g14890
		TDP1	At5g15170
iv) Gap-filling and nick sealing		XRCC1	At1g80420
		SAV6	At5g26680
		PARP1	At2g31320
		PARP2	At4g02390
		Pol δ	See Table 2
		Pol ε	See Table 2
		LIG1	See Table 2

[a] Genes encoding bifunctional glycosylases are listed first, followed by monofunctional glycosylases

[b] *AGI* Arabidopsis genome initiative

[c] AGI locus code for components involved in DNA metabolic pathways is shown in Table 2

[d] Bifunctional DNA glycosylases with associated β-elimination activity are not detailed

DNA glycosylases hydrolyze the N-glycosidic bond of the damaged base, leaving an AP site. Different DNA glycosylases exist, each one with broad or narrow substrate specificities. Enzymes can be classified as monofunctional or bifunctional. Monofunctional glycosylases perform base excision only, whereas bifunctional glycosylases also have lyase activity. Biochemical mechanisms of DNA glycosylases have been the subject of recent reviews [33, 34]. (2) Cleavage of the sugar–phosphate backbone at the AP site. Cleavage is catalyzed either by an AP endonuclease or by the intrinsic AP lyase activity of a bifunctional DNA glycosylase. AP endonucleases and lyases generate different types of DNA termini. AP endonucleases release a 3′ OH and a 5′ deoxyribose–phosphate moiety (5′ dRP), while AP lyases release a 3′ phospho a,β-unsaturated aldehyde (3′ PUA) or 3′ phosphate by β- or βδ-elimination reactions, respectively, and a 5′ phosphate. (3) Processing of unconventional ends to conventional 3′ OH and 5′ phosphate moieties. This step involves different enzymes depending on whether the modified terminus is processed from the 5′ or 3′ end. The intrinsic dRP lyase activity of DNA polymerase β removes the 5′ dRP; the 3′ phosphodiesterase activity of the AP endonuclease eliminates the 3′ PUA, whereas polynucleotide kinase 3′ phosphatase (PNKP) processes the 3′ phosphate moiety. Additional SSB end cleaning enzymes are aprataxin and tyrosyl DNA phosphodiesterases (TDP) that process abortive ligation or topoisomerase reactions, respectively [35]. (4) Gap-filling and nick sealing. The short-patch or single nucleotide (SN) BER involves the incorporation of only a single nucleotide by the DNA polymerase β. The long-patch (LP) BER requires DNA synthesis mediated by DNA polymerases δ and ε and assisted by proliferating cell nuclear antigen (PCNA). The newly synthesized DNA displaces the downstream 5′ DNA end to form a flap structure containing 2–13 nucleotides. The displaced strand is then processed by a flap endonuclease (FEN). The choice of the pathway depends on the specificity of the DNA glycosylase and the proliferation status of the cell. Recent findings suggest that DNA polymerase λ can substitute for DNA polymerase β

Table 2 Components involved in DNA metabolic pathways in *A. thaliana*

Gene	EXO1	PCNA	POL δ	POL ε	RFC	LIG1
AGI locus code	At1g29630	At1g07370	At1g09815	At1g08260	At1g21690	At1g08130
		At2g29570	At2g42120	At2g27120	At1g63160	At1g49250
			At5g63960	At5g22110	At1g77470	
					At5g22010	
					At5g27740	

in filling single-nucleotide gaps and can perform gap filling in long-patch BER [36]. Poly ADP-ribose polymerase 1 (PARP1) and X-ray cross-complementation group 1 (XRCC1) also have roles in BER. PARP1 protects SSBs from the formation of more deleterious double-strand breaks (DSBs) [35] and XRCC1 is involved in the organization of BER-proficient multiprotein complexes [37]. Finally, the nick is sealed by a DNA ligase.
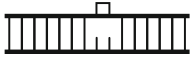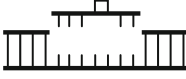
BER in plants

Most of the BER proteins found in animal cells have been described in plants [38, 39]. Tables 1B and 2 show an overview of described factors involved in BER mechanism in *A. thaliana*. Plants contain several DNA glycosylases: 8-oxoguanine DNA glycosylase (OGG1), formamidopyrimidine DNA glycosylase (FPG), NTH, and uracil DNA glycosylase (UNG) [38–44]. The enzymes OGG1 and FPG recognize oxidized purines; NTH, oxidized pyrimidines and UNG, U:G mismatches. Both NTH homologs (NTH1 and NTH2) were co-localized to nucleoids within *Arabidopsis* chloroplasts, thus indicating the existence of a BER pathway in these organelles to deal with photo-oxidative stress [44]. Plant genomes, in contrast to mammals, also encode specific 5-methyl-cytosine (5-meC) glycosylases that are involved in DNA demethylation [DEMETER (DME) and Repressor of Silencing 1 (ROS1)] [45–48]. These proteins show preference for 5-meC as substrate but can also process T:G mispairs. It has been reported that ROS1 has a very low turnover [46], with the recognition of pyrimidine modifications being the rate-limiting step [49]. An additional activity against T:G mispairs is displayed by MBD4L (methyl binding 4 DNA glycosylase-like) [50]. This enzyme was demonstrated to act on both U:G and T:G mispairs and to excise both U and T more efficiently at a CpG context. Roles of OGG1, ROS1 and MBDL4 in Arabidopsis tolerance to oxidative DNA damage have also been described using mutant or overexpression plants [51–53]. Other downstream proteins of the BER pathway have been characterized in plants. The Arabidopsis genome contains three genes encoding AP endonuclease-like proteins: APE1L (abasic endonuclease-1-like), APE2 (abasic endonuclease 2) and ARP (abasic endonuclease-redox

