Plant Signaling & Behavior

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/kpsb20

Lack of RNase H2 activity rescues HU-sensitivity of WEE1 deficient plants
Thomas Eekhoutab, Pooneh Kalhorzadehab & Lieven De Veylderab
a Department of Plant Systems Biology; Flanders Institute for Biotechnology (VIB); Ghent, Belgium
b Department of Plant Biotechnology and Bioinformatics; Ghent University; Ghent, Belgium
Accepted author version posted online: 15 Apr 2015.

To cite this article: Thomas Eekhout, Pooneh Kalhorzadeh & Lieven De Veylder (2015) Lack of RNase H2 activity rescues HU-sensitivity of WEE1 deficient plants, Plant Signaling & Behavior, 10:4, e1001226
To link to this article: http://dx.doi.org/10.1080/15592324.2014.1001226

PLEASE SCROLL DOWN FOR ARTICLE

Taylor & Francis makes every effort to ensure the accuracy of all the information (the “Content”) contained in the publications on our platform. However, Taylor & Francis, our agents, and our licensors make no representations or warranties whatsoever as to the accuracy, completeness, or suitability for any purpose of the Content. Any opinions and views expressed in this publication are the opinions and views of the authors, and are not the views of or endorsed by Taylor & Francis. The accuracy of the Content should not be relied upon and should be independently verified with primary sources of information. Taylor and Francis shall not be liable for any losses, actions, claims, proceedings, demands, costs, expenses, damages, and other liabilities whatsoever or howsoever caused arising directly or indirectly in connection with, in relation to or arising out of the use of the Content.

This article may be used for research, teaching, and private study purposes. Any substantial or systematic reproduction, redistribution, reselling, loan, sub-licensing, systematic supply, or distribution in any form to anyone is expressly forbidden. Terms & Conditions of access and use can be found at http://www.tandfonline.com/page/terms-and-conditions
Lack of RNase H2 activity rescues HU-sensitivity of WEE1 deficient plants

Thomas Eekhout1,2, Pooneh Kalhorzadeh1,2, and Lieven De Veylder1,2,*

1Department of Plant Systems Biology; Flanders Institute for Biotechnology (VIB); Ghent, Belgium; 2Department of Plant Biotechnology and Bioinformatics; Ghent University; Ghent, Belgium

Keywords: Arabidopsis, hydroxyurea, replication stress, RNase H2, WEE1

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 (WEE1KO) 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 WEE1KO 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. WEE1 is a tyrosine kinase that interferes with cell cycle progression, probably through inhibitory phosphorylation of cyclin-dependent kinases (CDKs). WEE1KO plants are phenotypically normal when grown under normal conditions, but are hypersensitive to replication stress. Replication stress can be triggered by adding hydroxyurea (HU) to the growth medium, a chemical inhibiting the ribonucleotide diphosphate 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. Nevertheless, due to much higher cellular NTP 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 WEE1KO 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
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 1](image1.png)

**Figure 1.** trd2-1 partially rescues WEE1KO 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.

![Figure 2](image2.png)

**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.
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, 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 coexpression 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 WEE1KO plants under replication stress. Loss of RNase H2 activity in trd1-1 and trd2-1 tolerates incorporation of rNMPs into the genome. We hypothesize that this incorporation of rNMPs, possibly through the retention of Okazaki fragments, partially restores the replication kinetics of WEE1KO 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.

Acknowledgments
We are grateful to Dr. Frantisek Baluska for inviting us to submit this communication. The authors thank Annick Bleys for help in preparing the manuscript.

Funding
This work was supported by grants of the Research Foundation Flanders (G.0C72.14N) and the Interuniversity Attraction Poles Program (IUAP P7/29 “MARS”), initiated by the Belgian Science Policy Office. T.E. is indebted to the Agency for Innovation by Science and Technology in Flanders for a predoctoral fellowship.

References
3. Joyce CM. Choosing the right sugar: how polymerases select a nucleotide substrate. Proc Natl Acad Sci U S A 1997; 94:1619-22; PMID:9050827; http://dx.doi.org/10.1073/pnas.94.5.1619

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).