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Lack of RNase H2 activity rescues HU-sensitivity of WEE1 deficient plants

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Because of their sessile lifestyle, plants have developed extensive mechanisms to safeguard their genetic information from one generation to the next. The WEE1 kinase is one of the guardians of genome integrity, being important during S-phase progression under replication stress. Knock-out plants for *WEE1* (*WEE1*^{KO}) show a hypersensitive response when grown on replication-inhibiting drugs. Recently, we reported the identification of a mutant in the *RNase H2A* gene that could partially complement this replication phenotype. Here, we present the identification of a second member of the RNase H2 complex, RNase H2B, being able to complement the root growth phenotype of *WEE1*^{KO} plants. We additionally show that deletion of a conserved domain in RNase H2B leads to loss of interaction with the RNase H2C subunit, likely explaining the loss of activity of the RNase H2 complex.

During their growth, plants are subjected to environmental and endogenous factors that can cause damage to their DNA, hereby compromising their genetic information. To prevent this, plants have developed checkpoints that arrest the cell cycle upon occurrence of DNA breaks or replication problems, granting the cell time to repair the damage. Ataxia Telangiectasia Mutated (ATM) and ATM- and RAD3-related (ATR) are 2 conserved protein kinases that act as sensors of different types of DNA damage and transduce DNA stress signals to downstream effectors. One of these downstream effectors is the WEE1 kinase, which has been shown to be active during the S-phase.[1] WEE1 is a tyrosine kinase that interferes with cell cycle progression, probably through inhibitory phosphorylation of cyclin-dependent kinases (CDKs). *WEE1*^{KO} plants are phenotypically normal when grown under normal conditions, but are hypersensitive to replication stress.[2] Replication stress can be triggered by adding hydroxyurea (HU) to the growth medium, a chemical inhibiting the ribonucleotide reductase enzyme, in this way limiting the pool of available deoxyribonucleotide triphosphates (dNTPs) necessary for efficient DNA replication by the DNA polymerases.

The substituent at the 2′ position of ribonucleotide triphosphates (rNTPs) is larger than that of dNTPs (OH compared to H), allowing DNA polymerases to distinguish dNTPs from rNTPs by sugar-type discrimination.[3] Nevertheless, due to much higher cellular rNTP levels, rNTPs are steadily incorporated into the genome during replication.[4,5] However, because of their reactive 2′OH group, incorporated ribonucleotide monophosphates (rNMPs) are more prone to strand cleavage, leading to elevated genome instability.[4] To counteract this problem, organisms have developed a ribonucleotide excision repair pathway, which is initiated by the ribonucleases H. These enzymes consist of 2 different types, the type I (RNase H1) that need at least 4 consecutive ribonucleotides for recognition, and the type II (RNase H2) that are able to cut even a single ribonucleotide in a DNA-RNA duplex.[6] Ribonuclease H2 consists of 3 subunits in most eukaryotic species: a catalytic subunit A, and the 2 regulatory subunits B and C.

In previous work, we used EMS mutagenesis and next-generation sequencing-based gene mapping to identify mutants that complement the *WEE1*^{KO} replication phenotype in the presence of HU.[7] This resulted in the identification of the *trd1-1* mutant, which holds a point mutation in the catalytic domain of the RNase H2 subunit A gene. The same mutagenesis approach resulted in additional complementing mutants, one named *trd2-1* (**Fig. 1A**). The causative mutation was mapped to the lower half of chromosome 4. Next-generation sequencing (NGS)-based gene mapping identified a single base-pair change in the *At4g20325* gene, resulting in a splice donor site mutation. The *At4g20325* gene is annotated as the B subunit of the RNase H2 protein complex and has already been partially characterized in our previous work.[7]

To analyze the nature of the mutation, RT-PCR was performed on root meristem cDNA. Two splice variants could be detected, of which one retained the intron following the splice

