

REVIEW

The RAD2 Family of Nucleases in Higher Plants

Tomoyuki FURUKAWA^{1,2*} and Hiroaki SHIMADA^{2,3}

¹ Department of Plant Biology, University of California, Davis (Davis, CA 95616, USA)

² Tissue Engineering Research Center, Tokyo University of Science
(Noda, Chiba 278–8510, Japan)

³ Department of Biological Science and Technology, Tokyo University of Science
(Noda, Chiba 278–8510, Japan)

Abstract

The RAD2 family of nucleases is a group of essential nucleases involved in 3R mechanisms (DNA replication, DNA repair and DNA recombination). The RAD2 family consists of three classes (XPG/class I, FEN-1/class II and EXO1/class III). Recent advances in plant genome projects have revealed that in addition to plant counterparts belonging to each class of the RAD2 family higher plants have a fourth set of RAD2 family members that constitute a new class in the RAD2 family (SEND-1/class IV). DNA damage generated by endogenous or exogenous factors cause mutations that threaten the survival of plants if they are misrepaired or not repaired, which results in low crop yields. Understanding the functions of plant RAD2 family members in DNA repair is important for agricultural technology enabling an enhancement of resistance to genotoxic stresses caused by DNA damages. In this review, recent advances in our understanding of plant RAD2 family members are discussed.

Discipline: Biotechnology

Additional key words: DNA repair, rice (*Oryza sativa* L. cv. Nipponbare)

Introduction

DNA is constantly damaged by both endogenous and exogenous factors⁸. Endogenous factors include DNA replication errors, oxidative damages by reactive oxygen species generated mostly in organelles, and hydrolytic damage caused by hydrolysis of the glycosylic bond between purine bases and the DNA backbone resulting in loss of purine bases. Exogenous factors include alkylation damage caused by mutagens, ultraviolet (UV)-induced damages such as the cyclobutane pyrimidine dimer (CPD) and the pyrimidine (6–4) pyrimidone dimer (the 6–4 photoproduct), and DNA single strand and double strand breaks caused by ionizing radiation. Efficient and accurate DNA repair mechanisms exist in plants to repair damaged DNA. A great variety of enzymes (e.g. nucleases, DNA polymerases, DNA binding proteins, helicases, and other accessory proteins) are employed in DNA repair mechanisms in a well-coordinated manner. The RAD2 family of nucleases plays a critical role in the incision of damaged DNA through their substrate-specific

exo- and endonuclease activities³⁸. The RAD2 family of nucleases identified in animals and yeasts consists of three classes, XPG/class I, FEN-1/class II and EXO1/class III. Members of the family are classified according to their molecular mass and the location of two highly conserved nuclease domains called XPG-N and XPG-I. While the XPG-N domain is always situated near the N-terminus, the position of the XPG-I domain varies among members of the RAD2 family (Fig.1). The complete sequencing of the two plant genomes (rice and *Arabidopsis*) has revealed that higher plants have RAD2 homolog genes corresponding to each of the three previously described classes^{15,28,39} (Table 1). Interestingly, two novel nucleases, *OsSEND-1* (*Oryza sativa* Single strand DNA endonuclease-1) and *OsGEN-L* (*O. sativa* GEN (XPG-like endonuclease)-like (*OsGEN-L*), have been isolated from rice as new RAD2 family members composing a new class in the RAD2 family (SEND-1/class IV)^{14,41}. These findings lead us to hypothesize that higher plants not only have DNA repair pathways similar to those found in animals or yeasts, but also plant-specific DNA repair pathways in which new RAD2 members are involved. To

