SNM-dependent recombinational repair of oxidatively induced DNA damage in *Arabidopsis thaliana*

Jean Molinier⁺, Marie-Eve Stamm & Barbara Hohn

Friedrich Miescher-Institute for Biomedical Research, Basel, Switzerland

Two different roles for SNM (sensitive to nitrogen mustard) proteins have already been described: the SNM1/PSO2-related proteins are involved in the repair of the interstrand crosslink (ICL) and the ARTEMIS proteins are involved in the V(D)J recombination process. Our study shows that an *Arabidopsis* SNM protein, although structurally closer to the SNM1/PSO2 members, shares some properties with ARTEMIS but also has novel characteristics. *Arabidopsis* plants defective for the expression of AtSNM1 did not show hypersensitivity to the ICL-forming agents but to the chemotherapeutic agent bleomycin and to H₂O₂. AtSNM1 mutant plants are delayed in the repair of oxidative damage and did not show enhancement of the frequency of somatic homologous recombination on exposure to H₂O₂ and to the bacterial elicitor flagellin, both inducing oxidative stress, as observed in the control plants. Therefore, our results suggest the existence, in plants, of a novel SNM-dependent recombinational repair process of oxidatively induced DNA damage.

Keywords: *Arabidopsis*; SNM; homologous recombination; oxidative stress

EMBO reports (2004) 5, 994–999. doi:10.1038/sj.embor.7400256

INTRODUCTION

All living organisms are constantly exposed to environmental stresses that cause damage. Plants, because of their sedentary lifestyle, are especially susceptible to damage caused by environmental factors. To preserve genome integrity, plants use different DNA repair pathways, such as direct repair, base excision repair (BER), nucleotide excision repair (NER) or mismatch repair (MMR). Homologous recombination (HR) and non-homologous end joining (NHEJ) are the two pathways used for repairing DNA double-strand breaks (DSBs). HR contributes to the preservation of genome integrity, whereas NHEJ seals DNA ends directly and can lead to sequence alterations (Vonarx *et al*, 1998; Britt, 1999).

Inter/intrastrand crosslinks (ICLs) are a highly toxic form of DNA lesion. ICLs can be induced by UV radiation, furocoumarins and chemotherapeutics (Dronkert & Kanaar, 2001). ICLs prevent DNA strand separation, in turn blocking transcription, replication and chromosomal segregation, and thus triggering cell apoptosis (Li & Moses, 2003). Many processes are involved in ICL repair, suggesting interactions between different repair pathways. The initial step of ICL repair involves the removal of the lesion by the NER machinery (McHugh *et al*, 2000). In yeast and mammals, HR also has an important role in the repair of ICLs (Jachymczyk *et al*, 1981; de Silva *et al*, 2000). Therefore, the balance between the NHEJ and HR pathways is not only critically important in the repair of DSBs but also has a strong influence on the outcome of ICL repair.

The SNM1 (sensitive to nitrogen mustard) protein is involved in ICL repair in yeast (Wolter *et al*, 1996). The mammalian and yeast SNM-related proteins are nuclear proteins containing a conserved metallo- β -lactamase domain (Callebaut *et al*, 2002; Ma *et al*, 2002; Li & Moses, 2003). This domain is essential for the nuclease activity of SNM1 and is required for repair of DSBs resulting from ICL removal (Li & Moses, 2003). Mouse and human SNM1 homologues are also involved in the repair of ICL-mediated damage (Dronkert *et al*, 2000).

One of the human SNM-related proteins is also known as ARTEMIS. Mutations in ARTEMIS are associated with severe immunodeficiency syndrome due to a defect in V(D)J recombination. ARTEMIS interacts with the DNA-dependent protein kinase and is responsible for opening the hairpin and for overhang processing during the V(D)J recombination process (Ma *et al*, 2002). ARTEMIS mutants are also hypersensitive to DNA-damaging agents such as bleomycin (BLM) and γ -rays (Moshous *et al*, 2001). ARTEMIS therefore seems to have a general role in NHEJ, in addition to a specific role in V(D)J joining. Taken together, these studies suggest that SNM-related proteins have a variety of roles in DSB repair in different organisms.

