

Buying time: FASCIATA1 deficiency rescues *wee1* plants from replication stress by delaying mitosis

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Cells have a remarkable ability to faithfully duplicate and segregate the genome, even when replication is challenged by various types of DNA damage. The fidelity of chromosome segregation relies on the DNA damage response (DDR), a checkpoint system that activates DNA repair genes and blocks cell cycle progression until the damage is repaired. The checkpoint kinase WEE1 has emerged as a core component in the plant DDR, mainly from studies in *Arabidopsis* (*Arabidopsis thaliana*). In this issue of *Plant Physiology*, Eekhout et al. investigate how both WEE1-dependent and -independent signaling pathways contribute to completion of DNA replication in the presence of stress.

In plants, as in other eukaryotes, two closely related sensor kinases, ATAXIA TELANGIECTASIA MUTATED (ATM) and ATM AND RAD3-RELATED (ATR), initiate DNA damage signaling (Figure 1). ATM is responsible for processing double-stranded breaks, a particularly dangerous type of DNA damage that must be repaired before the cell enters mitosis. ATR is more broadly required for sensing extended tracts of single-stranded DNA, a common consequence of replication stress during S phase. Downstream of ATM/ATR, the two pathways converge on the master regulator SUPPRESSOR OF GAMMA RESPONSE 1 (SOG1), which is a direct substrate of ATM and responsible for transcriptional activation of a broad range of DNA repair and cell cycle genes (Ogita et al., 2018). Inactivation of SOG1 generally blocks both ATM- and ATR-dependent signaling (Figure 1) with severe consequences for DNA damage tolerance and genome stability (Yoshiyama et al., 2009).

How does the DDR slow down cell cycle progression when it senses DNA damage? In animals, the connection between DNA damage sensing and the cell cycle relies heavily on ATM/ATR-mediated inactivation of the CELL DIVISION

CYCLE 25 (CDC25) family of phosphatases, which leads to inhibition of the main mitotic inducer CYCLIN-DEPENDENT KINASE 1 (CDK1). This, in turn, stabilizes replication forks that have encountered a block and prevents firing of new replication origins until the block has been lifted. CDC25 also plays a central role, together with the WEE1 kinase, for normal timing of cell cycle progression in the absence of stress.

Plants lack a CDC25 homolog, and the *Arabidopsis* homolog of WEE1 is dispensable for cell cycle progression in non-stressed conditions (De Schutter et al., 2007), highlighting the divergence between animal and plant cell cycle regulation. However, *Arabidopsis* WEE1 becomes critical during DNA replication stress, as shown in plants grown in the presence of the replication-inhibitory drug hydroxyurea (HU). The roots of HU-grown WEE1-deficient plants are extremely short, with a reduced meristem, extensive cell death, and advanced vascular differentiation (De Schutter et al., 2007; Cools et al., 2011; Kalhorzadeh et al., 2014). Recent studies have shown that, rather than directly inhibiting CDK activity by inhibitory phosphorylation, which is an important mechanism in animals, *Arabidopsis* WEE1 may indirectly control CDK activity through stabilization of CDK inhibitor proteins (Pan et al., 2021) and alternative splicing of cyclin transcripts (Wang et al., 2021).

Eekhout et al. investigated the relationship between WEE1 and ATM/ATR signaling during replication stress in *Arabidopsis*. They began by screening for suppressors of the *wee1-1* mutant with improved root growth in the presence of HU and found a loss-of-function mutation in the FASCIATA 1 (FAS1) gene. FAS1 is a conserved member of the protein complex that loads histones onto newly replicated chromosomes in S phase, and FAS1-deficiency causes