protein) [54]. All three enzymes were found to exhibit AP endonuclease activity in vitro [55], with ARP representing the major activity in Arabidopsis cell extracts [56]. Based on the available DNA sequence data, a wheat homolog of AtAPE1L has been cloned and characterized [57]. Orthologues of human PNKP (named AtZDP), TDP1, XRCC1 and DNA ligase I were also functionally characterized in Arabidopsis [42, 55, 56, 58–61]. Finally, it has been demonstrated that AP sites may be processed in plants through both SN- and LP-BER [41] and that DNA ligase I restores the continuity of the repaired DNA strand during both SN- and LP-BER [56]. Recent studies identified and characterized a gene named *SAV6* (shade avoidance mutant), a homolog of human *FEN1* [62]. Compared to the animal FEN1, SAV6 shows both flap and gap endonuclease activities, but lacks exonuclease activity.

Nucleotide excision repair

Nucleotide excision repair (NER) is responsible for the processing of bulky helix-distorting damage, such as CPDs and 6-4PPs induced by UV irradiation. Deficiencies in NER are associated with several human autosomal recessive disorders, namely xeroderma pigmentosum (XP), cockayne syndrome (CS) and trichothiodistrophy (TTD) among others. XP, CS and TTD have provided the names of some of the genes involved in the pathway (XPA through XPG, XPV, CSA, CSB and TTDA). NER is a multistep process which comprises four steps (Table 3A). (1) Damage recognition. Two different modes of damage recognition coexist: global genome NER (GG-NER) and transcription-coupled NER (TC-NER). GG-NER detects damage occurring throughout the genome and is specifically initiated by a heterotrimeric XPC-HR23B-CEN2 complex (XPC and HR23A/HR23B are the human homologs of yeast and plant RAD4 and RAD23 proteins, respectively), with the assistance in some cases of DDB (damaged DNA-binding) protein complex. DDB, a heterodimeric complex comprising DDB1 and DDB2 subunits, specifically binds CPDs and 6-4PPs with moderate or high affinity, respectively, and stimulates significantly XPC binding to UV-damaged lesions. GG-

Table 3 Overview of steps (A) and *A. thaliana* required factors (B) involved in NER

A		**B**	
Step	**Schematic representation**	**Gene**	**AGI locus code** [a,b]
i) Damage recognition		RAD4	At5g16630
		RAD23A	At1g16190
		RAD23B	At1g79650
		RAD23C	At3g02540
		RAD23D	At5g38470
		CEN2	At4g37010
		DDB1A	At4g05420
		DDB1B	At4g21100
		DDB2	At5g58760
		CSA	At1g27840
			At1g19750
		CHR8	At2g18760
		CHR24	At5g63950
ii) Formation of a stable preincision complex around the damage site		XPB1	At5g41370
		XPB2	At5g41360
		UVH6	At1g03190
		TFIIH1	At1g55750
			At1g61420
		GTF2H2	At1g05055
		TFIIH3	At1g18340
		TFIIH4	At4g17020
		TTDA	At1g12400
			At1g62886
		CDKD;1	At1g73690
		CDKD;2	At1g66750
		CDKD;3	At1g18040
		CYCH;1	At5g27620
		MAT1	At4g30820
iii) Excision of the damaged nucleotide		UVH3	At3g28030
		UVH1	At5g41150
		ERCC1	At3g05210
		RPA	See Table 4
iv) Completion of DNA synthesis followed by ligation		PCNA	See Table 2
		RFC	See Table 2
		Pol δ	See Table 2
		Pol ε	See Table 2
		LIG1	See Table 2

[a] *AGI* Arabidopsis genome initiative

[b] AGI locus code for components involved in DNA metabolic pathways is shown in Tables 2 and 4