The study of the closest *Arabidopsis* homologue of the yeast and mammalian SNM1 protein also suggests a broad role of SNM1 in DSB repair in plants. However, contrary to expectation, the AtSNM1-deficient plants were not hypersensitive to the two ICL-forming agents cisplatin and mitomycin C (MMC), but showed a moderate sensitivity to the chemotherapeutic agent BLM and to hydrogen peroxide (H₂O₂). In AtSNM1 mutant plants exposed

Friedrich Miescher-Institute for Biomedical Research, Maulbeerstrasse 66, 4058 Basel, Switzerland

⁺Corresponding author. Tel: +41 61 697 61 00; Fax: +41 61 697 39 76;

E-mail: jean.molinier@fmi.ch

Received 12 May 2004; revised 28 July 2004; accepted 23 August 2004; published online 24 September 2004

to H₂O₂ and to the bacterial elicitor flagellin, both inducing oxidative stress, the frequency of somatic HR (HRF) was not found to be enhanced as compared with the control plants. We therefore suggest the existence in plants of an SNM1-dependent recombinational repair process of oxidatively induced DNA damage.

RESULTS AND DISCUSSION
Characterization of AtSNM1

The *Arabidopsis* SNM protein (AtSNM1) studied is predicted to be 484 amino acids (aa) long. SNM homologues range from 484 aa long in *Arabidopsis* to 1,040 aa long in mammals. SNM proteins contain a conserved metallo-β-lactamase domain (Fig 1A). This domain, although variable in size, always retains a conserved motif of 10aa, which has been shown to be essential for SNM1 function in *Saccharomyces cerevisiae* (Li & Moses, 2003). In the species mentioned, the region surrounding the metallo-β-lactamase domain shares 20–90% identity at the protein level.

The *Arabidopsis* genome encodes three SNM-like proteins sharing 36 and 32% identity among them (Fig 1B). AtSNM1 shares around 48% identity with the mammalian and *Drosophila* SNM1 proteins known to be involved in ICL repair. Therefore, expression of the AtSNM1 gene was expected to be induced on exposure to the two ICL-inducing agents cisplatin and MMC, and SNM1-defective plants were expected to be sensitive to the two ICL-inducing agents as in yeast and mammals (Wolter et al, 1996; Dronkert et al, 2000).

Expression profile of AtSNM1

The expression profile of the AtSNM1 gene was first analysed in wild-type (WT) plants exposed to MMC and cisplatin. Surprisingly, neither MMC nor cisplatin influenced AtSNM1 expression in the first 24 h following the treatment (data not shown). Plants were exposed to BLM, which is known to induce DSBs by means of an oxidation-mediated reaction and also other DNA oxidative lesions (Povirk & Steighner, 1989), to UVC, methyl methane sulphonate

Fig 1 | Sequence comparison between several SNM1 proteins. (A) Multiple alignment of the conserved metallo-β-lactamase motif of representative members of the SNM-related proteins. Identical amino acids are shaded in pink; similar amino acids are shaded in dark blue (uppercase) and amino acids sharing 30% conservation are shaded in blue (lowercase). (B) Unrooted phylogenetic tree of the metallo-β-lactamase motif. Branch lengths shown are proportional to the calculated evolutionary distances (clustalX program). At, *Arabidopsis thaliana*; Dm, *Drosophila melanogaster*; Hs, *Homo sapiens*; Mm, *Mus musculus*; Os, *Oryza sativa*; Sc, *Saccharomyces cerevisiae*; Sp, *Schizosaccharomyces pombe*.

Fig 2 | Molecular characterization of AtSNM1 expression and T-DNA insertion lines. (A) RT-PCR analysis of AtSNM1 expression in WT plants after exposure to BLM (10^{-7} M), MMS (40 ppm), H₂O₂ (0.6 mM), xylanase (20 mg l⁻¹) and active flagellin (0.3 μ M). (B) Genomic organization of the AtSNM1 locus and position of the two T-DNA insertions in the first exon (grey boxes) in the *Atsnm1-1* plants and in the 3' untranslated region in the *Atsnm1-2* plants. Primers 1, 2, 5', RB and LB were used for genotyping *Atsnm1-1* plants and primers 3, 4 and LB were used for genotyping *Atsnm1-2* plants. The sequences of the T-DNA/plant genome junctions are available in supplementary information online. (C) RT-PCR analysis of AtSNM1 expression in *Atsnm1-1* and *Atsnm1-2* plants after exposure to BLM (10^{-7} M) and H₂O₂ (0.6 mM).