*Corresponding author: e-mail tmfurukawa@ucdavis.edu

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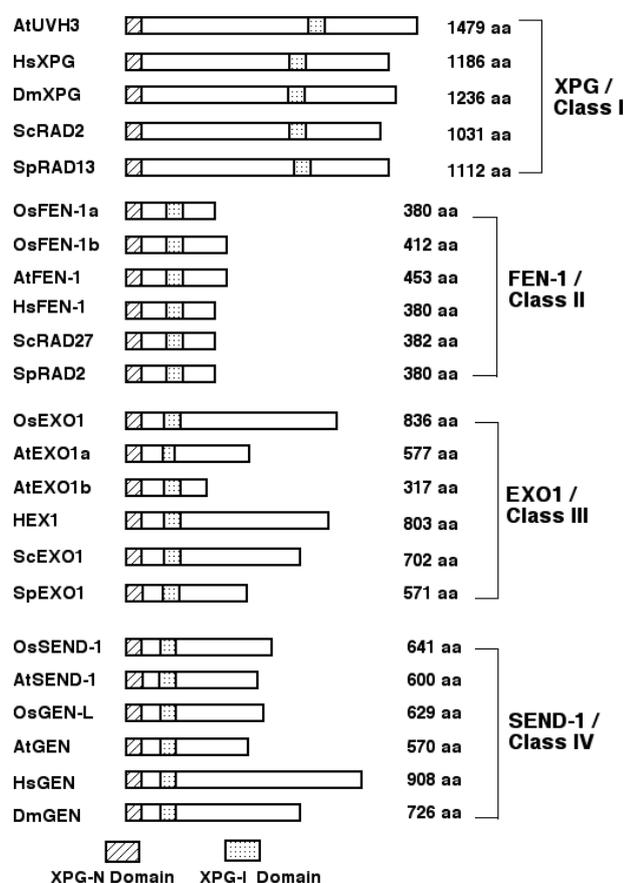


Fig. 1. Comparison of eukaryotic RAD2 family proteins

The XPG domains (XPG-N domain and XPG-I domain) are indicated with hatching or stippling, respectively. Accession numbers for each protein sequence; HsXPG (CAA49598), DmXPG (AAD50779), ScRAD2 (CAA97287), SpRAD13 (CAA19011), ScRAD27 (P26793), SpRAD2 (CAB36991), HEX1 (AAC32259), ScEXO1 (NP_014676), SpEXO1 (NP_596050), HsGEN (NP_872431), DmGEN (BAC57447). Accession numbers for plant RAD2 family proteins are summarized in Table 1.

date, however, little is known about plant RAD2 family members. In this review, we focus on research regarding the molecular basis of the RAD2 nuclease family and its function in higher plants.

XPG/class I

Xeroderma pigmentosum G (XPG) has two nuclease activities: a magnesium-dependent single-strand DNA endonuclease activity^{17,45} and a structure-specific endonuclease activity that cleaves at the 3'-side of the damage-containing bubble structure of DNA formed during nucleotide excision repair (NER)^{10,12,44} (Fig. 2(a)). XPG also

