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Original Paper

Tissue-specific and DNA damage-responsive expression of the *Arabidopsis RAD51* gene promoter in transgenic *Arabidopsis* and tobacco

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Abstract As is the case with other organisms, plants respond to genotoxic stresses by expressing DNA repair genes upon DNA damage. To uncover the mechanisms involved in regulated expression of the DNA damage-responsive gene, we investigated the tissue specific expression and DNA damage-responsiveness of the *Arabidopsis RAD51* (*AtRAD51*) gene promoter in *Arabidopsis* and tobacco. Transgenic *Arabidopsis* and tobacco plants harboring *AtRAD51* promoter- β-glucuronidase (GUS) were used to study the detailed expression pattern of the *AtRAD51* gene. A histochemical GUS assay of bleomycin- or UV-treated plants showed that the *AtRAD51* promoter in young tissues is actively expressed particularly in meristematic cells of the root and shoot apex of seedlings. In the absence of genotoxic stress, GUS activities were detected only at very low levels in these same organs. In mature plants, the *AtRAD51* promoter is mainly expressed in flower bud, sepal, stigma, later anther, pedicel when treating with DNA damaging agent. The expression patterns of reporter assays were consistent with the *AtRAD51* mRNA accumulation pattern. These results suggest that the regulated expression of the *AtRAD51* gene is controlled mainly at the level of transcription directed primarily by the promoter function of the gene.

Key words: *Arabidopsis RAD51* promoter, DNA damage, tissue-specific expression.

Genotoxic stress in all living organisms induces DNA damage, which, if not repaired, can lead to mutation accumulation or cell death. After exposure to genotoxic stresses, eukaryotic cells are repaired by cell cycle arrest and the activation of transcription of specific genes induced by DNA damage (Zhou and Elledge 2000).

In higher eukaryotes, when double-strand breaks (DSBs) occur by radiomimetic chemicals and ionizing irradiation etc., DNA damage is repaired using either a homologous recombination (HR) or a non-homologous end joining (NHEJ). HR, which occurs via the pairing of homologous DNA sequences, is an essential process for the stability and integrity of the genome (Jackson 2002). On the other hand, the regulation of HR is particularly important for efficient gene targeting in plant cells (Puchta 2002; Britt and May 2003). As a key factor of HR-related proteins, RAD51 has been known to search homology and has a strand exchange activity. *RAD51* gene was found in yeast mutants sensitive to irradiation, and conserved in higher eukaryotes. So far, *RAD51* homologues have been identified in animals, fungi and plants. Many studies have shown that these proteins share common properties in different systems. Expression of *RAD51* is induced after gamma irradiation in yeast and *Coprinus cinereus*, and cell-cycle regulated in vegetative cells of yeast and mouse (Shinohara et al. 1993; Stassen et al. 1997). In mice and humans, RAD51 proteins are specifically found as nuclear foci in cells undergoing meiotic recombination and as foci associated with the chromosomes at the onset of synapsis and synaptonemal complex formation (Daboussi et al. 2002). Moreover, *RAD51* expression is induced by ionizing radiation, X-ray and γ-irradiation etc., which cause DNA damage in *Arabidopsis* (Klimyuk and Jones 1997; Doutriaux et al. 1998; Osakabe et al. 2002).

In recent studies, analyses of RAD51-like proteins have also shown the involvement of these *RAD51* paralogues in meiosis and recombinational repair (Bleuvard et al. 2004; Li et al. 2004; Bleuvard et al. 2005; Abe et al. 2005). Analysis of *Arabidopsis* orthologs of BRCA2, a protein whose mutations are involved in breast cancer in humans showed that *Arabidopsis* RAD51 proteins interact with BRCA2 (Siaud et al. 2004; Dray et al. 2006). RNAi constructs

Abbreviations: Fluc, firefly luciferase; GUS, β-glucuronidase; RT-PCR, reverse transcription polymerase chain reaction

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aimed at silencing the BRCA2 genes or the RAD51 gene at meiosis triggered the same reproducible sterility phenotype, which was associated with dramatic meiosis alterations, suggesting that homologous recombination is highly disturbed in these meiotic cells.

Investigation into developmental regulation of genes involved in DNA repair is necessary to elucidate the biological properties of DNA repair as a defense mechanism against DNA damage. Plant development differs from that of animals in that the differentiation of germline tissue occurs very late in plant development; thus the maintenance of genome integrity during plant development seems especially important in view of the transmission of the intact genome from the somatic cells to the germline. Also in plants, as plant cells are bound by cell walls and tumors cannot metastasize, the induction of tumors by DNA damage rarely give rise to a lethal event. One might therefore expect, in plants, that the expression of repair genes required for the removal of DNA damage would be limited to rapidly dividing cells and germline tissues. The regulated expression of DNA repair genes, *AtLIG4*, *AtGR1*, *AtPARP1* and *AtRAD51*, all of which have been shown an increase of transcript level after treatment with DNA-damaging agents in dividing cells of seedlings and hypothesized to be involved in DNA damage repair and/or cell cycle regulation (Deveaux et al. 2000; Doucet-Chabeaud et al. 2001; Hefner et al. 2005). Unlike in other organisms, however, little is known about mechanisms that regulate gene expression in response to genotoxic stress in plants.

As a part of our efforts to uncover the mechanisms involved in regulated expression of the DNA damage-responsive genes in response to genotoxic stress in plants, we carried out this study focusing on the *RAD51* homologue from *Arabidopsis* (*AtRAD51*) because expression profile and the genomic DNA sequence information are available (Urban et al.1996; Maeda et al. 2004). Recent works of DNA repair-related plant genes involved in response to treatment with DNA damaging agents revealed that the induction level of *AtRAD51* gene is one of the best among the up-regulated genes that respond to DNA damage (Chen et al. 2003; Molinier et al. 2005). In order to investigate the DNA damage response of *AtRAD51* promoter, a radiomimetic bleomycin, which is known to induce mostly DSBs (Menke et al. 2001), and the UV-B radiation, which is reported to result in increased frequencies of homologous recombination in plant (Ries et al. 2000), were employed as DNA damaging agents. Using transgenic *Arabidopsis* and tobacco plants harboring *AtRAD51* promoter-reporter fusion genes, we investigated the expression pattern of the *AtRAD51* promoter. Reporter gene assays of bleomycin- or UV-treated plants and the tissue-specific expression of the *AtRAD51* gene revealed the promoter function of the *AtRAD51* gene.

