博士論文番号：1281205
(Doctoral student number)

# Studies on DNA damage response during lateral root formation in *Arabidopsis thaliana*

## シロイヌナズナの側根形成における DNA 損傷応答に関する研究

**La Ode Muhammad Muchdar Davis**

Nara Institute of Science and Technology
Graduate School of Biological Sciences
Supervisor : Prof. Masaaki Umeda

(Submitted on 2016/08/10)

# Contents

**Abbreviations** ⋯ **3**

**Abstract** ⋯ **5**

**Introduction** ⋯ **9**

**Materials and Methods** ⋯ **26**

**Results** ⋯ **31**

**Discussion** ⋯ **63**

**Acknowledgements** ⋯ **71**

**References** ⋯ **73**

## Abbreviations

ACR4 : *Arabidopsis* crinkly 4
AFB : auxin-related F-box
AHK : *Arabidopsis* histidine kinase
AHP : *Arabidopsis* histidine phosphotransfer
ARF : auxin response factor
ARR : *Arabidopsis* response regulator
ATM : ataxia telangiectasia mutated
ATR : ataxia telangiectasia and Rad3 related
Aux/IAA : auxin/indole-3-acetic acid
BA : benzyladenine
BIR : break-induced repair
CDK : cyclin-dependent kinase
CHASE : cyclases/histidine kinases associated sensory extracellular
CKX1 : cytokinin oxidases/dehydrogenases 1
CYC : cyclin
CYP735A1/A2 : cytochrome P450 monooxygenases 735 A1/A2
DHJ : double holliday junction
DNA : deoxyribonucleic acid
DR5 : direct repeat 5
DSB : double-strand break
EMS : ethyl methanesulfonate
GARP : GOLDEN2, ARR-B, Psr1
GUS : β-glucuronidase
HR : homologous recombination
HU : hydroxyurea
iP : isopentenyladenine
iPRP : isopentenyl adenosine-5'-phosphate
IPT : isopentenyl transferase
LAX3 : like Aux1 3
LBD/ASL : lateral organ boundaries domain/asymmetric leaves2-like

LOG : lonely guy
LR : lateral root
LRFC : lateral root founder cell
LRP : lateral root primordium
MES : 2-(N-morpholino)ethanesulfonic acid
MMC : mitomycin C
MMS : methyl methanesulfonate
MRN : Mre11-Rad50-Nbs1
MS : Murashige and Skoog
NAC : NAM/ATAF/CUC
NHEJ : non-homologous end joining
PIN : pin-formed
ROS : reactive oxygen species
SDSA : synthesis-dependent strand annealing
SE : standard error of the mean
SIM : siamese
SMR : siamese-related
SOG1 : suppressor of gamma response 1
SSB : single-strand break
ssDNA : single-stranded DNA
TIR1 : transport inhibitor response 1
*t*Z : *trans*-zeatin
*t*ZRP : *trans*-zeatin ribotide-phosphate
UV : ultraviolet

バイオサイエンス研究科　博士論文要旨

| | | | |
|---|---|---|---|
| 所属<br>（主指導教員） | Laboratory of Plant Growth Regulation<br>(Prof. Masaaki Umeda) | | |
| 氏名 | La Ode Muhammad Muchdar Davis | 提出 | 平成 28 年 8 月 10 日 |
| 題目 | Studies on DNA damage response during lateral root formation in *Arabidopsis thaliana* | | |

Plants are inevitably exposed to various stresses throughout their lifetimes. Abiotic stresses, such as high salinity, osmotic stress, drought, strong light illumination, and heavy metals, produce reactive oxygen species (ROSs) in cells, resulting in the breakage of genomic DNA. Furthermore, naturally occurring endogenous by-products of cell metabolism and ultraviolet light block replication fork, and ionizing radiation and radiomimetic drugs cause DNA lesions, thereby generating DNA single-strand breaks (SSBs) and double-strand breaks (DSBs). Because the maintenance of genome integrity is crucial for survival under various environmental conditions, plants have a signaling pathway that senses DNA lesions and transduces the signal to trigger cellular responses to DNA damage. Plants sense DNA damage through the cellular sensor kinases ATAXIA-TELANGIECTASIA MUTATED (ATM) and ATM AND RAD3-RELATED (ATR). ATM is activated by DSBs, whereas ATR responds to SSBs and DNA replication fork blocking. ATM and ATR phosphorylate and activate the plant-specific NAC transcription factor SUPPRESSOR OF GAMMA RESPONSE 1 (SOG1). Phosphorylated SOG1 induces the expression of downstream genes involved in DNA repair and cell cycle regulation. Root development is controlled in a plastic manner to

cope with fluctuating environmental conditions. Since lateral roots (LRs) contribute to water and nutrient uptake from soil and account for the majority of plant root mass, control of LR formation is crucial in the adaptation of plant growth to environmental conditions. However, the underlying mechanism controlling LR formation in response to environmental stresses has remained largely unknown.

To analyze the response of LR formation to DNA damage, I treated *Arabidopsis* seedlings with the radiomimetic reagent zeocin, which causes DSBs. I found that DSBs inhibits LR formation. To reveal whether the inhibition of LR formation in response to DNA damage is an active process involving ATM/ATR-SOG1 pathways, I observed the response of *atm-2*, *atr-2*, and *sog1-1* mutants. Surprisingly, compared with the wild-type, *sog1-1* and *atm-2*, but not *atr-2*, showed hypersensitivity to zeocin in LR formation. Furthermore, zeocin treatment severely inhibits cell division for LR primordium (LRP) formation in *sog1-1* and *atm-2*. Since DSB signals are transmitted through the ATM–SOG1 pathway, I speculated that DNA damage may be highly accumulated in *atm-2* and *sog1-1* mutants, thereby inhibiting cell division. To test this hypothesis, expression of a DNA repair gene was monitored in LRP. Interestingly, the expression of several DNA repair genes, *RAD51*, *BRCA1, RAD17*, and *PARP2*, was induced by DNA damage in wild-type but not significantly in *sog1-1*. These results suggest that ATM-activated SOG1 is essential for the induction of DNA repair genes, thus maintaining genome integrity and allowing continuous cell division during LRP development.

The phytohormone cytokinin is known to inhibit LR initiation. When wild-type seedlings were treated with bioactive cytokinins, LR density significantly decreased. This tendency was highly similar to that of zeocin-treated seedlings, suggesting that DNA damage inhibits LR formation by modulating cytokinin signaling. To examine whether the cytokinin signaling is altered in LRP after DNA damage, I observed the expression of the cytokinin signaling marker gene *ARR5*. Zeocin treatment dramatically increased *ARR5* expression in LRP, indicating that the cytokinin signaling is enhanced in LRP in response to DNA damage. Furthermore, I found that zeocin induced several cytokinin biosynthesis genes in a SOG1-dependent manner, thereby activating cytokinin signaling in the LRP. To determine whether the activation of cytokinin signaling is involved in the inhibition of LR formation under DNA damage conditions, I observed the zeocin response of plants defective in cytokinin biosynthesis or signaling, such as *ipt3-2;5-1;7-1, 35S::CKX1* or *arr1-3;12-1,* respectively. I found that reduction of LR density in response to zeocin treatment was observed in *ipt3-2;5-1;7-1, arr1-3;12-1*, and *35S::CKX1* less frequently than in the wild-type. Overall, these results indicate that an increase in cytokinin signaling is crucial for the inhibition of LR formation in response to DNA damage.

My data demonstrate that SOG1-mediated regulation of DNA repair and cytokinin signaling plays a key role for maintenance of genome integrity in LRP and inhibition of LR formation under genotoxic stress. Both DNA repair and cytokinin signaling can be crucial for maintaining the ability of plants to produce LR after

recovery from genotoxic stress. It is known that various environmental stresses often produce ROS and cause the breakage of genomic DNA, implying that the above mechanism also underlies programmed response to other stresses in controlling overall root growth.

# Introduction

## DNA damage and genotoxic stress

Plants constantly suffer DNA damage throughout their lifetime. DNA damage (*e.g.*, breakage in DNA sugar-phosphate backbone, nucleotide mismatch, base losses and chemical modifications) introduces mutations in genetic information and causes genome instability (Aguilera & García-Muse 2013). In plants, DNA damage is caused by exogenous factors from the environment such as UV and gamma ray irradiations, and various kind of genotoxic substances, such as DNA alkylating, crosslinking and radiomimetic agents (Britt 1996; Tuteja et al. 2001). In addition, DNA damage is also generated by endogenous factors such as the faulty of DNA polymerase during replication, spontaneous DNA depurination and the naturally occurring metabolic by-products in the form of reactive oxygen species (ROS) and various reactive intermediates (Britt 1996; Møller et al. 2007; Tripathy & Oelmüller 2012). Furthermore, environmental stresses such as osmotic stress, high salinity, metal toxicity, pollutant, heat and high light stress, nutrient deprivation and pathogen attacks have been also shown to cause overproduction of ROS to a level beyond the scavenging capacity of the cell (Mittler 2002). Elevated reactive oxygen radicals pose a threat to genomic integrity since they can oxidize and damage genomic DNA, resulting in serious consequences for the cell (Apel & Hirt 2004).

By-products of cellular metabolism in the form of ROS and natural alkylating agents such as S-adenosylmethionine and N-nitrosamines can cause modification in DNA bases (Britt 1996; Fu et al. 2012). ROS-induced oxidative lesions such as 8-

hydroxyguanine and uracil are mutagenic, while other lesions such as thymine glycol and its degradation product, urea, block DNA synthesis (Britt 1996). In contrast, alkylating agents modify DNA base by transferring alkyl group to the nitrogen and the extracyclic oxygen atoms of the base (Fu et al. 2012).

Based on the number of its reactive sites, alkylating agent is divided into two categories, monofunctional and bifunctional agents. Monofunctional alkylating agents such as methylmethanesulfonate (MMS) and ethylmethanesulfonate (EMS) contain one active site that can react with DNA base, resulting in formation of a simple alkylated adduct (Manova & Gruszka 2015; Kurowska et al. 2011; Sikora et al. 2011, Fu et al. 2012). Simple alkylated adducts, such as $N^7$-methylguanine is prone to spontaneous depurination and subsequent mutagenesis, meanwhile $N^3$-methyladenine is highly toxic, as it blocks DNA replication (Shrivastav et al. 2010; Fu et al. 2012). Bifunctional alkylating agents including nitrogen mustards and mitomycin C (MMC), contain two reactive groups that can bind to separate DNA bases (Fu et al. 2012). When a base from one strand is covalently bond to another base on the opposing strand of the DNA duplex, DNA interstrand crosslink formed (Noll et al. 2006). Covalent linkage between the interstrand-crosslinked bases prevents DNA strands separation during replication and transcription (Noll et al. 2006; Deans & West 2011).

In contrast to interstrand crosslinks, DNA intrastrand crosslinks are formed when the bases in the same strand of a DNA double helix covalently crosslink each other (Deans & West 2011). Cisplatin, an anti-cancer drug, predominantly produces DNA intrastrand crosslinks lesions, which account for the drug cytotoxicity (Noll et al. 2006; Rabik &

Dolan 2007). In addition, cisplatin produces small proportion of interstrand crosslink adducts (Jung & Lippard 2007; Kartalou & Essigmann 2001).

Despite their much less occurrence than all other type of DNA damage, the breakage of DNA backbone either single or double-strand breaks (SSBs or DSBs), causes high risk to genome integrity, leading to chromosome rearrangement and loss (Aguilera & García-Muse 2011). DNA strand breaks are primarily generated when DNA replication encounters obstacles such as various DNA damage lesions, protein barriers, heterochromatin, non-B DNA structures, and transcribed genes (Zeman & Cimprich 2014). Secondly, they are caused by the ROS-induced oxidative damage in sugar phosphate backbone of DNA (Lieber, 2010). Thirdly, DNA strand breaks can be induced by ionizing radiation. Ionizing radiation exerts high energy transmitted in the form of electromagnetic waves such as gamma-rays and X-rays, or particles such as alpha, beta and heavy ion particles (Alizadeh et al. 2015).

**DNA damage responses**

Because the maintenance of genome integrity is crucial for survival under various environmental conditions, plants have a signaling pathway that senses DNA lesions and transduces the signal to trigger cellular responses to DNA damage (Figure 1-1) (Ciccia & Elledge 2010; Hu et al. 2016). Similar to mammals and yeasts, plants sense DNA damage through the sensor kinases ATAXIA-TELANGIECTASIA MUTATED (ATM) and ATM AND RAD3-RELATED (ATR) (Garcia et al. 2003; Culligan et al. 2004). ATM is activated by DSBs, whereas ATR responds to SSBs and DNA replication fork blocking.

ATM and ATR phosphorylate the plant-specific NAC transcription factor SUPPRESSOR OF GAMMA RESPONSE 1 (SOG1) (Yoshiyama et al. 2013; Sjogren et al. 2015). Phosphorylation on the consensus SQ motifs activates SOG1 to induce the transcription of downstream genes, which are associated with cell cycle regulation, DNA repair, and programmed cell death (Yoshiyama et al. 2013, Furukawa et al. 2010).

Rapid transcriptional changes are one of the prominent responses to DNA damage in plants. The upregulated genes mainly consist of genes involved in genome maintenance and metabolism (*e.g.*, ribonucleotide reductase, DNA polymerases δ and ε, and RPA-like genes), chromatin structure and maintenance (*e.g.*, *SYN2*), and DNA repair (*e.g.*, *BRCA1, RAD51, RAD17, PARP1* and *PARP2*). Meanwhile, the downregulated genes are mostly involved in mitotic cell cycle regulation (*e.g.*, *CYCB1;2, CDKB1;2, CDKB2;1*) and cytokinesis (*e.g.*, *KNOLLE*) (Culligan et al. 2006).

**DNA repair**

Repair of DSBs has two main mechanisms, homologous recombination (HR) and non-homologous end-joining (NHEJ) (Chapman et al. 2012) (Figure 1-2). HR uses homologous sequences as the template for repair, while NHEJ-mediated repair does not require homologous sequences. Utilization of NHEJ and HR for DSB repair are sequential and depend on the cell cycle. Namely, NHEJ is the main mechanism for DSB repair in somatic plant cells, and is associated with random integration of DNA segments into plant genomes (Puchta & Fauser 2014). In contrast, HR is more prevalent during the S-to-G2 phases, since sister chromatids produced during DNA replication are utilized as

the homologous template for recombination (Puchta 2005).

In HR-mediated DSB repair, DSBs are recognized and bound by the Mre11-Rad50-Nbs1 (MRN) complex. Recently, RAD17 has been shown to facilitate recruitment of the MRN complex at DSB sites (Wang et al. 2014). MRN complex catalyzes DSB end resection to generate 3'-OH overhang tail (Symington 2014). These single-strand DNAs (ssDNAs) are secured by REPLICATION PROTEIN A (RPA) proteins. BRCA2 and BRCA1 act as mediator proteins that displace RPA from ssDNA and promote formation of RAD51-ssDNA nucleofilament. RAD51-ssDNA nuclueofilament plays a key role in homology search and invasion of homologous DNA strand (Heyer et al. 2010). After RAD51-dependent strand invasion, an intermediate structures called displacement loop (D-loop) is formed between the invading 3' DNA overhang strand and the homologous recipient strand. DNA polymerase then proceeds to synthesize new DNA on the invading 3' DNA end, and a Holliday junction is formed (Heyer et al. 2010). Following this, DNA synthesis occurs on the invading strand, restoring the strand on the homologous chromosome that was displaced during strand invasion.