NER in mammalian cells has been covered in recent reviews [63, 64]. TC-NER is responsible for the recognition of lesions in the transcribed strand of active genes and is initiated by RNA polymerase stalled at a lesion, with the help of specific factors CSA, CSB, and XPA binding protein 2 (XAB2) whose function is not clear. Many aspects of mammalian TC-NER can be found in some recent reviews [64–66]. (2) Formation of a stable preincision complex around the damage site. After DNA recognition, GG-NER and TC-NER converge into the same pathway. The transcription factor II H (TFIIH) is recruited to the lesion. TFIIH is a multiprotein complex composed of ten subunits arranged in two functional subcomplexes: the core subcomplex containing the DNA helicase XPB, p62, p52, p44, p34 and p8 (TTDA) and the CAK [cyclin dependent kinase (CDK)-activating kinase] subcomplex containing CDK7, cyclin H (CYCH) and the assembly factor ménage-à-trois-1 (MAT1); both subcomplexes are bridged by the DNA helicase XPD [67, 68]. The result of the TFIIH activity is the partial unwinding of the DNA duplex that leads to the recruitment of XPA, RPA (replication protein A), and the endonuclease XPG. Then, XPA interacts and recruits the second endonuclease, ERCC1 (excision repair cross complementing 1)–XPF. (3) Excision of the damaged nucleotide. The damaged nucleotide is removed by dual incisions 5′ and 3′ to the lesion by endonucleases XPF and XPG, respectively, generating a 24–32 oligonucleotide single-strand fragment containing the damaged site. There

Table 4 RPA proteins in *A. thaliana*

Gene	*RPA1*	*RPA2*	*RPA3*
AGI locus code	At2g06510 (A)	At2g24490 (A)	At3g52630 (A)
	At5g08020 (B)	At3g02920 (B)	At4g18590 (B)
	At5g45400 (C)		
	At5g61000 (D)		
	At4g19130 (E)		

is a defined order of the two incisions. The 5′ incision by the heterodimer ERCC1–XPF is performed first; repair synthesis is initiated followed by the 3′ incision by XPG [69]. (4) Completion of DNA synthesis followed by ligation. The repair synthesis is carried out by DNA polymerases δ, ε, or κ depending on chromatin accessibility of the damaged site [70]. NER is completed by nick sealing by DNA ligase I or IIIα.

NER in plants

This pathway has been primarily studied in Arabidopsis and rice [7]. In Arabidopsis, four genes, namely *RAD23*, *DDB1*, *CSA* and *XPB* have been duplicated (Table 3B) [7, 71]. Interestingly, *RAD23* was also found to be duplicated in rice, Populus and Sorghum and many times in Arabidopsis [7, 71]. Although Arabidopsis mutants affecting the expression of individual *RAD23* genes exhibited pleiotropic developmental defects (*rad23b*) or no obvious phenotype (*rad23a*, *rad23c*, and *rad23d*), higher-order mutant combinations indicated that the RAD23 family is essential in Arabidopsis [72]. The two DDB1 homologs, DDB1A and DDB1B, were expressed throughout plant development with DDB1B showing the lowest levels [73, 74] but playing a crucial role because *ddb1b* null alleles appear lethal ([73] and references therein). Overlapping expression patterns were also observed for the two CSA homologs. Both homologs share significant sequence identity and likely form heterotetramers in plants [75]. Decreased levels of several of these genes, namely *DDB1A*, *DDB1B*, *CSA* or *XPB1* affected UV plant tolerance [75–79]. A difference in sensitivity upon exposure of UV radiation was also observed for plants defective in *DDB2*, *UVH6* (defective in the human XPD homolog) or *CSB* homologs (*CHR8* and *CHR24*) [76, 77, 80–82]. It should be mentioned that some plant homologs of the human XP genes were initially named after the UV hypersensitive (uvh) phenotype of the mutants. On the other hand, overexpression of *DDB1A* conferred increased resistance to UV-induced DNA damage [78].

The interaction of XPC-CEN2 (AtRAD4-AtCEN2) and validation of some in silico predicted TFIIH subunit transcripts were also reported in plants [7, 83–87]. Human

TFIIH components p62, p44, p34 and p52 correspond to TFIIH1, TFIIH2 (AtGTF2H2), TFIIH3 and TFIIH4 in *A. thaliana*, while CDK7 is encoded by three CDKDs (Table 3B). Homologs of the human RPA complex, heterotrimer composed of three associated subunits RPA1, RPA2 and RPA3, were also functionally characterized in Arabidopsis [88]. However, in contrast to other eukaryotes, multiple genes encode the RPA subunits in *A. thaliana* (Table 4) [88, 89]. A phylogenetic tree based on RPA1 protein sequences revealed three evolutionary groups [90]. Group A is composed of RPA1A; group B, of RPA1B and RPA1D and group C, of RPA1C and RPA1E proteins. These authors propose that group C includes proteins involved in DNA damage repair [90]. No apparent XPA homolog exists in plants [10].

