Mechanistic insights into DNA damage recognition and checkpoint control in plants

Josephine Herbst[1,2,3], Qian-Qian Li[1,2,3] and Lieven De Veylder[1,2]*

[1]Department of Plant Biotechnology and Bioinformatics, Ghent University, 9052 Gent, Belgium.

[2]Center for Plant Systems Biology, VIB, 9052 Gent, Belgium.

[3]These authors contributed equally: Josephine Herbst, Qian-Qian Li.

* e-mail: lieven.deveylder@psb.vib-ugent.be

The plant DNA damage response (DDR) pathway safeguards genomic integrity by rapid recognition and repair of DNA lesions that, if unrepaired, may cause genome instability. Most frequently, DNA repair goes hand in hand with a transient cell cycle arrest, which allows cells to repair the DNA lesions before engaging into a mitotic event, but consequently also affects plant growth and yield. Through the identification of DNA damage-response proteins and cell cycle regulators that react to DNA double-strand breaks or replication defects, it has become clear that these form highly interconnected networks. Those networks operate both at the transcriptional and post-transcriptional level and include liquid-liquid phase separation and epigenetic mechanisms. Strikingly, whereas the upstream DDR sensors and signaling components are well conserved across eukaryotes, some of the more downstream located effectors are diverged in plants, likely to suit unique lifestyle features. Additionally, DDR components display functional diversity across ancient plant species, dicots and monocots. Observed resistance of DDR mutants towards high salinity, aluminum toxicity, phosphate limitation and seed aging indicates that gaining knowledge about the plant DDR may offer solutions to combat climate change and the associated risk for food insecurity.

Introduction

As the world's population continues to grow and the effects of climate change become more apparent, maintaining food security is one of the greatest challenges of our time. Rising temperatures, prolonged droughts and floods have a dramatic impact on crop yield and agricultural productivity. Due to their immobility, one consequence of the exposure of plants to these emerging extreme and fluctuating environmental conditions are DNA lesions that in turn influence cell cycle progression and cell viability, affecting growth and yield. Indeed, a plentitude of exogenous and endogenous factors disrupts genomic integrity[1], leading to various DNA lesions like single- and double-strand breaks, (oxidative) base damages, crosslinks and pyrimidine dimers. Fortunately, DNA damage is rapidly counteracted by a highly complex signaling network, converged in the term DNA damage response (DDR). Three important steps are needed for a fully functional and efficient DDR (Fig. 1). The damaged DNA must be rapidly recognized, initiating a signaling cascade (step 1) that needs to be transmitted (step 2) towards effectors (step 3). Those effectors eventually activate DNA repair, pause the cell cycle or arrest proliferation of the affected cells by either killing them or pushing them into differentiation. Strikingly, although extensive studies across the tree of life revealed that many DDR factors are conserved over eukaryotes, especially the effectors and the final responsive components appear to have diverged between animals and plants.

In all eukaryotes, the onset of the DDR pathway depends on the type of DNA damage, positioned around either one of two phosphoinositide 3-kinase-related kinases, ATAXIA TELANGIECTASIA MUTATED (ATM) and ATAXIA TELANGIECTASIA AND RAD3-RELATED (ATR). ATM is primarily activated by DNA double-strand breaks (DSBs) that are recognized by a protein complex composed of MRE11-RAD50-NBS1 (MRN). Through dimerization, the MRN complex tethers the break ends together (Fig. 1) and resections the lose 5' ends at the DNA break side via its endo- and exonuclease activity, thus producing 3' overhangs needed for further repair[2]. The NBS1 subunit recruits inactive ATM dimers, which are subsequently released as activated ATM monomers[3].

ATR is predominantly activated by DNA single-strand breaks (SSBs) and stalled replication forks, also typified as replication stress. During replication stress, stretches of single-stranded DNA (ssDNA) emerge due to continued unwinding of the DNA helix in the absence of new strand biosynthesis. This emerging ssDNA is bound by replication protein A (RPA)[4-6] and RAD17. RPA subsequently recruits the ATR-interacting protein (ATRIP), which ultimately forms a stable complex with ATR[7-10]. Once ATR is activated, further association with the DNA topoisomerase II binding protein (TopBP1) is triggered, which stimulates the autoactivation of ATR, at least in yeast and human cells[11]. The RAD9-RAD1-HUS1 (9-1-1) complex can also activate ATR[7,12,13] (Fig. 1).

Once activated, mammalian ATM and ATR phosphorylate the tumor suppressor protein p53, a pivotal DDR master regulator that triggers the onset of DNA repair, cell cycle regulation and

apoptosis[14-16]. Whereas plants have homologs of the DNA damage-sensing components, including ATM and ATR, they lack p53, but they rely on SUPPRESSOR OF GAMMA RADIATION 1 (SOG1). Although this plant-specific transcription factor does not share any sequence similarity with p53[17,18], SOG1 acts as a functional homolog, being activated by ATM/ATR[19,20]. Following phosphorylation, SOG1 activates the expression of a wide variety of target genes, being predominantly involved in DNA repair and cell cycle checkpoint activation[21,22]. In addition to SOG1, E2F transcription factors and the WEE1 kinase are crucial regulators of the DDR[23,24], building complex regulatory networks operating at both the transcriptional and post-transcriptional level. Altogether, this network provides a quality control mechanism preventing cells with damaged DNA from entering mitosis.

To honor the 20th anniversary of the identification of SOG1[17], marking the starting point of plant-specific DDR research, we give an overview of the plant DDR, focusing on recent discoveries in DNA damage sensing, DNA repair mechanisms and cell cycle checkpoints activated in somatic cells in response to DNA single- and double-strand breaks. In addition, we highlight what is known about the DDR in crop plants and ancient species and summarize the importance of DDR during environmental stress responses.

A. DNA damage-sensing and repair mechanisms

I) One nucleofilament to rule them all: RAD51 recruitment and function

One of the earliest events after the occurrence of DSBs is the phosphorylation of the conserved H2A.X histone variant by ATM near the damaged site, giving rise to γ-H2A.X[25]. The accumulation of γ-H2A.X is the major signal for the DDR initiation. Recently, the first plant γ-H2A.X reader was identified, being the γ-H2A.X-INTERACTING PROTEIN (XIP)/BRCT-domain protein 3 (BCP3)[26,27] (Fig. 2a). XIP/BCP3 interacts specifically with γ-H2A.X via its dual BREAST CANCER SUSCEPTIBILITY GENE 1 (BRCA1) carboxyl-terminal (BRCT) domain, a known phospho-protein-binding platform[26,28]. Intriguingly, the XIP/BCP3-paralog BCP4 was found to interact with γ-H2A.X in a similar way, suggesting that BCP4 could be another γ-H2A.X reader[29]. Once XIP/BCP3 is bound to γ-H2A.X, it leads to the recruitment of the recombinase RAD51, which subsequently forms larger nucleoprotein filaments at the break site. These RAD51 filaments are required for DNA break repair through the process of error-free, template-based homologous recombination (HR), by aiding the identification of intact homologous sequences and the invasion of those double-stranded templates. In addition to XIP/BCP3, the cell cycle inhibitory RETINOBLASTOMA (Rb)-RELATED1 (RBR1) protein and the unknown DNA DAMAGE RESPONSE MUTANTS 2 (DDRM2) protein promote RAD51 recruitment to the damaged site[30,31]. Intriguingly, RAD51 recruitment depends also on its phosphorylation by complexes consisting of a plant-specific B1-type cyclin-dependent kinase (CDKB1) and a B1-type cyclin (CYCB1). As the activity of these CDKB1/CYCB1 complexes is the highest after completion of DNA replication, hence at the moment

when sister chromatids are available, this mechanism may has evolved as an elegant system to repair DNA preferentially by HR instead of by error-prone non-homologous end-joining, thus linking DNA repair with the cell cycle status[32].

Many recruiting and interacting factors of RAD51, including RBR1 and DDRM2, partially colocalize in foci[30,31,33]. Live imaging of interactions indicate that the formation of RAD51-containing foci is a highly dynamic and tightly spatiotemporally controlled process[30,31,33-35]. By tracking chromosome mobility, it was found that RAD51 foci movement is the highest during the earliest steps of HR and during the S/G2-phase transition, most likely facilitating the search for homologous sequences[35]. RAD51 mobility is impaired in the *sog1* mutant, indicating a tight correlation between SOG1 function and DNA repair foci formation[35]. This mechanism is likely facilitated by DDRM2, which is a direct target of SOG1[31]. A process that facilitates the spatial organization of repair foci is liquid-liquid phase separation (LLPS), in which membrane-free structures form condensates when their concentrations reach a critical threshold[36]. The nuclear lamin-like CROWDED NUCLEI (CRWN) 1 and 2 proteins enable factors with a low or no phase separation potential, like the RAD51 paralog RAD51D, to form condensates at damaged DNA[36]. The formation of those "DNA repair bodies" might be needed for an efficient repair of DNA lesions.

II) It´s getting complex: SMC5/6

The repair of damaged DNA requires large-scale rearrangements of the chromatin structure. Critical factors for such rearrangements are the evolutionarily conserved STRUCTURAL MAINTENANCE OF CHROMOSOME (SMC) proteins. While SMC1 and SMC3 are important components of the cohesin-complex (keeping replicated sister chromatids close until they are bipolarly attached to the spindle), SMC2 and SMC4 are required to form condensin (folding chromosomes into compact structures that can be more easily distributed during mitosis and meiosis). Differently, the SMC5/6 complex is a versatile component of the DDR network and is recruited to damaged DNA sites to promote DNA repair through HR[37]. Similar to other SMC-containing complexes, the SMC5/6 complex consists of several NON-SMC ELEMENTS (NSEs). The recruitment of SMC5/6 depends on DSB-induced RNAs (diRNAs) that arise from active transcription at both DSB fragment ends, especially in repeat-rich regions (Fig. 2b)[38]. The produced diRNAs are recognized and bound by the RNA-interference component ARGONAUTE 2 (AGO2) and the resulting diRNA-AGO2 complex specifically targets the DSB site and recruits the double-stranded RNA-binding protein INVOLVED IN DE NOVO2 (IDN2)[38,39]. IDN2 further recruits CELL DIVISION CYCLE 5 (CDC5), which attracts the transcriptional coactivator ALTERATION/DEFICIENCY IN ACTIVATION2B (ADA2b) and ultimately recruits the SMC5/6 complex at the DSB to facilitate DNA repair[39-41]. One function of the SMC5/6 complex is to recruit the POLYMERASE-ASSOCIATED FACTOR 1 (PAF1) complex (PAF1C). Once recruited, PAF1C attracts the ubiquitin ligases HISTONE MONO-

UBIQUITINATION 1 (HUB1) and HUB2, resulting in the mono-ubiquitination of H2B and ultimately the facilitation of DSB repair via HR[42].

The stepwise assembly of the SMC5/6-recruiting complex is further stabilized in a blue light-dependent manner by cryptochrome photoreceptors, enhancing the interaction between ADA2b and SMC5[43]. The resulting condensed complex might serve as a hub for DNA repair factors[39,43-45], which could also undergo LLPS, facilitated by the SMC5/6 subunit SUPPRESSOR OF NONEXPRESSER OF PATHOGENESIS-RELATED GENES1 INDUCIBLE 1 (SNI1) via its interaction with the nuclear lamin-like proteins CRWN1 and 2[36]. This LLPS could not only strengthen the distinct protein interactions but might also protect the loose DNA ends. Another SMC5/6 complex subunit, the E3-sumo ligase NSE2, triggers the proteasomal degradation and therefore the release of proteins trapped in DNA–protein crosslinks[46].