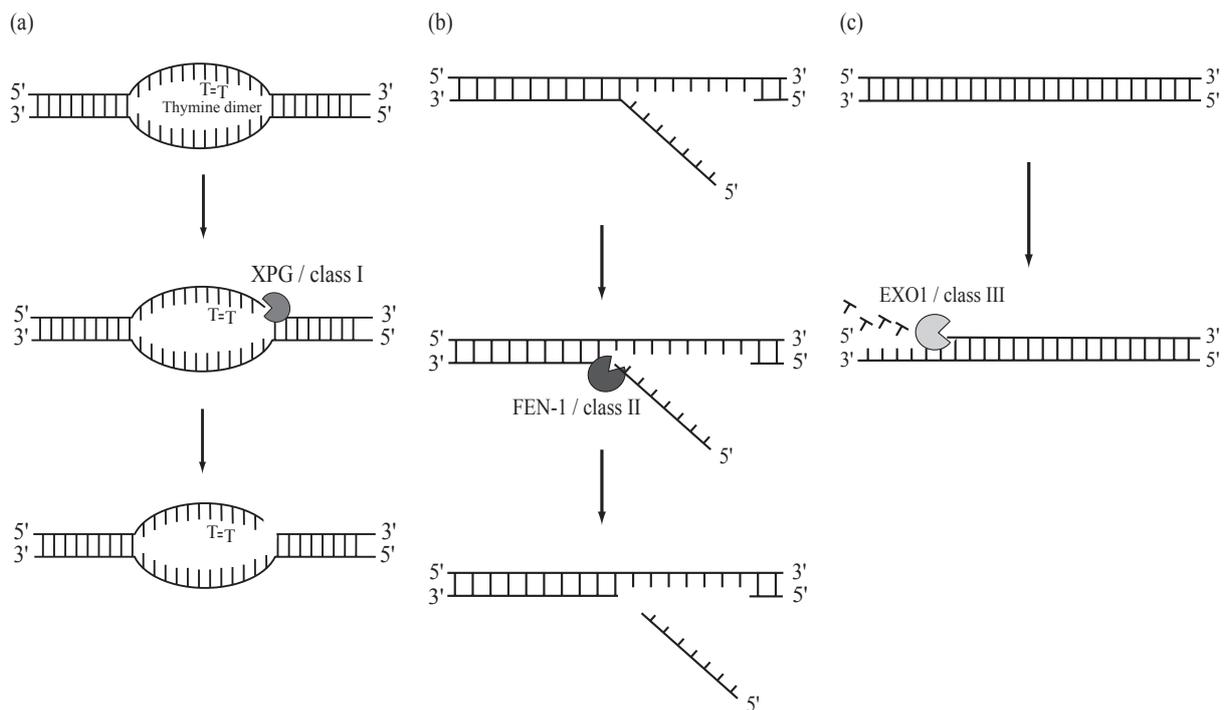
functions in transcription-coupled repair (TCR), an alternative NER pathway used to repair an oxidative-damaged base (e.g. 8-oxoguanine (8-oxoG) and thymine glycol (Tg; 5,6-dihydroxy-5,6-dihydrothymine)). In TCR, XPG enhances the binding activity of mammalian NTH1 (*E. coli* nth endonuclease III-like 1) proteins, a DNA glycosylase possessing both DNA glycosylase and AP lyase activities against 5,6-dihydroxyuracil (DHU)^{3,21} and Tg^{6,32}. The yeast RAD2 protein (ScRAD2; *Saccharomyces cerevisiae* RAD2) possesses similar nuclease activities; 5'-3' exonuclease activity¹⁶, single-strand DNA endonuclease activity¹⁶, and a structure-specific endonuclease activity¹⁸. An abnormal developmental phenotype and hypersensitivity to DNA damaging agent are observed in XPG deficient animals. XPG knockout mice show hypersensitivity to UV, predisposition for skin cancer with extremely high frequency, and abnormal development such as small body size and short life span¹⁹. The *Drosophila mus201* mutant expressing a truncated *DmXPG* is hypersensitive to UV, X-rays, nitrogen mustard, and DNA alkylating agents such as methylmethanesulfonate (MMS)^{9,48,57}. In plants, *Arabidopsis* UVH3 (*AtUVH3*) was identified from a series of analyses of *Arabidopsis* radiation-sensitive mutants²⁴ and was confirmed as a plant counterpart of the XPG gene³⁹. The *uvh3/uvh3* homozygous mutant displays increased sensitivity to both UV-C and oxidative damage caused by hydrogen peroxide. In addition, the *uvh3/uvh3* mutant shows an early senescence phenotype. These results indicate that *AtUVH3* is employed both during DNA repair and the normal developmental process in plants.

FEN-1/class II

Flap endonuclease-1 (FEN-1) is a structure-specific nuclease possessing two nuclease activities: An endonuclease activity that removes 5'-flap structures of DNA and a 5' to 3' double-stranded DNA exonuclease activity. FEN-1 plays a critical role in the removal of flap structures containing damaged DNA in long-patch base excision repair (BER) as well as in the removal of RNA primers in Okazaki-fragment maturation during DNA replication^{1,20,38,42} (Fig. 2 (b)). Several papers on *FEN-1* knockout animals have been published to date. *FEN-1* knockout chicken cells are sensitive to MMS oxidative DNA damage⁴⁰. *FEN-1* knockout mice abort during early embryogenesis³³. Mice harboring a mutation in *FEN-1* are predisposed to autoimmunity, chronic inflammation and cancers⁶⁰. A yeast deficient strain for *Saccharomyces cerevisiae* RAD27, the yeast homologue of *FEN-1* gene, shows a temperature sensitive phenotype due to an accumulation of non-removed RNA primers in the lag-