Mismatch repair

The mismatch repair (MMR) system is best known for its role in the recognition and correction of single base–base mismatches and unpaired nucleotides that arise through replication errors, deamination of 5-methylcytosine, and recombination between divergent sequences. Eukaryotic MMR has been studied in great detail and has been covered in several recent reviews [2, 91–94]. Also, an overview of the history of eukaryotic MMR has been recently reported [95]. The pathway involves several steps (Table 5A). (1) Lesion recognition by MutS proteins. In eukaryotes, MutS proteins function as heterodimers composed of related, but distinct MutS homologs (MSH) subunits. To date, eight MSH subunits were discovered [96], of which MSH2, MSH3 and MSH6 are involved in nuclear MMR in human cells. These MSHs subunits assemble as MSH2-MSH6 (MutSα) and MSH2-MSH3 (MutSβ). MutSα recognizes base–base mismatches and short insertion/deletion loops (IDLs) while MutSβ mediates the repair of IDLs up to 16 nucleotides [97]. The molecular basis of substrate specificities has been investigated. A series of crystal structures of human MutSα in complex with different DNA substrates showed an extensive interaction of a conserved phenylalanine residue in the MSH6 subunit with the DNA mispair [98]. MSH3 lacks the phenylalanine residue that makes protein-mispair contact. Instead, MutSβ binds the sugar–phosphate backbone of the IDL [99]. DNA is severely bent and the unpaired bases are flipped out and solvent exposed as revealed by the crystal structure of human MutSβ complexed with DNA containing IDLs of varying size [99]. In addition to crystal reports, abilities of MutSα and MutSβ to process base–base mismatches and IDLs were explored. The results indicate that MutSα and MutSβ differ in DNA substrate recognition and that the MutSβ–IDL interaction is greatly stimulated by an excess

Table 5 Overview of steps (A) and *A. thaliana* required factors (B) involved in MMR

A

B

Step	Schematic representation	Gene	AGI locus code [a,b]
i) Lesion recognition		*MSH2*	At3g18524
		MSH3	At4g25540
		MSH6	At4g02070
		MSH7	At3g24495
ii) DNA repair complex assembly		*MLH1*	At4g09140
		PMS2	At4g02460
iii) Strand discrimination		*RFC*	See Table 2
		PCNA	See Table 2
iv) Strand excision and resynthesis		*EXO1*	See Table 2
		RPA	See Table 4
		POL δ	See Table 2

[a] *AGI* Arabidopsis genome initiative

[b] AGI locus code for components involved in DNA metabolic pathways is shown in Tables 2 and 4

amount of MutSα [97]. Besides DNA-binding activity, all MutS proteins contain highly conserved ABC-ATPase domains that are essential for MMR ([100] and references therein). Communication between the DNA and the ATP-binding/hydrolysis sites appears to play an important role in the molecular functions of both MutSα and MutSβ [97–99, 101]. In addition to the significant differences between MutSα and MutSβ in substrate recognition and ATP binding and hydrolysis, MutSα differs from MutSβ in the manner that it interacts with PCNA, with MutSα-PCNA complex playing only a limited role in mismatch removal but MutSβ-PCNA complex being highly relevant to small IDLs processing [102]. Finally, current evidence suggests that mammalian MSH2 and MSH6 are transcriptionally and translationally up-regulated in a cell cycle-dependent manner [103, 104], but MSH3 is not cell cycle regulated [104]. (2) DNA repair complex assembly. Subsequent to mismatch binding, MutL proteins are recruited to the MutS proteins/DNA complex in an ATP-dependent manner. Like MutS proteins, MutL proteins function as heterodimers in eukaryotes. The major activity is associated with MutLα (heterocomplex of MLH1-PMS2 in humans but MLH1-PMS1 in yeast and plants). Each subunit of MutLα contains a highly conserved N-terminal region and a weakly conserved C-terminal domain connected by a long flexible linker arm. The N-terminal regions bind and hydrolyze ATP and bind to DNA ([100] and references therein). The C-terminal domains are essential for dimerization [92]. In addition, the C-terminal domains of the PMS subunits have a metal-binding site and an endonuclease activity that is required for MMR [105]. Linker arms vary in length and sequence and are important for MutLα–DNA interactions [