B. DNA damage checkpoint control mechanisms

To pause cell proliferation following DNA damage or replication defects, various DDR mechanisms interfere with the molecular cell cycle machinery. Although prominent regulators controlling cell proliferation in response to DNA damage have been identified[47], only recently it has become clear how these regulators are activated and how they affect different pathways to arrest cell cycle progression. The WEE1 kinase is a key component of the DNA damage checkpoint in response to replication defects, such as caused by application of hydroxyurea (HU) that blocks dNTP biosynthesis needed for new DNA synthesis. WEE1 operates downstream of ATR and both *wee1* and *atr* mutants are hypersensitive to HU[48,49]. Curiously, whereas mammalian and yeast WEE1 predominantly arrest the cell cycle by phosphorylating cyclin-dependent kinases (CDKs)[50,51], the Arabidopsis counterpart appears to be more promiscuous. Indeed, although the A-type CDKA;1 is a likely WEE1 target[48], the mutation of its potential WEE1 phospho-target sites does not affect the DDR, indicating that WEE1 might also phosphorylate other targets[52]. Through screening for *atr* suppressor mutants under replication stress conditions, several non-CDK targets of WEE1 were identified, including the ubiquitin E3 ligase F-BOX-LIKE17 (FBL17), being a key player of cell cycle progression mediating the ubiquitination and subsequent degradation of the CDK inhibitory class of KIP-RELATED PROTEINs (KRPs)[53-55]. WEE1-mediated phosphorylation of FBL17 triggers its destruction, and hence accumulation of the KRPs, contributing to a cell cycle arrest under replication stress conditions (Fig. 3). Interestingly, a phosphomimick FBL17 variant can still be degraded in a WEE1-dependent manner, indicating that WEE1 stimulates FBL17 degradation both through a direct and indirect mechanism. Accordingly, WEE1 phosphorylates the anaphase-promoting complex/cyclosome (APC/C) co-activator subunit APC10, which enhances the interaction between FBL17 and the APC/C E3 ubiquitin ligase, mediating the

degradation of FBL17 (Fig. 3)[56]. Notably, besides its function in cell cycle regulation, FBL17 co-localizes with γ-H2A.X at DSB sites in an RBR1-dependent manner. Thus, FBL17 is speculated to support the repair of DNA lesions through ubiquitination of yet to be identified DDR proteins[57].

Another identified direct target of WEE1 is PLEIOTROPIC REGULATORY LOCUS (PRL1), a core subunit of the MOS4-associated complex (MAC) involved in alternative splicing. WEE1-dependent phosphorylation of PRL1 promotes its ubiquitylation and subsequent degradation, leading to intron retention within mRNA transcripts of cell cycle genes, including the D-type cyclins *CYCD1;1* and *CYCD3;1*[45]. The translated aberrant CYCD proteins are likely unable to activate CDK activity, resulting in an arrest of cell cycle progression in response to replication stress (Fig. 3). PRL1 can also be a subunit of Cullin4-based E3 ubiquitin ligase $CRL4^{PRL1}$ to mediate the polyubiquitination and degradation of TSO2, a subunit of the ribonucleotide reductase that catalyzes the formation of dNTPs from ribonucleotides. Thus, WEE1-dependent PRL1 degradation can promote dNTP biosynthesis to resolve replication stress (Fig. 3)[58].

Screening for *wee1* suppressor mutants also helped in understanding the mechanisms that fail in the absence of a functional replication checkpoint. For example, *wee1* HU hypersensitivity can be rescued by ribonuclease H2 (RNAH2) mutations that trigger an increased incorporation of ribonucleotides (rNTPs) in the replicated DNA, likely substituting the insufficient level of dNTPs and illustrating that a shortage of the latter activates WEE1 activity. Strikingly, *wee1 rnah2* double mutants display small base-pair deletions, highlighting that rNTP incorporation in the genome must be avoided to maintain genome stability[59]. Therefore, the RNase H2 complex plays a key role in retaining genome stability under conditions that limit dNTP availability. Likewise, suppression of HR through knockout of *XRCC2* and *RAD51C* suppresses the HU hypersensitive phenotype of the *wee1* mutant[59], indicating that recombination under replication-deficient conditions might account for genome instability. Similarly, a mutation in *FASCIATA1* (*FAS1*), encoding a subunit of the chromatin assembly factor 1 complex, rescues *wee1* and *atr* HU hypersensitivity. The *fas1* mutation leads to telomere dysfunction and formation of DSBs, activating an ATM- and SOG1-dependent G2/M checkpoint, which may give cells of the *fas 1 wee1* double mutant the time to repair the replication stress-induced DNA damage, (possibly) explaining the rescue[60]. Similarly, a mutation in the catalytic subunit of DNA polymerase α can rescue the HU hypersensitivity of *wee1,* likely because of a slowed down replication rate that matches up with the HU-induced decrease in dNTP levels. Thus, genome stability and chromatin structure are important for DNA damage checkpoint activation, strongly suggesting that the inability to match DNA unwinding with replication speed accounts for the activation of a WEE1-dependent checkpoint[61].

Similar to *wee1*, *sog1* mutants are hypersensitive to HU. Importantly, *wee1 sog1* double mutants display an additive phenotype in the presence of HU, indicating that SOG1 and WEE1 independently control the replication stress checkpoint[62]. Not surprisingly, given its importance in DNA

damage control, SOG1 is controlled at multiple levels: not only is its activity controlled through phosphorylation by both ATM and CASEIN KINASE 2 (CK2)[20,63], but also by the Snf-related kinase SnRK1, a central integrator of energy signaling that responds to the available AMP/ATP ratio[64], providing a potential mechanism that links a low energy status with cell cycle progression (Fig. 3). SOG1 activity also partially depends on WEE1, as another target of WEE1, GENERAL CONTROL NONDEREPRESSIBLE 20 (GCN20), inhibits the translation of *SOG1* transcripts. Upon replication stress, WEE1-dependent phosphorylation of GCN20 results in its ubiquitination and degradation, thus relieving the translational inhibition of SOG1 (Fig. 3)[65]. Conversely, *WEE1* is a SOG1 target gene[21], implying a WEE1/SOG1 positive feedback loop as a reason for cell cycle arrest under replication stress[65]. Additionally, SOG1 protein stability is positively controlled by DNA DAMAGE RESPONSE MUTANT 1/SUMO-TARGETED UBIQUITIN E3 LIGASE 2 (DDRM1/STUBL2) that ubiquitinates SOG1 at multiple sites (Fig. 3)[66].

Whereas WEE1 controls an intra-S-phase cell cycle checkpoint[67], SOG1 possesses the ability to impose a G2/M arrest in response to DSBs through the transcriptional activation of the SIAMESE-RELATED (SMR) CDK inhibitor genes *SMR5* and *SMR7*[68], as well as two SOG1-related transcription factor genes, *ANAC044* and *ANAC085*[69] (Fig. 4). Both act on the repressive MYB (Rep-MYB) transcription factors MYB3R3/5 that suppress the expression of G2/M-specific genes, such as B-type cyclins. On the one hand, SMR5 and SMR7 inhibit CDK activity needed for proteolytic turnover of the Rep-MYBs[70], whereas ANAC044 and ANAC085 stabilize the same repressors in a yet to be determined post-transcriptional manner. Combined, both pathways result in the accumulation of Rep-MYBs in response to DSBs, resulting in a cell cycle arrest (Fig. 4).

Next to WEE1 and SOG1, E2F transcription factors are expected to play an important role in the checkpoint control, given their role as transcriptional activators of DNA replication and repair genes[23]. Within Arabidopsis, canonical E2FA and E2FB are considered as transcriptional activators, whereas E2FC is generally considered as a repressor[71]. Through copurification, RBR1, E2FB, E2FC and ANAC044 were found to be part of the DREAM (Dimerization partner, RB-like, E2F and Multivulval class B) complex, being a multi-subunit transcriptional complex that controls the expression of cell cycle-related genes[72,73] (Fig. 4). Although this explains a transcriptional repressive role for ANAC044, the relationship between E2F transcription factors and the DDR appears to be intricate. Whereas E2FB is needed to halt the cell cycle in response to DNA crosslinks, correlated with its potential role in a repressive DREAM complex[72], its activity is also required to allow progression through the G2/M transition under replication stress conditions[24]. Additionally, although SOG1 is not part of the DREAM complex, it shares many transcriptional targets with the E2Fs. Accordingly, E2Fs operate antagonistically or synergistically with SOG1 to control cell cycle progression and DDR gene expression during replication stress[24]. Among the antagonistically regulated genes, *ANAC085* and *ANAC044* can be found, suggesting that E2FB could dampen a SOG1-dependent cell cycle arrest, whereas those

genes under synergistic control may allow fine-tuning of gene expression levels according to the replication stress intensity (Fig. 4). In such model, E2F activity might account for basal induction levels of DNA repair and checkpoint genes during the S-phase, when cells are expected to be the most sensitive to replication inhibitory stresses, whereas SOG1 might account for further activation in response to fork stalling.

Also Tesmin/TSO1-like CXC domain-containing proteins (TCX5/6) were identified as components of the DREAM complex[72]. The TCX5/6-containing DREAM complex precludes DNA hypermethylation and prevents excessive cell proliferation[74], suggesting that DREAM complexes regulate the epigenetic landscape in plants. Consistently, BTE1 (BARRIER OF TRANSCRIPTION ELONGATION 1) was identified as another DREAM complex subunit. BTE1 interacts with WDR5A, the component of the Complex Proteins Associated with Set1 (COMPASS)-like complex that represses the transcription of target genes via inhibiting H3K4me3 deposition and RNA polymerase II elongation[75]. Interestingly, more than a thousand genes that are occupied by BTE1 are also bound by E2FA, indicating that E2FA could work cooperatively with components of the DREAM complex to regulate gene expression (Fig. 4).

Interestingly, E2F transcription factor activity can be repressed by the above-mentioned SMC5/6 complex subunit SNI1, resulting in the transcriptional repression of E2F target genes through the recruitment of histone deacetylases[76]. The resulting elimination of E2F activity not only restores root growth, probably by allowing cell cycle progression in the presence of DNA damage, but also suppresses the observed cell death of stem cells. This ATM-, ATR- and SOG1-mediated cell death of predominantly vascular stem cells is observed in many DDR mutants and can be induced by radiomimetic drugs (such as bleomycin and zeocin). It has been put forward as an efficient process to safeguard genome integrity by preventing the distribution of DNA-damaged cells across the organism[77], although the exact executers of this cell death program are still unknown.

Other potential regulators of the DDR are long non-coding RNAs (lncRNAs). In humans, DDR-related lncRNAs gained attention due to their oncogenic character[78]. In Arabidopsis, over 90% of the X-ray-induced lncRNAs depend on ATM activity[79]. Meta- and comparative genome sequencing approaches underline the potential of plant lncRNAs to be involved in the DDR[80,81]. The lack of certain lncRNAs reduces the repair capacity for DSBs[80]. Moreover, the lncRNA *LINDA* is not only important for a proper transcriptional response to DNA damage, but is also necessary for the recovery of root meristems following loss of stem cells by the above-mentioned cell death program[81]. Because lncRNAs are not well-conserved, lncRNAs are excellent candidates for species-specific fine-tuning of the DDR.