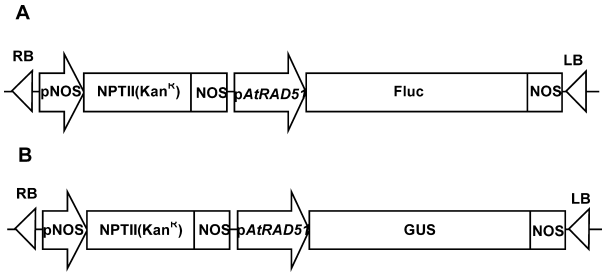

Figure 1. Schematic representation of promoter-reporter fusion constructs. *AtRAD51*::Fluc (A) and *AtRAD51*::GUS (B) were used in this study. pAtRAD51, a 0.7 kb fragment of the *AtRAD51* gene promoter; NOS, nos-terminator sequence from the pBI221 vector; Fluc, modified firefly luciferase gene; GUS, β-glucuronidase gene.

Materials and methods

Construction of the AtRAD51 promoter-reporter fusions

For the construction of the promoter-GUS reporter gene fusion, a promoter fragment from a luciferase fusion plasmid, pBI121*AtRAD51*::Fluc (Figure 1A; Maeda et al. 2004), was digested by *Nco*I, filled-in with Klenow enzyme and then digested with *Hin*dIII. Isolated promoter fragments were inserted into *Sma*I-*Hin*dIII site of pBluescript II KS+. The inserts were excised by *Hin*dIII-*Bam*HI digestion and then inserted into *Hin*dIII/*Bam*HI site of PBI121 vector, which contained the GUS-coding sequence followed by the NOS terminator (Figure 1B).

Transformation and regeneration of transgenic plants

The binary vector containing GUS fusion construct was introduced into *Agrobacterium tumefaciens* strain LBA4404 via electroporation. *In planta* transformation of *Arabidopsis* was performed by the floral dip method with a slightly modified procedure of Bechtold et al. (1993). Transformation of *Nicotiana tabacum* cv. SR1 was carried out as described previously (Maeda et al. 2004).

Treatment with DNA damaging agents

Plants were treated with or without DNA damaging agents, bleomycin (25 μg ml^{-1}) and UV-B (0.5 mW cm^{-2}, 30 min). In *Arabidopsis* seedlings harboring the *AtRAD51*::Fluc construct, bioluminescence was detected in shoot and root meristem within 1 h, increased rapidly at 3 h and expressed maximally at 6 h with the treatment of bleomycin (data not shown). In case of UV treatment, bioluminescence level showed a peak at 12 h. Thus, we visualized the tissue-specific expression after 6 h and 12 h of bleomycin and UV treatment, respectively. For bleomycin treatment, plants were incubated in MS liquid medium with bleomycin (25 μg ml^{-1}) for 6 h and then washed with MS liquid medium. For the UV irradiation experiments, plants were transferred to MS solid medium and exposed to UV-B irradiation (0.5 mW cm^{-2}, 30 min) using BX-15 UV illuminator (ATTO, Tokyo), which emits most of their energy within the UV-B range (290–320 nm) with an emission peak at

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312 nm. The UV dose was measured using a UVR-400 UV meter (Iuchi, Tokyo). Following UV-B irradiation, the plates were cultured for 12 h in complete darkness to prevent photorepair.

RNA isolation and RT-PCR

Total RNA from plant tissues was extracted following the procedure described previously (Davison and Furner 1999; http://genome-www.stanford.edu//comguide/chap_4.../2_RNA_mini_extraction.htm). A 1 μg aliquot of RNA was reverse transcribed using AMV reverse transcriptase XL in the presence of dNTPs and in a final volume of 20 μl with random oligonucleotides. PCR was performed with 10 μl of the reverse transcription reaction in a final volume of 50 μl, in the presence of dNTPs and Taq polymerase (Takara). RT-PCR was performed using total RNA with two *AtRAD51* mRNA-specific primers; *AtRAD51*-cod.5′; GGAGCAGCGTAGAAACCAGA-ATGC and *AtRAD51*-cod.3′; GGCCTGAATGTTCCCTCA-GCATCA. The specific primers, F-*EF1*α; TCGAGACCA-CCAAGTACTACTGC and R-*EF1*α; ATCATACCAGTCTCA ACACGTCC of the constitutively expressed *EF-1*α (elongation factor 1α) gene were used as an internal control.

Enzymatic assay of GUS activity

Histochemical assays for GUS activity were performed as described by Jefferson et al. (1987), with some modifications (Gallagher 1992). Organs of mature plants or entire seedlings were treated first with 70% ethanol for 1 min at room temperature, washed three times with 0.1 M sodium phosphate buffer, pH 7.2, and incubated for 48 h in staining buffer (1mM X-Gluc, 0.5 mM potassium ferricyanide, 0.5 mM potassium ferrocyanide, and 100 mM sodium phosphate, pH 7.2) at 37°C in the dark. Chlorophyll was removed by incubation in 70% ethanol at room temperature. GUS-stained material was directly observed using a Nikon SMZ-U stereoscopic microscope and photographed with a model H-III camera (Nikon). Quantitative kinetic analysis of GUS activity was conducted using fluorometry according to the method of Jefferson et al. (1991) using ARVO SX plate reader (Perkin Elmer). The GUS activity was expressed as units of GUS enzyme per microgram of total protein in each sample.