Table 1. Summary of plant RAD2 family members

Name	Class	Plant	Chr.	Nucleotide Accession No.	Protein Accession No.	Publication
<i>OsUVH3</i>	I	Rice	3	–	–	Found in the rice genome sequence
<i>AtUVH3</i>	I	<i>Arabidopsis</i>	3	AF312711	At3g28030	[39]
<i>OsFEN-1a</i>	II	Rice	5	AB021666	BAA36171	[27]
<i>OsFEN-1b</i>	II	Rice	3	AB080084	BAC98428	[29]
<i>AtFEN-1</i>	II	<i>Arabidopsis</i>	5	NM_180546	At5g26680	Predicted from the <i>Arabidopsis</i> genome sequence
<i>OsEXO1</i>	III	Rice	1	AB179769	BAD60834	[15]
<i>AtEXO1a</i>	III	<i>Arabidopsis</i>	1	AY113038	At1g18090	Predicted from the <i>Arabidopsis</i> genome sequence
<i>AtEXO1b</i>	III	<i>Arabidopsis</i>	1	AY140055	At1g29630	Predicted from the <i>Arabidopsis</i> genome sequence
<i>OsSEND-1</i>	IV	Rice	8	AB074260	BAB72003	[14]
<i>AtSEND-1</i>	IV	<i>Arabidopsis</i>	3	NM_114749	At3g48900	Predicted from the <i>Arabidopsis</i> genome sequence
<i>OsGEN-L</i>	IV	Rice	9	AB158320	BAD93194	[29]
<i>AtGEN</i>	IV	<i>Arabidopsis</i>	1	NM_100069	At1g01880	Predicted from the <i>Arabidopsis</i> genome sequence

**Fig. 2. Summary of cleavage sites of each RAD2 family protein**

The arrows indicate the site of cleavage. (a): A cleavage of the bubble structure of DNA containing thymine dimer by the XPG/class I protein. (b): A cleavage of the 5'-flap structure of DNA by the FEN-1/class II protein. (c): A cleavage of the double strand DNA by the EXO1/class III protein.

ging DNA strand during replication³⁰, hypersensitivity to DNA damaging agents, and a high rate of spontaneous mutations^{25,46,52}. *FEN-1* is also suggested to be involved in homologous recombination (HR), an important DNA double-strand break repair pathway^{26,56}. There have been several reports about plant *FEN-1* homologues. A plant

FEN-1 protein purified from the inflorescence of cauliflower (*Brassica oleracea* var. *botrytis*) possesses both 5'-flap endonuclease and 5' to 3' exonuclease activities²⁷. Two rice *FEN-1* homologue genes (*OsFEN-1a* and *OsFEN-1b*) have been identified and their functional differences investigated by way of a functional complementa-

tion assay using a yeast $\Delta rad27$ null mutant^{28,30}. The OsFEN-1a recombinant protein possesses both 5'-flap endonuclease and 5' to 3' exonuclease activities; this nuclease activity is enhanced by rice proliferating cell nuclear antigen (OsPCNA) protein^{28,29}. Localization of OsFEN-1a and OsPCNA proteins during mitosis and meiosis has been analyzed using their specific antibodies²⁹. They colocalize in the nuclei of cells undergoing interphase (G1, S and G2 phase) and telophase at M phase. On the other hand, they localize close to the chromatin at the pachytene stage during meiotic prophase, suggesting their involvement in small amount of DNA synthesis and DNA recombination during meiosis.