(MMS) or H₂O₂, which causes oxidative damage. UV-C did not influence AtSNM1 expression in the first 24 h following the treatment, whereas its expression was found to be induced 24 h after BLM treatment and 2 h after MMS and H₂O₂ treatments (Fig 2A). AtSNM1 expression was also found to be induced on exposure to the elicitors xylanase (6 h) and flagellin (2 h; Fig 2A), which provoke an oxidative burst and defence responses (Enkerli *et al*, 1999; Felix *et al*, 1999). Reactive oxygen species (ROS), such as H₂O₂, mediate the plant response to pathogen attack (for review, see Apel & Hirt, 2004) and cause oxidative DNA damage (Sekiguchi & Hayakawa, 1998). These expression data confirm microarray analyses of WT *Arabidopsis* plants exposed to several different genotoxic and environmental stresses (Molinier *et al*, 2004c). We, therefore, suggest a role for AtSNM1 in the response to genotoxic stress, including that arising from pathogen attack.

Characterization of AtSNM1-defective plants

To define better the role of AtSNM1 in DNA repair, two *Arabidopsis* SNM1 insertion lines were characterized. In line *snm1-1*, the T-DNA is integrated in the first exon, 22 nucleotides downstream of the ATG codon (Fig 2B). In line *snm1-2*, the T-DNA is integrated in the 3' untranslated region (3'UTR), 66 nucleotides downstream of the stop codon (Fig 2B). Contrary to our expectation, no significant differences in development were

noticed between the *Atsnm1* plants and the control plants exposed to MMS or the two ICL-inducing agents MMC and cisplatin (data not shown). Although SNM1 functions in ICL repair are partially conserved between yeast and mammals, the relationship between SNM1 structure and function seems to be different in plants, suggesting that this particular AtSNM1 protein is not essential for the general ICL repair process in *Arabidopsis*. AtSNM proteins may have redundant functions in the cellular response to DNA-damaging agents or may differ in tissue specificity of DNA repair or may be involved in other cellular processes. The involvement of the two other *Arabidopsis* SNM-like proteins in ICL repair remains to be investigated.

AtSNM1 transcripts remain undetectable in *snm1-1* and *snm1-2* mutant plants following exposure to BLM (24 h) or H₂O₂ (2 h; Fig 2C). These results confirm that *snm1-1* and *snm1-2* lines are defective in AtSNM1 expression. Plants in which RNA-mediated interference (RNAi) was used to downregulate the SNM1 steady-state level also did not produce detectable levels of AtSNM1 under any condition tested (supplementary information online).

SNM1-defective plants were retarded in growth at 10^{-7} and 10^{-6} M BLM compared with the control (Fig 3A). In yeast and mice, hypersensitivity to BLM was observed in ARTEMIS-defective cells but not in SNM1-defective cells (Wolter *et al*, 1996; Moshous *et al*, 2001; Rooney *et al*, 2003). As AtSNM1 is not closely related to the mammalian ARTEMIS proteins and as V(D)J recombination or a process resembling it has not been described in plants, the main function of AtSNM1 might be in an alternative repair process.

A moderate sensitivity of the mutant plants compared with the control plants was also noticed in the presence of 0.6 and 1.2 mM H₂O₂ (Fig 3B). Hypersensitivity of SNM1-deficient cells to agents inducing oxidative damage has not been reported in other organisms.

Taken together, these results suggest a nonessential role for AtSNM1 in the repair process of BLM- and H₂O₂-induced DNA damage. Therefore, to determine whether AtSNM1 has a direct role in the repair of DNA damaged by ROS, the repair efficiency of *Atsnm1* plants was evaluated using cell extracts and an *in vitro* DNA repair assay (Li *et al*, 2002). A linearized plasmid was treated with methylene blue plus visible light, which causes oxidative damage (mainly 8-oxo-G), that serves as a substrate for the DNA-synthesis-dependent repair such as BER of oxidative DNA damage (Li *et al*, 2002). A significant delay in the incorporation of digoxigenin (DIG)-labelled dUTP in the mutant plants compared with the control was observed (supplementary information online). These data suggest that *Atsnm1* plants are delayed but not impaired in DNA-synthesis-dependent repair of oxidative DNA damage.

Therefore, we suggest that AtSNM1 encodes a protein involved in the processing of DNA lesions formed after exposure to oxidative stress.