Visualization of luciferase activity

In vivo luciferase expression was visualized using bioluminescence imaging. Luciferase activity was visualized in living plants as light emitting sectors at 10 min in dark after spraying of 0.1mM D-luciferin potassium salt (Promega), which was applied to whole plants, and observed using a VIM camera system (Hamamatsu Photonics). Photons were collected for 40 min to obtain a well-defined image of bioluminescent tissues.

Results

GUS expression of the AtRAD51 promoter in Arabidopsis seedlings

In 7- and 14-day-old seedlings, a high-level of GUS activity was observed in seedlings transformed with *CaMV35S*::GUS (Figure 2A, E), but, very low GUS activities were observed in seedlings containing the GUS reporter gene under the control of the *AtRAD51* promoter (Figure 2B, F). In response to treatment with DNA-damaging agents, strong GUS staining was observed in the shoot apical meristem, root meristem and lateral root primodia of seedling, but not in other tissues (Figure 2B, C, D, F, G, H). Non-transformed *Arabidospsis* plants were used as a negative control and they did not reveal any pattern of X-Gluc staining (data not shown).

To confirm the induction of the *AtRAD51* promoter in *Arabidopsis* upon treatment with bleomycin or UV, we also conducted quantitative fluorometric GUS assays. As shown in Figure 3A, induction of the *AtRAD51* promoter in response to DNA damage was observed. Transgenic plants harboring *CaMV35S*::GUS exhibited 150-fold higher GUS activity compared with bleomycin-treated *AtRAD51* transgenic plants. However, no significant influence on expression levels by DNA damage was observed under the conditions of this study. To confirm endogenous gene expression in response to DNA damage, we tested RT-PCR detection of *AtRAD51* mRNA from *Arabidopsis* seedlings treated with or without DNA-damaging agent. As shown in Figure 3B, a significant increase in *AtRAD51* mRNA levels was observed in treated plants.

In order to supplement the GUS reporter assay data for UV-treated seedlings, we employed the LUC reporter assay to visualize spatiotemporal expression pattern of the *AtRAD51* promoter in seedlings (Figure 4). Fourteen-day-old seedlings, harboring the *AtRAD51*::Fluc construct, were used for bioluminescent imaging of LUC activity *in vivo*. The spatial pattern of *AtRAD51* expression detected by LUC assay was identical to that of the histochemical localization of GUS activity.

Analysis of the tissue-specific activity of the AtRAD51 promoter in mature Arabidopsis plants

In *CaMV35S*::GUS transgenic *Arabidopsis* plants, no tissue specificity was observed under the conditions exploited in this study (Figure 5A, E). On the other hand, marked tissue specificity of the *AtRAD51*-GUS was observed in mature plants. In roots of mature plants, strong GUS staining could be seen in root tips and lateral root primordia of BM treated plants (Figure 5B, C, D). GUS activity of *AtRAD51* was also observed in young bud without treatment of DNA damaging agent (Figure 5F). After treatment with DNA damaging agent, the strong GUS staining in mature plants, compared with non-treated samples, were observed in flowers (Figure 5G, H, I, J, K). In closed, unfertilized flowers, GUS activity was detected in sepal and stigma (Figure 5H, I). In open, fertilized flowers expression was observed in the sepal, stigma, anther, and pedicel (Figure 5J, K).

To investigate the induction of the promoter, we performed a quantitative fluorometric GUS assay in different tissues of flowering plants. As shown in Figure

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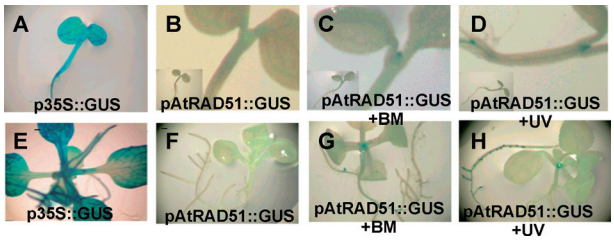

Figure 2. Histochemical localization of GUS activity directed by the *AtRAD51* promoter in 7 (A–D)- and 14 (E–H)- day-old transgenic *Arabidopsis* seedlings. (A, E) p35S::GUS as a positive control. GUS activity was strongly expressed in whole tissues; (B, F) pAtRAD51::GUS without DNA damaging agent; (C, G) pAtRAD51::GUS treated with UV; (D, H) pAtRAD51::GUS treated with bleomycin. GUS activity was induced in shoot and root meristem by treatment with DNA damaging agents, bleomycin (25 μg ml^{-1}, 6 h) or UV-B (0.5 mW cm^{-2}, 30 min).

