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AtMMS21 regulates DNA damage response and homologous recombination repair in *Arabidopsis*

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ABSTRACT

DNA damage is a significant problem in living organisms and DNA repair pathways have been evolved in different species to maintain genomic stability. Here we demonstrated the molecular function of AtMMS21, a component of SMC5/6 complex, in plant DNA damage response. Compared with wild type, the *AtMMS21* mutant plants show hypersensitivity in the DNA damaging treatments by MMS, cisplatin and gamma radiation. However, *mms21-1* is not sensitive to replication blocking agents hydroxyurea and aphidicolin. The expression of a DNA damage response gene *PARP2* is upregulated in *mms21-1* under normal condition, suggesting that this signaling pathway is constitutively activated in the mutant. Depletion of *ATAXIA-TELANGIECTASIA MUTATED* (*ATM*) in *mms21-1* enhances its root growth defect phenotype, indicating that *ATM* and *AtMMS21* may play additive roles in DNA damage pathway. The analysis of homologous recombination frequency showed that the number of recombination events is reduced in *mms21-1* mutant. Conclusively, we provided evidence that AtMMS21 plays an important role in homologous recombination for DNA damage repair.

1. Introduction

DNA damage occurs in all organisms and it is induced by endogenous metabolism or exogenous environment. DNA damage has severe effects on genomic information maintenance and chromosome structure stability, possibly resulting in abnormal development, cancer and cell death. To reduce the impact from genotoxic stress, the living organisms have evolved effective pathways for DNA damage repair [1,2]. Double-strand break (DSB), a type of DNA damage, happens frequently during DNA replication and meiosis [3]. Cells always start a response to DSB by cell cycle control, DNA repair or programmed cell death [4]. In multicellular organisms, a DNA repair machine is evolved to clear the errors from DSBs. DSB is repaired by non-homologous end-joining (NHEJ) or homologous recombination (HR), dependent on the feature of DNA damage and cell cycle stage [5,6]. In plant meristems, the maintenance of genomic information is important for the sequential organogenesis, and HR is always used to repair the DSBs in mitotic cells [7,8].

Unlike NHEJ which simply ligates free ends of DSB, HR mediated repair does not introduce genomic mutations. During the HR process, several proteins are recruited onto DSBs. PARP1/2 plays as a sensor for DSBs and initiates the recruitment of MRN (MRE11–Rad50–NBS1) complex on DSBs [9,10]. A kinase ATM (ATAXIA-TELANGIECTASIA MUTATED), one of key signal transducers in the DNA damage response, is also recruited early onto DSBs and phosphorylates the histone variant H2AX and other signaling proteins to sequentially stimulate the expression of downstream genes [11]. After protein recruitment and DSB processed, RAD51 promotes strand invasion of the broken sister chromatid into the intact sister chromatid. When DNA synthesis completed, the joint DNA molecule is resolved to finish DSB repair [12]. Different from ATM, an ATM related kinase ATR (ATM/RAD3-RELATED) involved in DNA damage pathway is always activated by single-stranded DNA damage. Both ATM and ATR are conserved in plants and play distinct and additive roles in DNA damage response [13–15].

The structural maintenance of chromosomes (SMC) protein family is essential for chromosomal architecture and organization [16,17]. There are six eukaryotic SMC proteins, which heterodimerize with one another to form three types of complexes:

Abbreviations: DSB, double-strand break; NHEJ, non-homologous end-joining; HR, homologous recombination; ATM, ATAXIA-TELANGIECTASIA MUTATED; ATR, ATM/RAD3-RELATED; SMC, structural maintenance of chromosomes; DAG, days after germination; SDSA, synthesis-dependent strand annealing.

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cohesin (SMC1/3), condensin (SMC2/4), and SMC5/6. A number of experimental evidence indicated that SMC5/6 mainly plays a role in HR in DNA damage repair [18]. Mutation of the SMC5/6 complex component resulted in hypersensitivity to DNA damaging agents and decreased capacity to repair DSBs in different species [17,19]. The SMC5/6 complex has been shown to be recruited to DSBs on sister chromatids in yeast, supporting that SMC5/6 mediates DSB repair by HR mechanism [20]. MMS21, a SUMO E3 ligase, is a critical subunit in the SMC5/6 complex and sumoylates several other subunits in the complex to regulate DNA repair and recombination [21,22]. SUMO ligase activity of MMS21 is required for DNA repair and several critical proteins involved in DNA repair, such as the cohesin subunit SCC1 and the DNA repair protein TRAX [21,23] have been reported as substrates of MMS21. In previous studies, AtMMS21 has been identified as a SUMO E3 ligase which regulates the maintenance of root stem cell niche in *Arabidopsis* [24,25]. AtMMS21 is required for the expression of stem cell niche related transcription factors and the normal cellular organization of meristem by preventing cell death. There is evidence that AtMMS21 may also be involved in DNA damage responses in root development [26].

Here, we demonstrated the function of SUMO E3 ligase AtMMS21 in DNA damage response and recombination repair. Our data showed that mutation of *AtMMS21* increased sensitivity to DNA damaging agents and the constitutive activation of DNA damage response in *mms21-1* may be mediated by ATM. The result from DNA recombination frequency assay suggested that AtMMS21 may participate in DNA damage repair by HR pathway. This study would provide more information to understand the molecular mechanism of SUMOylation in DNA damage repair in plants.