EXO1/class III

Exonuclease-1 (EXO1) was originally purified from *Schizosaccharomyces pombe* as an exonuclease whose activity is increased during meiosis⁵⁰. EXO1 homologue genes are highly conserved in eukaryotes^{13,35,51,59}. The RAD2 domain of the human EXO1 protein possesses both 5' to 3' exonuclease and flap-structure specific endonuclease activities^{34,36} (Fig. 2 (c)). EXO1 is suggested to function in DNA mismatch repair (MMR) since the interaction between the EXO1 protein and other DNA mismatch repair proteins (MSH2, MLH1, MSH3 and MSH2) is confirmed^{2,43,47,51}. Besides interaction with MMR proteins, human EXO1 protein interacts with Werner syndrome protein (WRN)⁴⁹ and RECQ1¹¹ helicases belonging to RECQ family of helicases involved in DNA repair. EXO1 knockout mutants have been generated in mice and yeasts. EXO1^{-/-} deficient mice cells show a significant high mutation rate due to the reduction of MMR activity⁵⁸. Mice EXO1 mutant exhibits reduced survivability, sterility due to loss of chiasmata during meiosis, decreased class-switch recombination of immunoglobulin genes and changes in the characteristics of somatic hypermutation^{4,57}. Yeast EXO1 deletion mutants, on the other hand, display weak sensitivity to MMS, reduction in the processing of DNA double strand breaks during meiosis, and an increase in the frequency of meiotic crossing over in meiosis⁵⁴. It has also been suggested that EXO1 may be involved in meiotic and mitotic recombination¹³, DSB processing^{37,53}, Okazaki fragment processing⁵², and maintenance of telomeres and replication forks^{5,61}. Recently, a plant homologue of EXO1 has been isolated from rice and termed *OsEXO1*¹⁵. OsEXO1 interacts with other rice DNA repair proteins: DNA polymerase λ (OsPol λ), replication protein A 70kDa subunit b (OsRPA70b), and replication protein A 32 kDa subunit (OsRPA32). These four DNA repair genes (*OsEXO1*, *OsPol λ* , *OsRPA70b*, and *OsRPA32*) are highly expressed in meristems where

DNA replication is active and not in non-proliferating tissues such as mature leaves, implying their collaboration in DNA repair and replication^{15,31,55}.

SEND-1/class IV

Recently, two novel nucleases, *OsSEND-1* and *OsGEN-L*, have been identified from rice^{14,41} (Fig. 1 and Table 1). *OsSEND-1* was found as a gene with high homology to other RAD2 family genes by searching rice expressed sequence tag (EST) clones¹⁴, while *OsGEN-L* was found through analysis of Ac-tagged rice lines⁴¹. They share the highest amino acid sequence homology each other in the XPG domains among rice RAD2 family members, and phylogenetic analysis using the XPG-I domains of each RAD2 family member protein shows that they belong to class IV of the RAD2 nuclease family⁴¹. A counterpart of *OsSEND-1* is not found in any eukaryotes outside of the plant kingdom, suggesting that *OsSEND-1* is a plant-specific RAD2 family member. Contrary to *OsSEND-1*, *OsGEN-L* homologue genes are found in plants and animals^{22,23}. Biochemical analysis of their recombinant proteins demonstrates that they possess nuclease activities (*OsSEND-1*; single-strand DNA endonuclease activity¹⁴, *OsGEN-L*; flap endonuclease activity⁴¹); however their substrate specificity remains unidentified. *OsSEND-1* and *OsGEN-L* show different expression patterns in various rice tissues. *OsSEND-1* is strongly expressed in meristems and young leaves, weakly in panicles, and not in mature leaves¹⁴. *OsGEN-L* is constitutively expressed in roots, leaves and flower buds⁴¹. During anther development, *OsGEN-L* expression is up-regulated in the post-meiotic stages⁴¹. RNA interference (RNAi) technology was performed to examine the function of *OsSEND-1* and *OsGEN-L*. *OsSEND-1* is suggested to play an important role in DNA repair pathways. DNA damaging treatments such as UV-B irradiation, MMS and hydrogen peroxide induce *OsSEND-1* expression¹⁴. The rice *OsSEND-1*-RNAi transgenic plants display shorter root length compared to that of wild type when they are cultivated on the solid MS medium containing MMS (ms in preparation). On the other hand, repression of *OsGEN-L* expression by RNAi causes male sterility due to abnormal development of the microspore at the early uninucleate stage⁴¹. Taken together, these findings suggest that these RAD2 members play important roles in DNA repair and other cellular events such as microspore development in rice.