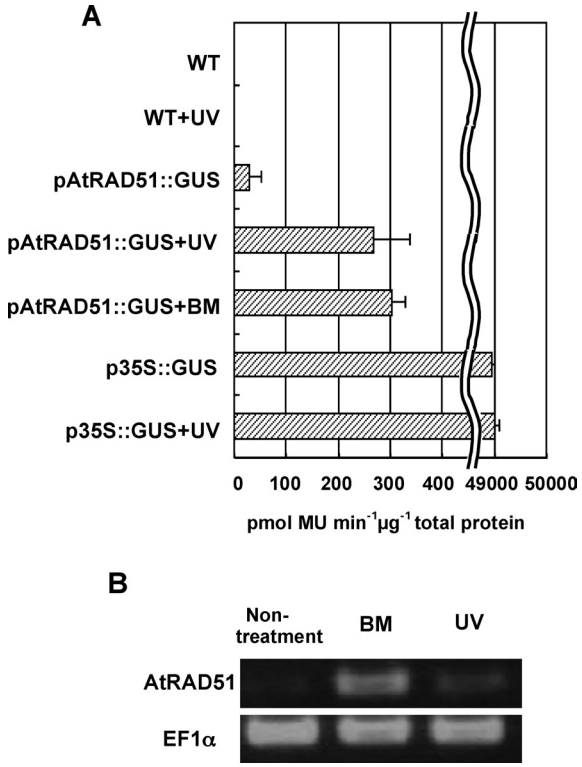

Figure 3. Expression of *AtRAD51* in 14-day-old seedlings of *Arabidopsis* plants. (A) Quantitative kinetic analysis of GUS activity using fluorometric assay (Jefferson et al. 1991). GUS activity was induced by treatment with DNA-damaging agents, bleomycin (25 μg ml^{-1}, 6 h) or UV-B (0.5 mW cm^{-2}, 30 min). The mean background GUS activity of 53 (SD 2) pmol min^{-1} μg^{-1} of WT was subtracted from all measurements shown. Error bars represent standard deviation of the means of 3 independent experiments; (B) RT-PCR of transgenic *AtRAD51*::GUS *Arabidopsis* plants. Total RNA was isolated from non-treated, bleomycin- or UV- treated plants.

6A, GUS activity was relatively low in leaves and higher activities were detected in buds and roots. A clear induction of GUS activity was observed in each tissue by the treatment of DNA damaging agent. To confirm the endogenous gene induction, we also performed RT-PCR analysis for *AtRAD51* mRNA in the mature *Arabidospsis*

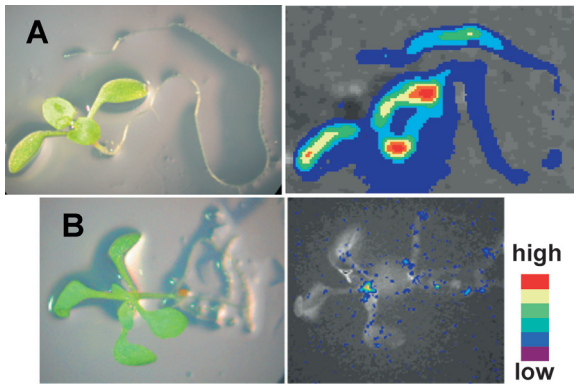

Figure 4. Fluc activity directed by the promoter reporter fusion constructs in 14-day-old transgenic *Arabidopsis* seedlings. (A) p35S::Fluc control. Fluc activity was strongly expressed in whole tissues; (B) pAtRAD51::Fluc with treatment of UV-B (0.5 mW cm^{-2}, 30 min). The AtRAD51 promoter expression monitored by Fluc activity is similar to the spatial pattern of histochemical localization of GUS.

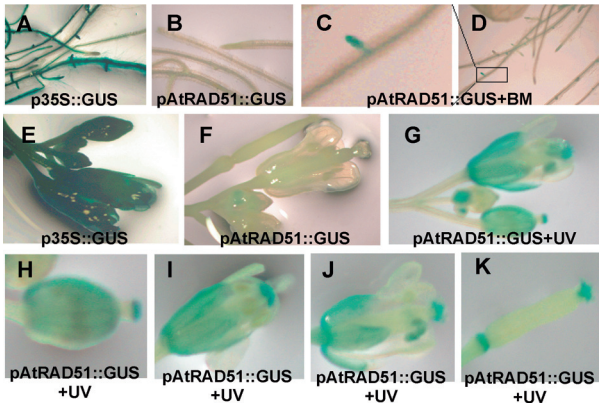

Figure 5. Histochemical localization of GUS activity directed by the *AtRAD51* promoter in mature (A–K) transgenic *Arabidopsis* plants. (A, E) p35S::GUS positive control. GUS activity was strongly expressed in whole tissues; (B, F) pAtRAD51::GUS without DNA damaging agent; (G–K) pAtRAD51::GUS treated with UV; (A, D) pAtRAD51::GUS treated with bleomycin. GUS activity was induced in shoot and root meristem by treatment with DNA damaging agents, bleomycin (25 μg ml^{-1}, 6 h) or UV-B (0.5 mW cm^{-2}, 30 min). Weak staining was induced in young floral bud (F) without treatment by DNA damaging agent. GUS activity was induced in young bud (G, H) sepal and stigma in closed flower (I), sepal, mature anther and stigma in open flower (J) and stigma in silique (K) by treatment with UV (0.5 mW cm^{-2}).

plants (Figure 6B). Consistent with the fluorometric assay, *AtRAD51* expression was high in buds and roots, and increased in response to treatment with DNA damaging agent. Taken together, our results demonstrate that the *AtRAD51* promoter directs tissue specific expression and is induced in response to DNA damage.

Pattern of GUS expression of the AtRAD51 promoter in transgenic AtRAD51::GUS tobacco plants

Maeda et al. (2004) reported that the *AtRAD51* promoter is able to direct gene expression in tobacco cells in

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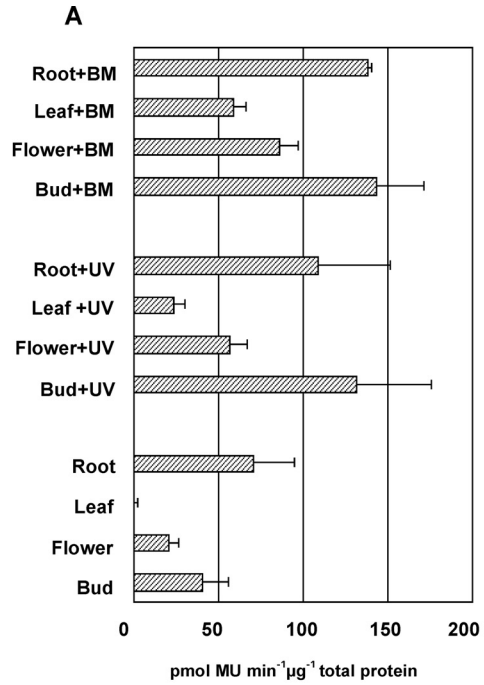

Figure 6. Expression of *AtRAD51* in various tissue of mature *AtRAD51*::GUS *Arabidopsis* plants. (A) Quantitative kinetic analysis of GUS activity using fluorometric assay (Jefferson et al. 1991). GUS activity was induced by treatment with DNA damaging agents, bleomycin (25 μg ml^{-1}, 6 h) or UV-B (0.5 mW cm^{-2}, 30 min). Mean background GUS activity of 26 (SD 4) pmol min^{-1} μg^{-1} of WT was subtracted from all measurements shown. Error bars represent standard deviation of the means of 2 independent experiments; (B) RT-PCR of transgenic *AtRAD51*::GUS *Arabidopsis* plants. Total RNA was isolated from non-treated, bleomycin- or UV- treated plants. Expression levels of bud, flower, leaf and root in mature plants for *AtRAD51* and *EF1α* determined by RT-PCR.