In contrast to HR, NHEJ pathway directly joins the broken ends of DSB in an error-prone manner without homologous sequences, frequently resulting in sequence modifications (insertions, deletions or substitutions) and DNA segment translocation (Chapman et al. 2012). Despite prone to error, NHEJ is predominantly chosen for DSB repair (Manova & Gruszka 2015; Puchta 2005). In NHEJ, heterodimer proteins KU70 and KU80 bind and bridge the ends of DSB, aligning the DNA ends and at the same time protecting them from degradation. DSB ends do not always possess 3' hydroxyl and 5'

phosphate termini that are compatible for direct ligation. In fact, most of DSBs have chemical modifications at or near the ends that need to be removed or processed so that rejoining can proceed (Povirk et al. 2012; Davis & Chen 2013). The KU complex acts as scaffolds for the recruitment of DNA-PKc, a phosphoinositide 3-kinase (PI3K) family protein, needed for recruitment of various processing proteins (Davis & Chen 2013). Artemis, one of the DSB end processing enzyme, possesses a nuclease activity to resect DSB overhangs and to generate ligatable ends. Finally, the blunt-end ligation is mediated by X-ray repair cross-complementing protein (XRCC)–DNA ligase 4 (LIG4) proteins complex (Manova & Gruzska 2015). In addition, discontinuous gaps that remain after alignment of DSBs with overhangs are typically filled in by gap-filling DNA polymerases μ and λ (Davis & Chen 2013).

**Lateral root organogenesis**

Root development is controlled in a plastic manner to cope with fluctuating environmental conditions. Since lateral roots (LRs) contribute to water and nutrient uptake from soil and account for the majority of plant root mass, control of LR formation is a crucial survival strategy for plants under stressful conditions (Malamy 2005). LR formation is inhibited in response to salt/osmotic stress (Van der Weele et al. 2000; Deak & Malamy 2005; Duan et al. 2013), herbicide-induced oxidative stress and Fe, and Zn toxicity (Li et al. 2015; Richard et al. 2011; Jain et al. 2013).

LRs are formed post-embryonically along the primary root, followed by ramification (tertiary, quaternary, and further branching) (Nibau et al. 2008) (Figure 1-3).

Oscillation of auxin response in the protoxylem of primary roots controls the regular distribution and left-right alternating pattern of LRs (De Smet et al. 2012; Moreno-Risueno et al. 2010). LRs are originated from the pericycle, which is located between the endodermis and the central vasculature of primary roots. When the auxin response reaches the maximum oscillation peak, the xylem-pole pericycle cells are primed; local auxin accumulation triggers the specification of a subset of pericycle cells into LR founder cells (LRFCs) (Dubrovsky et al. 2008). After this specification, LRFC division is activated to form LR primordium (LRP). During this initiation step, nuclei in a pair of LRFCs migrate toward the common cell wall, leading to asymmetric cell division that generates two small daughter cells flanked by two larger cells (Malamy & Benfey 1997; Casimiro et al. 2001; De Rybel et al. 2010). This asymmetric cell division is followed by a series of anticlinal cell division, resulting in the formation of a single layered first-stage LRP. Afterwards, LRP rapidly develops into another seven distinct stages through successive cell divisions. The first three rounds of periclinal division form LRPs at stage II, III and IV characterized by two, three and four cell layers, respectively (Malamy & Benfey 1997). Subsequent periclinal, tangential, and anticlinal divisions generate dome-shaped LRP at stage V that progressively penetrates into the endodermis and the cortex (Malamy & Benfey 1997). From stage VI, LRP acquires a putative vascular cell layer surrounded by three outer layers, which correspond to the epidermis, the cortex, and the endodermis (Malamy & Benfey 1997). Transition from stage VII to VIII is primarily driven by cell expansion, and thereafter, LRs emerge from the epidermis of the primary root (Malamy & Benfey 1997).

**The role of auxin in LR development**

Auxin controls various aspects of plant development. Indole-3-acetic acid (IAA) is the common naturally occurring auxin in plants, which can exist in the free active form or the conjugated inactive intermediates (Zhao 2012). Auxin response is transcriptionally regulated by the AUXIN RESPONSE FACTORs (ARFs) protein family. Under low auxin conditions, transcriptional repressors of Auxin/INDOLE ACETIC ACID (Aux/IAA) family proteins form a heterodimer with ARFs, thereby blocking ARFs from efficiently binding to the promoters of auxin responsive genes. Auxin is perceived by the F-box protein, TRANSPORT INHIBITOR RESPONSE 1/AUXIN SIGNALING F-BOX PROTEINS (TIR1/AFBs), which is included in the E3 ligase SKP1–Cul1–F-box (SCF). When auxin is abundant, auxin promotes the interaction between TIR1/AFBs and Aux/IAAs, leading to ubiquitylation and proteasome-mediated degradation of Aux/IAA. Aux/IAA degradation releases ARFs from repressive complex, allowing transcriptional regulation of target genes and eventually enhancing auxin response (Wang & Estelle 2014). Interestingly, different TIR1/AFB–Aux/IAA complexes display different binding affinities to auxin. Due to this property, specific Aux/IAAs are degraded at different thresholds of auxin concentration (Wang & Estelle 2014).

Auxin controls LR initiation, LRP development and LR emergence via multiple auxin-signaling modules. An auxin signaling module consists of a pair of Aux/IAA and ARF proteins which regulate auxin-responsible genes (Lavenus et al. 2013). During LR initiation, IAA28–ARF5, –ARF6, –ARF7, –ARF8, and –ARF19 modules are required for priming of LR founder cells (Lavenus et al. 2013). The IAA14/SLR–ARF7 and –

ARF19 modules control the expression of *LATERAL ORGAN BOUNDARIES-DOMAIN/ASYMMETRIC LEAVES2-LIKE* (*LBD/ASL*) family to promote polarization and coordinated nuclear migration of two neighboring LR founder cells, and to trigger LR initiation (De Rybel et al. 2010; Goh et al. 2012; Fukaki et al. 2002; Fukaki et al. 2005; Okushima et al. 2005). The IAA12–ARF5 has been also shown to successively control LR initiation together with IAA14/SLR–ARF7 and –ARF19 (De Smet et al. 2010). Patterned cell division in the early stage is important to establish functional LRPs. Leucine-rich repeat receptor-like kinase ARABIDOPSIS CRINCKLY 4 (ACR4), which is downstream of the IAA14-dependent auxin signaling pathway, specifies cell identity and controls formative division of LRP cells by repressing ectopic division of surrounding pericycle cells (De Smet et al. 2008). Another factor PUCHI, an AP2-like transcription factor, regulates cell division patterning at the flanks of the LRP through auxin-dependent signaling (Hirota et al. 2007).

Auxin transport conducted by PIN-FORMED (PIN)-mediated auxin efflux, and AUXIN RESISTANT 1 (AUX1) and LIKE AUX1 (LAX)-dependent auxin influx shapes auxin gradient in the LRP. Proper auxin gradient is crucial for controlling LR development and determining LR density and position (Benková et al. 2003; Marchant et al. 2002; Laskowski et al. 2008; Swarup et al. 2008). In order to emerge, LRPs must grow through three tissue layers of the primary root, namely the endodermis, the cortex and the epidermis, thus accommodation to these overlying layers is also crucial for LR development. SHY2-dependent auxin perception in the overlying endodermal cells is required for biomechanical change in cell shape, size and barrier feature, thus allowing

LRP penetration through endodermis (Vermeer et al. 2014). Furthermore, sequential induction of *PIN3* and *LAX3* is instrumental to properly direct the auxin flux to the cortex and epidermal cells overlying the LRP. This coordinated auxin focusing is required for induction of cell wall remodeling enzymes to loosen the cortex and the epidermis, thereby facilitating LRP growth and emergence (Swarup et al. 2008; Peret et al. 2013).

**Cytokinins and its function during LR development**

Phytohormone cytokinins play pivotal roles in various aspects of plant growth and development, including cell proliferation, cell differentiation and organogenesis, as well as in plant immunity and stress response (Hwang et al. 2012). Cytokinins are adenine derivatives with distinct substitutions attached to the N6 position of the adenine ring (Kieber & Schaller 2014). In *Arabidopsis*, N6-(Δ2-isopentenyl) adenine (iP) and trans-zeatin (tZ) free bases are known as major bioactive cytokinins (Sakakibara 2006). In addition, inactive cytokinin conjugates exist in the form of cytokinin ribosides, ribotides, and glucose derivatives (Sakakibara 2006). The initial step of cytokinin biosynthesis is N6-prenylation of adenosine 5′ -phosphates catalyzed by adenosine phosphate-isopentenyltransferase (IPT), which produces iP-riboside 5′ -phosphates (iPRPs; Kakimoto 2001; Takei et al. 2001). The trans-hydroxylation of the prenyl side chain of iPRPs is catalyzed by the cytochrome P450 enzymes CYP735A1 and CYP735A2, producing tZ-riboside 5′ -phosphates (tZRPs). The cytokinin-activating enzyme LONELY GUY (LOG) then converts iP- and tZ-riboside 5′-monophosphate to iP and tZ, respectively (Kurakawa et al. 2007; Kuroha et al. 2009).

In *Arabidopsis thaliana*, cytokinin signal transduction occurs via multi-step phosphotransfer system which consists of membrane-localized sensor kinase ARABIDOPSIS HISTIDINE KINASE (AHK), histidine-containing phosphotransfer protein ARABIDOPSIS HISTIDINE-CONTAINING PHOSPHOTRANSFER (AHP), and type-B ARABIDOPSIS RESPONSE REGULATOR (ARR) (Hwang et al. 2012; Kieber & Schaller 2014). Cytokinin receptors AHKs are hybrid kinases that contain the N-terminal histidine kinase domain and the C-terminal receiver domain. Cytokinin binding to the transmembrane CHASE domain of AHKs induces autophosphorylation at the histidine residue in the kinase domain, which subsequently triggers transfer of the phosphoryl group from the kinase domain to the aspartic acid residue in the receiver domain (Kieber & Schaller 2014). AHPs act downstream of AHKs to relay the phosphoryl group from AHKs to type-B ARRs. AHPs move from the cytosol to the nucleus and relay the phosphoryl group to nuclear-localized type-B ARRs. The phosphorylated type-B ARRs bind to the *cis* element in cytokinin-responsive genes and regulate their expression. Type-B ARRs are characterized by the N-terminal receiver domain, which perceives the phosphosignal from AHPs, and the large C-terminal extension consisting of Myb-like DNA binding domain (GARP domain), activation domain, and putative nuclear localization signal. The function of type-B ARRs is regulated by the interplay between the DNA binding domain and the receiver domain. The receiver domain negatively regulates the activity of type-B ARRs, whereas the phosphorylation of the receiver domain releases this inhibition (Kieber & Schaller 2014).

It has been known that exogenous cytokinin application results in fewer LRs,

while mutants or transgenic plants with reduced cytokinin level and signaling have an increased number of LRs (Li et al. 2006; Miyawaki et al. 2006; Mason et al. 2005; Werner et al. 2003; Laplaze et al. 2007). Fine-tuning of the expression of cytokinin biosynthesis genes in the LR founder cells regulates the distance between primordia (Chang et al. 2015). Cytokinins antagonize auxin function during LR development. While proper auxin gradient formation is essential not only for LR initiation but also for LR development and emergence (Benkova et al. 2003; Swarup et al. 2008), cytokinins negatively regulate LR development by interfering the transcription of the auxin efflux carrier *PIN* genes and by promoting lytic degradation of the PIN proteins, thereby disrupting the formation of an auxin gradient around LR founder cells and LRP (Laplaze et al. 2007; Marhavý et al. 2011). Moreover, cytokinins have an inhibitory effect on LRP development in a stage-dependent manner; namely, young primordium is more sensitive to cytokinins than that of advanced stages. In the early stage, auxin gradient is easily disrupted by exogenous cytokinin application. On the other hand, as the LRP develops into more advanced stages, stable and robust auxin gradient is getting established, thus LRP is less affected by cytokinins (Bielach et al. 2012). The role of cytokinins in regulating cell division and organogenesis by controlling the *PIN* expression has been a recurrent theme both in primary and lateral root (Dello loio et al. 2008; Pernisová et al. 2009; Ruzicka et al. 2009; Zhang et al. 2011; Marhavý et al. 2011).

**Objectives of the research**

DNA damage is relevant problem to plant productivity. For instance, aluminum

(Al) toxicity which severely affects crop production all over the world is known to cause DNA damage (Kochian et al. 2015; Larsen et al. 1997; Sjogren et al. 2015). Furthermore, environmental stresses are well known to cause over-accumulation of ROS, which is also detrimental to DNA integrity (Mittler et al. 2002; Apel & Hirt 2004). However, our understanding on how the DNA damage signal is interpreted by plant cell and manifested biologically as growth and developmental response is still very limited.

In this study, I found that DNA damage not only inhibited primary root growth but also suppressed LR formation, indicating that DNA damage response may regulate overall root system. Plant root system is determined by primary root growth and LR formation. Primary root growth is mainly driven by the cell division in the pre-existing root apical meristem, while LR development is a post-embryonic organogenesis event involving the formation of new meristem from a subset of pericycle cells in the primary root (Petricka et al. 2012; De Smet et al. 2006). LRs constitute the majority of plant root system and their formation is highly influenced by environmental factors, thus the control of LR formation hold a great importance in determining root system architecture in response to ever changing external conditions (Nibau et al. 2008; Benková et al. 2010). Given this pivotal role of LR formation and the relevance of DNA damage as a real problem to plant productivity, it is important to study the DNA damage response in LR formation. I aim to reveal the molecular mechanism of inhibition of LR formation in response to DNA damage and to understand the biological importance of such response during LR organogenesis.

In this study, I investigated the LR response of model plant *Arabidopsis*

*thaliana*. I revealed that DSB, DNA alkylation and crosslinks damage inhibits LR formation. Furthermore, I revealed that SOG1-dependent DNA damage signaling is involved in the induction of cytokinin biosynthesis genes. LR formation was less inhibited by DSBs in cytokinin biosynthesis and signaling mutants, suggesting that plants actively elevate cytokinin levels to inhibit LR formation in response to DNA damage. In parallel, SOG1-mediated signaling pathway induced DNA repair genes independently from that of cytokinin biosynthesis genes. Based on these findings, I propose that DNA damage signaling separately controls DNA repair and cytokinin level, thereby maintaining genome integrity during LR development.