[106] and references therein]. The presence of these linkers has been associated with large asymmetric conformational changes following sequential ATP binding and hydrolysis [107]. (3) Strand discrimination. Identification and discrimination of the newly synthesized DNA strand in eukaryotes are attributed to strand breaks involved in the leading- and lagging-strand synthesis. Replication factor C (RFC) specifically recognizes 3′ termini at the replication fork and loads PCNA asymmetrically onto these sites, with the same side facing towards the strand discontinuity [108]. PCNA loaded at these breaks is required for MutLα endonuclease activation, apparently through protein–protein interaction [109]. Once activated, MutLα incises the strand containing the preexisting strand break due to the loading polarity of PCNA [109]. MutLα endonuclease can also be activated on DNA that contains a MutSβ recognizable lesion but, while MutSα can interact independently with MutLα and PCNA, interactions of MutSβ with MutLα and PCNA were found to be mutually exclusive [102]. These results suggest important differences in the repair mechanisms of single base–base mismatches and IDLs. (4) Strand excision and re-synthesis. Multiply incised molecules are substrates for the 5′–3′ activity of MutSα-activated exonuclease I (Exo1) [110]. Once the mismatch is excised, MutSα and RPA regulate Exo1 activity that leads to excision termination [111]. The repair reaction is completed by a correct DNA re-synthesis by DNA polymerase δ, followed by ligation. In the absence of Exo1, MMR can still be detected suggesting the existence of one or more Exo1-independent modes of mismatch repair. The mechanism most likely involves a strand displacement synthesis by DNA polymerases δ that is strongly stimulated by RPA

Table 6 Overview of steps (A) and *A. thaliana* required factors (B) involved in HR

A		B	
Step	**Schematic representation**	**Gene**	**AGI locus code** [a,b]
i) DNA end resection and nucleoprotein filament formation		MRE11	At5g54260
		RAD50	At2g31970
		NBS1	At3g02680
		COM1	At3g52115
		RECQ4A	At1g10930
		RAD51	At5g20850
		RAD51B	At2g28560
		RAD51C	At2g45280
		RAD51D	At1g07745
		XRCC2	At5g64520
		XRCC3	At5g54750
		BRCA2A	At4g00020
		BRCA2B	At5g01630
		RAD54	At3g19210
ii) DNA homology search and strand invasion		RAD51	At5g20850
		RAD54	At3g19210
iii) DNA heteroduplex extension		POL δ	See Table 2
		PCNA	See Table 2
		RFC	See Table 2
iv) Resolution of the D-loop SDSA		SRS2	At4g25120
		FANCM	At1g35530
DSBR		EME1A	At2g21800
		EME1B	At2g22140
		MUS81	At4g30870
		GEN1	At1g01880
		SEND1	At3g48900
HJ dissolution		RECQ4A	At1g10930
		TOP3α	At5g63920
		RMI1	At5g63540

[a] *AGI* Arabidopsis genome initiative

[b] AGI locus code for components involved in DNA metabolic pathways is shown in Table 2

[112]. Exo1-dependent and independent MMR has been the subject of a recent review [113].

MMR in plants

Arabidopsis and other plants encode homologs of MutS and MutL proteins found in other eukaryotic organisms, but also contain a unique MSH polypeptide (MSH7) [114, 115] (Table 5B). MSH7 forms a heterodimer with MSH2 and the protein complex is designated MutSγ. Quantitative analysis of transcript levels of representative genes (*MSH2* and *PMS1*) of the pathway indicated that these genes show a higher expression in calli than in seedlings from *A. thaliana*, thus confirming the essential genome maintenance function performed by MMR system in rapidly dividing tissues [116, 117]. Further knowledge of

the role of a given plant MMR protein came from in vivo studies. Using a yeast system, we have found that AtMutSγ is able to specifically recognize G/T, A/C, T/C, G/A, T/T, or A/A mismatches and/or neighboring sequences containing T [118] and that the expression of AtMutLα affected the repair of IDLs in microsatellite sequences [119]. The functionality of plant MutS and MutL homologs was also assessed using *A. thaliana* (reviewed in [114]), tomato [120], rice [121] and Nicotiana [122] mutant plants. Suppression of *MSH2, PMS1* or *MLH1* was associated with microsatellite instability, an increase in homeologous recombination relative to wild-type plants and a broad spectrum of mutant phenotypes in their progenies. Inactivation of *MSH7* led a modest increase in the frequency of recombination between *Solanum lycopersicum* and a distantly related species (*Solanum lycopersicoides*) [120], while in *A. thaliana*, the inactivation of *MSH7* did not affect the rates of somatic homologous or homeologous recombination, but influenced the rates of meiotic recombination [123]. Finally, somewhat comparable to mammalian proteins, we demonstrated that *msh2* mutant plants showed an altered expression pattern of cell cycle marker genes after induced DNA damage in comparison to wild-type plants and we experimentally validated *MSH6* as an E2F target gene [124].

Double-strand break repair

Double-strand break (DSB) repair uses two main strategies: a homology-dependent, error-free homologous recombination repair (HR); a potentially error-prone, classical, Ku-dependent non-homologous end-joining (C-NHEJ) and a recently identified error-prone repair pathway, named alternative non-homologous end-joining pathway (A-NHEJ). The choice between these pathways depends on the phase of the cell cycle and the initiation of DNA end resection [125–127].