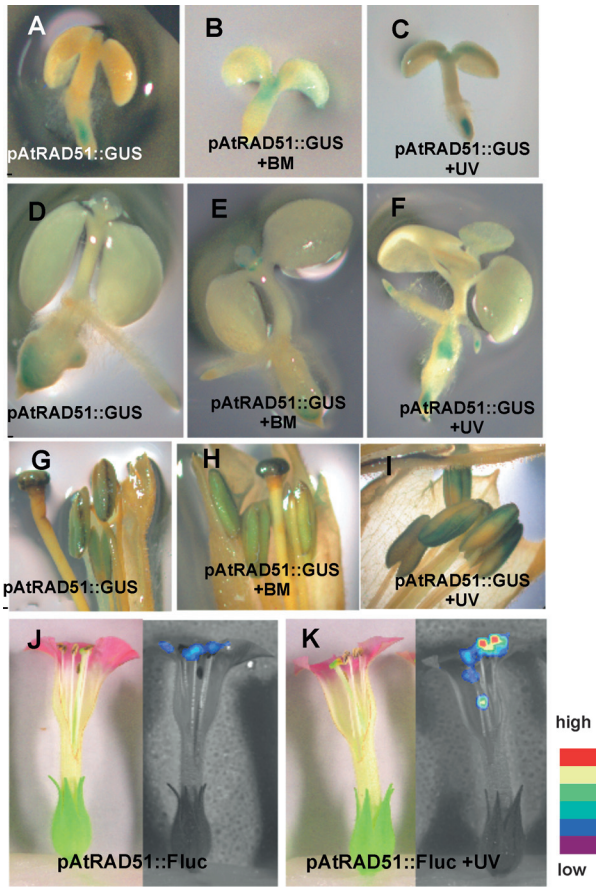

Figure 7. Expression of *AtRAD51* in 7 (A–C), 14-day-old (D–F) and mature (G–K) transgenic *AtRAD51*::GUS (A–I) and *AtRAD51*::Fluc (J–K) tobacco plants. (A, D, G, J) transgenic plants without DNA damaging agent; B, E and H, transgenic plants treated with bleomycin; (C, F, I, K) pAtRAD51::GUS plants treated with UV. GUS activity was induced in shoot and root meristem of seedling and anther of flower by treatment with DNA damaging agents, bleomycin (25 μg ml^{-1}, 6 h) or UV-B (0.5 mW cm^{-2}, 30 min). *AtRAD51* expression monitored by Fluc activity is identical to the spatial pattern of histochemical localization of GUS.

response to DNA damage. To further investigate tissue-specific expression of the *AtRAD51* promoter in tobacco, we generated transgenic tobacco plants harboring *AtRAD51*::GUS. In the histochemical assay of 7- or 14-day-old seedlings, strong staining was observed in the shoot apical meristem, root meristem and lateral root primordia, whereas no expression was observed in cotyledons or hypocotyls (Figure 7A, B, C, D, E, F). The GUS staining of transgenic tobacco flowers indicated preferential high expression of the *AtRAD51* promoter activities in anthers (Figure 7G, H, I). We also analyzed the Fluc activity to visualize the expression pattern of the *AtRAD51* promoter Fluc fusion in transgenic tobacco flowers (Figure. 7J, K). The spatial pattern of *AtRAD51* expression detected by Fluc assay was identical to that of the histochemical localization of GUS activity. Unlike *Arabidopsis*, even without treatment with a DNA-damaging agent, the *AtRAD51* promoter in tobacco plants showed considerably high expression levels. Transgenic plants harboring *CaMV35S*::GUS and *CaMV35S*::Fluc showed neither DNA damage response nor tissue specificity under the conditions of this study (not shown).

To obtain quantitative data, we also carried out a fluorometric GUS assay using 3-week-old and flowering tobacco plants (Figure 8, 9). In tobacco leaves, the *AtRAD51* promoter showed clear induction in response

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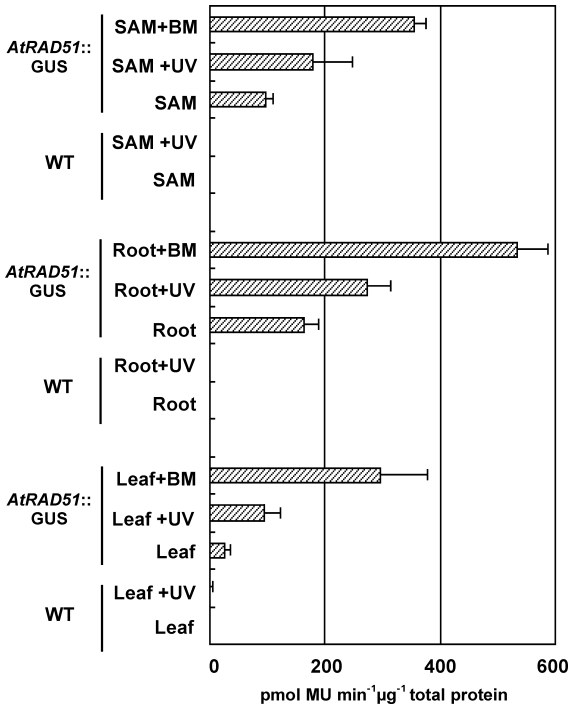

Figure 8. GUS activity in 3 weeks old wild-type and *AtRAD51*::GUS tobacco plants. Quantitative kinetic analysis of GUS activity was conducted using fluorometric assay (Jefferson et al. 1991). GUS activity was induced by treatment with DNA damaging agents, bleomycin (25 μg ml^{-1}, 6 h) or UV-B (0.5 mW cm^{-2}, 30 min). SAM, shoot apical meristem. Mean background GUS activity of untreated each tissue of WT was subtracted from all measurements shown. Error bars represent standard deviation of the means of 2–4 independent experiments.