Another emerging mechanism controlling the plant DDR is autophagy, in mammals best known for its role in eliminating dysfunctional protein complexes and organelles[82]. Autophagy was initially

described as a rather non-selective destruction process, but there is increasing evidence that selective autophagy represents an important mechanism in many cellular responses, including the DDR. Key mammalian DDR components, including ATM and p53, have been identified as regulators of autophagy (reviewed in[83,84]). Interestingly, several plant autophagy-related (ATG) genes are critical for the response to DNA damage, as their corresponding mutants are hypersensitive to replication stress, DSBs and DNA crosslinks[85]. KNOTEN1 (KNO1) was identified as a potentially important factor mediating autophagy in response to DNA damage, promoting HR through targeting the RECQ MEDIATED INSTABILITY 1 (RMI1) protein for degradation via autophagy[85].

C. DDR in crops and ancient species

Despite the conserved nature of key DDR regulators, species-specific adaptations can be found. The moss *Physcomitrium patens* is an ancient land plant diverged from green algae. Recent studies identified its key DDR components, including one functional ATM (PpATM) and ATR (PpATR) kinase. A knockout in either results in a growth phenotype, with *Ppatm* mutants progressing faster through developmental transitions, whereas *Ppatr* mutants display an inhibition in these transitions, resulting in smaller gametophores, as well as sporadic cell death[86,87]. These data illustrate a role for both kinases during vegetative development, which is contrasting to their Arabidopsis counterparts. Under genotoxic conditions, the PpATR kinase appears to be the dominant checkpoint regulator[86]. Again, this strongly deviates from Arabidopsis, in which ATM is the main factor controlling the response to DSBs[88]. The reason for this differential use in checkpoint regulators between species is currently unknown. Strikingly, within *P. patents*, DSBs trigger the reprogramming of differentiated leaf cells into stem cells, a process that depends on PpATR-dependent transcriptional activation of *STEMIN* transcription factor genes[87].

As outlined above, SOG1 plays a key role in the transcriptional activation of the DDR, with homologs being identified in eudicots, monocots and gymnosperms, as well as ancient flowering plants. No *SOG1*-like genes are found in unicellular and colonial algae[89,90], placing its origin in nonvascular plants. This includes *P. patents*, in which two *SOG1* genes, *PpSOG1a* and *PpSOG1b,* were identified to redundantly act in the DDR[91,92]. Similar to Arabidopsis, the *Physcomitrium sog1a sog1b* double mutant shows increased resistance to genotoxic treatments[92]. Additionally, PpSOG1-dependent genes are enriched for similar Gene Ontology terms as observed for Arabidopsis, including DNA repair and cell cycle control. Moreover, both SOG1 protein sequences hold potential ATM/ATR phosphorylation sites, a feature shared with their Arabidopsis counterpart[92]. As major difference between both species, *PpATR* is found among the target genes of PpSOG1. Accordingly, *PpATR* transcript levels strongly increase by gamma irradiation[92], whereas Arabidopsis *ATR* expression is not

affected by genotoxic agents. This suggests the existence of a positive feedback loop that has been lost in Arabidopsis and of which the importance in the moss is currently unknown.

The DDR network also shows divergence in monocot versus eudicot species, which may relate to differences in genome size and chromatin organization. These differences may influence the accessibility of DNA repair proteins to damaged sites, potentially affecting the dynamics of DNA damage-sensing and -repair processes. Contrary to Arabidopsis, maize *atr* but not *atm* mutants show fertility defects that might be due to severe replication stress during the early stages of embryo and endosperm development[93]. Conversely, ATR deficiency does not affect the fertility in barley (*Hordeum vulgare*), but *Hvatr* mutants show a shorter root system than the wild type under normal conditions, which might be related to the appearance of DNA nicks and breaks in 60% of root meristem nuclei[94]. Given that both barley and maize have large genome sizes and contain complex repetitive regions[95,96], these characteristics may increase the susceptibility of their genomes to replication stress and DNA breaks, implying that the DDR pathway plays a more essential role in crops with large genomes than those with small genomes.

Similar to *P. patens*, rice holds two putative SOG1 orthologs, OsSOG1 and SOG1-like (OsSGL), both holding multiple potential ATM/ATR phosphorylation sites. However, whereas in *P. patens* both genes appear to operate redundantly, in rice only the *Ossog1* mutant displays a DNA damage-sensitive phenotype and inhibition of DNA repair genes following DNA damage[97]. Accordingly, *OsSGL* appears to be an OsSOG1 target gene, with its contribution to the DDR still to be determined. Together, these studies reveal some conservation and divergence of SOG1 function in different plant species. However, DDR components in crops and ancient plants need further investigation in the future.

D. Importance of the DDR in response to environmental stresses

Environmental extremes, including high temperatures, industrial soil pollution and the wash out of nutrients by erosion, are challenging for sessile organisms. Especially in the light of climate change, it is important to understand the impact of those extremes on plant survival and genome integrity in order to maintain plant growth and seed yield. Important sensors and signal integrators for environmental changes are the plant organelles, especially the chloroplasts. Like nuclear DNA, chloroplast DNA is susceptible to reactive oxygen species (ROS)-induced DNA damage that can lead to chloroplast dysfunction, which in turn can trigger an ROS burst[98-100]. Important guardians of the chloroplast genome are the cooperatively working ssDNA-binding proteins WHIRLY1 (WHY1), WHY3 and RecA1, a prokaryotic homolog of RAD51[101-103] (Fig. 5). Although chloroplast dysfunction does not directly cause nuclear DNA damage, it affects the status of nuclear gene expression through retrograde signaling, in which a plastid-derived signal controls the expression of nuclear-encoded genes.

Accordingly, SOG1-dependent activation of *SMR5* and *SMR7* can be observed in response to impaired chloroplast functioning or high-light intensities, impinging on cell cycle checkpoints[68,104,105]. An essential retrograde signaling element was identified in the CHLOROPLAST AND NUCLEUS DUAL-LOCALIZED PROTEIN 1 (CND1) protein. CND1 promotes the initiation of nuclear DNA replication through its association with prereplication complexes at the replication origins and facilitates chloroplast genome stability by interaction with WHY1[106].

Chloroplasts are also important sensors for heat: WHY1 and WHY2 are induced by heat[107,108], and WHY1 is a confirmed factor for thermotolerance in tomato[108]. Heat causes the accumulation of ROS in many cellular compartments, including the nucleus[109], where it causes DNA damage[110]. Moreover, if the environmental temperature exceeds a certain threshold, plant fertility decreases[111,112], while telomere truncation and mutation rates increase[113,114]. With a higher frequency of local heatwaves that tend to last longer[115], this is an alarming observation that could decrease seed production. Thermotolerance can be acquired through the E3 ubiquitin ligase HIGH EXPRESSION OF OSMOTICALLY RESPONSIVE GENES 1 (HOS1). Upon heat exposure, HOS1 accumulates, leading to the transcriptional activation of the DNA helicase RECQ2 [107], which in turn is involved in overcoming stalled replication forks[116]. Additionally, the accumulation of HOS1 causes the induction of other DDR genes, including *ATM* and *WHY2* (Fig. 5). ATM ensures chromosome stability by efficient repair of DSBs under heat stress, as *atm* mutants exhibit increased chromosome fragmentation[117]. Besides the effect on ATM, heat induces the expression of *ANAC044* and *ANAC085*, causing the accumulation of Rep-MYBs and ultimately a G2/M cell cycle arrest[69,118], potentially supported by the heat-induced expression of the cell cycle inhibitory genes *SMR3* and *SMR5*[68].

Heat-triggered accumulation of ROS and DNA damage accelerates seed aging, resulting in decreased seed vigor and seedling viability[119,120]. Aging in general is driven by the accumulation of DNA damage, which is associated with increased programmed cell death and telomere shortening[121,122]. During seed dormancy, the activity of the DDR machinery minimizes the consequences of accumulating DNA damage over time[121,123]. Moreover, the age-associated accumulation of DNA damage is counteracted by a lag-phase during seed germination, during which the DDR machinery ensures repair of occurred damage and consequently, the survival of the emerging seedling[121,123]. Later in a plant's life, during senescence, ATM suppresses plant aging by activating DNA repair and simultaneously suppressing age-associated transcription factors[122], underlining the importance of an active DDR during the whole life cycle.

Seedling survival and plant growth are affected not only by seed age but also by the environment, especially by a balanced availability of nutrients. E.g., the lack of nutrients, such as phosphate (Pi), inhibits root growth due to impaired cell elongation, accompanied by a cell cycle arrest and loss of stem cell identity, a phenomenon also described as stem cell exhaustion[19,63,125]. To

counteract low Pi availability, plants secrete organic acids into the rhizosphere to dissolve metal-bound Pi, leading to the release of Pi and iron (Fe^{3+}). Fe^{3+} is converted into Fe^{2+} that subsequently reacts with hydrogen peroxide, leading to the generation of hydroxyl radicals[126]. The accumulation of hydroxyl radicals initiates the accumulation of callose at plasmodesmata, and consequently the disruption of cell-to-cell communication[125]. Additionally, ROS induce *SMR5* and *SMR7* expression, thus evoking cell cycle checkpoints[127]. Accordingly, stem cell exhaustion during phosphate starvation can be rescued by knockout of their upstream regulators ATM or SOG1[63].

Another Pi-chelating metal is aluminum (Al^{3+}), exemplified by the usage of high Pi fertilizers to cope with aluminum toxicity. Accordingly, symptoms of Al^{3+} toxicity and Pi deficiency are comparable, and both stresses share common response pathways[19]. However, the stem cell exhaustion phenotype caused by Al^{3+} is mainly due to the intrinsic genotoxicity of Al^{3+} that non-covalently binds DNA, affecting DNA unwinding[128] and consequently triggering replication stress. The replication stress activates ATR and SOG1, leading to cell cycle arrest, which in turn prevents potentially impaired DNA to be passed on to daughter cells[19,63,94,129,130].

Two other metals affecting DNA integrity are boron ($B(OH)_3$) and cadmium. An excess of $B(OH)_3$ inhibits root growth and cell division[131,132] and generates ROS-mediated oxidative damage[133]. Moreover, $B(OH)_3$ increases the accessibility of chromatin, making it more susceptible to DNA damage caused by ROS[134,135]. The accumulation of ROS in response to $B(OH)_3$ is mediated by the NAC-type transcription factor NAC103, which is degraded by the 26S proteasome to avoid the accumulation of DSBs[136]. Cadmium on the other hand inhibits plant growth by interfering with cell division via the SOG1-mediated expression of *SMR4, SMR5* and *SMR7*[137,138]. SOG1 does furthermore interfere with the redox-potential of the cells by inducing the expression of important components of the oxidative signaling and ROS scavenging pathways[137].

Outlook

The world record of global temperature in 2023 once again showed us the alarming reality of climate change, triggering weather extremes that endanger food security. It is pivotal to understand the consequences of these environmental threats on the plant's genomic stability, as well as how this instability affects growth and yield. Clearly, impairing cell cycle checkpoints allows to cope with high salinity, Al^{3+} toxicity, Pi limitations and seed ageing, but currently the long-term consequences of bypassing the DNA surveillance mechanisms on later developmental stages or offspring plants are unclear. Moreover, cross-species comparisons indicate that finding an overall solution to deal with environmental-induced loss of genome integrity may be challenging, given the reported differences observed between ancient species, Arabidopsis and crops. Clearly, the genotoxic stress tolerance phenotypes observed for Arabidopsis DDR mutants need to be tested within agricultural species.