Conclusion

Recent advances in plant genome sequencing have

revealed that plants have DNA repair genes functionally identical to animals as well as plant-specific DNA repair genes²². Understanding the functions of these DNA repair genes helps us answer the questions regarding when, where and how plants repair their damaged-DNA and whether plants have evolved a plant-specific DNA repair pathway. In this review, the importance of members of the RAD2 nuclease family is emphasized because they play critical roles not only in incision of damaged-DNA among various DNA repair pathways, but also in developmental processes. It has been suggested that three plant RAD2 classes (class I, class III and class IV) collaborate in the UV-damaged DNA repair pathway. Plants utilize two different pathways, “photorepair” and “dark repair” (also termed NER), for repair of UV-damaged DNA^{8,22}. Comparison of gene expressions of plant DNA repair genes in nonproliferating organs versus proliferating organs by microarray analysis indicates that photorepair is the main UV-damaged DNA repair pathway in nonproliferating organs while NER is the primary UV-damaged DNA repair pathway in proliferating organs³¹. The XPG protein functions in NER as described in the XPG/class I section (see above and Fig. 2(a)). On the other hand, expression of both *OsEXO1* and *OsSEND-1* genes is induced by UV irradiation^{14,15}. Neither of which complements *ScRAD2* functions (unpublished data), indicating a role for these genes in an alternative dark repair pathway (Fig. 3). Recently, it has been reported that transgenic rice plants overexpressing *OsSEND-1* and UV-damaged DNA binding protein 2 (*UV-DDB2*) had an enhanced tolerance for UV-B irradiation²². This finding suggests that an overexpression of DNA repair genes could give plants increased resistance to genotoxic stresses caused by DNA damage, which increases crop yields under a harsh environment. However, a detailed picture of the role of RAD2 family members in plant repair and developmental processes still remain unclear. A deeper understanding of this important family and its functional partners could make a great contribution to the advancement of agricultural technology.

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Note added in proof: Recently, class IV RAD2 family nucleases in human (GEN1) and yeast (YEN1) have been identified as holiday junction resolvases, suggesting their essential roles during HR. (Ip, S.C. et al. (2008) Identifi-

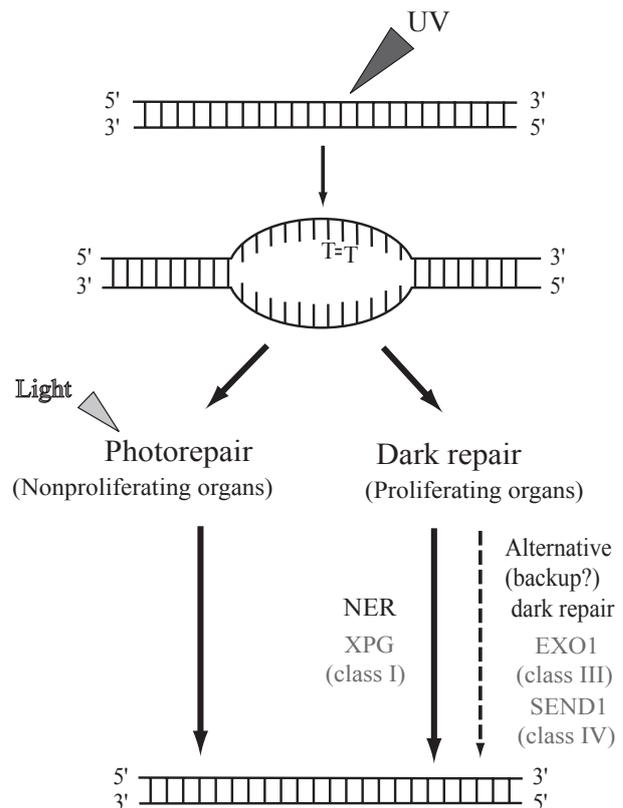


Fig. 3. Model of UV-damaged DNA repair pathways in plants

Relative contribution of each repair pathway in repair of UV-damaged DNA is represented by width of the arrows.

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