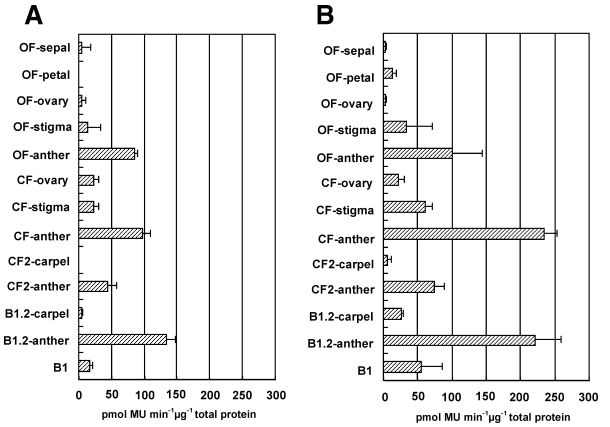

Figure 9. GUS activity in flowers of *AtRAD51*::GUS tobacco plants. Quantitative kinetic analysis of GUS activity was conducted using a fluorometric assay (Jefferson et al., 1991). GUS activity was measured in flowering tobacco plants without (A) or with treatment (B) of UV-B (0.5 mW cm^{-2}, 30 min). B1, 0.3–1 cm of bud; B1.2, 1,2 cm of bud; CF2, 2 cm of closed flower; CF, closed flower just before opening the flower; OF, open flower. Mean background GUS activity of WT in treated or untreated conditions was subtracted from all measurements shown. Error bars represent standard deviation of the means of 2 independent experiments.

to treatment with a DNA-damaging agent. Although low levels of GUS expression were detected from untreated root and SAM samples, induction of the *AtRAD51* promoter in UV- or bleomycin-treated root, as also observed in the histochemical assay, was evident in these organs (Figure 8). To further investigate the expression of the *AtRAD51* promoter in tobacco, we examined GUS activity in floral organs of the *AtRAD51*::GUS tobacco. As shown in Figure 9A, high GUS activities were detected in the anther at various stages of flower development. On the other hand, relatively low activities were detected from the sepal, petal, ovary, stigma and carpel. Although increases in GUS activities were relatively modest, similar levels of induction of *AtRAD51* promoter activity in response to UV-B treatment were observed in these samples (Figure 9B).

Discussion

Many works have been reported on gene regulation of DNA repair genes in response to DNA damage (Chen et al. 2003; Molinier et al. 2005). However, the promoter functions of DNA repair genes that direct tissue specific and DNA damage-responsive gene expression are not necessarily clear. To investigate in detail the regulated expression of the *AtRAD51* gene promoter we conducted a series of experiments using transgenic *Arabidopsis* and tobacco. Because the expression of the *AtRAD51* gene is relatively low, we used GUS reporter gene fusion as a method with higher sensitivity to investigate the tissue specificity of *AtRAD51* gene. To evade problems associated with GUS assay, we also exploited Fluc reporter assay for monitoring the *AtRAD51* promoter activity (Uknes et al.1993).

In this study, bleomycin and UV were employed as DNA damaging agents. A radiomimetic agent, bleomycin, which is known to induce SSBs and DSBs (Menke et al. 2001), was used because of its versatility and low cellular toxicity. Molinier et al. (2005) also reported the enhancement of the somatic HR frequency and the up-regulation of the HR-related genes with the treatment of bleomycin in *Arabidopsis*. We also exposed samples with UV, which induced DNA damage resulting in cyclobutane pyrimidine dimmers and (6-4) photoproduct that can be directly repaired by photolyase, or, in the absence of light, by nucleotide excision repair (NER). In *Arabidopsis*, a defect in direct repair or NER induces the use of HR repair process (Britt 1999; Molinier et al. 2005). UV-B radiation has been reported to increase the frequency of homologous recombination in plants (Ries et al. 2000). In the present study, bleomycin and UV also induced the expression of the *AtRAD51* promoter effectively in *Arabidopsis* and tobacco.

In transgenic *Arabidopsis* seedling, *AtRAD51*::GUS expression was observed in the shoot apical meristem,

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root meristem and lateral root primordia of seedlings, whereas no expression was observed in cotyledons or hypocotyls. The meristematic cells give rise to additional organs and eventually produce gametes. The meristem would then stop the cell division cycle to provide time for repair following DNA damage, and then accumulate a series of DNA repair proteins including AtRAD51. On the other hand, in mature plants, the *AtRAD51* promoter activity was detected in young flower buds without treatment with a DNA damaging agent (Figure 5F). In the absence of DNA-damaging agents, the *AtRAD51* gene showed a very low expression level in leaves but relatively high expression levels in young flower buds and roots were detected. These results are consistent with the fact that leaves consist largely of non-dividing cells, while roots and flower buds undergo active cell divisions. After exposure to DNA-damaging agents, the expression of *AtRAD51* in all tissues was up-regulated. Because the AtRAD51 protein is considered to play a role in mitotic as well as meiotic DNA recombination and repair, relatively high expression levels of the *AtRAD51* promoter in young flower buds and roots are consistent with the fact that these organs are contain cells with high mitotic activity (Doutriaux et al. 1998). Treatment with a DNA-damaging agent increased the *AtRAD51* promoter expression in the flower of mature *Arabidopsis* plants, compared with non-treated ones. It was highly induced in sepal and stigma of closed, unfertilized flowers and in sepal, stigma and anther of open, fertilized flowers. These results may suggest that the amount of AtRAD51 protein molecules may be important for DNA recombination repair in these organs. Li et al. (2004) reported that *AtRAD51* expression showed the increased level in young anthers before meiosis and stronger in later anther at the time of male meiosis by using RNA *in situ* hybridization. Consistent with an important function of the *AtRAD51* in meiosis, our results also indicated a strong expression of the promoter in later anther. On the other hand, GUS staining was not observed in anther of closed flowers of *Arabidopsis* in this study. This might be due to the failure of penetration of chromogenic substrate X-Gluc.