Recombinational repair of ROS-induced DNA damage

In yeast and mammals, SNM-related proteins were shown to be involved in the processing of ICL-induced DNA DSBs and in the V(D)J recombination process, respectively (Wolter *et al*, 1996; Dronkert *et al*, 2000; Ma *et al*, 2002). To determine whether the studied AtSNM1 protein could have a role in DSB repair, AtSNM1-defective plants were monitored for recombinational

Fig 3 | Sensitivity of AtSNM1-defective plants to DNA-damaging agents. In all, 4-day-old *in vitro*-germinated AtSNM1-defective plants (*snm1-1* and *snm1-2*) and control plants were incubated in liquid culture medium in the presence of increasing doses of BLM (A) or H₂O₂ (B). Relative weight ((weight treated/weight untreated) × 100) was evaluated 4 days after exposure to BLM (10^{-6} and 10^{-7} M) or H₂O₂ (0.6 and 1.2 mM). A total of 18 plants was used per experiment. Two experiments were performed for all treatments; data were aggregated.

DNA repair using an *in planta* recombination assay (Swoboda et al, 1994; Molinier et al, 2004a).

Somatic HRF was monitored in RNAi plants and in *snm1-1* plants crossed with *Arabidopsis* lines containing recombination substrates, after exposure to the DNA-damaging agents UVC, MMS, cisplatin, MMC, BLM and H₂O₂. Somatic HRF was increased to the same extent in WT and AtSNM1-defective plants treated with UVC, BLM, MMS, cisplatin and MMC (data not shown) when compared with untreated control plants.

To test the biological relevance of AtSNM1-mediated repair of oxidatively induced DNA damage, H₂O₂ and a bacterial elicitor mimicking natural stress responses were used. FLG22, a 22 aa-long synthetic peptide derived from flagellin, the most abundant protein of bacterial flagella, is known to elicit plant defence responses and to induce a rapid oxidative burst (Felix et al, 1999). As a control, a 22 aa-long peptide derived from *Agrobacterium tumefaciens* was used. It differs from the active FLG22 by 1 aa and does not induce oxidative burst and defence responses (Felix et al, 1999).

Somatic HRF was enhanced by five- to tenfold after treatments of control plants with H₂O₂ and active FLG22 (Fig 4A,B). In contrast, in both RNAi and insertional mutant plants, somatic HRF remained almost unchanged after treatments (Fig 4A,B). These results thus suggest that HR-mediated repair of these two ROS-inducing treatments specifically requires the activity of SNM1, whereas the repair of UVC, cisplatin or MMC DNA damage is independent of this activity. One scenario that may explain the specific role of AtSNM1 in recombinational DNA repair borrows ideas from the nucleolytic activity of SNM proteins (for reviews, see Jeggo & O'Neill, 2002; Li & Moses, 2003). If AtSNM1 can process DSB intermediates as suggested for yeast and mammalian homologues, we might predict it to be of special importance in reacting to oxidative lesions within single-stranded DNA overhangs. The role of SNM1 in somatic HR may be of special importance for the evolution of disease resistance genes, as they can be arranged as clustered loci (for reviews, see Hulbert et al, 2001; Leister, 2004). Oxidative damage, occurring during pathogen infection, may speed up the generation of new resistance gene assortments (Lucht et al, 2002; Kovalchuk et al, 2003). The work presented here clearly documents the existence of an SNM1-dependent repair process of ROS-induced DNA damage in *Arabidopsis*.

METHODS

Plant material and growth conditions. *Arabidopsis thaliana* plants (Columbia ecotype) were used for all experiments. Plants containing a T-DNA integrated in the SNM1 locus (At3g26680) were obtained from the SAIL collection (Torrey Mesa Research Institute, USA). *In vitro*-cultured plants were germinated on GM medium (MS salts (Duchefa, The Netherlands), 1% sucrose, 0.8% agar, pH 5.8) and grown in a culture chamber under a 16 h/8 h photoperiod (20 °C/16 °C).

Molecular characterization of AtSNM1 insertional mutants. The mutant plants, *snm1-1* and *snm1-2*, carrying T-DNAs in the SNM1 locus, were characterized using PCR. Primers 1 and 2, specific for the WT allele, and primers LB/2 and RB/5', specific for the T-DNA, were used to identify *Atsnm1-1*. Primers 3 and 4 for the WT allele and primers LB2 and 3, specific for the T-DNA, were used to identify *Atsnm1-2* (Fig 2B). Primer sequences are available in supplementary information online.