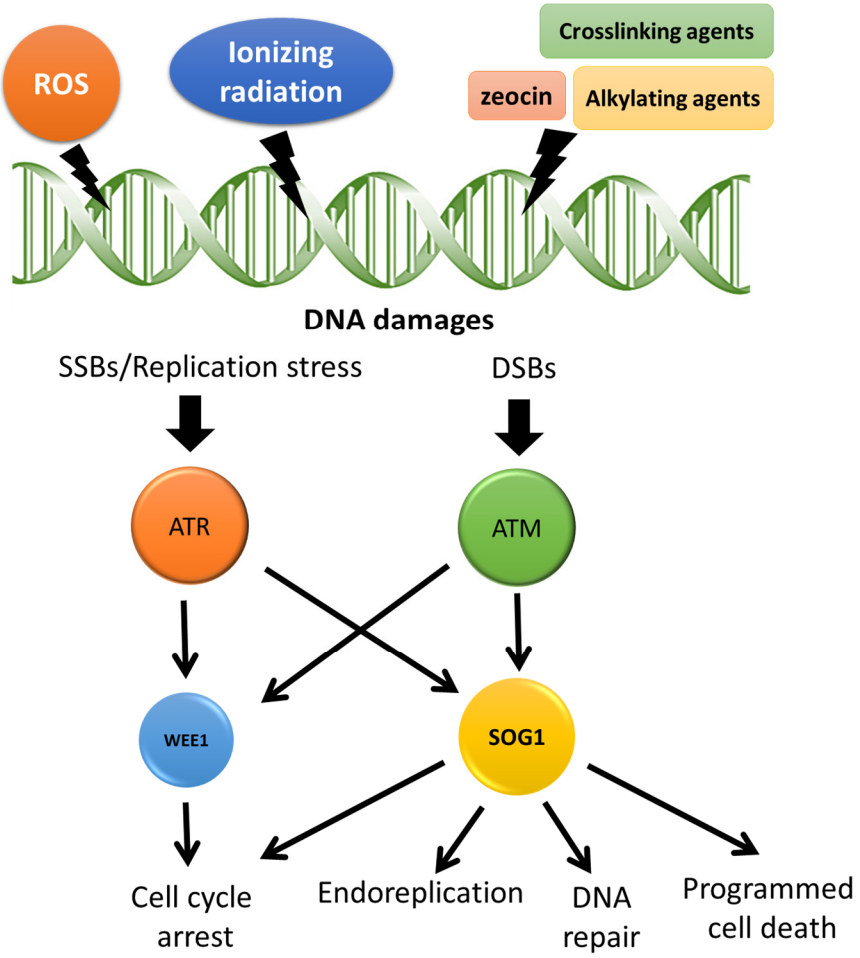



**Figure 1-1.** DNA damage response in plants

DNA damage activates DNA damage signaling. SSBs and replication stress activate ATR-dependent signaling, while DSBs activate ATM-mediated pathway. Both ATM and ATR upregulate WEE1 expression and in turn, WEE1 phosphorylates CDK to arrest cell cycle. ATM and ATR phosphorylation activate SOG1 transcription factor which is important for transcriptional regulation of downstream genes involved in cell cycle arrest, endoreplication, DNA repair and programmed cell death.

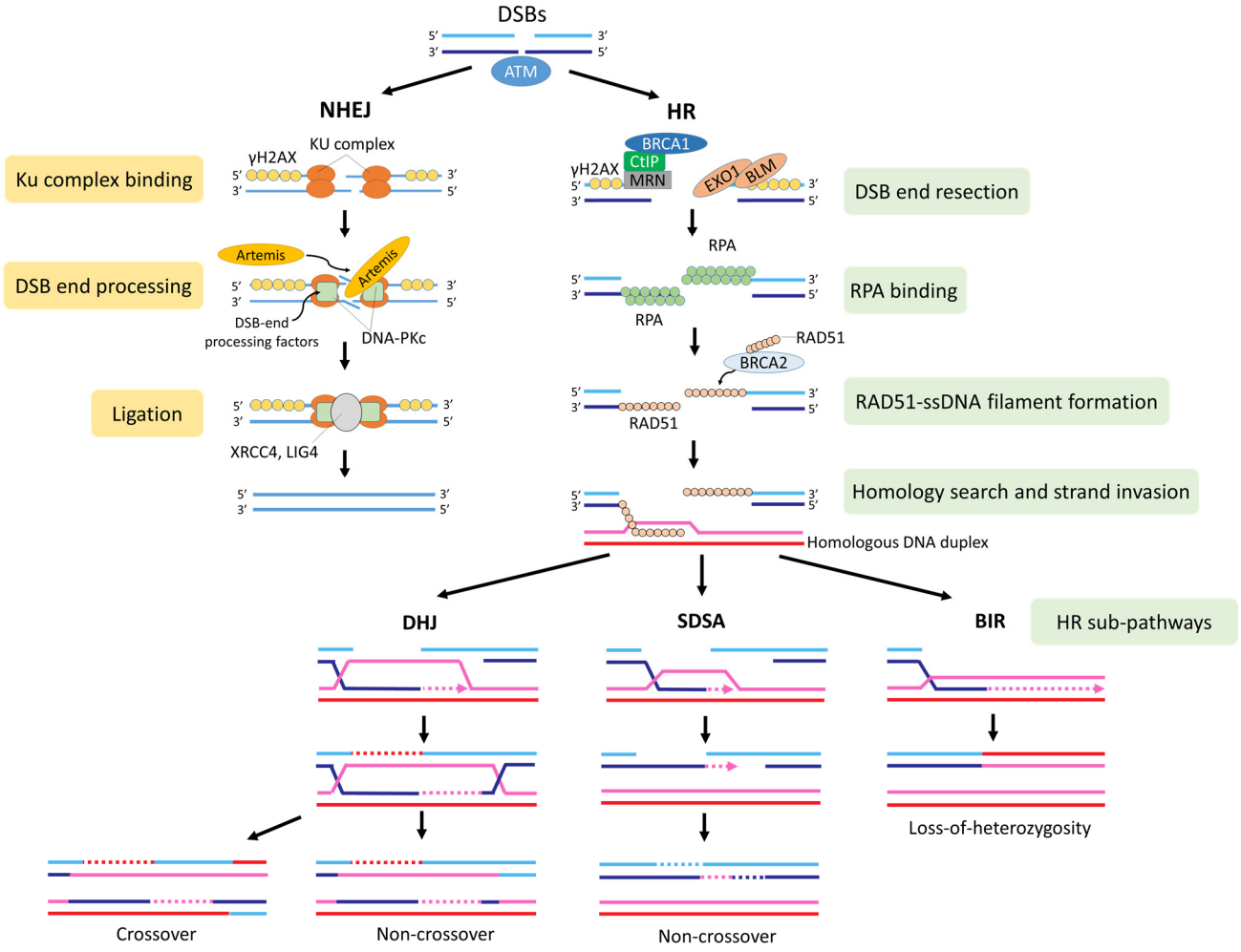



**Figure 1-2.** DSB repair pathway

DSBs induce ATM activation, and ATM phosphorylates H2AX, generating $\gamma$ H2AX foci that facilitate recruitment of multiple DNA repair proteins. DSBs are repaired through NHEJ or HR-mediated pathway. NHEJ pathway directly joins the broken ends of DSB without homologous sequences. In NHEJ pathway, DSB ends are bound and secured by KU complex. The DSB ends are then processed and ligated. In HR-mediated repair pathway, DSB ends are resected into 3'OH single-stranded overhangs. RPA binds and secures the ssDNA. BRCA2 mediates the replacement of RPA with RAD51 protein, leading to formation of RAD51-ssDNA nucleofilaments. RAD51 catalyzes homology search and strand invasion of homologous chromosome/sister chromatid. HR pathway branches into several routes, namely double holiday junction (DHJ), synthesis-dependent strand annealing (SDSA) and break-induced replication (BIR) sub-pathways. Figure is modified from Chowdury et al. 2013 and Heyer et al. 2010.

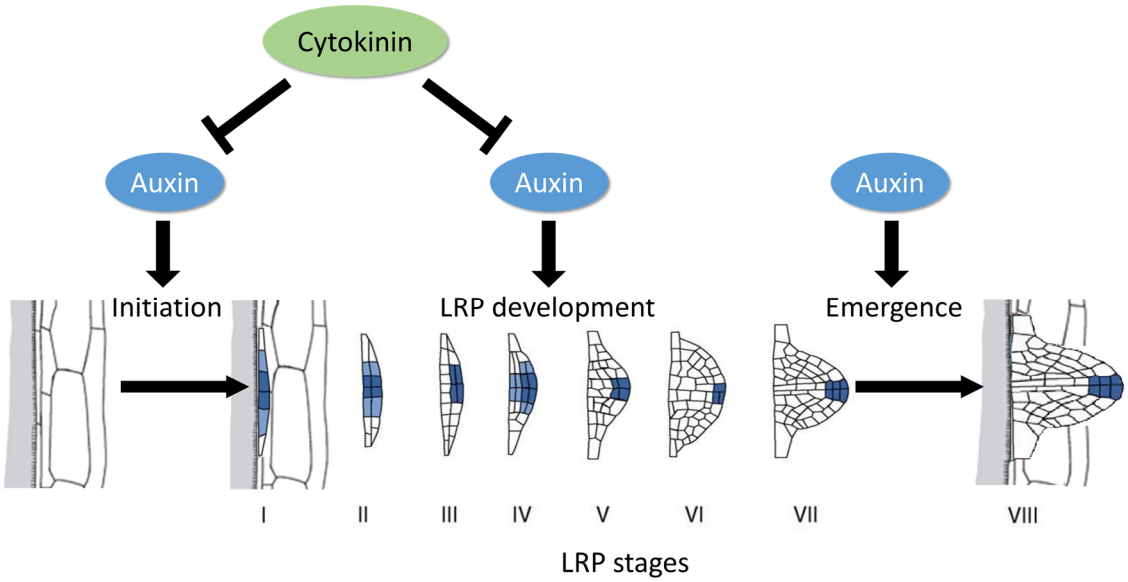



**Figure 1-3.** LR organogenesis

LR organogenesis in *Arabidopsis* consists of three major events, LR initiation, LRP development, and LR emergence. LRP initiated from a subset of pericycle cells undergoes a series of patterned cell divisions that progressively develop from stage I up to stage VIII primordium and eventually emerges as mature LR. Local auxin accumulation in LRP (indicated as blue color in this figure) is crucial for proper LR development. In contrast, cytokinin inhibits this process by affecting auxin accumulation. Figure is modified from Péret et al. 2009.

# Materials and Methods

## Plant growth conditions

*Arabidopsis thaliana* (ecotype Columbia-0) plants were grown vertically under continuous light conditions at 22°C on Murashige and Skoog (MS) plates (0.5 × MS salts, 0.5 g/L 2-(*N*-morpholino)ethanesulfonic acid (MES), 1% sucrose, and 1.2% phytoagar, pH 6.3). For DNA damage and cytokinin experiments, five-day-old seedlings were transferred onto medium containing DNA-damaging agents: zeocin (Invitrogen), bleomycin, MMC, cisplatin, HU (Wako), or MMS (Nacalai Tesque); or cytokinins: kinetin (Sigma) or benzyladenine (Wako).

## Plant materials and constructs

*tir1-1* (Ruegger et al. 1998), *sog1-1* (Yoshiyama et al. 2009), *atm-2* (Garcia et al. 2003), *atr-2* (Culligan et al. 2004), *ipt3-2;5-1;7-1* (Miyawaki et al. 2006), *arr1-3;12-1* (Mason et al. 2005), *35S::CKX1* (Werner et al. 2003), *ARR5::GUS* (D'Agostino et al. 2000), and *DR5::GUS* (Ulmasov et al. 1997) have been described previously.

The promoter sequence of *RAD51* was amplified from *Arabidopsis* genomic DNA by polymerase chain reaction (PCR) with 5′-AAAAAGCAGGCTTTAGCGTCAAGTAGTTGG-3′ and 5′-AGAAAGCTGGGTTTCTCTCAATCAGAGC-3′ primers and cloned into the pDONR™221 (Invitrogen) entry vector by BP recombination reaction according to the manufacturer's instructions (Invitrogen). To generate the *pRAD51:GUS* construct, the

entry clone was mixed with the pGWB3 destination vector (Nakagawa et al. 2009) and was subjected to LR recombination reaction according to the manufacturer's instructions (Invitrogen). All constructs were transferred into the *Agrobacterium tumefaciens* GV3101 strain harboring plasmid pMP90. The obtained strains were used to generate stably transformed *Arabidopsis* with the floral dip transformation method (Clough & Bent 1998).

**Quantitative RT-PCR**

Total RNA was extracted from *Arabidopsis* root with a Plant Total RNA Mini Kit (Favorgen Biotech Corp.). First-strand complementary DNA (cDNA) was prepared from total RNA with ReverTra Ace® (Toyobo) according to the manufacturer's instructions. For quantitative PCR, a THUNDERBIRD SYBR qPCR Mix (Toyobo) was used with 100 nM primers and first-strand cDNAs. PCR was performed on a LightCycler 480 Real-Time PCR System (Roche) with the following conditions: 95°C for 5 min; 45 cycles at 95°C for 10 sec, 60°C for 10 sec, and 72°C for 15 sec. *ACTIN2* (At3g18780) was used as a reference gene. Primer sequences are listed in Table 2-1.

**GUS staining**

Seedlings were incubated in GUS staining solution (100 mM sodium phosphate, 1 mg/mL 5-bromo-4-chloro-3-indolyl β-D-glucuronide, 0.5 mM ferricyanide, and 0.5 mM ferrocyanide [pH 7.4]) in the dark at 37°C. The samples were cleared with a clearing solution (chloral hydrate, glycerol, and water [8 g:1 mL:1 mL]) and observed under a

light microscope (Olympus).

**Root growth analysis**

For root growth experiments, seedlings were grown vertically on square plates. Root tips were marked on the plates every 24 h. The plates were photographed, and root growth was measured with ImageJ software (http://rsb.info.nih.gov/ij/) by calculating the distance between successive marks along the root axis.

**Table 2-1.** Primers used for qRT-PCR

| Genes | Primer sequences |
|---|---|
| *ACT2* | 5’- CTGGATCGGTGGTTCCATTC -3’ |
| | 5’- CCTGGACCTGCCTCATCATAC -3’ |
| *IPT2* | 5’- AGGCTCCTTCGTCGTCAA -3' |
| | 5’- CCATGATTCTTCAGATTTGCTTAATA -3' |
| *IPT3* | 5’- CGGGTTCGTGTCTGAGAGAG -3’ |
| | 5’- CTGACTTCCTCAACCATTCCA -3’ |
| *IPT5* | 5’- AGTTACAGCGATGACCACCA -3’ |
| | 5’- GGCAGAGATCTCCGGTAGG -3’ |
| *IPT7* | 5’- ACTCCTTTGTCTCAAAACGTGTC -3’ |
| | 5’- TGAACACTTCTCTTACTTCTTCGAGT -3’ |
| *IPT9* | 5’- TGGATTGTATCTGCGATGGTT -3’ |
| | 5’- TGGGCCTCAGCGATAACTT -3’ |
| *LOG1* | 5’- GAACTCGGAACCGAACTGG -3’ |
| | 5’- TCAAACCCATTAAACCAATGC -3’ |
| *LOG2* | 5’- TTTGAAGAGTTGTTGGAAGTCATC -3’ |
| | 5’- TCCATCCACGTTCAATAGTCC -3’ |
| *LOG3* | 5’- TGATGCTTTTATTGCCTTACCA -3’ |
| | 5’- CCACCGGCTTGTCATGTAT -3’ |
| *LOG4* | 5’- GTTTGATGGGTTTGGTTTCG -3’ |
| | 5’- CACCGGTCAACTCTCTAGGC -3’ |
| *LOG5* | 5’- ATGGGTTTGGTCTCACAAGC -3’ |
| | 5’- CTCCGGTTATCTCTTTGTCCA -3’ |
| *LOG6* | 5’- CAATGGGAACAAAGCTAGTTATCAA -3’ |
| | 5’- AAGATCAATCTTCCTCATCATCACCA -3’ |

| | |
|---|---|
| *LOG7* | 5’- CATGTTCTAGGGGTCATTCCA -3’ |
| | 5’- CTCCGATGGTCTCACCAGTT -3’ |
| *LOG8* | 5’- ATTGCACTCCCTGGAGGTTA -3’ |
| | 5’- CCCATCAACATTCAATAGACCA -3’ |
| *CYP735A1* | 5’- GGCCTTCCCTCAGTCGAT -3’ |
| | 5’- TTCAAATGCCATCCTTGGTAG -3’ |
| *CYP735A2* | 5’- GAACAGCTCTCAAGTCTTACTTCGT -3’ |
| | 5’- TCAAATGCCATTCTTGGTAAAA -3’ |
| *RAD17* | 5’- CTAGTGCGACTCAAGAAGAC -3’ |
| | 5’- GCCTGTATTTGTCAACCCAC -3’ |
| *RAD51* | 5’- GATCACGGGAGCTCGATAAA -3’ |
| | 5’- GCGGAACTCACCATATAACTCTG -3’ |
| *BRCA1* | 5’- TCTTGCTCAGGGCTCACAGTTGAAG -3’ |
| | 5’- TTTCCCCTCCAAGATTGCCATCATG -3’ |
| *PARP2* | 5’- AGCCTGAAGGCCCGGGTAACA -3’ |
| | 5’- GCTGTCTCAGTTTTGGCTGCCG -3’ |

# Results

## DNA damage inhibits LR formation

To observe the response of LRs to DNA damage, I treated *Arabidopsis* seedlings with the radiomimetic reagent zeocin, which causes DSBs (Huang et al. 1981). When 5-day-old seedlings were transferred onto zeocin-containing medium and grown for seven days, primary root growth was retarded (Figure 3-1 A, B) as reported previously (Adachi et al. 2011), but LR formation was also inhibited compared with that in the untreated control (Figure 3-1 C). The reduction in LR density (the number of emerged LRs per primary root length) was dependent on zeocin concentration (Figure 3-1 D). This result indicates that DSBs inhibit LR formation.