A previous study with Fluc reporter assay indicated that the *AtRAD51* promoter is able to direct expression in response to DNA damage in transgenic tobacco (Maeda et al. 2004). We also investigated tissue-specific expression of the *AtRAD51* promoter in tobacco in this study. In seedlings of *AtRAD51*::GUS transgenic tobacco plants, the expression pattern of the *AtRAD51* promoter was similar to that in *Arabidopsis*, suggesting that not only in response to DNA damage, tissue specificity of *AtRAD51* promoter is also conserved in tobacco. On the other hand, transgenic tobacco flowers showed relatively high expression of the *AtRAD51* promoter in anthers of all stages (Figure 7, 9). This observation is in contrast to the fact that the promoter was active only in anthers of open flower in *Arabidopsis* (Figure 5, 6). Further investigations including the expression analysis of DNA damage-responsive genes in tobacco will be necessary to study these differences in regulated expression of the *AtRAD51* promoter in *Arabidopsis* and tobacco. The present study showed that the expression patterns directed by the *AtRAD51* promoter were essentially the same in *Arabidopsis* and tobacco indicating that the regulatory mechanisms involved in the *AtRAD51* promoter is conserved between two species, at least to some extent.

The mammalian Rad51 proteins are specifically found in cells, where mitotic or meiotic recombination takes place, such as thymus, spleen, ovary and testis (Morita et al. 1993; Shinohara et al. 1993). Unlike *Arabidopsis* and yeast, the mammalian *RAD51* gene is not substantially induced at the level of transcription in response to genotoxic stresses (Chen et al. 1997; Vispe et al. 1998), but the protein only re-localizes to nuclear foci (Daboussi et al. 2002). The numerous experiments performed with mutant cells defective in the RAD51 homologues have shown that they play roles in somatic recombination, DNA repair and chromosome stability. Although the function of RAD51 has been well studied in organisms from yeast to humans, large gaps remain on our knowledge of its role in plants. Recently, the plant genes involved in DNA repair have been identified in plants and many of them are highly induced by the treatment with DNA damaging agents (Chen et al. 2003; Garcia et al. 2003; Molinier et al. 2005). The involvement of these genes in cell growth regulation has also been demonstrated (Deveaux et al. 2000; Hefner et al. 2005). The results on regulated expression of the *AtRAD51* promoter obtained in the present study were consistent with those reports on DNA damage responsiveness of DNA repair genes in plants.

Analysis of promoters for DNA repair genes including *RAD51* from yeast revealed a number of regulatory boxes, speculating to be involved in transcriptional regulation following irradiation (Mercier et al. 2001). Increased expression of the human paralogue *RAD51B* after treatment with DNA damaging agents is assumed to be mediated by 'consensus' promoter binding sites for both the AP2 and p53 proteins (Peng et al. 1998). These consensus regulatory boxes identified in other organisms were not present in the *AtRAD51* promoter. In plants, the search for *cis*-regulatory elements within the putative promoter region of several genotoxic stress revealed some putative consensus sequences (Chen et al. 2003). However, no studies on a DNA damage-responsive promoter have been conducted. The *AtRAD51* promoter characterized in this study could be an ideal starting point for further investigations on the promoter function of the DNA damage-responsive genes in higher plants.

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Identification of the *cis*-regulatory elements of the promoter critical for expression in response to DNA damage will lead to the identification and isolation of *trans*-acting factors involved in the transcriptional regulation of the *AtRAD51* promoter. These findings will help elucidate the specific regulation of gene expression by DNA damage, tissue specificity and the mechanisms involved in response to DNA damage in higher plants.

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References

Abe K, Osakabe K, Nakayama S, Endo M, Tagiri A, Todoriki S, Ichikawa H, Toki S (2005) Arabidopsis RAD51C gene is important for homologous recombination in meiosis and mitosis. *Plant Physiol* 139: 896–908

Bechtold N, Ellis J, Pelletier G (1993) *In planta Agrobacterium*-mediated gene transfer by infiltration of adult *Arabidopsis thaliana* plants. *C R Acad Sci Paris Life Sciences* 316: 1194–1199

Bleuyard JY, White CI (2004) The *Arabidopsis* homologue Xrcc3 plays an. essential role in meiosis. *EMBO J* 23: 439–449

Bleuyard JY, Gallego ME, Savigny F, White CI (2005) Differing requirements for the *Arabidopsis* Rad51 paralogs in meiosis and DNA repair. *Plant J* 41: 533–545

Britt AB (1999) Molecular genetics of DNA repair in higher plants. *Trends plant Sci* 4: 20–25

Britt AB, May GD (2003) Re-engineering plant gene targeting. *Trends plant Sci* 8: 90–95

Chen F, Nastasi A, Shen Z, Brenneman M, Crissman H, Chen DJ (1997) Cell cycle-dependent protein expression of mammalian homologs of yeast DNA double-strand break repair genes Rad51 and Rad52. *Mutat Res* 384: 205–211

Chen IP, Haehnel U, Altschmied L, Schubert I, Puchta H (2003) The transcriptional response of Arabidopsis to genotoxic stress-a high-density colony array study (HDCA). *Plant J* 35: 771–786