Homologous recombination

HR is favored during the S/G2 phase of the cell cycle, when sister chromatids are available as homologous templates [128, 129]. This mechanism was the subject of several recent reviews [130–132]. In this pathway, four steps are required (Table 6A). (1) DNA end resection and nucleoprotein filament formation. Initial processing of the ends involves the trimeric complex MRE11-RAD50-NBS1 (MRN) and the CtBP (C-terminal binding protein) interacting protein (CtIP) [133, 134]. Further resection involves the 5′–3′ exonuclease EXO1 or the combined helicase/nuclease activities of the BLM/DNA2 (Bloom Syndrome RecQ Like Helicase/DNA replication helicase/nuclease2)

[135]. The 3′ single-strand DNA (ssDNA) tails generated are initially bound by ssDNA-binding protein RPA [136]. Subsequently, RPA is replaced by another ssDNA-binding protein, RAD51, which forms a nucleoprotein filament on the DNA. The replacement of RPA by RAD51 requires several mediator proteins, such as BRCA2 (protein encoded by the breast cancer-associated gene 2) and RAD54 [137–139]. Human cells have two RAD54 homologs, RAD54 and RAD54B, which display similar biochemical activities [140]. Evidence also suggests that RAD51 paralogs, such as XRCC2, XRCC3, RAD51B, RAD51C, and RAD51D, function in promoting and/or stabilizing RAD51 nucleofilaments [131, 141]. However, unlike RAD51 that self-assembles, RAD51 paralogs form at least four different heterodimeric and heterotetrameric complexes. (2) DNA homology search and strand invasion. The RAD51 nucleoprotein filament begins searching for a homologous DNA sequence and then invades the intact double-stranded DNA molecule to form a heteroduplex DNA structure termed the displacement loop (D-loop). D-loop formation is stimulated by RAD54 [131, 132]. Besides ssDNA binding, RAD51 also interacts with dsDNA and associates with the newly formed heteroduplex DNA. (3) DNA heteroduplex extension. The invading strand in the D-loop structure is then extended by several components of the replication machinery, namely DNA polymerase δ, PCNA and RFC [142]. (4) Processing of the D-loop. HR can take several different steps: synthesis-dependent strand annealing (SDSA), Holliday junction (HJ) resolution (the DSBR model) and HJ dissolution [130, 132]. SDSA involves the displacement of the newly synthesized strand by DNA helicase(s), followed by strand annealing, DNA synthesis and ligation. DSBR is characterized by the capture of the second end of the DSB, formation of a double HJ (dHJ) structure and resolution by specialized resolvases (e.g., GEN1, MUS81-EME1, SLX1-SLX4). Alternatively, the dHJ can be dissolved by the BTB complex consisting of BLM helicase, DNA topoisomerase IIIα and BLAP75. Regulation of HJ processing enzymes has been covered in recent reviews [143, 144]. Depending on the pathway employed for HJ processing, different recombination outcomes are generated. SDSA and HJ dissolution lead to the formation of non-crossover while HJ resolution contributes to the formation of non-crossover or crossover. The formation of non-crossover recombinants is promoted in mitotic cells, in contrast with that occurring in meiotic cells.

An alternative process that involves recombination between regions of homology at both sides of the break can also occur. This process, known as single-strand annealing (SSA), requires HR proteins involved in DNA end resection and annealing and also RPA, but is independent of RAD51 [130–132, 145]. After DNA end resection, the 3′

ssDNA tails generated can reveal complementary sequences on both sides of the break. These sequences anneal forming a branched structure. The 3′ tails are then removed and the nicks are ligated. This process inevitably results in a deletion at the repair junction.

Classical non-homologous end-joining

C-NHEJ functions throughout the cell cycle but is dominant in G1 and G2 [129]. Several recent reviews have focused on C-NHEJ [146–150]. Three different steps are involved in this pathway. (1) DNA end recognition and tethering. The initial step is the recognition and binding of DSBs by the Ku protein (heterodimer of KU70-KU80 in eukaryotes) in a sequence-independent manner. Recent evidence suggests that only two Ku molecules are present at a DSB in vivo, presumably one at each end of the DSB [151]. The Ku-DNA complex then interacts with the DNA-dependent protein kinase (DNA-PK) catalytic subunit (DNA-PKcs) forming a very stable and functional DNA-PK complex. This complex remains tethered to the end and prevents end access. The DNA-PK activated protein undergoes extensive autophosphorylation in vivo [152]. This results in a conformational change that opens the central DNA-binding cavity and releases the autophosphorylated DNA-PKcs from DNA ends [152]. Structures and functions of the Ku protein and the DNA-PK have been recently reviewed [153–155]. (2) DNA end processing. Depending on the nature of the DNA damage, one or more enzyme activities are involved in DNA end processing. For example, the 5′-dRP/AP lyase activity of the Ku protein excises abasic or 5′-dRP/AP sites [156, 157], the tyrosyl DNA phosphodiesterase activity of TDP2 hydrolyzes 5′ phosphotyrosyl-DNA bonds [158], and the 3′-DNA phosphatase and 5′-DNA kinase activities of the polynucleotide kinase/phosphatase (PNKP) generate ligatable 5′-phosphates and 3′-hydroxyl ends [159, 160], among others. In many cases, recruitment of these proteins depends on their direct interaction with Ku [154]. (3) Ligation of the DSB. The final step is carried out by DNA ligase IV, which functions in complex with its cofactors XRCC4 and XLF (XRCC4 like factor, also known as Cernunnos) [150]. Recently, a new protein named PAXX (PAralog of XRCC4 and XLF) has been reported to promote Ku-dependent DNA ligation in vitro [161].