LR is developed from LRP through a series of cell divisions according to well-defined processes (Casimiro et al. 2003; Péret et al. 2009). After its initiation, LRP rapidly develops through successive cell divisions. The first three rounds of periclinal division form LRPs at stage II, III and IV, which are characterized by two, three and four cell layers, respectively (Malamy & Benfey 1997). Subsequent periclinal, tangential, and anticlinal divisions generate dome-shaped LRP at stage V that progressively penetrates into the endodermis and the cortex (Malamy & Benfey 1997). From stage VI onward, LRP already possesses a putative vascular cell layer surrounded by three outer layers, which correspond to the epidermis, the cortex, and the endodermis (Malamy & Benfey 1997). Transition from stage VII to VIII is primarily driven by cell expansion, and thereafter, LRs emerge from the epidermis of the primary root (Malamy & Benfey 1997).

To gain insight into how DSBs affect LRP development, I observed the distribution of each LRP stage after zeocin treatment (Figure 3-2). In the absence of zeocin, more than 50% of the observed events were in emerged stage. Zeocin treatment reduced the ratio of emerged LR by half, while that of LRP at the first stage increased more than twofold (Figure 3-2). Furthermore, frequency of LR initiation was reduced upon zeocin treatment (data not shown). These results indicate that DSBs inhibit the progression of LRP development at stage I and LR initiation, while about 80% of LRP still progress into later stages.

To examine whether other types of DNA damage also inhibit LR formation, seedlings were treated with bleomycin, methyl methanesulfonate (MMS), cisplatin, mitomycin C (MMC), or hydroxyurea (HU). Bleomycin, a radiomimetic drug belonging to the same class as zeocin, causes DSBs (Povirk 1996). MMS is an alkylating agent that methylates guanine and adenine bases, causing base mispairing and replication blocks (Beranek 1990; Llorente et al. 2008). MMC generates interstrand cross-links on DNA, whereas cisplatin preferentially forms intrastrand cross-links (Eastman 1985; Rink et al. 1996). HU inhibits deoxyribonucleotide production, thereby causing stalled replication fork (Wang & Liu 2006; Saban & Bujak 2009). These DNA damaging agents inhibited primary root growth in a concentration-dependent manner (Figure 3-3). Interestingly, bleomycin, MMS, cisplatin, and MMC inhibited LR formation (Figure 3-4 A–D), whereas HU treatment had no significant effect on LR density although primary root growth was retarded (Figure 3-3 E, Figure 3-4 E). These results suggest that DSBs, DNA alkylation, and DNA cross-links, but not DNA replication stress, inhibit LR formation.

**DNA damage up-regulates cytokinin signaling in LRP**

Phytohormone auxin and cytokinins antagonistically regulate LR development. Auxin promotes LR initiation and LRP development, whereas cytokinin inhibits those processes (Lavenus et al. 2013; Laplaze et al. 2007; Marhavy et al. 2011). I therefore investigated the involvement of these two phytohormones in LR inhibition in response to DNA damage. As for auxin, I assessed LR response of the *TIR1*-defective mutant. TIR1 is an F-box protein that perceives auxin and functions in complex with an SCF E3 ligase to promote proteasome-mediated degradation of the auxin signaling repressor Aux/IAAs, thereby activating auxin signaling (Wang & Estelle 2014). The *tir1* mutant showed less LR density compared with the wild-type under control conditions (Figure 3-5 B). Following zeocin treatment, *tir1* showed even more reduction in LR density compared with that of wild-type (Figure 3-5 A, B). For instance, upon 5 μM zeocin treatment, the LR density in wild-type was only reduced by 59%, while that of *tir1* was more severely reduced by 73%, indicating that *tir1* is hypersensitive to zeocin (Figure 3-5 B). This result suggests that auxin plays a role in LR inhibition in response to DNA damage.

I then examined the effects of cytokinins on LR formation. When wild-type seedlings were treated with bioactive cytokinins, such as 300 nM kinetin or 40 nM benzyladenine (BA), LR density significantly decreased (Figure 3-6), supporting previous observations (Laplaze et al. 2007). Detailed analysis of LRP development showed that compared with the untreated control, cytokinin-treated seedlings showed a greater than twofold increase in the ratio of LRP arrested at stage I (Figure 3-6 C). Note

that this tendency is highly similar to that of zeocin-treated seedlings (Figure 3-6), suggesting that DNA damage inhibits LR formation by modulating cytokinin signaling.

To examine whether the cytokinin signaling is altered in LRP after DNA damage, I observed the expression of the cytokinin signaling marker gene *ARR5*. It has been shown that cytokinin treatment activates the GUS reporter gene expression driven by the *ARR5* promoter (D'Agostino et al. 2000). As shown in Figure 3-7, *pARR5::GUS* expression was detected in the central cylinder, but not in LRP, in the absence of zeocin. However, zeocin treatment dramatically increased GUS expression in LRP, indicating that the cytokinin signaling is enhanced in LRP in response to DNA damage.

**DNA damage actively induces cytokinin biosynthesis genes**

Although cytokinin signaling was activated in LRP under DNA damage, previous microarray data showed that the expression of genes involved in cytokinin signal transduction was not altered by DSBs-inducing gamma ray irradiation (Table 3-1). Therefore, I hypothesized that cytokinin biosynthesis is up-regulated in response to DNA damage. I used qRT-PCR to measure the expression levels of cytokinin biosynthesis genes *IPT*s (*IPT2*, *3*, *5*, *7*, *9*), *LOG*s (*LOG1*, *2*, *3*, *4*, *5*, *6*, *7*, *8*) and *CYP735A*s (*CYP735A1*, *2*). The results showed that transcript levels of *IPT2*, *IPT7*, *IPT9*, *LOG2*, *LOG3*, *LOG4*, *LOG5*, *LOG7*, and *LOG8* were increased in roots after treatment with 5 μM or 10 μM zeocin (Figure 3-8).

ATM is activated by DSBs, whereas ATR responds to SSBs and DNA replication stress (Garcia et al. 2003; Culligan et al. 2004). ATM and ATR then

phosphorylate and activate the plant-specific transcription factor SOG1 (Yoshiyama et al. 2013; Sjogren et al. 2015). The ATM-SOG1 pathway regulates hundreds of genes involved in cell cycle arrest, stem cell death, early onset of endoreplication, and DNA repair (Culligan et al. 2006; Yoshiyama et al. 2009). To determine whether the SOG1-mediated pathway is required for upregulation of cytokinin biosynthesis genes, I examined the expression of *IPT2*, *IPT7*, *IPT9*, *LOG3*, and *LOG4* which are upregulated in wild-type upon zeocin treatment, in the *sog1-1* mutant carrying a missense mutation in the NAC domain (Yoshiyama et al. 2009). The qRT-PCR results showed that the induction of the expression of these genes by zeocin treatment was suppressed in *sog1-1* (Figure 3-9 A, B), indicating that the induction of cytokinin biosynthesis genes is a programmed response to DNA damage through the SOG1-dependent pathway.

Induction of cytokinin biosynthesis genes leads to an increase in cytokinin level (Sun et al. 2003; Sakakibara et al. 2005). Indeed, measurement of the content of bioactive cytokinins, iP and tZ, revealed that zeocin treatment significantly increased the iP level in wild-type, whereas such an increase was absent in *sog1-1* (Supplemental Figure 3-1; data was taken by Dr. H. Sakakibara in RIKEN). Altogether, these results indicate that DNA damage actively upregulates cytokinin biosynthesis in the root tip.

**Elevated cytokinin signaling is crucial for the inhibition of LR formation in response to DNA damage**

To determine whether the activation of cytokinin signaling is involved in the inhibition of LR formation under DNA damage conditions, I observed the zeocin response

of plants defective in cytokinin biosynthesis or signaling. *ipt3-2;5-1;7-1* has mutations in three major *IPT* genes, and is known to produce a very low amount of cytokinins (Miyawaki et al. 2006). I used this mutant because *IPT7* is one of the cytokinin biosynthesis genes induced by zeocin treatment (Figure 3-8, Figure 3-9). As a cytokinin signaling mutant, *arr1-3;12-1* was used in which type-B response regulators ARR1 and ARR12 are defective, thus signaling through the two-component pathway is weakened (Mason et al. 2005). I also used a transgenic line overexpressing cytokinin oxidase-dehydrogenase 1 (*CKX1*) under the cauliflower mosaic virus 35S promoter (*35S::CKX1*), in which endogenous active cytokinins are actively degraded by CKX1 (Werner et al. 2003). As shown in Figure 3-10, reduction of LR density in response to zeocin treatment was observed less frequently in *ipt3-2;5-1;7-1, arr1-3;12-1*, and *35S::CKX1* than in the wild-type. In *ipt3-2;5-1;7-1* and *35S::CKX1*, LR density was not reduced further at zeocin concentrations higher than 2.5 μM (Fig. 3-10 D, E). However, in *arr1-3;12-1*, it was reduced dependently on zeocin concentration up to 7.5 μM (Fig. 3-10 F). *Arabidopsis* possesses eleven type-B response regulators, and at least several of them have similar activities in cytokinin signaling (Hill et al. 2013). Therefore, it is likely that not only ARR1 and ARR12 but also other type-B response regulators are involved in transmitting the cytokinin signal enhanced by DNA damage. As a result, compared with *ipt3-2;5-1;7-1* and *35S::CKX1*, *arr1-3;12-1* displays more sensitive phenotype to zeocin. Overall, my results indicate that an increase in cytokinin production and subsequent signaling is crucial for the inhibition of LR formation in response to DNA damage.

**Cell division during LR formation is severely inhibited in *sog1* and *atm* mutants**

I have shown that the upregulation of cytokinin biosynthesis genes is an active response to DSBs in roots. However, it is still unknown whether inhibition of LR formation in zeocin-treated wild-type plants is an outcome of this active response to DNA damage. As mentioned above, DNA damage signaling in plants is mediated by the ATM/ATR-SOG1 pathway. Therefore, to reveal whether the inhibition of LR formation is a programmed response to DSBs, I first observed the LR response of *sog1-1* mutant to zeocin treatment. Surprisingly, compared with the wild-type, *sog1-1* showed hypersensitivity to zeocin in LR formation (Figure 3-11 A, B), whereas primary root growth was tolerant to zeocin (Figure 3-12) as reported previously (Adachi et al. 2011).

Even though zeocin-induced inhibition of LR formation is an active response to DNA damage, the *sog1-1* mutation might cause the accumulation of DNA damage, thereby masking suppression of the LR phenotype. To gather hints about this scenario, I observed the distribution of LRP stages in wild-type and *sog1-1*. In wild-type seedlings, zeocin treatment reduced the ratio of emerged LR by half, while that of LRP at the first stage increased more than twofold (Figure 3-13 A), indicating that even though LR development was inhibited at early stages, many LRP can develop into mature stage. By contrast, most LRP formation was arrested from stage I to IV in *sog1-1* mutant, and no emerged LR was observed (Figure 3-13 B). This result suggests that zeocin treatment severely inhibits cell division for LRP formation in *sog1-1*. Indeed, when *sog1-1* was treated with zeocin, periclinal and anticlinal divisions did not occur uniformly during LRP development (Figure 3-14). The cells constituting the zeocin-treated LRP in *sog1-1* were

irregular in size and shape. Furthermore, the boundaries between each cell layers in those primordia are not clearly defined, resulting in abnormally shaped LRP (Figure 3-14).

I also observed the response of *atm-2* and *atr-2* knockout mutants to zeocin. Similar to *sog1-1*, *atm-2* showed zeocin-induced arrest of LRP development at early stages (Figure 3-13 C). On the contrary, I observed emerged LR in *atr-2* mutant (Figure 3-13 D), suggesting that cell division was not inhibited as severely as that in *sog1-1* or *atm-2* mutants. In contrast to the wild-type, zeocin treatment increased the ratio of LRP at stage III to VIII, rather than stage I, in *atr-2*, implying that ATR may be participated in the inhibition of LRP development upon DNA damage.

**SOG1-mediated control of genome integrity is important for LR formation**

The above results indicate that zeocin-induced DSBs severely inhibit cell division during LRP development in *sog1-1* and *atm-2* mutants. Since DSB signals are transmitted through the ATM–SOG1 pathway (Yoshiyama et al. 2013), DNA damage may be highly accumulated in *atm-2* and *sog1-1* mutants, thereby inhibiting cell division. To test this hypothesis, expression of a DNA repair gene was monitored in LRP. RAD51 participates in DSBs repair via HR, and its expression is up-regulated by DNA damage (Osakabe et al. 2002; Abe et al., 2005). In wild-type seedlings carrying the *pRAD51::GUS* reporter gene, zeocin treatment increased β-D-glucuronidase (GUS) expression in LRP (Figure 3-15 A). By contrast, a trace level of GUS expression was observed in *sog1-1* even in the presence of zeocin (Figure 3-15 A). Measurement of *RAD51* transcripts with quantitative reverse transcription-polymerase chain reaction (qRT-PCR) supported the

results of the GUS expression experiments (Figure 3-15 B, C). Similarly, the expression of other DNA repair genes, *BRCA1, RAD17*, and *PARP2* (Block-Schmidt et al. 2011; Heitzeberg et al. 2004; Jia et al. 2013), was induced by DNA damage in wild-type but not significantly in *sog1-1* (Figure 3-15 B, C). These results suggest that ATM-activated SOG1 is essential for the induction of DNA repair genes, thus maintaining genome integrity during LRP development. Note that primary root growth in *sog1-1* was not inhibited, rather faster than that in the wild-type, in the presence of zeocin (Figure 3-12), suggesting that compared with the LRP, the primary root meristem is more resistant to accumulating DNA damage for unknown reasons.

To examine whether the zeocin-treated LRPs still retain the ability to develop into LRs, 2.5 or 5 μM zeocin-treated seedlings were transferred onto medium without zeocin and measured the number of emerged LR. In wild-type, LR density was gradually increased after transfer to zeocin-free medium; about 20 % increase in LR density was observed within five days after transfer (Figure 3-16 A). In *sog1-1* mutant, however, LR formation was not recovered within five days (Figure 3-16 B), suggesting that irregularly formed LRP in *sog1-1* could not restart cell division even in the absence of zeocin. These data indicate that SOG1-mediated maintenance of genome integrity plays a key role in preserving LRPs.