Thanks to the revolutionary advances in genome editing techniques, the generation of corresponding DDR mutants in diverse species is only limited by their genome-editing and propagation efficiency. Interestingly, CRISPR/Cas9-based genome editing in immortalized human retinal pigment epithelial cells was found to trigger a cell cycle arrest that could be overcome through p53 inhibition[139]. Hence, it is possible that DDR mutants may not only offer a solution to combat climate instability by directly mitigating a DNA damage- or replication stress-activated cell cycle arrest, but also indirectly contribute to increasing genome-editing frequencies or the development of novel genome-editing applications.

Acknowledgments

The authors thank Annick Bleys, Marieke Dubois and Jefri Heyman for critical reading and editing of the manuscript. This work was supported by a grants of the Research Foundation Flanders (projects G011420N and G0A6J24N).

Author Contributions

L.D.V led the project. J.H, Q.-Q.L. and L.D.V. wrote the manuscript. J.H and Q.-Q.L. prepared the figures. All of the authors read and approved the final manuscript.

Ethics declaration

The authors declare no competing interests.

References

1 Pedroza-Garcia, J. A., Xiang, Y. & De Veylder, L. Cell cycle checkpoint control in response to DNA damage by environmental stresses. *Plant Journal* **109**, 490-507 (2022). https://doi.org:10.1111/tpj.15567

2 Langerak, P., Mejia-Ramirez, E., Limbo, O. & Russell, P. Release of Ku and MRN from DNA ends by Mre11 nuclease activity and Ctp1 is required for homologous recombination repair of double-strand breaks. *PLoS Genet* **7**, e1002271 (2011). https://doi.org:10.1371/journal.pgen.1002271

3 Lee, J. H. & Paull, T. T. ATM activation by DNA double-strand breaks through the Mre11-Rad50-Nbs1 complex. *Science* **308**, 551-554 (2005). https://doi.org:10.1126/science.1108297

4 Aklilu, B. B., Soderquist, R. S. & Culligan, K. M. Genetic analysis of the Replication Protein A large subunit family in Arabidopsis reveals unique and overlapping roles in DNA repair,

meiosis and DNA replication. *Nucleic Acids Research* **42**, 3104-3118 (2014). https://doi.org:10.1093/nar/gkt1292

5 Yates, L. A. *et al.* A structural and dynamic model for the assembly of Replication Protein A on single-stranded DNA. *Nature Communications* **9**, 5447 (2018). https://doi.org:10.1038/s41467-018-07883-7

6 Eschbach, V. & Kobbe, D. Different replication protein A complexes of *Arabidopsis thaliana* have different DNA-binding properties as a function of heterotrimer composition. *Plant & Cell Physiology* **55**, 1460-1472 (2014). https://doi.org:10.1093/pcp/pcu076

7 Zou, L. & Elledge, S. J. Sensing DNA damage through ATRIP recognition of RPA-ssDNA complexes. *Science* **300**, 1542-1548 (2003). https://doi.org:10.1126/science.1083430

8 Sweeney, P. R., Britt, A. B. & Culligan, K. M. The Arabidopsis ATRIP ortholog is required for a programmed response to replication inhibitors. *Plant Journal* **60**, 518-526 (2009). https://doi.org:10.1111/j.1365-313X.2009.03975.x

9 Cortez, D., Guntuku, S., Qin, J. & Elledge, S. J. ATR and ATRIP: partners in checkpoint signaling. *Science* **294**, 1713-1716 (2001). https://doi.org:10.1126/science.1065521

10 Namiki, Y. & Zou, L. ATRIP associates with replication protein A-coated ssDNA through multiple interactions. *Proceedings of the National Academy of Sciences of the United States of America* **103**, 580-555 (2006). https://doi.org:10.1073/pnas.0510223103

11 Kumagai, A., Lee, J., Yoo, H. Y. & Dunphy, W. G. TopBP1 activates the ATR-ATRIP complex. *Cell* **124**, 943-955 (2006). https://doi.org:10.1016/j.cell.2005.12.041

12 Heitzeberg, F. *et al.* The Rad17 homologue of *Arabidopsis* is involved in the regulation of DNA damage repair and homologous recombination. *Plant Journal* **38**, 954-968 (2004). https://doi.org:10.1111/j.1365-313X.2004.02097.x

13 Zou, L., Liu, D. & Elledge, S. J. Replication protein A-mediated recruitment and activation of Rad17 complexes. *Proceedings of the National Academy of Sciences of the United States of America* **100**, 13827-13832 (2003). https://doi.org:10.1073/pnas.2336100100

14 Banin, S. *et al.* Enhanced phosphorylation of p53 by ATM in response to DNA damage. *Science* **281**, 1674-1677 (1998). https://doi.org:10.1126/science.281.5383.1674

15 Canman, C. E. *et al.* Activation of the ATM kinase by ionizing radiation and phosphorylation of p53. *Science* **281**, 1677-1679 (1998). https://doi.org:10.1126/science.281.5383.1677

16 Tibbetts, R. S. *et al.* A role for ATR in the DNA damage-induced phosphorylation of p53. *Genes & Development* **13**, 152-157 (1999). https://doi.org:10.1101/gad.13.2.152

17 Preuss, S. B. & Britt, A. B. A DNA-Damage-Induced Cell Cycle Checkpoint in Arabidopsis. *Genetics* **164**, 323-334 (2003). https://doi.org:10.1093/genetics/164.1.323

18 Yoshiyama, K. O., Conklin, P. A., Huefner, N. D. & Britt, A. B. Suppressor of gamma response 1 (*SOG1*) encodes a putative transcription factor governing multiple responses to DNA damage. *Proceedings of the National Academy of Sciences of the United States of America* **106**, 12843-12848 (2009).

19 Sjogren, C. A., Bolaris, S. C. & Larsen, P. B. Aluminum-dependent terminal differentiation of the Arabidopsis root tip is mediated through an ATR-, ALT2-, and SOG1-regulated transcriptional response. *Plant Cell* **27**, 2501-2515 (2015). https://doi.org:10.1105/tpc.15.00172

20 Yoshiyama, K. O. *et al.* ATM-mediated phosphorylation of SOG1 is essential for the DNA damage response in *Arabidopsis*. *EMBO Reports* **14**, 817-822 (2013). https://doi.org:10.1038/embor.2013.112

21 Bourbousse, C., Vegesna, N. & Law, J. A. SOG1 activator and MYB3R repressors regulate a complex DNA damage network in *Arabidopsis*. *Proceedings of the National Academy of Sciences of the United States of America* **115**, E12453-E12462 (2018). https://doi.org:10.1073/pnas.1810582115

22 Ogita, N. *et al.* Identifying the target genes of SUPPRESSOR OF GAMMA RESPONSE 1, a master transcription factor controlling DNA damage response in *Arabidopsis*. *Plant Journal* **94**, 439-453 (2018). https://doi.org:10.1111/tpj.13866

23 Vandepoele, K. *et al.* Genome-wide identification of potential plant E2F target genes. *Plant Physiology* **139**, 316-328 (2005). https://doi.org:10.1104/pp.105.066290
24 Nisa, M. *et al.* Distinctive and complementary roles of E2F transcription factors during plant replication stress responses. *Molecular Plant* **16**, 1269-1282 (2023). https://doi.org:10.1016/j.molp.2023.07.002
25 Waterworth, W. M. *et al.* Phosphoproteomic analysis reveals plant DNA damage signalling pathways with a functional role for histone H2AX phosphorylation in plant growth under genotoxic stress. *Plant Journal* **100**, 1007-1021 (2019). https://doi.org:10.1111/tpj.14495
26 Fan, T. *et al.* Arabidopsis γ-H2A.X-INTERACTING PROTEIN participates in DNA damage response and safeguards chromatin stability. *Nature Communications* **13**, 7942 (2022). https://doi.org:10.1038/s41467-022-35715-2
27 Vladejic, J. *et al.* Analysis of BRCT5 domain-containing proteins reveals a new component of DNA damage repair in Arabidopsis. *Front Plant Sci* **13**, 1023358 (2022). https://doi.org:10.3389/fpls.2022.1023358
28 Yu, X., Chini, C. C. S., He, M., Mer, G. & Chen, J. The BRCT Domain Is a Phospho-Protein Binding Domain. *Science* **302**, 639-642 (2003). https://doi.org:10.1126/science.1088753
29 Lorković, Z. J., Klingenbrunner, M., Cho, C. H. & Berger, F. Identification of plants functional counterparts of the metazoan Mediator of DNA Damage Checkpoint 1. Preprint at *bioRxiv* https://doi.org:10.1101/2023.05.19.541430 (2023)
30 Biedermann, S. *et al.* The retinoblastoma homolog RBR1 mediates localization of the repair protein RAD51 to DNA lesions in *Arabidopsis*. *EMBO Journal* **36**, 1279-1297 (2017). https://doi.org:10.15252/embj.201694571
31 Yu, C. *et al.* The multi-BRCT domain protein DDRM2 promotes the recruitment of RAD51 to DNA damage sites to facilitate homologous recombination. *New Phytologist* **238**, 1073-1084 (2023). https://doi.org:10.1111/nph.18787
32 Weimer, A. K. *et al.* The plant-specific CDKB1-CYCB1 complex mediates homologous recombination repair in Arabidopsis. *EMBO J* **35**, 2068-2086 (2016). https://doi.org:10.15252/embj.201593083
33 Horvath, B. M. *et al. Arabidopsis* RETINOBLASTOMA RELATED directly regulates DNA damage responses through functions beyond cell cycle control. *EMBO Journal* **36**, 1261-1278 (2017). https://doi.org:10.15252/embj.201694561
34 Kurzbauer, M.-T. *et al.* ATM controls meiotic DNA double-strand break formation and recombination and affects synaptonemal complex organization in plants. *Plant Cell* **33**, 1633-1656 (2021). https://doi.org:10.1093/plcell/koab045
35 Meschichi, A. *et al.* The plant-specific DDR factor SOG1 increases chromatin mobility in response to DNA damage. *EMBO Reports* **23**, e54736 (2022). https://doi.org:10.15252/embr.202254736
36 Yin, C., Sun, A., Guo, T., Mao, X. & Fang, Y. Arabidopsis lamin-like proteins CRWN1 and CRWN2 interact with SUPPRESSOR OF NPR1-1 INDUCIBLE 1 and RAD51D to prevent DNA damage. *Plant Cell* **35**, 3345-3362 (2023). https://doi.org:10.1093/plcell/koad169
37 Watanabe, K. *et al.* The STRUCTURAL MAINTENANCE OF CHROMOSOMES 5/6 complex promotes sister chromatid alignment and homologous recombination after DNA damage in *Arabidopsis thaliana*. *Plant Cell* **21**, 2688-2699 (2009). https://doi.org:10.1105/tpc.108.060525
38 Wei, W. *et al.* A role for small RNAs in DNA double-strand break repair. *Cell* **149**, 101-112 (2012). https://doi.org:10.1016/j.cell.2012.03.002
39 Jiang, J. *et al.* A diRNA-protein scaffold module mediates SMC5/6 recruitment in plant DNA repair. *Plant Cell* **34**, 3899-3914 (2022). https://doi.org:10.1093/plcell/koac191
40 Lai, J. *et al.* The transcriptional coactivator ADA2b recruits a structural maintenance protein to double-strand breaks during DNA repair in plants. *Plant Physiology* **176**, 2613-2622 (2018). https://doi.org:10.1104/pp.18.00123