Alternative non-homologous end-joining pathway

A-NHEJ increases as cells enter S-phase [162]. The molecular mechanism of A-NHEJ has recently been reviewed [163–165]. A-NHEJ occurs in the absence of key C-NHEJ factors and can involve alignment of microhomologous sequences; thus, this pathway is also known as

microhomology-mediated end-joining (MMEJ). A-NHEJ shares with C-NHEJ similar reaction steps. (1) DNA end recognition and tethering. DSBs are efficiently bound by PARP1 under Ku defective conditions [166]. In fact Ku and PARP1 compete for DNA end binding, and Ku also limits poly ADP-ribose (PAR) synthesis and ssDNA production in response to DSBs [166, 167]. Upon DSB binding, PARP1 gets activated and subsequently auto-poly(ADP-ribosyl)ated. Auto-modification of PARP1 results in a decreased affinity for nucleosomes and in a relaxed local chromatin structure, which facilitates chromatin remodeling for DNA repair [168, 169]. Overviews of the involvement of PARP1 in DNA repair have been published [170–172]. (2) DNA end processing. PARP1-mediated PAR synthesis then triggers recruitment of the MRN complex to initiate incompatible end resection [173, 174]. (3) Ligation of DNA ends. MRN interacts with the DNA ligase IIIα/XRCC1 complex and stimulates the joining of DNA ends [175]. Joining junctions involve microhomologies and deletions [175]. A second pathway independent of microhomologies that relies on DNA ligase I has also been suggested [176].

DSB repair in plants

Knowledge of recombination mechanisms in plants has significantly advanced in recent years, especially because DSB repair emerged as an important tool to achieve controlled modifications of plant genomes (for recent reviews see [177–183]). Most of the HR proteins have been identified and characterized in *A. thaliana* (Table 6B). Plants with decreased transcript levels of genes involved in the initial processing of the ends, namely *MRE11* [22], *RAD50* [22], *NBS1* [184] and *COM1* (a homolog of the human *CtIP*, [185]) displayed hypersensitivity (measured as a reduction in root growth, fresh weight or development of true leaves) to some genotoxic compounds [mitomycin (MMC) or methylmethanesulfonate (MMS), agents that produce inter-strand DNA crosslinks or that methylates DNA, respectively]. Interaction between NBS1 and MRE11 has been reported for *A. thaliana*, maize and rice [184, 186]. The NBS1 region involved in the interaction was further characterized in *A. thaliana* and defined towards the C-terminus [184]. Homologs of BLM, DNA2 and RPA are also conserved in plants. The loss of *RECQ4A* (a homolog of mammalian BLM helicase, [187–189], *JHS1* (a homolog of human DNA2, [190] or *RPA1C* and *RPA1E* [89] led to hypersensitivity (measured as a reduction in fresh weight or root growth) in response to a particular DNA damaging agent [MMS; cisplatin (CPT, an agent that mainly induces intra-strand DNA crosslinks); hydroxyurea (an agent that inhibits the ribonucleotide reductase and consequently reduces the dNTP pool and stalls replication

forks); zeocin (an agent that induces DSBs) or ionizing radiation]. The *recq4A* mutant also exhibited an increased frequency of HR [187, 189] suggesting a role for the RECQ4A protein in HR regulation. Suppression of HR is dependent on both the N-terminal region and the helicase activity of the protein [189]. A hyper-recombination phenotype was also observed for the *jhs1* mutant, probably due to an increased expression of genes involved in DNA damage repair [190].