While *sog1-1* was hypersensitive to zeocin, cytokinin-related mutants were tolerant to zeocin (Figure 3-10) although cytokinin biosynthesis genes are induced via the SOG1-mediated pathway (Figure 3-9). To resolve this paradox, I hypothesized that SOG1 differentially regulates genes for DNA repair and cytokinin biosynthesis, and that

enhanced cytokinin production inhibits LR formation but not affects DNA repair. I tested this hypothesis by measuring the expression levels of DNA repair genes in *ipt3-2;5-1;7-1*. As shown in Figure 3-17, *RAD51*, *BRCA1*, *PARP2*, and *RAD17* were induced by zeocin treatment to a similar extent as that in wild-type. This result suggests that, in cytokinin-related mutant, damaged DNA is properly repaired, thereby exhibiting a tolerant phenotype to zeocin.

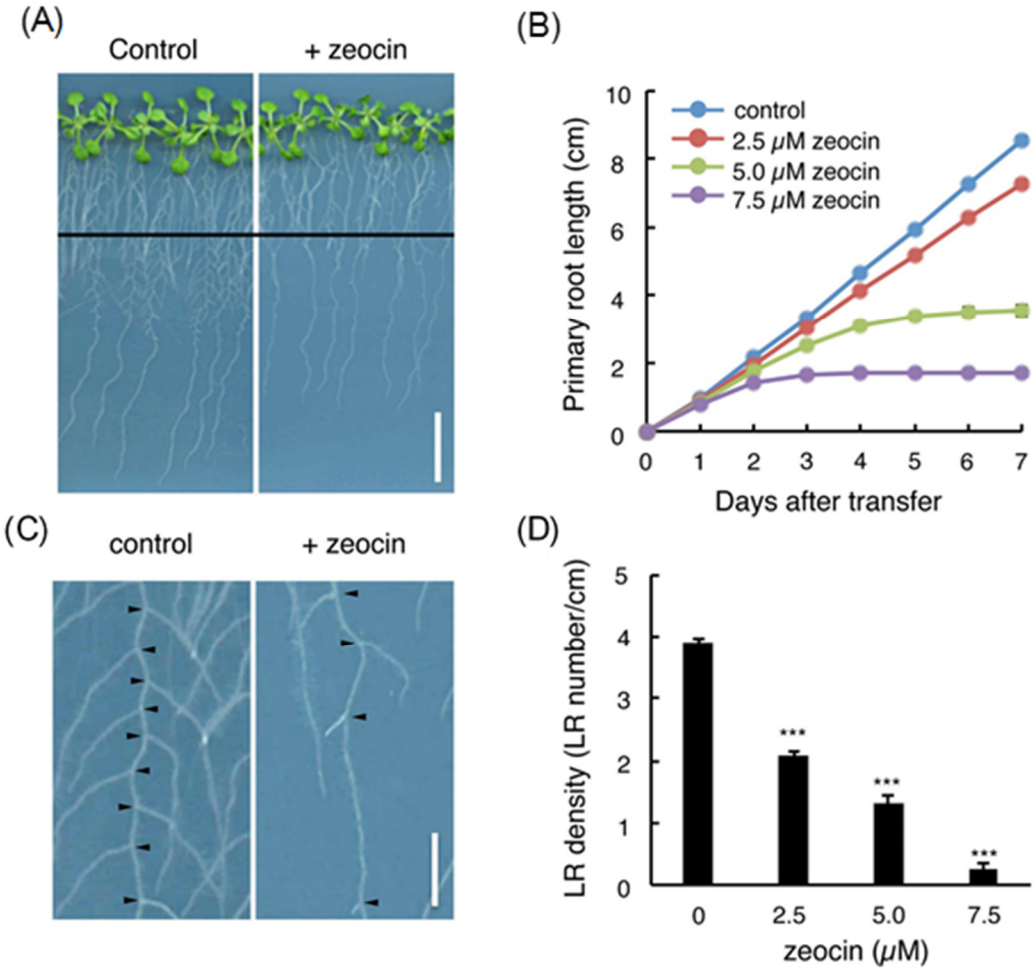



**Figure 3-1.** DNA damage inhibits LR formation.

(A) wild-type *Arabidopsis thaliana* (Col-0) seedlings grown in the absence or presence of zeocin. Five-day-old seedlings were transferred onto control medium (control) or medium containing 2.5 μM zeocin (+ zeocin) and grown for an additional seven days. Black lines indicate the positions of the root tips when the seedlings were transferred onto each medium. Bar = 2 cm. (B) Primary root growth of wild-type seedlings grown on the media containing the indicated concentration of zeocin. (C) LR phenotype of wild-type seedlings grown on control medium (control) or medium containing 2.5 μM zeocin (+ zeocin). Arrowheads indicate the positions of the LR along the primary roots. Bar = 0.5 cm. (D) LR density of wild-type seedlings grown on the media containing the indicated concentration of zeocin. Data are presented as means ± standard error (SE; n > 20). Significant differences between treated seedlings and the untreated control were determined with the Student's *t*-test: ***, $P < 0.001$.

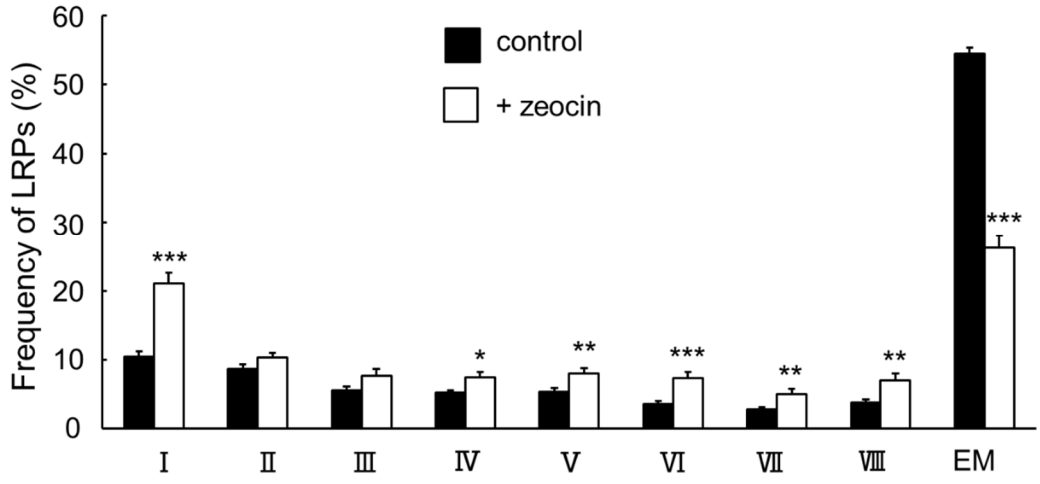



**Figure 3-2.** Stage distribution of LRP in response to zeocin treatment

Five-day-old *DR5::GUS* seedlings were transferred onto control medium or medium containing 2.5 μM zeocin (+ zeocin) and grown for an additional seven days. Data are presented as means ± SE (n > 10). Significant differences between treated seedlings and the untreated control were determined with the Student's *t*-test: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

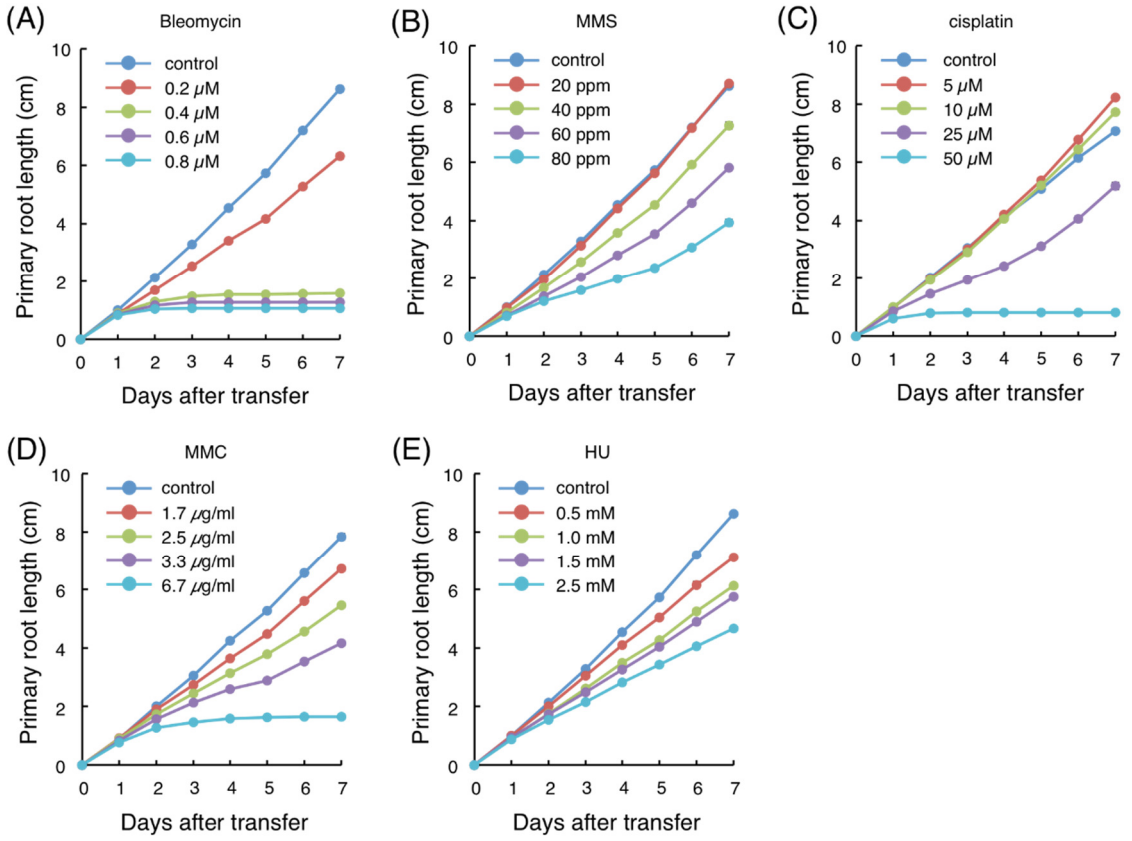



**Figure 3-3.** Growth of primary root treated with various DNA damaging agents.

Five-day-old wild-type seedlings were transferred onto control medium or medium containing the indicated concentrations of bleomycin (A), methyl methanesulfonate (MMS) (B), cisplatin (C), mitomycin C (MMC) (D), or hydroxyurea (HU) (E), and primary root length was measured. Data are presented as means ± SE (n = 14).

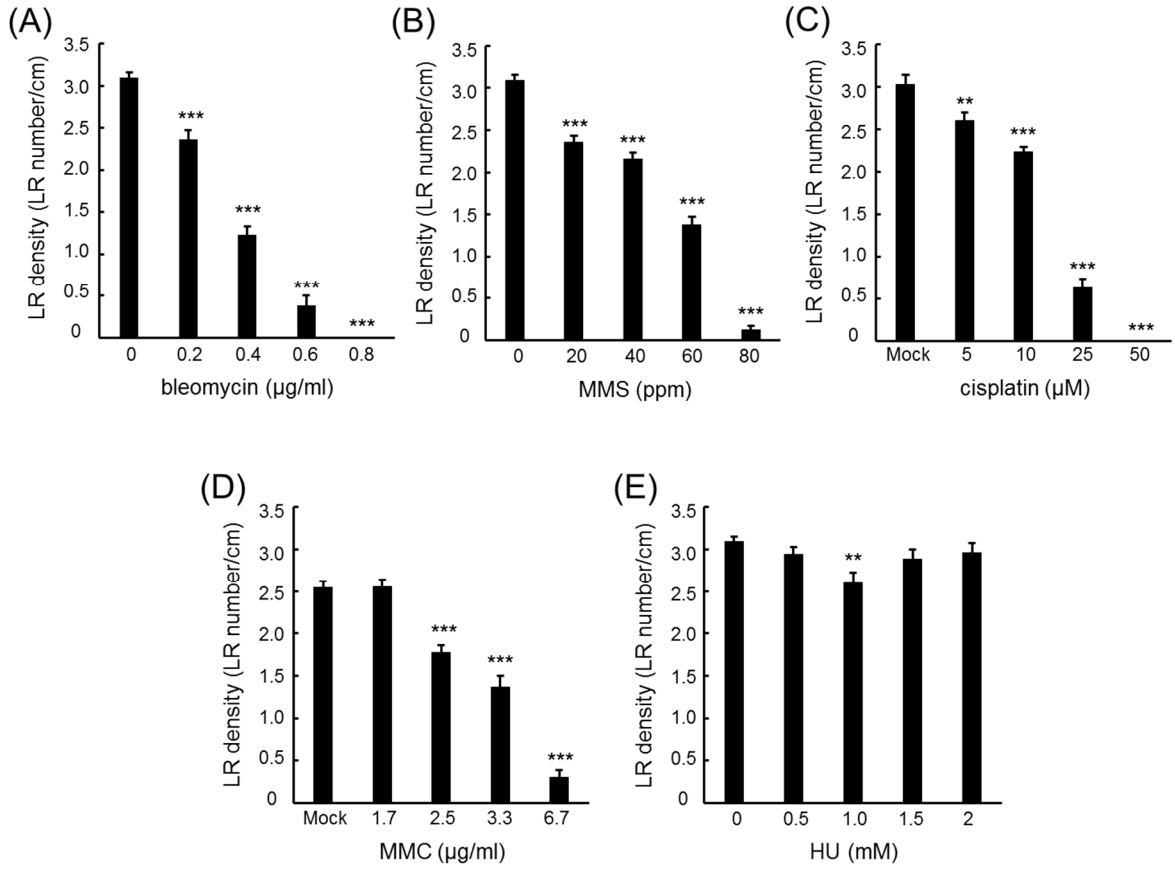



**Figure 3-4.** DNA double-strand breaks, alkylation, and cross-links, but not DNA replication stress, inhibit LR formation.

LR density after transfer onto medium containing various DNA-damaging agents. Five-day-old seedlings were transferred onto control medium or medium containing the indicated concentrations of bleomycin (A), methyl methanesulfonate (MMS) (B), cisplatin (C), mitomycin C (MMC) (D), or hydroxyurea (HU) (E) and grown for an additional seven days. Data are presented as means ± SE (n = 14). Significant differences between treated seedlings and the non-treated control were determined with the Student's t-test: **, *P < 0.01;* ***, *P < 0.001.*

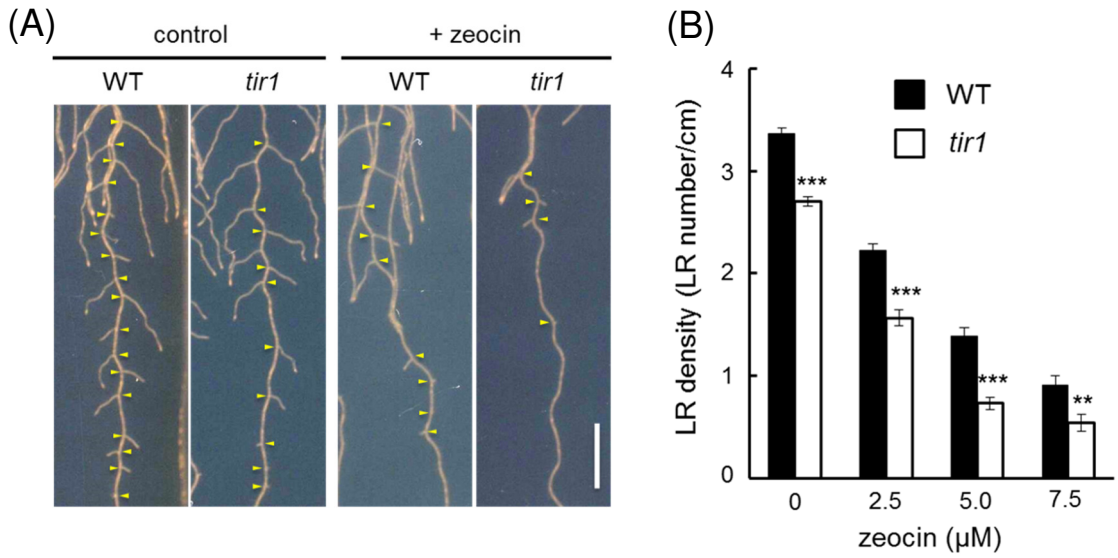



**Figure 3-5.** *tir1* mutant is hypersensitive to zeocin

(A) LR phenotype of *tir1* mutant seedlings. Five-day-old wild-type (WT) and *tir1* eedlings were transferred onto control medium (control) or medium containing 2.5 μM zeocin (+ zeocin), and grown for an additional seven days. Arrowheads indicate the positions of the LR along the primary roots. Bar = 0.5 cm. (B) LR density of WT and *tir1* after transfer onto control medium or medium containing the indicated concentrations of zeocin. Data are presented as means ± SE (n > 20). Significant differences between treated seedlings and the WT control were determined with the Student's *t*-test: **, $P < 0.01$; ***, $P < 0.001$.