41 Jiang, J. *et al.* A SWI/SNF subunit regulates chromosomal dissociation of structural maintenance complex 5 during DNA repair in plant cells. *Proceedings of the National Academy of Sciences of the United States of America* **116**, 15288-15296 (2019). https://doi.org:10.1073/pnas.1900308116

42 Li, C., Guo, Y., Wang, L. & Yan, S. The SMC5/6 complex recruits the PAF1 complex to facilitate DNA double-strand break repair in Arabidopsis. *EMBO J* **42**, e112756 (2023). https://doi.org:10.15252/embj.2022112756

43 Guo, T. *et al.* Photoexcited cryptochromes interact with ADA2b and SMC5 to promote the repair of DNA double-strand breaks in *Arabidopsis*. *Nature Plants* **9**, 1280-1290 (2023). https://doi.org:10.1038/s41477-023-01461-6

44 Liu, M. *et al.* IDN2 Interacts with RPA and Facilitates DNA Double-Strand Break Repair by Homologous Recombination in Arabidopsis. *Plant Cell* **29**, 589-599 (2017). https://doi.org:10.1105/tpc.16.00769

45 Wang, L. *et al.* The ATR-WEE1 kinase module inhibits the MAC complex to regulate replication stress response. *Nucleic Acids Research* **49**, 1411-1425 (2021). https://doi.org:10.1093/nar/gkaa1082

46 Dvorak Tomastikova, E. *et al.* SMC5/6 complex-mediated SUMOylation stimulates DNA-protein cross-link repair in Arabidopsis. *Plant Cell* **35**, 1532-1547 (2023). https://doi.org:10.1093/plcell/koad020

47 Gentric, N., Genschik, P. & Noir, S. Connections between the cell cycle and the DNA damage response in plants. *International Journal of Molecular Sciences* **22**, 9558 (2021). https://doi.org:10.3390/ijms22179558

48 De Schutter, K. *et al. Arabidopsis* WEE1 kinase controls cell cycle arrest in response to activation of the DNA integrity checkpoint. *Plant Cell* **19**, 211-225 (2007). https://doi.org:10.1105/tpc.106.045047

49 Culligan, K., Tissier, A. & Britt, A. ATR regulates a G2-phase cell-cycle checkpoint in *Arabidopsis thaliana*. *Plant Cell* **16**, 1091-1104 (2004). https://doi.org:10.1105/tpc.018903

50 Lundgren, K. *et al.* mik1 and wee1 cooperate in the inhibitory tyrosine phosphorylation of cdc2. *Cell* **64**, 1111-1122 (1991). https://doi.org:10.1016/0092-8674(91)90266-2

51 Watanabe, N., Broome, M. & Hunter, T. Regulation of the human WEE1Hu CDK tyrosine 15-kinase during the cell cycle. *EMBO Journal* **14**, 1878-1891 (1995). https://doi.org:10.1002/j.1460-2075.1995.tb07180.x

52 Dissmeyer, N. *et al.* Control of cell proliferation, organ growth, and DNA damage response operate independently of dephosphorylation of the *Arabidopsis* Cdk1 homolog CDKA;1. *Plant Cell* **21**, 3641-3654 (2009). https://doi.org:10.1105/tpc.109.070417

53 Kim, H. J. *et al.* Control of plant germline proliferation by SCF^{FBL17} degradation of cell cycle inhibitors. *Nature* **455**, 1134-1137 (2008). https://doi.org:10.1038/nature07289

54 Gusti, A. *et al.* The *Arabidopsis thaliana* F-box protein FBL17 is essential for progression through the second mitosis during pollen development. *PLoS One* **4**, e4780 (2009). https://doi.org:10.1371/journal.pone.0004780

55 Pan, T. *et al.* A novel WEE1 pathway for replication stress responses. *Nature Plants* **7**, 209-218 (2021). https://doi.org:10.1038/s41477-021-00855-8

56 Pan, T., Gao, S., Cui, X., Wang, L. & Yan, S. APC/C^{CDC20} targets SCF^{FBL17} to activate replication stress responses in *Arabidopsis*. *Plant Cell* **35**, 910-923 (2023). https://doi.org:10.1093/plcell/koac360

57 Gentric, N. *et al.* The F-Box-Like Protein FBL17 Is a Regulator of DNA-Damage Response and Colocalizes with RETINOBLASTOMA RELATED1 at DNA Lesion Sites. *Plant Physiol* **183**, 1295-1305 (2020). https://doi.org:10.1104/pp.20.00188

58 Bao, W. *et al.* Protein kinase ATR inhibits E3 ubiquitin ligase $CRL4^{PRL1}$ to stabilize ribonucleotide reductase in response to replication stress. *Cell Reports* **42**, 112685 (2023). https://doi.org:10.1016/j.celrep.2023.112685

59 Kalhorzadeh, P. *et al. Arabidopsis thaliana* RNase H2 deficiency counteracts the needs for the WEE1 checkpoint kinase but triggers genome instability. *Plant Cell* **26**, 3680-3692 (2014). https://doi.org:10.1105/tpc.114.128108
60 Eekhout, T. *et al.* G2/M-checkpoint activation in *fasciata1* rescues an aberrant S-phase checkpoint but causes genome instability. *Plant Physiology* **186**, 1893-1907 (2021). https://doi.org:10.1093/plphys/kiab201
61 Eekhout, T., Pedroza-Garcia, J. A., Kalhorzadeh, P., De Jaeger, G. & De Veylder, L. A mutation in DNA polymerase α rescues *WEE1^{KO}* sensitivity to HU. *International Journal of Molecular Sciences* **22**, 9409 (2021). https://doi.org:10.3390/ijms22179409
62 Hu, Z., Cools, T., Kalhorzadeh, P., Heyman, J. & De Veylder, L. Deficiency of the Arabidopsis helicase RTEL1 triggers a SOG1-dependent replication checkpoint in response to DNA cross-links. *Plant Cell* **27**, 149-161 (2015). https://doi.org:10.1105/tpc.114.134312
63 Wei, P. *et al.* Arabidopsis casein kinase 2 triggers stem cell exhaustion under Al toxicity and phosphate deficiency through activating the DNA damage response pathway. *Plant Cell* **33**, 1361-1380 (2021). https://doi.org:10.1093/plcell/koab005
64 Hamasaki, H. *et al.* SnRK1 kinase and the NAC transcription factor SOG1 are components of a novel signaling pathway mediating the low energy response triggered by ATP depletion. *Frontiers in Plant Science* **10**, 503 (2019). https://doi.org:10.3389/fpls.2019.00503
65 Chen, H. *et al.* The ATR-WEE1 kinase module promotes SUPPRESSOR OF GAMMA RESPONSE 1 translation to activate replication stress responses. *Plant Cell* **35**, 3021-3034 (2023). https://doi.org:10.1093/plcell/koad126
66 Wang, X. *et al.* A plant-specific module for homologous recombination repair. *Proceedings of the National Academy of Sciences of the United States of America* **119**, e2202970119 (2022). https://doi.org:10.1073/pnas.2202970119
67 Cools, T. *et al.* The *Arabidopsis thaliana* checkpoint kinase WEE1 protects against premature vascular differentiation during replication stress. *Plant Cell* **23**, 1435-1448 (2011). https://doi.org:10.1105/tpc.110.082768
68 Yi, D. *et al.* The *Arabidopsis* SIAMESE-RELATED cyclin-dependent kinase inhibitors SMR5 and SMR7 regulate the DNA damage checkpoint in response to reactive oxygen species. *Plant Cell* **26**, 296-309 (2014). https://doi.org:10.1105/tpc.113.118943
69 Takahashi, N. *et al.* A regulatory module controlling stress-induced cell cycle arrest in *Arabidopsis*. *Elife* **8**, e43944 (2019). https://doi.org:10.7554/eLife.43944
70 Chen, P. *et al. Arabidopsis* R1R2R3-Myb proteins are essential for inhibiting cell division in response to DNA damage. *Nature communications* **8**, 635 (2017). https://doi.org:10.1038/s41467-017-00676-4
71 Lammens, T., Li, J., Leone, G. & De Veylder, L. Atypical E2Fs: new players in the E2F transcription factor family. *Trends in Cell Biology* **19**, 111-118 (2009). https://doi.org:10.1016/j.tcb.2009.01.002
72 Lang, L. *et al.* The DREAM complex represses growth in response to DNA damage in *Arabidopsis*. *Life Science Alliance* **4**, e202101141 (2021). https://doi.org:10.26508/lsa.202101141
73 Zamora-Zaragoza, J., Klap, K., Sánchez-Pérez, J., Vielle-Calzada, J.-P. & Scheres, B. A phosphorylation code regulates the multi-functional protein RETINOBLASTOMA-RELATED1 in *Arabidopsis thaliana*. Preprint at *bioRxiv* https://doi.org:10.1101/2021.12.20.472892 (2023) (2021).
74 Ning, Y.-Q. *et al.* DREAM complex suppresses DNA methylation maintenance genes and precludes DNA hypermethylation. *Nature Plants* **6**, 942-956 (2020). https://doi.org:10.1038/s41477-020-0710-7
75 Wang, Y. *et al.* The *Arabidopsis* DREAM complex antagonizes WDR5A to modulate histone H3K4me2/3 deposition for a subset of genome repression. *Proceedings of the National Academy of Sciences of the United States of America* **119**, e2206075119 (2022). https://doi.org:10.1073/pnas.2206075119