Daboussi F, Dumay A, Delacote F, Lopez BS (2002) DNA double-strand break repair signaling: The case of RAD51 post-translational regulation. *Cellular Signaling* 5598: 52–59

Deveaux Y, Alonso B, Pierrugues O, Godon C, Kazmaier M (2000) Molecular cloning and developmental expression of AtGR1, a new growth-related Arabidopsis gene strongly induced by ionizing radiation. *Radiat Res* 154: 355–364

Doucet-Chabeaud G, Godon C, Brutesco C, de Murcia G, Kazmaier M (2001) Ionising radiation induces the expression of PARP-1 and PARP-2 genes in *Arabidopsis*. *Mol Genet Genomics* 265: 954–963

Doutriaux MP, Couteau F, Bergounioux C, White C (1998) Isolation and characterization of the RAD51 and DMC1 from Arabidopsis thaliana. *Mol Gen Genet* 257: 283–291

Dray E, Siaud N, Dubois E, Doutriaux MP (2006) Interaction between Arabidopsis Brca2 and its partners Rad51, Dmc1, and Dss1. *Plant Physiol* 140: 1059–1069

Gallagher SR (1992) *GUS protocols: using the GUS gene as a reporter of gene expression*. Academic press, inc.

Garcia V, Bruchet H, Camescasse D, Granier F, Bouchez D, Tissier A (2003) AtATM is essential for meiosis and the somatic response to DNA damage in plants. *Plant Cell* 15: 1–3

Hefner E, Huefner N, Britt AB (2005) Tissue-specific regulation of cell-cycle responses to DNA damage in Arabidopsis seedlings. *DNA Rep* 5: 102–110

Jackson SP (2002) Sensing and repairing DNA double-strand breaks. *Carcinogenesis* 23: 687–696

Klimyuk VI, Jones JDG (1997) AtDMC1, the Arabidopsis homologue of the yeast DMC1 gene, characterization, transposon-induced allelic variation and meiosis-associated expression. *Plant J* 11: 1–14

Li W, Chen C, Markmann-Mulisch U, Timofejeva L, Schmelzer E, Ma H, Reiss B, (2004) The Arabidopsis *AtRAD51* gene is dispensable for vegetative development but required for meiosis. *Proc Natl Acad Sci USA* 101: 10596–10601

Maeda T, Watakabe Y, Seo S, Takase H, Hiratsuka K (2004) Expression of the *AtRAD51* gene promoter in response to DNA damage in transgenic tobacco. *Plant Biotechnol* 21: 113–118

Menke M, Chen IP, Angelis KJ, Schubert I (2001). DNA damage and repair in *Arabidopsis thaliana* as measured by the comet assay after treatment with different classes of genotoxins. *Mut Res* 493: 87–93

Mercier G, DenisY, Marcb P, Picard L, Dutreix M (2001) Transcriptional induction of repair genes during slowing of replication in irradiated *Saccharomyces cerevisiae*. *Mut Res* 487: 157–172

Molinier J, Oakeley EJ, Niederhauser O, Kovalchuk I, Hohn B (2005) Dynamic response of plant genome to ultraviolet radiation and other genotoxic stress. *Mut Res* 571: 235–247

Morita T, Yoshimura Y, Yamamoto A, Murata K, Mori M, Yamamoto H, Matsushiro A (1993) A mouse homolog of the *Escherichia coli recA* and *Saccharomycies cerevisiae RAD51* genes. *Proc Natl Acad Sci USA* 90: 6577–6580

Osakabe K, Yoshioka T, Ichikawa H, Toki S (2002) Molecular cloning and characterization of RAD51-like genes from *Arabidopsis thaliana*. *Plant Mol Biol* 50: 71–81

Peng L, Rice M, Kmiec E (1998) Analysis of the human RAD51L1 promoter region and its activation by UV light. *Genomics* 54: 529–541

Puchta H (2002) Gene replacement by homologous recombination in plants. *Plant Mol Biol* 48: 173–182

Ries G, Heller W, Puchta H, Sandermann H, Seidlitz HK, Hohn B (2000) Elevated UV-B radiation reduces genome stability in plants. *Nature* 406: 98–101

Shinohara A, Ogawa H, Matsuda Y, Ushio N, Ikeo K, Ogawa T (1993) Cloning of human, mouse and fission yeast recombination genes homologous to RAD51 and recA. *Nature Genet* 4: 239–243

Siaud N, Dray E, Gy I, Gerard E, Takvorian N, and Doutriaux MP. (2004) Brca2 is involved in meiosis in *Arabidopsis thaliana* as suggested by its interaction with Dmc1. *EMBO J* 23: 1392–401

Stassen NY, Logsdon JM Jr, Vora GJ, Offenberg HH, Palmer JD, Zolan ME (1997) Isolation and characterization of rad51 orthologs from Coprinus cinereus and Lycopersicon esculentum, and phylogenetic analysis of eukaryotic recA homologs. *Curr Genet* 31: 144–157

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Uknes S, Dincher S, Friedrich L, Negrotto D, Williams S, Thompson-Taylor H, Potter S, Ward E, Ryals J (1993) Regulation of pathogenesis-related protein-1a gene expression tobacco. *Plant Cell* 5: 159–169

Vispe S, Cazaux C, Lesca C, Defais M (1998) Overexpression of Rad51 protein stimulates homologous recombination and increases resistance of mammalian cells to ionizing radiation. *Nucleic Acids Res* 26: 2859–2864

Urban C, Smith KN, Beier H (1996) Nucleotide sequences of nuclear tRNA(Cys) genes from Nicotiana and Arabidopsis and expression in HeLa cell extract. *Plant Mol Biol* 32: 549–552

Zhou BB, Elledge SJ (2000) The DNA damage response: putting checkpoints in perspective. *Nature* 408:433–439

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