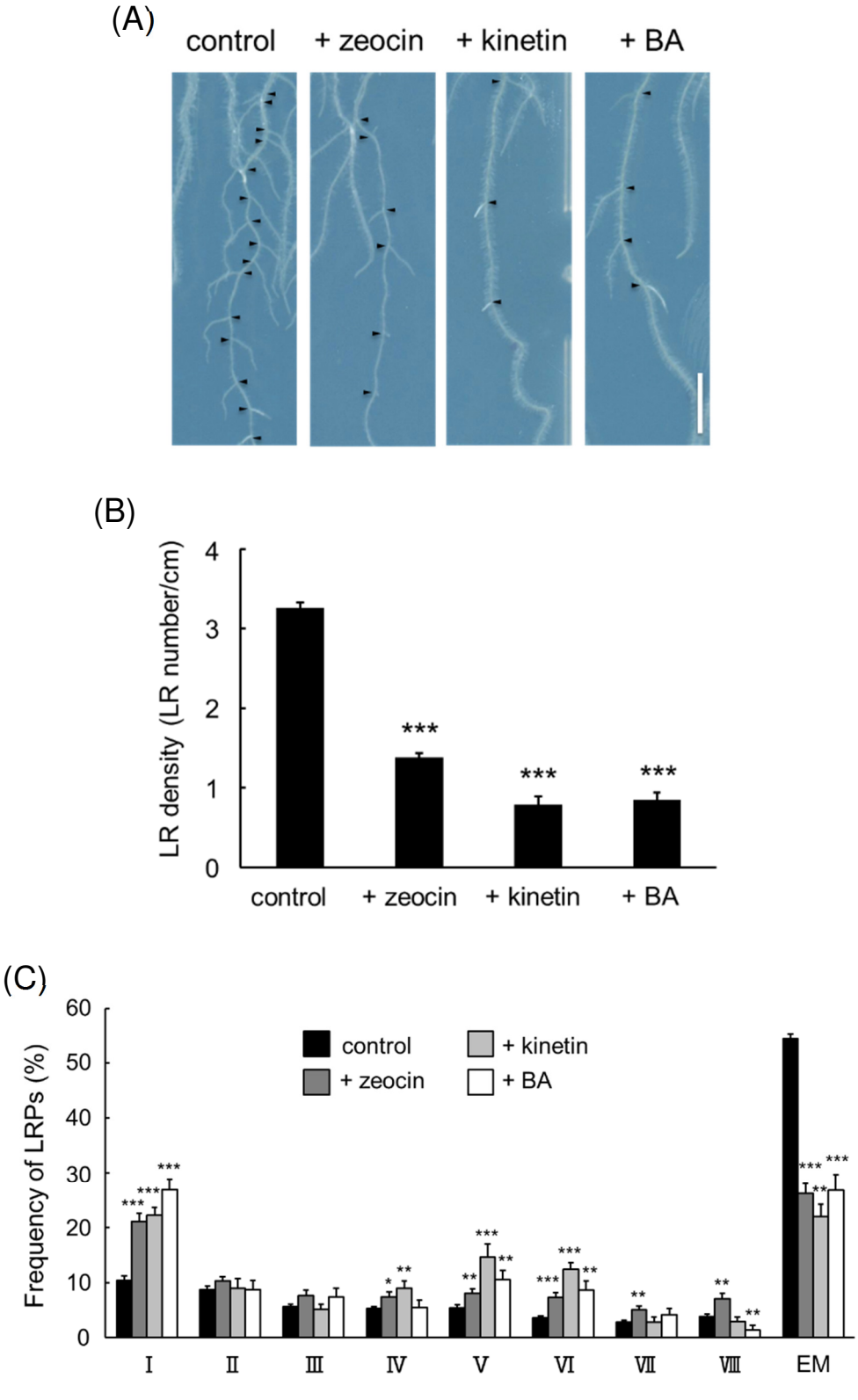

(A)
control
+ zeocin
+ kinetin
+ BA
(B)
LR density (LR number/cm)
0
1
2
3
4
***
***
***
control
+ zeocin
+ kinetin
+ BA
(C)
Frequency of LRPs (%)
0
10
20
30
40
50
60
control
+ zeocin
+ kinetin
+ BA
I
II
III
IV
V
VI
VII
VIII
EM

**Figure 3-6.** Cytokinin inhibit lateral root formation

(A) LR phenotype of zeocin- or cytokinin-treated seedlings. Five-day-old wild-type seedlings were transferred onto control medium (control) or medium containing 2.5 µM zeocin (+ zeocin), 300 nM kinetin (+ kinetin), or 40 nM benzyladenine (+ BA) and grown for an additional seven days. Arrowheads indicate the positions of the LR along the primary roots. Bar = 0.5 cm. (B) LR density after transfer onto control medium or medium containing 2.5 µM zeocin (+ zeocin), 300 nM kinetin (+ kinetin), or 40 nM benzyladenine (+ BA). Data are presented as means ± SE (n > 20). Significant differences between treated seedlings and the untreated control were determined with the Student's *t*-test: ***, $P < 0.001$. (C) Stage distribution of LRP in response to zeocin and cytokinin treatments. Five-day-old *DR5::GUS* seedlings were transferred onto control medium (control) or medium containing 2.5 µM zeocin (+ zeocin), 300 nM kinetin (+ kinetin), or 40 nM benzyladenine (+ BA) and grown for an additional seven days. Data are presented as means ± SE (n > 15). Significant differences between treated seedlings and the non-treated control were determined with the Student's *t*-test: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

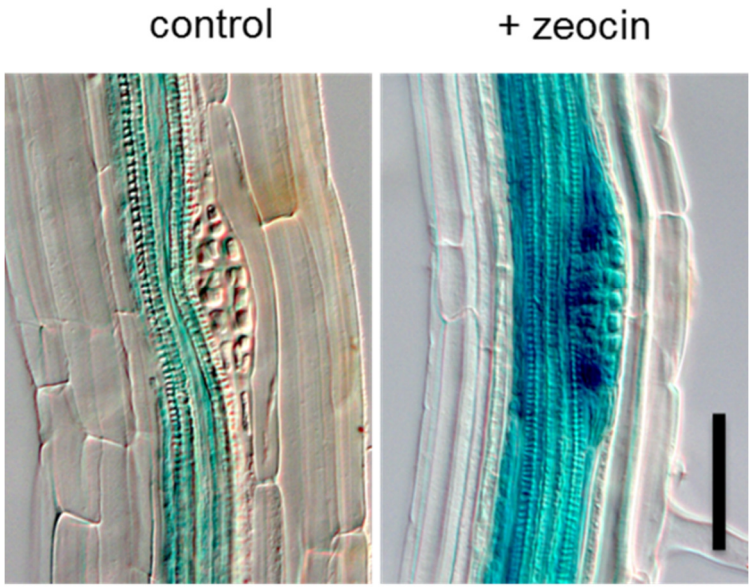



**Figure 3-7.** Cytokinin signaling is up-regulated in LRP in response to DNA damage.

LRP of wild-type seedlings harboring *pARR5:GUS*. Five-day-old seedling were transferred onto control medium (control) or medium containing 5 µM zeocin (+ zeocin), and GUS staining was conducted after 7 days. Bar = 50 µm.

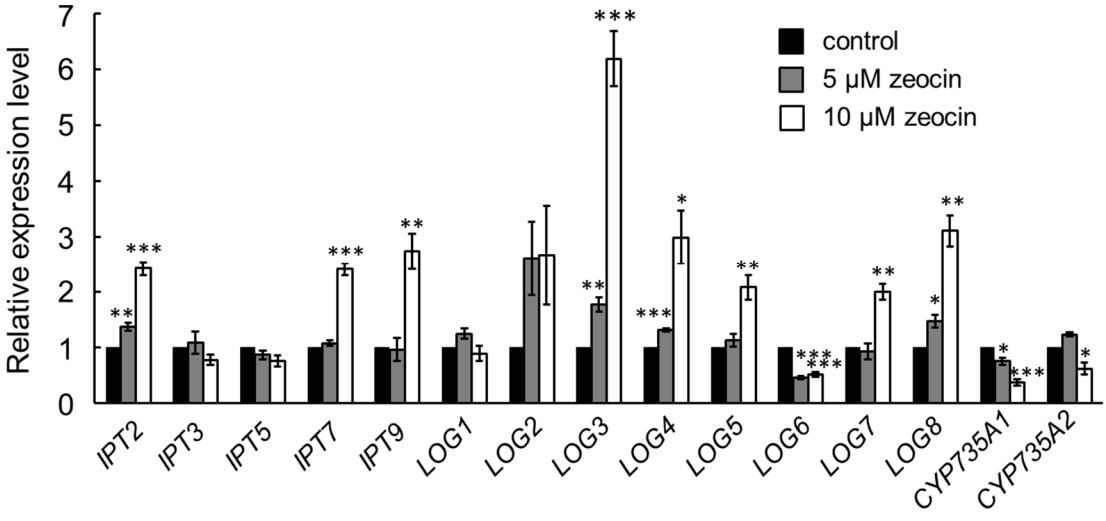



**Figure 3-8.** DNA damage induces cytokinin biosynthesis genes.

Transcript levels of cytokinin biosynthesis genes in roots. Five-day-old wild-type seedlings were transferred onto control medium or medium containing 5 or 10 μM zeocin for seven days. Total RNA was extracted from roots. The mRNA levels were normalized to that of *ACTIN2*, and are indicated as relative values, with that of the control set to 1. Data are presented as means ± SE (n = 3). Significant differences between treated seedlings and the control were determined with the Student's *t*-test: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

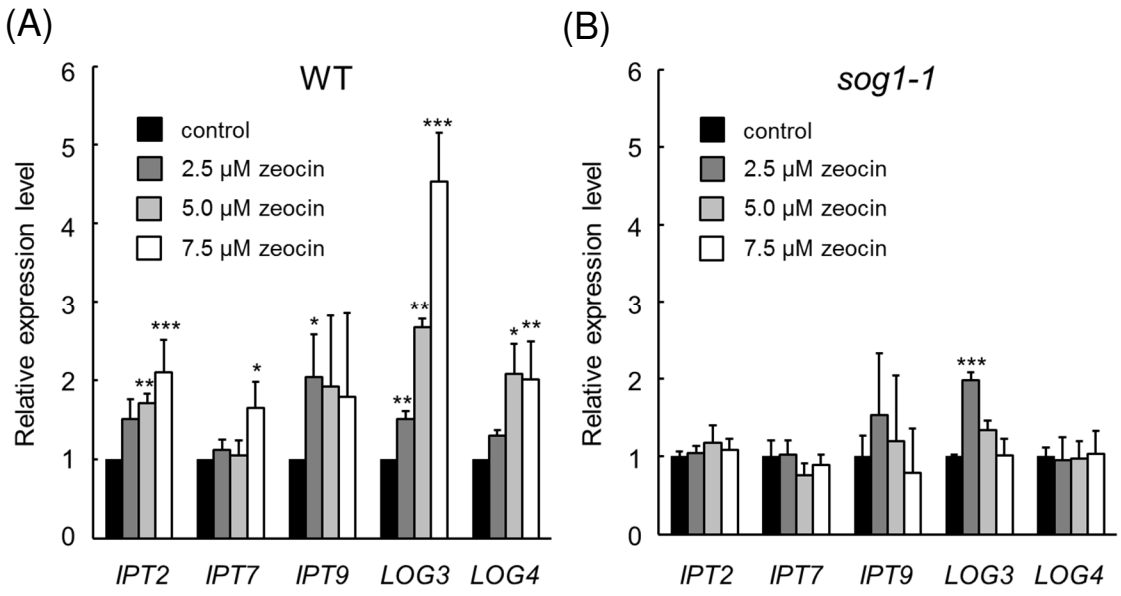



**Figure 3-9.** SOG1 is required for the induction of cytokinin biosynthesis genes.

Transcript levels of *IPT2*, *IPT7*, *IPT9*, *LOG3*, and *LOG4* in roots. Five-day-old wild-type (WT) (A) and *sog1-1* (B) seedlings were transferred onto control medium or medium containing the indicated concentrations of zeocin for seven days. Total RNA was extracted from roots. The mRNA levels were normalized to that of *ACTIN2* and are indicated as relative values, with that of the control set to 1. Data are presented as means ± SE (n = 3). Significant differences between treated seedlings and the control were determined with the Student's *t*-test: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