Molecular characterization of AtSNM1 expression. The expression pattern of the AtSNM1 gene was determined by PCR after reverse transcription of RNA (RT-PCR). Total RNA from either WT, *snm1-1*, *snm1-2* or RNAi plants (RNAi3 and RNAi4) was prepared using the RNeasy extraction kit (Qiagen, USA). RT was performed using 3 µg of total RNA, oligodT primer and the Reverse transcription kit (Amersham, USA). To confirm that the PCR amplifications were within the linear range, different dilutions of

Fig 4 | Somatic homologous recombination frequencies in AtSNM1-defective plants after H₂O₂ or flagellin treatments. (A) Controls (recombination lines IC9 or 1406), RNAi, null segregant (NS), Atsnm1-1 or Atsnm1-2 plants were exposed to increasing concentrations of H₂O₂ (0, 0.3, 0.6, 2.5 and 5 mM). (B) Controls (recombination lines IC9 or 1406), RNAi, null segregant (NS), Atsnm1-1 or Atsnm1-2 plants were grown in the presence of 0.3 μM of either inactive (control) or active FLG22. At 5 days after the treatment, the somatic HRF (average number of GUS + spots/plant) was monitored using the histochemical β-glucuronidase (GUS) assay. A total of 48 plants were used per experiment. At least two separate experiments were carried out for all treatments; data were aggregated.

the RT reaction mixture and several PCR cycles were used. Initial denaturation was carried out at 94 °C for 1 min followed by annealing for 1 min at 58 °C, and then amplification was performed for 30 s at 72 °C. For optimal amplification of SNM1 transcripts, the RT reaction was diluted twofold and 35 PCR cycles were used. For optimal detection of the actin transcripts, the RT reaction was diluted 25-fold and 30 PCR cycles were used. Primers 6 and 7 were used for revealing AtSNM1 expression and primers act2-5' and act2-3' were used as controls for actin. Primer sequences are available in supplementary information online.

RNAi construct. The pOEXhp vector (Molinier et al, 2004b) was designed for RNAi technology allowing expression of double-strand RNA. The second exon (210 base pairs (bp)) of the SNM1 gene (AGI At3g26680) was cloned into the pOEXhp vector. The resulting phpSNM1 plasmid was mobilized into *A. tumefaciens* and was used to transform IC9 plants carrying an intermolecular recombination substrate (Molinier et al, 2004a). HRF was measured in at least two independent experiments in the T2 generation of two independent transformants, RNAi3 and RNAi4, in batches of more than 60 plants. The insertional mutants *snm1-1* and *snm1-2* were crossed with the IC9 line (Molinier et al, 2004a)

or 1406 line (Gherbi et al, 2001) containing an inter- and intramolecular recombination substrate, respectively. Plants homozygous for both loci were used for further experiments.

Treatment of plants with DNA-damaging agents, xylanase and flagellin peptides. For BLM, MMS, cisplatin, MMC and H₂O₂ treatments, 4-day-old WT, *snm1-1*, *snm1-2*, IC9 and RNAi seedlings were transferred from solid MS medium into 96-well culture plates. Each well contained one seedling in 200 μl of liquid MS medium. The following day, the culture medium was removed and replaced by either 200 μl of BLM (Sigma, Switzerland; 0, 10⁻⁹, 10⁻⁸, 10⁻⁷, 10⁻⁶, 10⁻⁵ and 10⁻⁴ M), MMS (Fluka, Switzerland; 0, 20, 40, 60 and 100 ppm), cisplatin (Sigma, Switzerland; 0, 2.5, 5, 10 and 20 μM), MMC (Sigma; 0, 10⁻⁹, 10⁻⁸, 10⁻⁷, 10⁻⁶, 10⁻⁵ and 10⁻⁴ M) or H₂O₂ (Fluka; 0, 0.3, 0.6, 2.5 and 5 mM). The plates were incubated for 7 days in the culture chamber. For UVC irradiation (0, 10 and 30 kerg/cm², 254 nm), 4-day-old seedlings were irradiated with light provided by an HNS 55 W OFR lamp (Osram, France). Plants were allowed to grow for another 4 days and the resistance or sensitivity of *snm1-1*, *snm1-2* and RNAi plants was compared with that of WT and IC9 plants. For xylanase treatment, 10-day-old plants were sprayed with 20 mg/l of

xylanase from *Trichoderma viride* (Sigma) and 0.1% of extraxon (Ciba, Switzerland). For flagellin treatment, 5-day-old *Arabidopsis* plants were transferred into 24-well culture plates (two plants per well) in MS liquid medium. After 2 days, 0.3 µM of active or inactive FLG22 (Felix et al, 1999) was added to the culture medium, and plants were allowed to grow for another 7 days.