76 Wang, L., Chen, H., Wang, C., Hu, Z. & Yan, S. Negative regulator of E2F transcription factors links cell cycle checkpoint and DNA damage repair. *Proc Natl Acad Sci U S A* **115**, E3837-E3845 (2018). https://doi.org:10.1073/pnas.1720094115
77 Fulcher, N. & Sablowski, R. Hypersensitivity to DNA damage in plant stem cell niches. *Proc Natl Acad Sci U S A* **106**, 20984-20988 (2009). https://doi.org:10.1073/pnas.0909218106
78 Li, G., Deng, L., Huang, N. & Sun, F. The Biological Roles of lncRNAs and Future Prospects in Clinical Application. *Diseases* **9**, 8 (2021). https://doi.org:10.3390/diseases9010008
79 Wang, Z., Schwacke, R. & Kunze, R. DNA damage-induced transcription of transposable elements and long non-coding RNAs in *Arabidopsis* is rare and ATM-dependent. *Molecular Plant* **9**, 1142-1155 (2016). https://doi.org:10.1016/j.molp.2016.04.015
80 Durut, N. *et al.* Long non-coding RNAs contribute to DNA damage resistance in *Arabidopsis thaliana*. *Genetics* **225**, iyad135 (2023). https://doi.org:10.1093/genetics/iyad135
81 Herbst, J., Nagy, S. H., Vercauteren, I., De Veylder, L. & Kunze, R. The long non-coding RNA *LINDA* restrains cellular collapse following DNA damage in *Arabidopsis thaliana*. *Plant Journal* **in press** (2023). <https://www.ncbi.nlm.nih.gov/pubmed/37616189>.
82 Mortimore, G. E., Lardeux, B. R. & Adams, C. E. Regulation of microautophagy and basal protein turnover in rat liver. Effects of short-term starvation. *J Biol Chem* **263**, 2506-2512 (1988).
83 Juretschke, T. & Beli, P. Causes and consequences of DNA damage-induced autophagy. *Matrix Biol* **100-101**, 39-53 (2021). https://doi.org:10.1016/j.matbio.2021.02.004
84 Zhang, Y. *et al.* Autophagy-related Proteins in Genome Stability: Autophagy-Dependent and Independent Actions. *Int J Biol Sci* **18**, 5329-5344 (2022). https://doi.org:10.7150/ijbs.76134
85 Chen, P. *et al.* KNO1-mediated autophagic degradation of the Bloom syndrome complex component RMI1 promotes homologous recombination. *EMBO J* **42**, e111980 (2023). https://doi.org:10.15252/embj.2022111980
86 Martens, M., Horres, R., Wendeler, E. & Reiss, B. The importance of ATM and ATR in *Physcomitrella patens* DNA damage repair, development, and gene targeting. *Genes* **11**, 752 (2020). https://doi.org:10.3390/genes11070752
87 Gu, N. *et al.* DNA damage triggers reprogramming of differentiated cells into stem cells in Physcomitrella. *Nature Plants* **6**, 1098-1105 (2020). https://doi.org:10.1038/s41477-020-0745-9
88 Culligan, K. M., Robertson, C. E., Foreman, J., Doerner, P. & Britt, A. B. ATR and ATM play both distinct and additive roles in response to ionizing radiation. *Plant Journal* **48**, 947-961 (2006). https://doi.org:10.1111/j.1365-313X.2006.02931.x
89 Yoshiyama, K. O. SOG1: a master regulator of the DNA damage response in plants. *Genes & Genetic Systems* **90**, 209-216 (2016). https://doi.org:10.1266/ggs.15-00011
90 Yoshiyama, K. O., Kimura, S., Maki, H., Britt, A. B. & Umeda, M. The role of SOG1, a plant-specific transcriptional regulator, in the DNA damage response. *Plant Signaling & Behavior* **9**, e28889 (2014). https://doi.org:10.4161/psb.28889
91 Goffová, I. *et al.* Roles of RAD51 and RTEL1 in telomere and rDNA stability in *Physcomitrella patens*. *Plant Journal* **98**, 1090-1105 (2019). https://doi.org:10.1111/tpj.14304
92 Sakamoto, A. N. *et al.* SOG1, a plant-specific master regulator of DNA damage responses, originated from nonvascular land plants. *Plant Direct* **5**, e370 (2021). https://doi.org:10.1002/pld3.370
93 Pedroza-Garcia, J. A. *et al.* Maize ATR safeguards genome stability during kernel development to prevent early endosperm endocycle onset and cell death. *Plant Cell* **33**, 2662-2684 (2021). https://doi.org:10.1093/plcell/koab158
94 Szurman-Zubrzycka, M. *et al.* ATR, a DNA damage signaling kinase, is involved in aluminum response in barley. *Frontiers in Plant Science* **10**, 1299 (2019). https://doi.org:10.3389/fpls.2019.01299
95 Haberer, G. *et al.* Structure and architecture of the maize genome. *Plant Physiology* **139**, 1612-1624 (2005). https://doi.org:10.1104/pp.105.068718

96 Mascher, M. *et al.* A chromosome conformation capture ordered sequence of the barley genome. *Nature* **544**, 427-433 (2017). https://doi.org:10.1038/nature22043
97 Nishizawa-Yokoi, A. *et al.* SUPPRESSOR OF GAMMA RESPONSE 1 plays rice-specific roles in DNA damage response and repair. *Plant Physiology* **191**, 1288-1304 (2023). https://doi.org:10.1093/plphys/kiac490
98 Tripathi, D., Oldenburg, D. J. & Bendich, A. J. Oxidative and glycation damage to mitochondrial DNA and plastid DNA during plant development. *Antioxidants & Redox Signaling* **12**, 891 (2023). https://doi.org:10.3390/antiox12040891
99 Tripathi, D., Nam, A., Oldenburg, D. J. & Bendich, A. J. Reactive oxygen species, antioxidant agents, and DNA damage in developing maize mitochondria and plastids. *Frontiers in Plant Science* **11**, 596 (2020). https://doi.org:10.3389/fpls.2020.00596
100 Kumar, R. A., Oldenburg, D. J. & Bendich, A. J. Changes in DNA damage, molecular integrity, and copy number for plastid DNA and mitochondrial DNA during maize development. *Journal of Experimental Botany* **65**, 6425-6439 (2014). https://doi.org:10.1093/jxb/eru359
101 Maréchal, A. *et al.* Whirly proteins maintain plastid genome stability in *Arabidopsis*. *Proceedings of the National Academy of Sciences of the United States of America* **106**, 14693-14698 (2009). https://doi.org:10.1073/pnas.0901710106
102 Zampini, E., Lepage, E., Tremblay-Belzile, S., Truche, S. & Brisson, N. Organelle DNA rearrangement mapping reveals U-turn-like inversions as a major source of genomic instability in *Arabidopsis* and humans. *Genome Research* **25**, 645-654 (2015). https://doi.org:10.1101/gr.188573.114
103 Wang, W. *et al.* RNase H1C collaborates with ssDNA binding proteins WHY1/3 and recombinase RecA1 to fulfill the DNA damage repair in Arabidopsis chloroplasts. *Nucleic Acids Research* **49**, 6771-6787 (2021). https://doi.org:10.1093/nar/gkab479
104 Duan, S., Hu, L., Dong, B., Jin, H.-L. & Wang, H.-B. Signaling from plastid genome stability modulates endoreplication and cell cycle during plant development. *Cell Reports* **32**, 108019 (2020). https://doi.org:10.1016/j.celrep.2020.108019
105 Hudik, E. *et al.* Chloroplast dysfunction causes multiple defects in cell cycle progression in the Arabidopsis *crumpled leaf* mutant. *Plant Physiology* **166**, 152-167 (2014). https://doi.org:10.1104/pp.114.242628
106 Jin, H.-L. *et al.* Dual roles for CND1 in maintenance of nuclear and chloroplast genome stability in plants. *Cell Reports* **42**, 112268 (2023). https://doi.org:10.1016/j.celrep.2023.112268
107 Han, S.-H., Park, Y.-J. & Park, C.-M. HOS1 activates DNA repair systems to enhance plant thermotolerance. *Nature Plants* **6**, 1439-1446 (2020). https://doi.org:10.1038/s41477-020-00809-6
108 Zhuang, K. *et al.* WHIRLY1 regulates *HSP21.5A* expression to promote thermotolerance in tomato. *Plant & Cell Physiology* **61**, 169-177 (2020). https://doi.org:10.1093/pcp/pcz189
109 Babbar, R., Karpinska, B., Grover, A. & Foyer, C. H. Heat-induced oxidation of the nuclei and cytosol. *Frontiers in Plant Science* **11**, 617779 (2020). https://doi.org:10.3389/fpls.2020.617779
110 Velichko, A. K., Petrova, N. V., Kantidze, O. L. & Razin, S. V. Dual effect of heat shock on DNA replication and genome integrity. *Molecular Biology of the Cell* **23**, 3450-3460 (2012). https://doi.org:10.1091/mbc.E11-12-1009
111 Lloyd, A., Morgan, C., Franklin, F. C. H. & Bomblies, K. Plasticity of meiotic recombination rates in response to temperature in *Arabidopsis*. *Genetics* **208**, 1409-1420 (2018). https://doi.org:10.1534/genetics.117.300588
112 Ning, Y. *et al.* Heat stress interferes with formation of double-strand breaks and homolog synapsis. *Plant Physiology* **185**, 1783-1797 (2021). https://doi.org:10.1093/plphys/kiab012
113 Lee, J. R. *et al.* Dynamic Interactions of Arabidopsis TEN1: Stabilizing Telomeres in Response to Heat Stress. *Plant Cell* **28**, 2212-2224 (2016). https://doi.org:10.1105/tpc.16.00408

114 Belfield, E. J. *et al.* Thermal stress accelerates *Arabidopsis thaliana* mutation rate. *Genome Research* **31**, 40-50 (2021). https://doi.org:10.1101/gr.259853.119
115 Perkins-Kirkpatrick, S. E. & Lewis, S. C. Increasing trends in regional heatwaves. *Nature Communications* **11**, 3357 (2020). https://doi.org:10.1038/s41467-020-16970-7
116 Kobbe, D., Blanck, S., Demand, K., Focke, M. & Puchta, H. AtRECQ2, a RecQ helicase homologue from Arabidopsis thaliana, is able to disrupt various recombinogenic DNA structures in vitro. *Plant J* **55**, 397-405 (2008). https://doi.org:10.1111/j.0960-7412.2008.03511.x
117 Zhao, J. *et al.* ATM-mediated double-strand break repair is required for meiotic genome stability at high temperature. *Plant Journal* **114**, 403-423 (2023). https://doi.org:10.1111/tpj.16145
118 Zhao, L. *et al.* Transcriptional regulation of cell cycle genes in response to abiotic stresses correlates with dynamic changes in histone modifications in maize. *PLoS One* **9**, e106070 (2014). https://doi.org:10.1371/journal.pone.0106070
119 Fenollosa, E., Jene, L. & Munne-Bosch, S. A rapid and sensitive method to assess seed longevity through accelerated aging in an invasive plant species. *Plant Methods* **16**, 64 (2020). https://doi.org:10.1186/s13007-020-00607-3
120 De Vitis, M. *et al.* Seed storage: maintaining seed viability and vigor for restoration use. *Restoration Ecology* **28**, S249-S255 (2020). https://doi.org:10.1111/rec.13174
121 Waterworth, W. M., Latham, R., Wang, D., Alsharif, M. & West, C. E. Seed DNA damage responses promote germination and growth in *Arabidopsis thaliana*. *Proceedings of the National Academy of Sciences of the United States of America* **119**, e2202172119 (2022). https://doi.org:10.1073/pnas.2202172119
122 Li, Z. *et al.* ATM suppresses leaf senescence triggered by DNA double-strand break through epigenetic control of senescence-associated genes in *Arabidopsis*. *New Phytologist* **227**, 473-484 (2020). https://doi.org:10.1111/nph.16535
123 Waterworth, W. M., Footitt, S., Bray, C. M., Finch-Savage, W. E. & West, C. E. DNA damage checkpoint kinase ATM regulates germination and maintains genome stability in seeds. *Proceedings of the National Academy of Sciences of the United States of America* **113**, 9647-9652 (2016). https://doi.org:10.1073/pnas.1608829113
125 Müller, J. *et al.* Iron-dependent callose deposition adjusts root meristem maintenance to phosphate availability. *Developmental Cell* **33**, 216-230 (2015). https://doi.org:10.1016/j.devcel.2015.02.007
126 Zheng, Z., Wang, Z., Wang, X. & Liu, D. Blue light-triggered chemical reactions underlie phosphate deficiency-induced inhibition of root elongation of *Arabidopsis* seedlings grown in petri dishes. *Molecular Plant* **12**, 1515-1523 (2019). https://doi.org:10.1016/j.molp.2019.08.001
127 Reyt, G., Boudouf, S., Boucherez, J., Gaymard, F. & Briat, J.-F. Iron- and ferritin-dependent reactive oxygen species distribution: impact on *Arabidopsis* root system architecture. *Molecular Plant* **8**, 439-453 (2015). https://doi.org:10.1016/j.molp.2014.11.014
128 Latha, K. S., Anitha, S., Rao, K. S. J. & Viswamitra, M. A. Molecular understanding of aluminum-induced topological changes in CCG_{12} triplet repeats: relevance to neurological disorders. *Biochimica et Biophysica Acta - Molecular Basis of Disease* **1588**, 56-64 (2002). https://doi.org:https://doi.org/10.1016/S0925-4439(02)00133-3
129 Nezames, C. D., Sjogren, C. A., Barajas, J. F. & Larsen, P. B. The *Arabidopsis* cell cycle checkpoint regulators TANMEI/ALT2 and ATR mediate the active process of aluminum-dependent root growth inhibition. *Plant Cell* **24**, 608-621 (2012). https://doi.org:10.1105/tpc.112.095596
130 Zhang, Y. *et al.* The cell cycle checkpoint regulator ATR is required for internal aluminum toxicity-mediated root growth inhibition in *Arabidopsis*. *Frontiers in Plant Science* **9**, 118 (2018). https://doi.org:10.3389/fpls.2018.00118