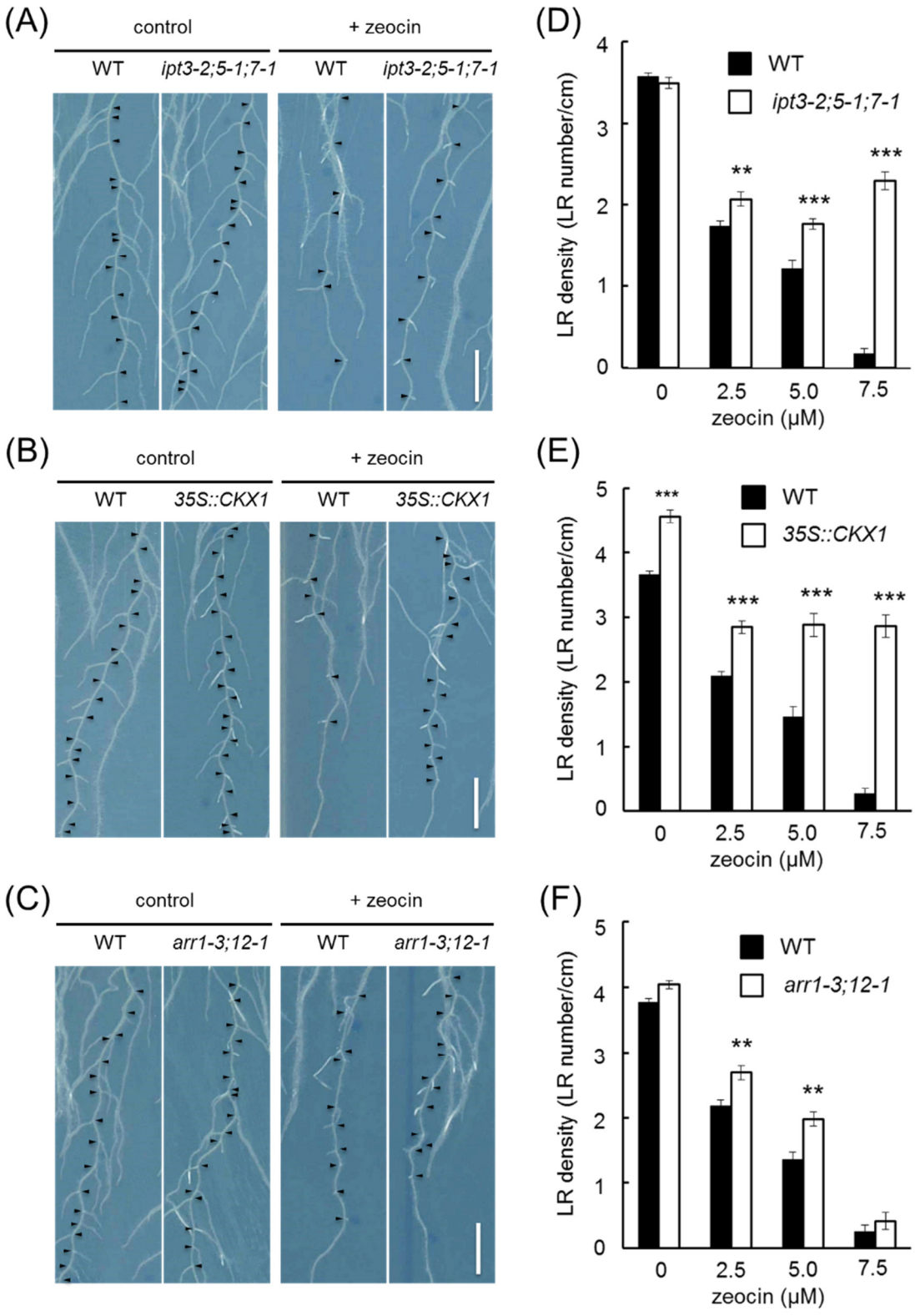

(A)
control
+ zeocin
WT
ipt3-2;5-1;7-1
WT
ipt3-2;5-1;7-1
(B)
control
+ zeocin
WT
35S::CKX1
WT
35S::CKX1
(C)
control
+ zeocin
WT
arr1-3;12-1
WT
arr1-3;12-1
(D)
WT
ipt3-2;5-1;7-1
LR density (LR number/cm)
zeocin (μM)
(E)
WT
35S::CKX1
LR density (LR number/cm)
zeocin (μM)
(F)
WT
arr1-3;12-1
LR density (LR number/cm)
zeocin (μM)

**Figure 3-10.** Cytokinin signaling is important for inhibition of LR formation in response to DNA damage.

(A–C) LR phenotype of cytokinin mutant seedlings. Five-day-old wild-type (WT), *ipt3-2;5-1;7-1* (A), *35S::CKX1* (B), and *arr1-3;12-1* (C) seedlings were transferred onto control medium (control) or medium containing 2.5 μM zeocin (+ zeocin), and grown for an additional seven days. Arrowheads indicate the positions of the LR along the primary roots. Bar = 0.5 cm. (D–F) LR density of wild-type (WT), *ipt3-2;5-1;7-1* (D), *35S::CKX1* (E), and *arr1-3;12-1* (F) after transfer onto control medium or medium containing the indicated concentrations of zeocin. Data are presented as means ± SE ($n > 20$). Significant differences between treated seedlings and the WT control were determined with the Student's *t*-test: **, $P < 0.01$; ***, $P < 0.001$.

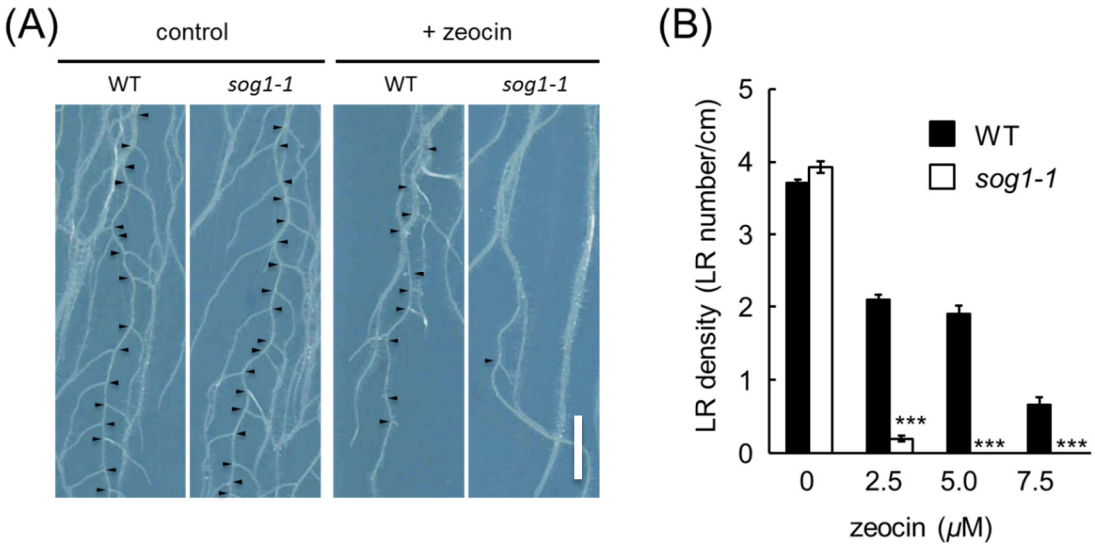



**Figure 3-11.** LR formation in *sog1-1* is hypersensitive to zeocin.

(A) LR phenotype of zeocin-treated *sog1-1* seedlings. Five-day-old wild-type (WT) and *sog1-1* seedlings were transferred onto control medium (control) or medium containing 2.5 μM zeocin (+ zeocin) and grown for an additional seven days. Arrowheads indicate the positions of the LR along the primary roots. Bar = 0.5 cm. (B) LR density of wild-type (WT) and *sog1-1* after transfer onto control medium or medium containing the indicated concentrations of zeocin. Data are presented as means ± SE ($n > 20$). Significant differences between treated seedlings and the WT control were determined with the Student's *t*-test: ***, $P < 0.001$.

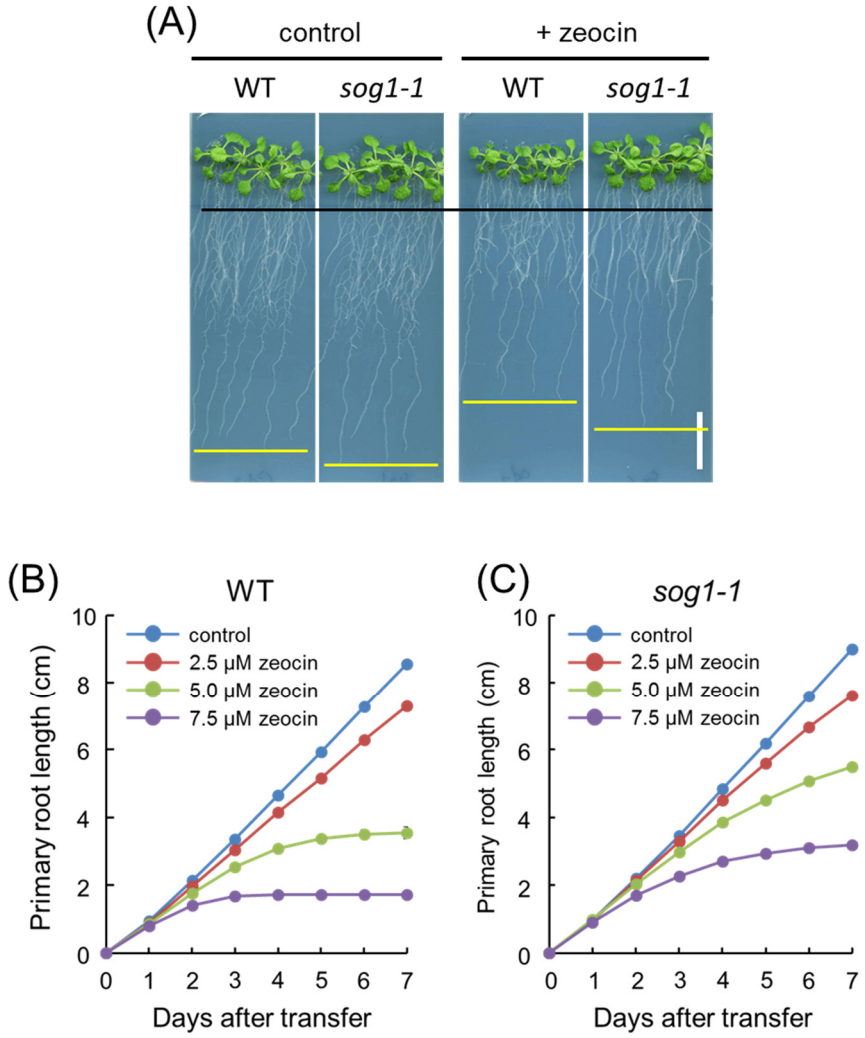



**Figure 3-12.** Primary root growth in *sog1-1* is tolerant to zeocin.

(A) Phenotype of zeocin-treated *sog1-1* seedlings. Five-day-old wild-type (WT) and *sog1-1* seedlings were transferred onto control medium (control) or medium containing 2.5 μM zeocin (+ zeocin) and grown for an additional seven days. Black lines indicate the positions of the root tips when the seedlings were transferred onto each medium. Yellow lines indicate the position of primary root tips. Bar = 2 cm. Root length of wild-type (WT) (B) and *sog1-1* (C) seedlings after transfer onto control medium or medium containing the indicated concentrations of zeocin. Data are presented as means ± SE (n > 20).

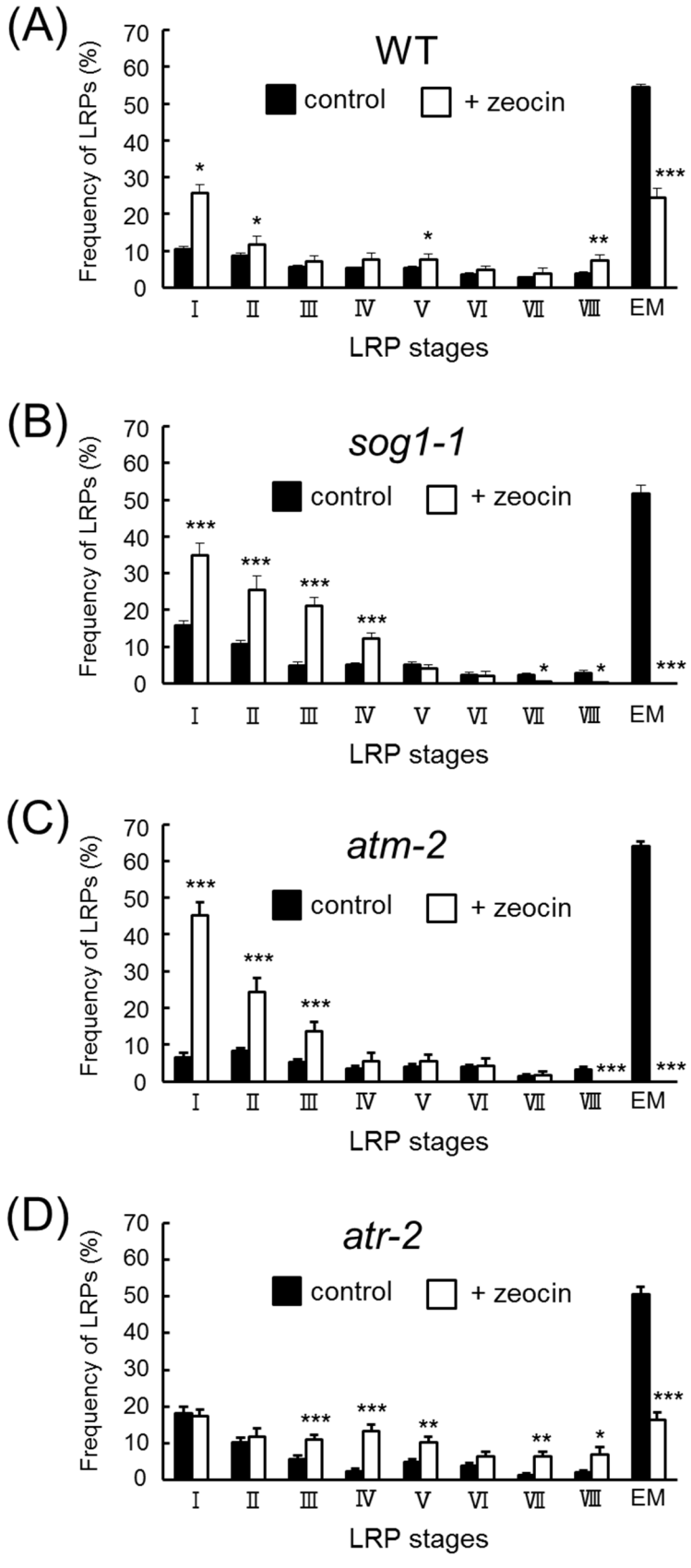

(A)
WT
control
+ zeocin
Frequency of LRPs (%)
LRP stages
(B)
sog1-1
(C)
atm-2
(D)
atr-2
I
II
III
IV
V
VI
VII
VIII
EM

**Figure 3-13.** Stage distribution of LRP in *sog1, atm* and *atr* mutants

Five-day-old *DR5::GUS* seedlings in wild-type (WT) (A), *sog1-1* (B) *atm-2* (C), and *atr-2* (D) backgrounds were transferred onto control medium (control) or medium containing 5.0 µM zeocin (+ zeocin), and grown for an additional seven days. Data are presented as means ± SE (n > 10). Significant differences between treated seedlings and the untreated control were determined with the Student's *t*-test: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

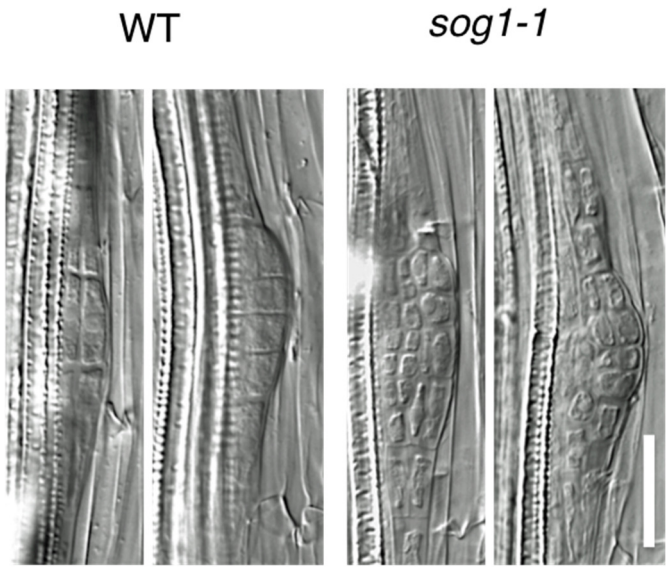



**Figure 3-14.** LRPs of zeocin-treated wild-type (WT) and *sog1-1* plants.

Five-day-old seedlings were transferred onto medium containing 2.5 μM zeocin and grown for an additional seven days. Bar = 25 μm.