In vitro DNA repair assay. The *in vitro* DNA repair assay was performed on cell extracts from the homozygous *snm1-1* and *snm1-2* plants and from the WT plant as a control following the method of Li et al (2002). The linearized pGEX plasmid was damaged with 10 µM methylene blue and visible light. A 25 µg portion of protein extracts was used per time point and mixed with 300 ng of damaged plasmid (pGEX) and 300 ng of nondamaged plasmid (pBSK) as control. The reaction was stopped after 30 min, 1 h or 2 h of incubation. Plasmids were purified using the Gel extraction kit (Qiagen). The agarose gel and DIG detection procedures were performed as described by Li et al (2002). Experiments were performed in duplicate with two independently prepared cell extracts.

Somatic homologous recombination assay. Somatic HR was monitored in RNAi plants and in insertional mutant lines crossed with the recombination lines 1406 and IC9 (Gherbi et al, 2001; Molinier et al, 2004a). The histochemical GUS assay was performed on 2-week-old *Arabidopsis* seedlings treated with BLM, MMS, cisplatin, MMC, H₂O₂, active and inactive FLG22 or UVC, as described by Jefferson et al (1987). HR was measured 4 days after each treatment. Somatic HRF was defined as total number of GUS-positive spots per total number of plants. Experiments were performed in triplicate with 24–48 plants per replicate.

Supplementary information is available at *EMBO reports* online (<http://www.emboreports.org>).

ACKNOWLEDGEMENTS

We thank P. Pelczar and N. Grimsley for critically commenting on the manuscript and all the members of our group for stimulating discussions. We also thank C. Zipfel for the flagellin peptides and V. Kalck for her technical support. J.M. was funded by the EU project PLANTREC No. QL2-CT-2001-01397. We acknowledge the Novartis Research Foundation for financial support.