Several studies have also addressed the functions of RAD51 and RAD51 paralogues in plants [191–194]. The *rad51*, *rad51c* or *xrcc3 A. thaliana* mutants showed a deficiency in SDSA [192]. Mutants impaired for the other three RAD51 paralogues (RAD51B, RAD51D, XRCC2) were also defective for spontaneous HR [191, 193, 195], although to differing extents [191, 193]. The *xrcc2* mutant was the most affected. A further reduction in the recombination rate has been observed in *rad51b/xrcc2* double mutant and *rad51b/rad51d/xrcc2* triple mutant line [193]. The triple mutant also exhibited a greater sensitivity to the DSB-inducing agent bleomycin (BLE) than the single and double mutants, suggesting a partial functional redundancy [194]. The redundancy of the two *AtBRCA2* genes has been also investigated [196–198]. Both *Atbrca2a* and *Atbrca2b* mutants showed hypersensitivity against CPT and ionizing irradiation, further stimulated in the *Atbrca2a/Atbrca2b* double-mutant line [196]. Another report indicated that the double mutant also showed hypersensitivity to MMC and a defect in somatic HR frequency [197].

The role of other factors involved in the process of strand exchange or in the stabilization of recombination intermediates has been elucidated using mutant plants. Loss of *RAD54* reduced SDSA and loss of *FANCM* reduced both SDSA and SSA [192]. Proteins involved in the resolution of the D-loop were characterized in vitro or in vivo. Recombinantly expressed SRS2 protein showed a functional 3′ to 5′ DNA helicase activity that was able to unwind nicked and partial HJ [199]. Biochemical properties of MUS81-EME1 complexes and GEN1 were also determined after cloning, heterologous expression and purification [200, 201]. Two functional *EME1* (*EME1A* and *EME1B*) and two functional *GEN1* [GEN1 (XPG-like endonuclease) and SEND1 (single-strand DNA endonuclease1)] homologs have been identified in *A. thaliana*. Both complexes, MUS81-EME1A and MUS81-EME1B, were able to cleave nicked HJs and, with a reduced efficiency, intact HJs [201]. Both GEN1 paralogs preferentially cleaved HJs near the junction point; slight differences in sequence preferences have been detected [200]. The role of MUS81 was also defined in vivo. The *mus81* mutant line showed a strong reduction in dry weight to MMC, CPT or MMS treatment [188]. Also, altered sensitivity (measured as a reduction in fresh weight) to

MMC and CPT was observed in *recq4A*, *top3A* and *rmi1* single mutant lines [202]. These three lines also showed an enhanced frequency of uninduced somatic HR [202].

In addition to the HR mechanism, KU-dependent and independent pathways have been also identified in plants. Arabidopsis encodes KU70 (At1g16970), KU80 (At1g48050), PARP1 (At2g31320) and PARP2 (At4g02390) [22, 203]. *A. thaliana* impaired in KU80 showed severe root developmental defects after γ irradiation [204, 205] and higher levels of DSBs [195]. As with the *ku80* mutant plant, the *xrcc1* mutant line also exhibited a hypersensitivity to γ irradiation [205]. Furthermore, analysis of DSB repair kinetics of *ku80* and *xrcc1* single mutant lines and *ku80/xrcc1* double mutant line indicated a more severe repair defect in the double mutant than in single mutant plants [205]. These observations thus confirm the existence of both KU-dependent and XRCC1-dependent pathways in plants [205]. Besides XRCC1, PARP is also involved in A-NHEJ. In contrast to humans where PARP1 is the major contributor to the PARP activity in response to DNA damage, PARP2 is the primary enzyme responsible for poly(ADP-ribosyl)ation under genotoxic stress in *A. thaliana* [203]. Plants impaired in both PARP1 and PARP2 displayed a strong reduction in root length and fresh weight after treatment with MMS [206]. However, growth of *parp1* and *parp2* single mutants was not more sensitive to MMS than wild-type seedlings [206]. Analysis of in vitro endjoining assays indicated that the *parp1/parp2* double mutant plant had twofold less end-joining products compared with the wild-type, while the *ku70* and *ku80* single mutant lines had almost fourfold more products than the wild-type [206]. These observations suggest a regulation of non-homologous recombination pathways. Studies in rice also reported that *ku70*, *ku80*, *lig4* or *xrcc3* mutants showed hypersensitivity to genotoxic agents and/or altered DSB repair [207, 208]. On the other hand, overexpression of OsRecQI4 (the AtRecQ4A counterpart in rice), OsExo1 and MtTpd2a enhanced DSB processing in rice and *Medicago truncatula*, respectively [209, 210]. No apparent DNA-PK, XLF, DNA ligase III or DNA polymerase β homologs exist in plants [24, 204, 205, 211].

Concluding remarks

Significant progress in plant DNA repair has been made in recent years. Sequence homology-based analysis allowed identification of many plant factors, not only in model organisms but also in some crop species. Most of them were also functionally validated. Thus, it is clear that DNA repair pathways are well conserved between plants and mammals. However, some activities have been particularly intensified in plants, probably related to a better adaptation

to variable environmental conditions, from which plants cannot escape, and/or to maintain genome stability over multiple generations. Although only limited information on DNA repair mechanisms from crop plants is available, the current advances in *A. thaliana* can well be translated to gain insights into the challenges of obtaining new varieties with novel traits.

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