131 Liu, D., Jiang, W., Zhang, L. & Li, L. Effects of Boron Ions on Root Growth and Cell Division of Broadbean (Vicia Faba L.). *Israel Journal of Plant Sciences* **48**, 47-51 (2000). https://doi.org:10.1560/c74e-vykd-fkyk-tqwk

132 Reid, R. J., Hayes, J. E., Post, A., Stangoulis, J. C. R. & Graham, R. D. A critical analysis of the causes of boron toxicity in plants. *Plant, Cell & Environment* **27**, 1405-1414 (2004). https://doi.org:10.1111/j.1365-3040.2004.01243.x

133 Cervilla, L. M., Blasco, B., Ríos, J. J., Romero, L. & Ruiz, J. M. Oxidative stress and antioxidants in tomato (*Solanum lycopersicum*) plants subjected to boron toxicity. *Annals of Botany* **100**, 747-756 (2007). https://doi.org:10.1093/aob/mcm156

134 Sakamoto, T. *et al.* Condensin II alleviates DNA damage and is essential for tolerance of boron overload stress in *Arabidopsis*. *Plant Cell* **23**, 3533-3546 (2011). https://doi.org:10.1105/tpc.111.086314

135 Sakamoto, T. *et al.* Proteasomal degradation of BRAHMA promotes boron tolerance in *Arabidopsis*. *Nature Communications* **9**, 5285 (2018). https://doi.org:10.1038/s41467-018-07393-6

136 Sotta, N. *et al. NAC103* mutation alleviates DNA damage in an *Arabidopsis thaliana* mutant sensitive to excess boron. *Frontiers in Plant Science* **14**, 1099816 (2023). https://doi.org:10.3389/fpls.2023.1099816

137 Hendrix, S. *et al.* Suppressor of gamma response 1 modulates the DNA damage response and oxidative stress response in leaves of cadmium-exposed *Arabidopsis thaliana*. *Frontiers in Plant Science* **11**, 366 (2020). https://doi.org:10.3389/fpls.2020.00366

138 Hendrix, S. *et al.* Cell cycle regulation in different leaves of Arabidopsis thaliana plants grown under control and cadmium-exposed conditions. *Environmental and Experimental Botany* **155**, 441-452 (2018). https://doi.org:10.1016/j.envexpbot.2018.06.026

139 Haapaniemi, E., Botla, S., Persson, J., Schmierer, B. & Taipale, J. CRISPR-Cas9 genome editing induces a p53-mediated DNA damage response. *Nature Medicine* **24**, 927-930 (2018). https://doi.org:10.1038/s41591-018-0049-z

Figures

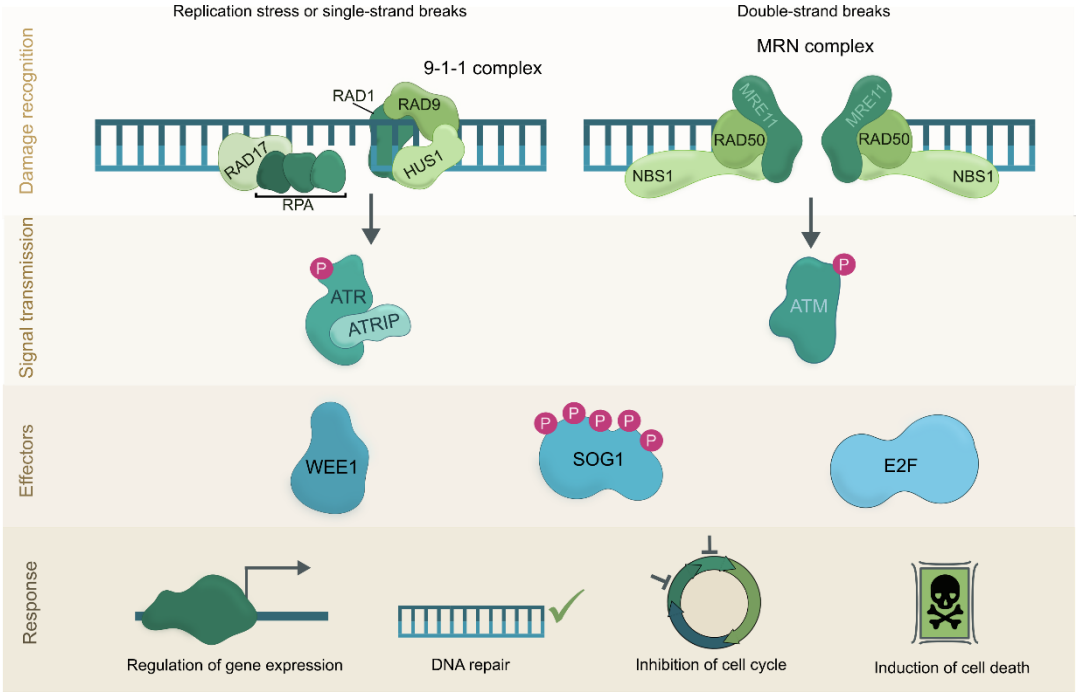

Fig. 1: Three step-based activation of the DDR, leading to an efficient DNA repair. Step 1: Recognition of the damaged DNA depends on the type of DNA lesions: while replication stress or single-strand breaks recognized by the RAD9-RAD1-HUS1 (9-1-1) complex or bound by replication protein A (RPA) and RAD17, double-strand breaks are recognized by the MRE11-RAD50-NBS1 (MRN) complex. Step 2: Signaling of the recognized DNA damage to the effectors also depends on the lesion type, activating either ATR or ATM. While ATR is activated in response to replication stress or single-strand breaks via the interaction with the ATR-interacting protein (ATRIP) and likely phosphorylation, ATM is activated by the NBS1 subunit in response to double-strand breaks. Step 3: Once ATR and ATM are active, they can, in plants, activate the DDR effectors WEE1, SOG1 and E2F. Those effectors trigger different cellular responses, leading to altered gene expression, effective DNA repair, inhibition of the cell cycle and induction of cell death.

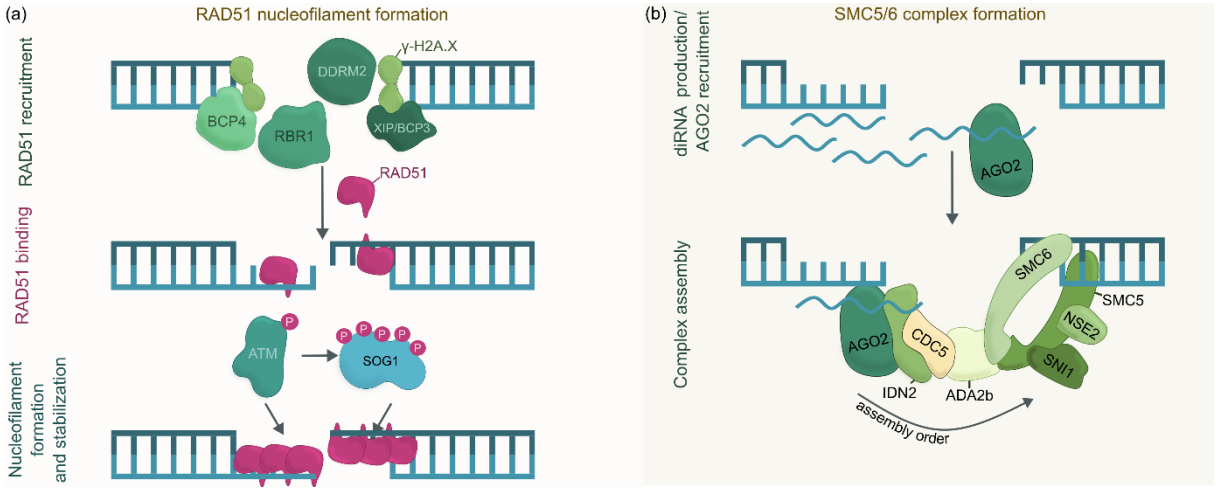

Fig. 2: Multistep formation of RAD51 nucleofilaments (a) and the active SMC5/6 complex (b). (a) RAD51 can be recruited by multiple proteins, including the γ-H2A.X readers XIP/BCP3, BCP4, RBR1 and DDRM2. After binding the break, RAD51 assembles into larger nucleofilaments, which can be further stabilized by SOG1 and ATM. (b) The association of the SMC5/6 complex to the damaged site is initiated by DSB-induced RNAs, which attract AGO2. Consequently, AGO2 triggers the ordered assembly of the complex needed for SMC5/6 recruitment at the damaged site.