REFERENCES

- Apel K, Hirt H (2004) Reactive oxygen species: metabolism, oxidative stress and signal transduction. *Annu Rev Plant Biol* **5**: 373–399
- Britt AB (1999) Molecular genetics of DNA repair in higher plants. *Trends Plant Sci* **4**: 20–24
- Callebaut I, Moshous D, Mornon JP, De Villartay JP (2002) Metallo-β-lactamase fold within nucleic acids processing enzymes: the β-CASP family. *Nucleic Acids Res* **30**: 3592–3600
- De Silva IU, Mc Hugh PJ, Clingen PH, Hartley JA (2000) Defining the roles of nucleotide excision repair and recombination in the repair of DNA interstrand cross-links in mammalian cells. *Mol Cell Biol* **20**: 7980–7990
- Dronkert MLG, Kanaar R (2001) Repair of DNA interstrand cross-links. *Mutat Res* **486**: 217–247
- Dronkert MLG, de Wit J, Boeve M, Vasconcelos ML, van Steeg H, Tan TLR, Hoeijmakers JHJ, Kanaar R (2000) Disruption of mouse SNM1 causes increased sensitivity to the DNA interstrand cross-linking mitomycin C. *Mol Cell Biol* **13**: 4553–4561
- Enkerli J, Felix G, Boller T (1999) The enzymatic activity of fungal xylanase is not necessary for its elicitor activity. *Plant Physiol* **121**: 391–397
- Felix G, Duran JD, Volko S, Boller T (1999) Plants have a sensitive perception system for the most conserved domain of bacterial flagellin. *Plant J* **18**: 265–276
- Gherbi H, Gallego ME, Jalut N, Lucht JM, Hohn B, White CI (2001) Homologous recombination *in planta* is stimulated in the absence of Rad50. *EMBO Rep* **2**: 287–291
- Hulbert SH, Webb CA, Smith SM, Sun Q (2001) Resistance gene complexes: evolution and utilization. *Annu Rev Phytopathol* **39**: 285–312
- Jachymczyk WJ, von Borstel RC, Mowat MRA, Hastings PJ (1981) Repair of interstrand cross-link in DNA of *Saccharomyces cerevisiae* requires two systems for DNA repair: the RAD3 system and the RAD51 system. *Mol Gen Genet* **182**: 196–205
- Jefferson RA, Kavanagh TA, Bevan MW (1987) GUS fusions: β-glucuronidase as a sensitive and versatile gene fusion marker in higher plants. *EMBO J* **6**: 3901–3907
- Jeggo P, O'Neill P (2002) The Greek goddess, Artemis, reveals the secrets of her cleavage. *DNA Repair* **1**: 771–777
- Kovalchuk I, Kovalchuk O, Kalck V, Boyko V, Filkowski J, Heinlein M, Hohn B (2003) Pathogen-induced systemic plant signal triggers DNA rearrangements. *Nature* **423**: 760–762
- Leister D (2004) Tandem and segmental gene duplication and recombination in the evolution of plant disease resistance genes. *Trends Genet* **20**: 116–122
- Li A, Schuermann D, Gallego F, Kovalchuk I, Tinland B (2002) Repair of damaged DNA by *Arabidopsis* cell extract. *Plant Cell* **14**: 263–273
- Li X, Moses RE (2003) The β-lactamase motif in Snm1 is required for repair of DNA double-strand breaks caused by interstrand crosslinks in *S. cerevisiae*. *DNA Repair* **2**: 121–129
- Lucht JM, Mauch-Mani B, Steiner HY, Metraux JP, Ryals J, Hohn B (2002) Pathogen stress increases somatic recombination frequency in *Arabidopsis*. *Nat Genet* **30**: 311–314
- Ma Y, Pannicke U, Schwarz K, Lieber R (2002) Hairpin opening and overhang processing by an Artemis/DNA-dependent protein kinase complex in nonhomologous end joining and V(D)J recombination. *Cell* **108**: 781–794
- McHugh P, Sones WR, Hartley JA (2000) Repair of intermediate structures produced at DNA interstrand cross-links in *S. cerevisiae*. *Mol Cell Biol* **20**: 3425–3433
- Molinier J, Ries G, Bonhoeffer S, Hohn B (2004a) Intersister and interhomolog recombination in *Arabidopsis thaliana*. *Plant Cell* **16**: 342–352
- Molinier J, Ramos C, Fritsch O, Hohn B (2004b) CENTRIN2 modulates homologous recombination and nucleotide excision repair in *Arabidopsis*. *Plant Cell* **16**: 1633–1643
- Molinier J, Oakley EJ, Niederhauser O, Kovalchuk I, Hohn B (2004c) Dynamic response of plant genome to ultraviolet radiation and other genotoxic stresses. *Mutat Res* (in press)
- Moshous D et al (2001) Artemis, a novel DNA double-strand break repair/V(D)J recombination protein, is mutated in human severe combined immune deficiency. *Cell* **105**: 177–186
- Povirk LF, Steighner RJ (1989) Oxidized apurinic/aprimidinic sites formed in DNA by oxidative mutagens. *Mutat Res* **214**: 13–22
- Rooney S, Alt FW, Lombard D, Whitlow S, Eckersdorff M, Fleming J, Fugmann S, Ferguson DO, Schatz DG, Sekiguchi J (2003) Defective DNA repair and increased genomic instability in Artemis-deficient murine cells. *J Exp Med* **197**: 553–565
- Sekiguchi M, Hayakawa H (1998), in *DNA Damage and Repair*, Nickoloff JA, Hoekstra MF (eds) Ch 6, pp 85–94. Totowa, Canada: Humana
- Swoboda P, Gal S, Hohn B, Puchta H (1994) Intrachromosomal homologous recombination in whole plants. *EMBO J* **13**: 484–489
- Vonarx EJ, Mitchell HL, Karthikeyan R, Chatterjee I, Kunz BA (1998) DNA repair in higher plants. *Mutat Res* **400**: 187–200
- Wolter R, Siede W, Brendel M (1996) Regulation of SNM1, an inducible *Saccharomyces cerevisiae* gene required for repair of DNA cross-links. *Mol Gen Genet* **250**: 162–168