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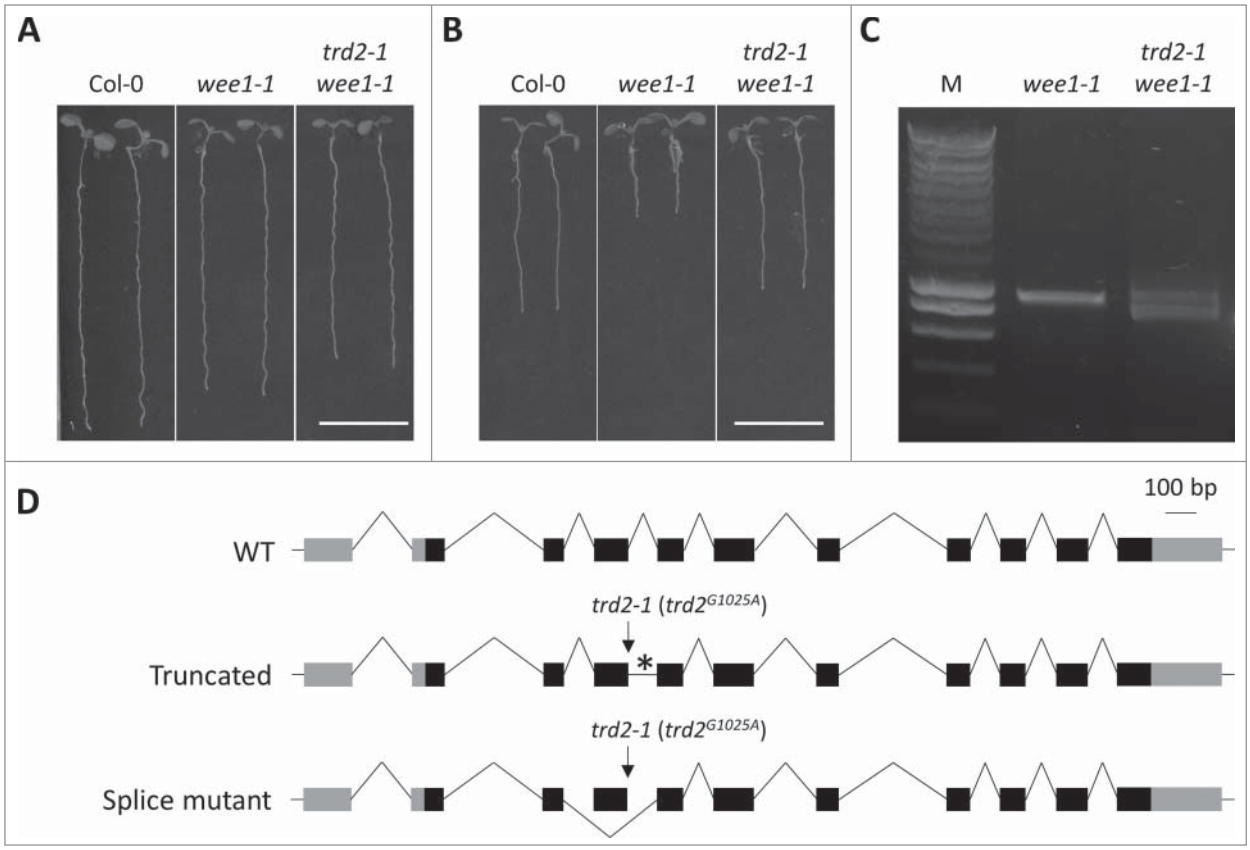

Figure 1. *trd2-1* partially rescues *WEE1*^{KO} hypersensitivity to HU. (**A**) and (**B**) Root growth of 7-day-old wild-type (WT, Col-0), *wee1-1*, and *trd2-1 wee1-1* mutant plants grown on control medium (A) or medium supplemented with 0.75 mM HU (B). Bar = 1 cm. (**C**) Splice variants of the *RNase H2B* transcript detected in *wee1-1* and *trd2-1 wee1-1* mutant plants by RT-PCR. M, SmartLadder 0.2–10 kb (Eurogentec). (**D**) Splice variants identified by sequencing. The position of the mutated base pair (*trd2-1*) is indicated with the arrow. The truncated form retains the fourth intron, causing a premature stop codon (indicated by *). The mutant form skips the third exon through alternative splicing.

donor site mutation, leading to a premature stop codon. The second variant showed a loss of the third exon because of alternative splicing (**Fig. 1B and C**). At the protein level, the loss of the third exon results in the deletion of 34 amino acids, which are conserved across RNase subunit B proteins of diverse species (**Fig. 2A**). The mammalian RNase H2 protein structure suggested that this part of the RNase H2B protein may be involved in the interaction with the C subunit. Therefore, a yeast 2-hybrid