2. Materials and methods

2.1. Plant materials and growth conditions

The *mms21-1* mutant and *35S:AtMMS21 Arabidopsis* (*Arabidopsis thaliana*; Columbia-0 ecotype) were isolated in our laboratory [24]. The following marker lines and mutants *atm-1* [27], *atr-2* [15]; *pPARP2:GUS* [28]; *pWEE1:GUS* [28]; *IU.GUS-8* [29]; *2 × 35S::I-SceI* [30] were obtained as described previously.

Seeds were surface sterilized for 2 min in 75% ethanol followed by 5 min in 1% NaClO solution, rinsed five times with sterile water, plated on Murashige and Skoog (MS) medium with 1% sucrose and 0.8% agar, and then stratified at 4 °C in the dark for 2 days. Plants were grown under long-day conditions (16 h of light/8 h of dark) at 22 °C.

2.2. DNA-damaging treatments

The DNA-damaging agents were purchased from Sigma and made as stock solutions following the product instructions. To detect the effects of DNA damaging agents on true leave growth, seedlings at 3 DAG were transferred onto MS agar plates containing different concentration of MMS (methyl methanesulfonate) or cisplatin. Photos were taken after 12 days and the true leaves of plants were counted after 12 days. The results presented are averages of 15–25 seedlings. The experiments were performed for three times with similar results. For the gamma irradiation assay, the seeds were irradiated at a dose of 50 or 200 Gy using a ^{60}Co source (Huada Radiation Company, Guangzhou, China). Seeds were imbibed in water at 4 °C for 3 days, irradiated and then immediately placed on MS plates for germination. For HU (hydroxyurea) and aphidicolin treatments, the seeds were germinated on MS plates containing 1 mM HU or 12 μg/mL aphidicolin, MS plates (for HU) and MS plates containing DMSO (for aphidicolin) were used in the control experiments. For IATM treatment, the seeds were germinated on MS plates containing 20 μM or 50 μM IATM (KU-55933, selleckchem) or MS plates containing same amount of DMSO as control. Photos were taken 5 days or 6 days after germination and the root lengths were analyzed by Digimizer image analysis software. The results were from 30 seedlings in replicated experiments with similar results. Data were expressed as means ± standard deviations. For comparisons between two groups, the Student's *t*-test (two-tailed) was used. A p value <0.05 was considered statistically significant.

2.3. Histological analysis

Histochemical analysis of GUS activity were performed in the seedlings including *PARP2:GUS* or *WEE1:GUS* in wild type or *mms21-1* mutant background. GUS staining solution (0.1 M sodium phosphate buffer (pH 7.0), 0.5 mM $K_3Fe(CN)_6$, 0.5 mM $K_4Fe(CN)_6$, 0.1% Triton X-100, 0.5 mg/mL X-Glucuronide) was made as described previously [28]. The seedlings were stained in GUS staining solution at 37 °C for 5–12 h, rinsed with water, and destained in 70% ethanol for microscopy.

2.4. Detection of homologous recombination frequency

To obtain the genotypes for the reporter assays, both *IU.GUS-8* and *2 × 35S::I-SceI* lines was crossed independently in the *mms21-1* mutant background. In the second generation after crossing, all homozygous plants required were identified by PCR based genotyping, then the reporter substrate and the I-SceI expressing construct were brought together by crossing the respective plants: either in the mutant or in the wild type background. The plants which are heterozygous for both the reporter and the I-SceI expressing construct but reside in a homozygous *mms21-1* mutant or the according wild type background were used in recombination assays. 2-Week-old seedlings were stained in GUS staining solution as described previously. The number of blue spots on the leaves was used to determine the recombination frequency.

3. Results

3.1. mms21-1 mutant shows increased sensitivity to MMS, cisplatin and gamma irradiation

In the previous study, it has been reported that the mutation of *AtMMS21* affects the amplification and differentiation of root stem cells [24,25] and the number of dead cells increased in the root of *AtMMS21* mutant [26]. To investigate whether AtMMS21 is involved in DNA repair, we detected the effect of DNA damaging agents MMS and cisplatin on the true leaf growth of *mms21-1* plants. Three days after germination, the seedlings were transferred onto the medium containing different concentration of MMS or cisplatin for 12 days. The result indicated that the true leaf production in *mms21-1* plants was inhibited by DNA damaging agents. When the plants were treated with 50 ppm MMS or 30 μm cisplatin, there was no true leaf in the *mms21-1* plants and the seedlings were dead after 12 days, however, the wild type plants could survive under this condition (Fig. 1A–C). At the same time, *AtMMS21* was overexpressed in *mms21-1* mutant to detect whether the sensitivity to DNA damage is really dependent on this gene. The treatment result showed that overexpression of *AtMMS21* was able to recover the *mms21-1* phenotype that the seedlings are supersensitive to DNA damaging agents MMS and cisplatin (Fig. 1D).

Because gamma irradiation also induces DSBs, the wild type and *mms21-1* seeds were treated by gamma radiation to determine whether AtMMS21 is also involved in this response. 5 days after irradiation, the root length was measured and the ratio between