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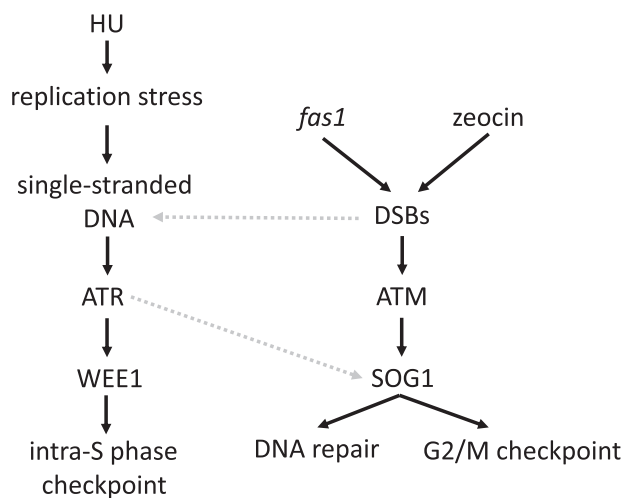


Figure 1 Model of DDR signaling in Arabidopsis. Replication stress, caused by HU, activates the ATR-WEE1-dependent intra-S phase checkpoint. Double-stranded breaks, caused pharmacologically by zeocin or by inactivation of the FAS1 gene, activate the ATM-dependent G2/M checkpoint. In the absence of WEE1, the intra-S phase checkpoint is silenced, causing hypersensitivity to the HU. This hypersensitivity is rescued by generation of DSBs, which causes a G2/M arrest that allows for completion of DNA replication before mitotic entry. Model adapted from Eekhout et al. (2021).

nucleosome assembly defects, resulting in increased formation of double-stranded breaks and activation of genes involved in DNA repair and recombination (Hisanaga et al., 2013). The duration of S phase was the same in wild-type and *fas1-8* plants, indicating the chromosomal abnormalities in the *fas1-8* mutant do not actually elicit a strong checkpoint response in S phase. Instead, the absence of FAS1 led to a much (~10 h) extended G2 phase, due to activation of the ATM-dependent G2/M checkpoint. The damage that leads to checkpoint activation in *fas1-8* may include double-stranded breaks in ribosomal DNA and telomere dysfunction.

The authors propose that this ATM-dependent checkpoint is in fact the reason for the rescue of *wee1-1* mutant plants grown on HU. According to their model, HU-grown WEE1-deficient roots suffer the catastrophic consequences of uncontrolled cell cycle progression with incompletely replicated chromosomes. Activation of the WEE1-independent G2/M checkpoint by deletion of FAS1 avoids these consequences by causing a premitotic delay long enough for the cell to complete DNA replication. The same effect was also obtained by treating the plants with the DNA-damaging drug zeocin, demonstrating double-stranded breaks are most likely responsible. The situation is analogous to the classical example of rescue of DNA damage checkpoint-deficient yeast cells by treatment with a microtubule poison that activates the spindle assembly checkpoint (Weinert and Hartwell, 1988).

To test their model, the authors deleted ATM in *fas1-8 wee1-1* plants and found this partially restored HU sensitivity, as expected if the ATM-dependent checkpoint is

responsible for processing the damage that occurs in the *fas1-8* mutant. However, the contribution of ATM was only partial, likely because ATR also plays a role, perhaps by sensing single-stranded DNA resulting from processing of double-stranded breaks. Accordingly, deletion of SOG1, which is known to integrate both ATM and ATR signaling, fully restored HU sensitivity to *fas1-8 wee1-1* plants.

In summary, this study highlights the role of WEE1 as a key intra-S-phase checkpoint regulator, required to delay S phase progression in the presence of replication stress. At the same time, Eekhout et al. show that, given enough time through activation of an alternative checkpoint, HU-treated *wee1-1* mutant plants can complete DNA replication before entering mitosis and thus maintain cell viability. This indicates WEE1 primarily functions as a link between DNA damage sensing and cell cycle regulation but is not required for completing DNA replication itself. Identifying the molecular steps leading to WEE1 activation and subsequent cell cycle delay is an important task for the understanding of how plants maintain genome integrity and DNA replication fidelity even under stressful conditions.

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