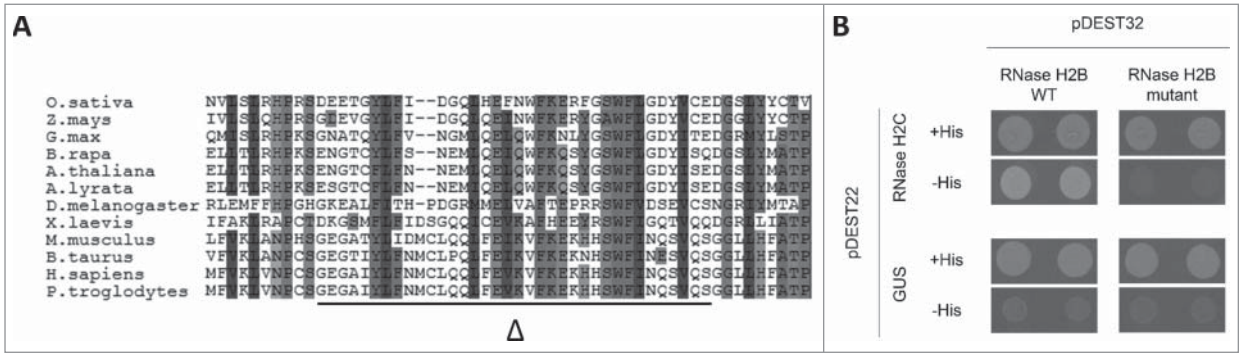

Figure 2. Deletion of a conserved region of the RNase H2B protein causes loss of interaction with the RNase H2C protein. (**A**) Sequence alignment of the RNase H2 subunit B from different species. The deleted region (Δ) is underlined. (**B**) Yeast 2-hybrid interactions between the C subunit and the wild-type or mutant B subunit of the RNase H2 complex. Interaction results in growth on medium -His. The GUS protein was used as negative control.

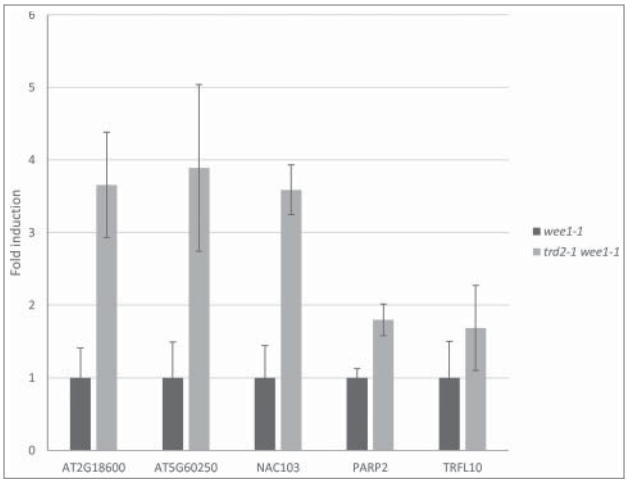

Figure 3. Loss of RNase H2 activity activates a DNA repair cluster. Relative expression levels of the indicated genes in 5-day-old *wee1-1* and *trd2-1 wee1-1* root tips. Expression levels in *wee1-1* were arbitrarily set to one. Data represent least square means ± SE (n = 2–3).

experiment was performed to analyze the interaction between the full-length and mutated RNase H2B protein with the C subunit. This screen confirmed the interaction of the wild-type B subunit with the C subunit,[7] whereas the mutated B subunit failed to bind to the C subunit (**Fig. 2B**).

In the *trd1-1* mutant, 7 genes were significantly upregulated based on an RNA-seq experiment, of which 5 appear in a coexpresssion cluster. To check whether these 5 genes were induced in the *trd2-1* mutant, qRT-PCR was performed on root tips of the *trd2-1* mutant grown under normal conditions. Except for the *TRFL10* gene, all genes were upregulated to a similar extent in the *trd2-1* mutant as in the *trd1-1* mutant (**Fig. 3**), confirming that the *trd2-1* mutation probably also results in a loss of function of the RNase H2 protein complex.

In conclusion, we identified a new RNase H2 mutant that rescues the hypersensitive phenotype of $WEE1^{KO}$ plants under replication stress. Loss of RNase H2 activity in *trd1-1 and trd2-1* tolerates incorporation of rNMPs into the genome.[7] We hypothesize that this incorporation of rNMPs, possibly through the retention of Okazaki fragments, partially restores the replication kinetics of $WEE1^{KO}$ plants by limiting the accumulation of single-stranded DNA that would otherwise be subjected to illegitimate recombination, leading to toxic structures.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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