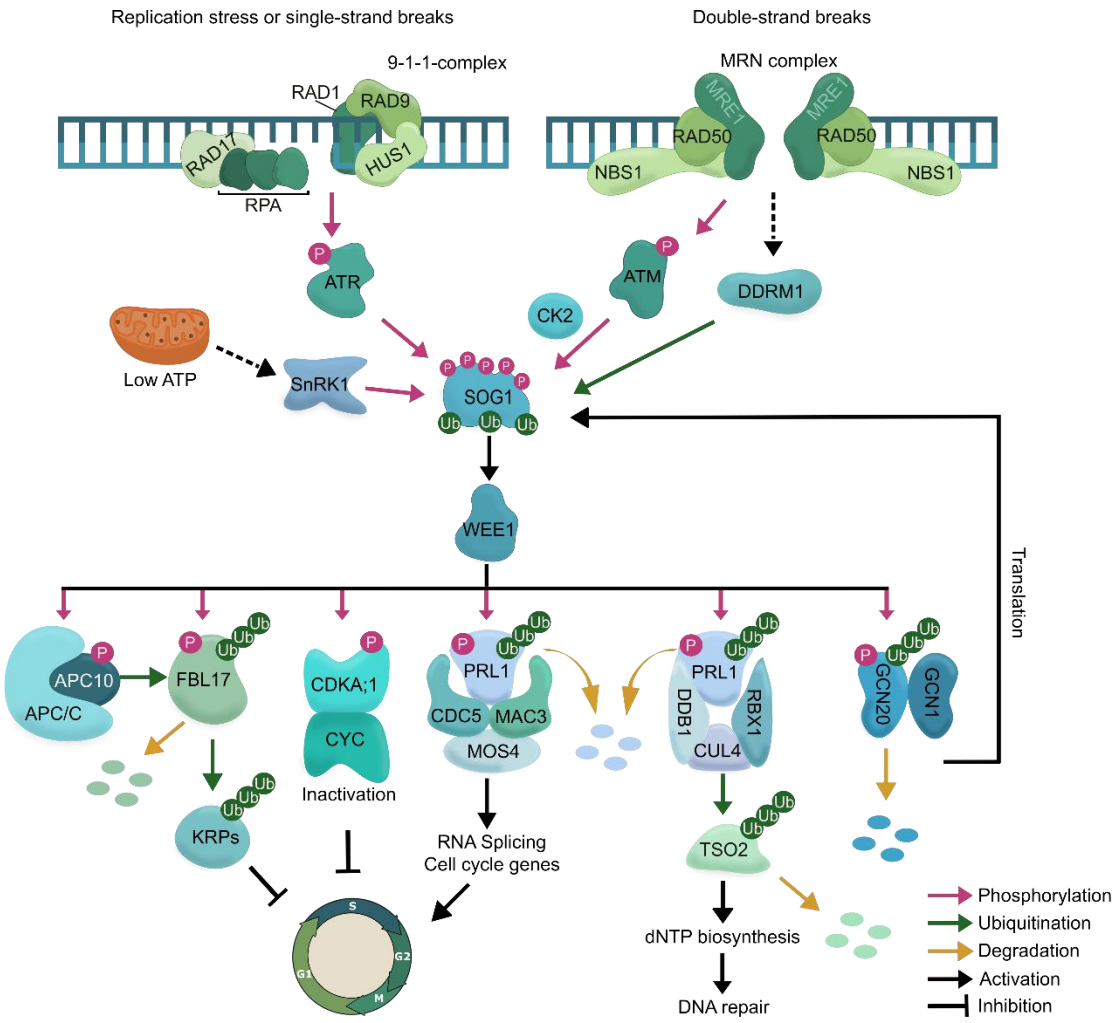

Fig. 3: The molecular mechanisms of the SOG1/WEE1-mediated DNA damage checkpoint. DNA single- and double-strand breaks predominantly trigger ATR and ATM activation, respectively, which then activate the SOG1 transcription factor by phosphorylation. CK2 is required for phosphorylation of SOG1 by ATM. SOG1 can also be phosphorylated by SnRK1 in the presence of low intracellular ATP levels. Additionally, SOG1 stability can be regulated by DDRM1 via ubiquitination upon DNA damage. WEE1 represents the key downstream target of SOG1 and phosphorylates many targets in response to replication defects, triggering a cell cycle arrest. WEE1 likely phosphorylates the A-type cyclin-dependent kinase CDKA;1 to inhibit its activity. Additionally, WEE1 phosphorylates FBL17 and APC10, which promotes the degradation of FBL17 that normally mediates the ubiquitination and degradation of KIP-RELATED PROTEIN (KRP) inhibitors, leading to the accumulation of KRPs and a cell cycle arrest. WEE1 phosphorylation of PRL1 promotes its degradation. PRL1 functions in two different protein complexes, and its degradation impairs correct splicing of cell cycle genes, contributing to a cell cycle arrest, and inhibits ribonucleotide reductase TSO2 degradation to promote dNTP biosynthesis and DNA repair. Moreover, WEE1 phosphorylates GCN20 to promote its degradation, which enhances the

translation of SOG1, thus establishing a WEE1-SOG1 positive feedback loop in response to DNA damage.

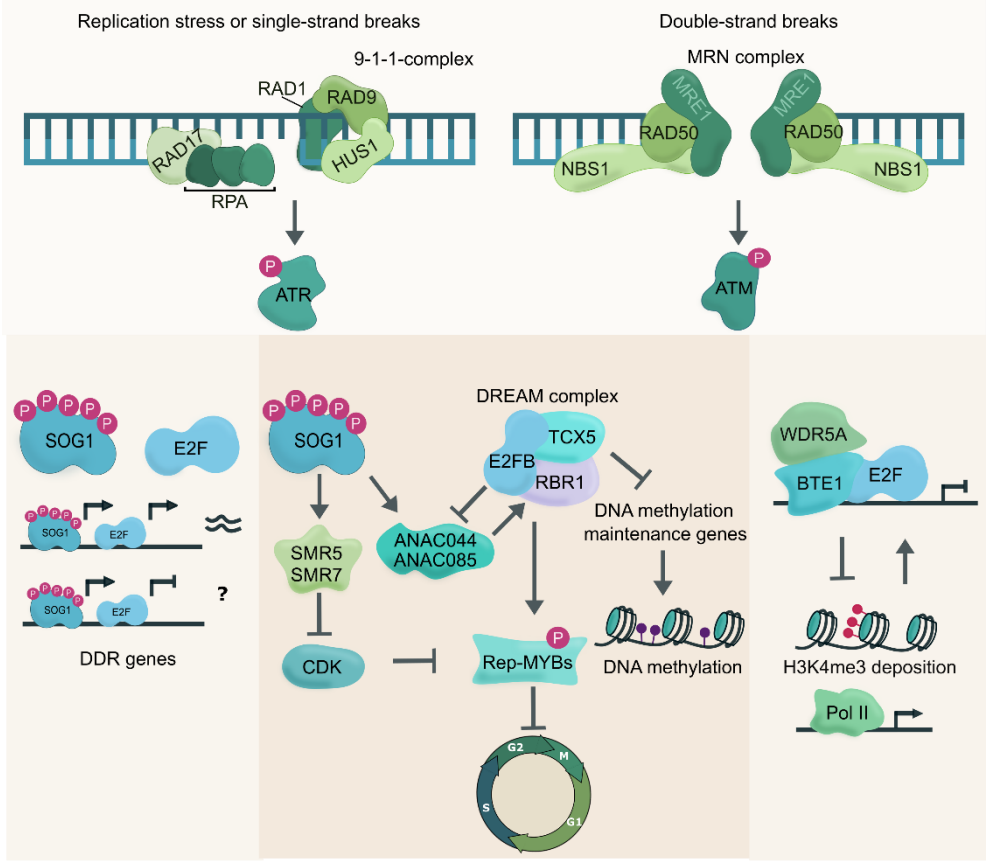

Fig. 4: The roles of DREAM complexes in the DNA damage checkpoint. E2F transcription factors are major components of the DREAM complexes and play a crucial role in the DDR. E2F and SOG1 antagonistically or synergistically control DDR gene expression under replication stress. Additionally, SOG1 can activate *SMR5* and *SMR7* gene expression, which inhibits CDK activity, resulting in Rep-MYBs accumulation that subsequently repress G2/M-specific genes. Further, as the targets of SOG1 and E2FB, ANAC044 and ANAC085, stabilize Rep-MYBs and interact with RBR1, a subunit of the transcriptional repressive DREAM complex, to repress cell cycle-related genes. Another component of DREAM complex is TCX5, which represses the expression of DNA methylation maintenance genes to preclude DNA hypermethylation. BTE1 is also a DREAM complex subunit, working together with E2F to regulate downstream gene expression. BTE1 also mediates the transcriptional repression function of the DREAM complex by direct interaction with WDR5A, which inhibits H3K4me3 deposition and polymerase II (Pol II) elongation at the target genes.

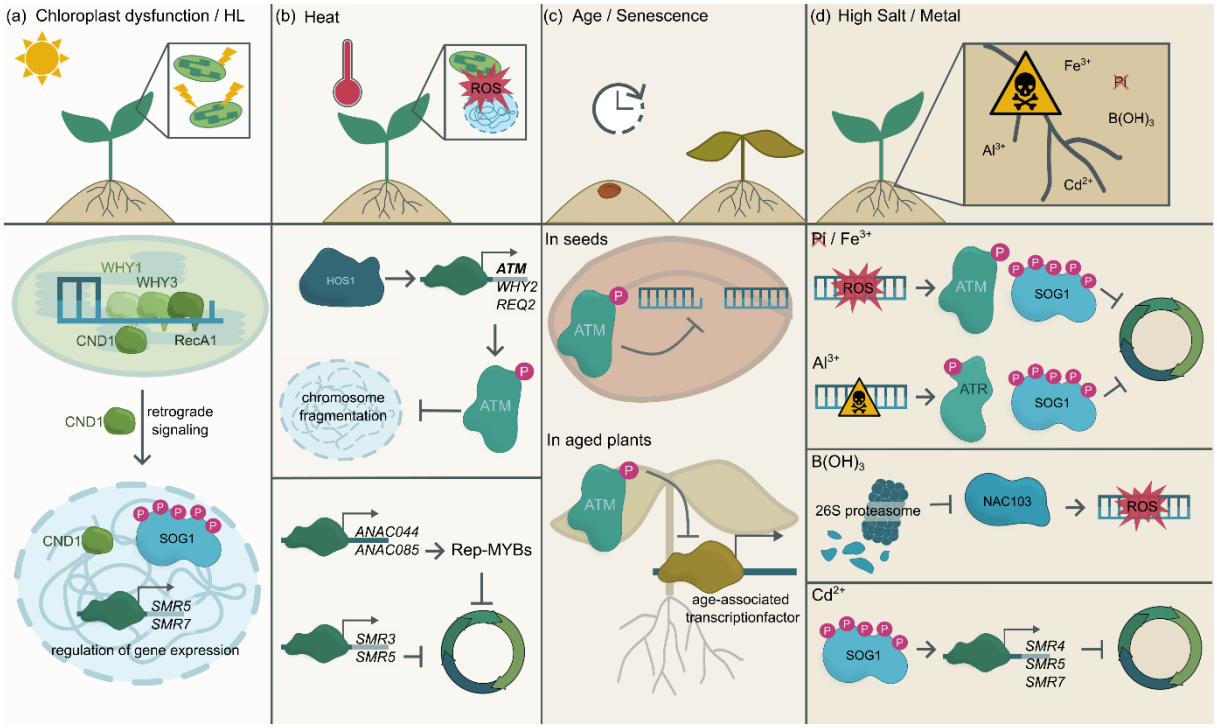

Fig. 5: The importance of a functional DDR during various environmental extremes. a,b Chloroplasts are important sensors for high light intensities (a) and heat (b). Under high light or chloroplast dysfunction, the ssDNA-binding proteins WHY1, WHY2 and RecA1 guard plastid genome integrity (a). The dual-localized CND1 protein interacts with WHY1 and furthermore promotes nuclear DNA replication. Additionally, HL induces the SOG1-dependent expression of *SMR5* and *SMR7*. (b) Heat triggers the accumulation of HOS1, which activate DDR-related genes like *WHY2*, *RECQ2* and *ATM*. ATM in turn suppresses heat-induced chromosome fragmentation. Heat also activates the gene expression of *ANAC044* and *ANAC085*, leading to the activation of Rep-MYBs and cell cycle inhibition, potentially in cooperation with *SMR3* and *SMR5*. (c) Age-associated accumulation of DNA damage in seeds can be counteracted by ATM. Moreover, during senescence, ATM represses age-associated transcription factors. (d) High salinity, inorganic phosphate (Pi) deficiency and accumulation of metals can create a toxic environment for plant roots. Pi deficiency, accompanied by an altered accumulation of iron (Fe^{3+}), triggers the generation of ROS and potentially ROS-induced DNA lesions, activating ATM and SOG1 that subsequently inhibit cell cycle progression. Aluminum (Al^{3+}) causes replication stress, which activates ATR and SOG1 and causes cell cycle arrest. Boron, in the form of boric acid ($B(OH)_3$), causes NAC103-mediated induction of DSBs, which is counteracted by the proteolytic degradation of NAC103 by the 26S proteasome. Cadmium (Cd^{2+}) activates SOG1, thus inducing a *SMR4*-, *SMR5*- and *SMR7*-mediated cell cycle arrest.