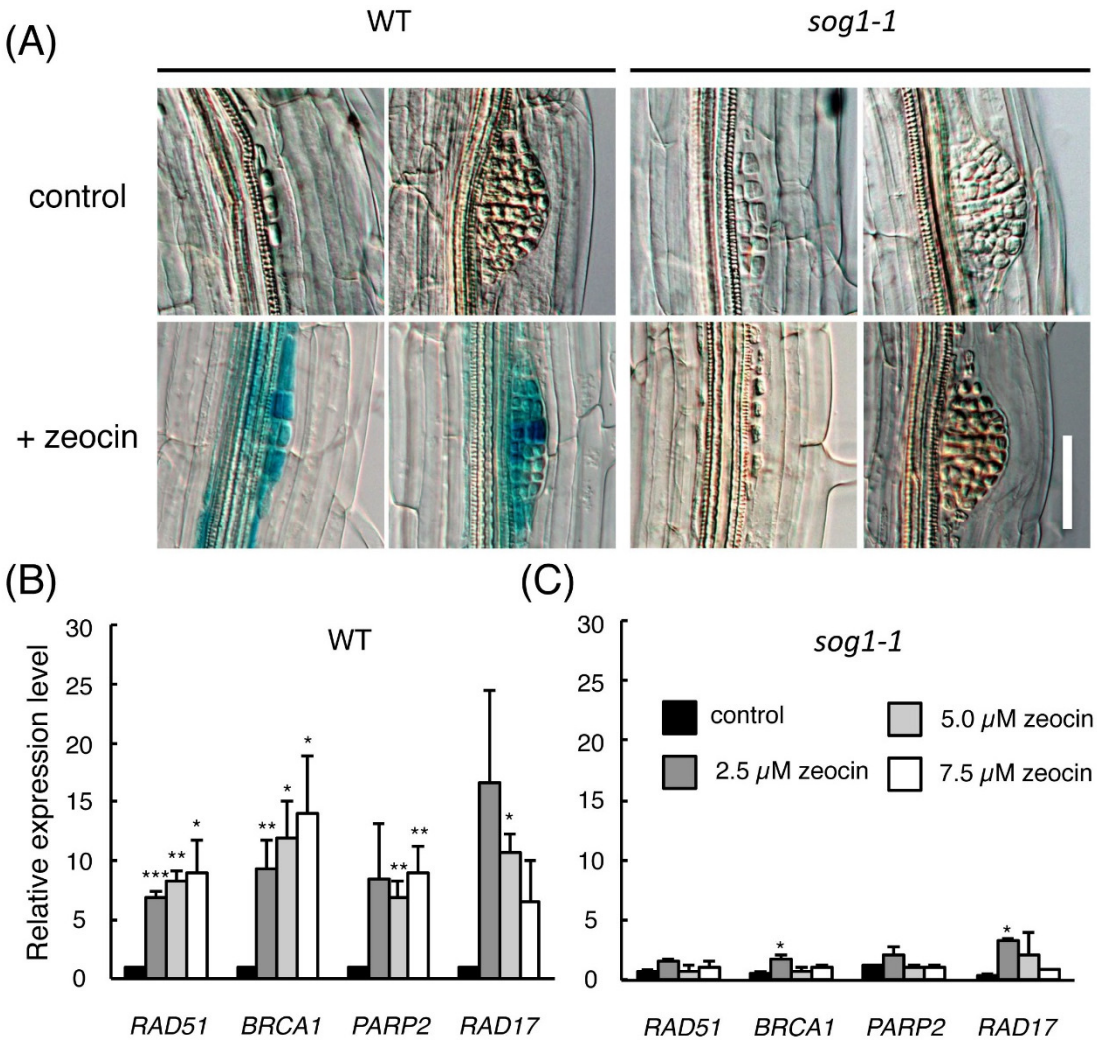



**Figure 3-15.** SOG1 is essential for the induction of DNA repair genes.

(A) LRP of wild-type (WT) and *sog1-1* seedlings harboring *pRAD51:GUS*. Five-day-old seedlings were transferred onto control medium (control) or medium containing 5 μM zeocin (+ zeocin), and GUS staining was conducted after 24 h. Bar = 50 μm. (B, C) Transcript levels of *RAD51*, *BRCA1*, *PARP2*, and *RAD17* in roots. Five-day-old wild-type (WT) (B) and *sog1-1* (C) seedlings were transferred onto control medium or medium containing the indicated concentrations of zeocin and grown for seven days. Total RNA was extracted from roots. The mRNA levels were normalized to that of *ACTIN2*, and are indicated as relative values, with that of the control set to 1. Data are presented as means ± SE (n = 3). Significant differences between treated seedlings and the control were determined with the Student's *t*-test: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

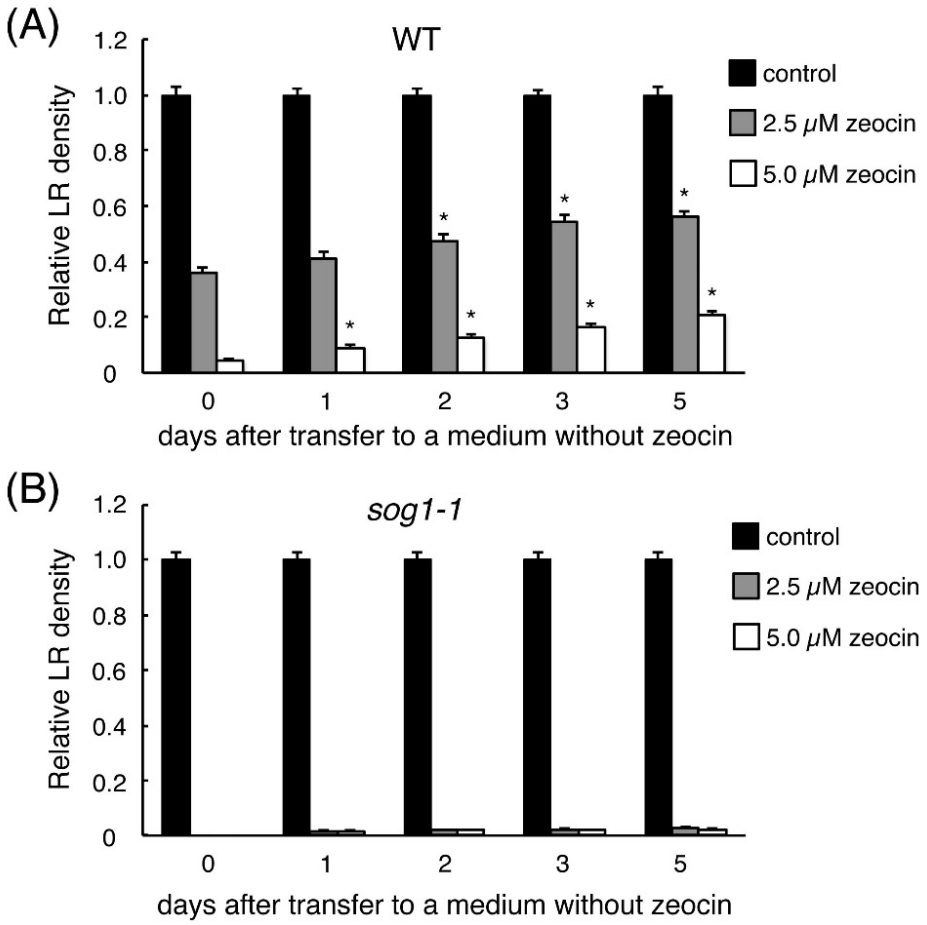



**Figure 3-16.** The zeocin-treated LRP in *sog1-1* has no ability to develop into LR.

LR density of zeocin-treated wild-type (WT) (A) and *sog1-1* (B) seedlings after transfer onto control medium. Five-day-old seedlings were transferred onto control medium (control) or medium containing 2.5 μM zeocin or 5.0 μM zeocin for five days, then transferred onto a control medium and grown for additional five days. LR density was normalized to that of the control and is indicated as a relative value, with that of the control set to 1.0. Data are presented as means ± SE ($n > 20$). Significant differences between values obtained before and after the plants were transferred to the control medium (day 0) were determined with the Student's *t*-test: *, $P < 0.001$.

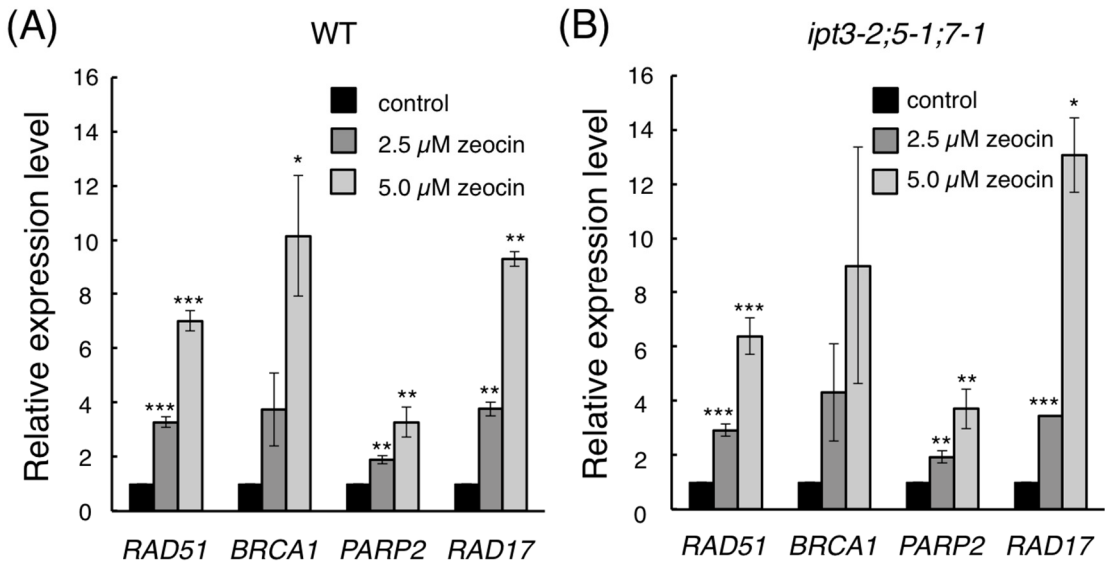


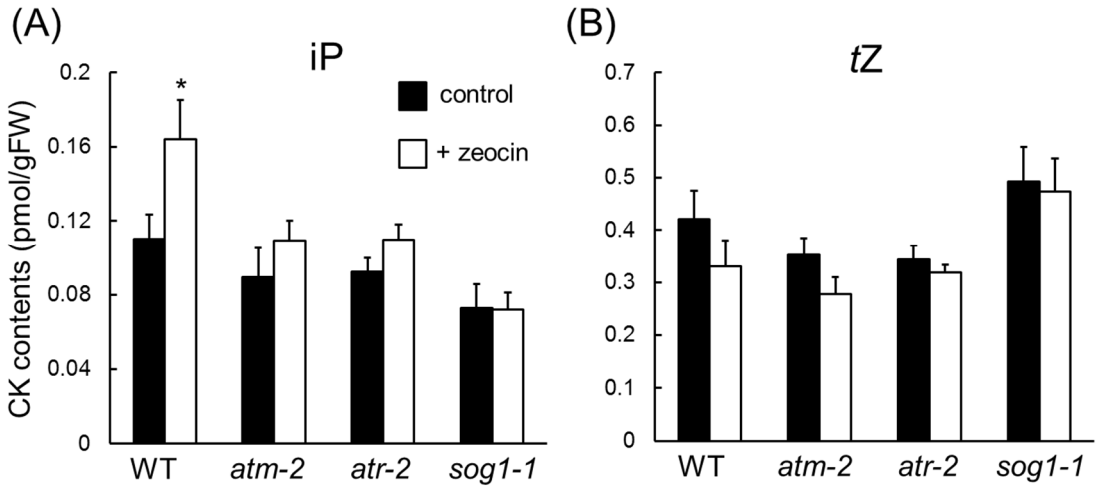

**Figure 3-17.** The induction of DNA repair genes upon zeocin treatment is not depend on cytokinin signaling.

Five-day-old wild-type (WT) (A) and *ipt3-2;5-1;7-1* (B) seedlings were transferred onto control medium or medium containing 2.5 or 5.0 μM zeocin and grown for seven days. Total RNA was extracted from roots. The mRNA levels were normalized to that of *ACTIN2*, and are indicated as relative values, with that of the control set to 1. Data are presented as means ± SD (n = 3). Significant differences between treated seedlings and the control were determined with the Student's *t*-test: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

**Table 3-1.** Transcript levels of cytokinin signaling genes after gamma ray irradiation

| AGI | Description | Culligan et al. (2006) | Yoshiyama et al. (2009) |
|---|---|---|---|
| AT1G27320 | *AHK3* | 0.79 | 0.84 |
| AT3G29350 | *AHP2* | 1.16 | 1.03 |
| AT5G39340 | *AHP3* | 1.11 | 1.14 |
| AT3G16360 | *AHP4* | 1.01 | 1.06 |
| AT1G03430 | *AHP5* | 1.72 | 1.31 |
| AT3G16857 | *ARR1* | 0.83 | 0.61 |
| AT4G16110 | *ARR2* | 0.94 | 0.88 |
| AT4G31920 | *ARR10* | 0.79 | 0.91 |
| AT1G67710 | *ARR11* | 0.98 | 0.63 |
| AT2G25180 | *ARR12* | 0.92 | 0.76 |
| AT2G01760 | *ARR14* | 0.88 | 0.94 |

Five-day-old wild-type seedlings were irradiated at 100 Gy and harvested 1.5 h after the end of the irradiation period. The numbers shown are normalized fold changes with respect to unirradiated plants. Data were obtained from Culligan et al. (2006) and Yoshiyama et al. (2009).




**Supplemental Figure 3-1.** Cytokinin level in root transition zone upon zeocin treatment (data was taken by Dr. H. Sakakibara in RIKEN).

Five-day-old wild-type (WT) *atm-2, atr-2,* and *sog1-1* seedlings were transferred onto control medium or medium containing 10 μM zeocin (+ zeocin) for 24 h. Cytokinins were extracted and determined from about 100 mg of primary root transition zone tissues as described previously using ultra-performance liquid chromatography–tandem mass spectrometry (AQUITY UPLC System/XEVO-TQS; Waters) with an octadecylsilyl column (AQUITY UPLC BEH C18, 1.7 μm, 2.1 × 100 mm; Waters). Data are presented as means ± SD (n = 3). Significant differences between treated seedlings and the control were determined with the Student's *t*-test: *, $P < 0.05$.

# Discussion

LRs are an important organ for water uptake and the absorption of nutrients from soil. Thus, precise control of LR formation is crucial for plants to adapt their growth to environmental conditions. In this study, I showed that DNA damage inhibited LR formation. My data demonstrated that SOG1-mediated DNA damage signaling elevated the expression of genes involved in DNA repair and cytokinin biosynthesis (Figure 4-1). Since LRP development was severely impaired by DNA damage in the *sog1-1* mutant, SOG1-mediated activation of DNA repair machineries seems crucial for maintaining genome integrity in LRP, thus for enabling LR formation after removal of genotoxic stress (Figure 3-16). Furthermore, my results indicated that SOG1-dependent enhancement of cytokinin signaling is required for the inhibition of LR formation under DNA damage conditions. There still remains a possibility that factors associated with cytokinin signaling respond to DNA damage, but my data clearly showed that genes for cytokinin biosynthesis are actively induced by zeocin treatment, suggesting that cytokinin content itself increased in LRP under DNA damage conditions. I found that induction of DNA repair genes was independent from that of cytokinin biosynthesis genes (Figure 3-17). This result explains that even though LR inhibition is an active process through the SOG1-mediated activation of cytokinin signaling, the impairment of the transcriptional activation of DNA repair genes in *sog1-1* mutant conceals the suppression of CK-dependent LR inhibition response. It is known that various environmental stresses often produce ROS and cause the breakage of genomic DNA (Mittler 2002; Apel & Hirt 2004).

Mutant and transgenic plants with reduced cytokinin content exhibit root growth resistance to salt stress (Nishiyama et al. 2011). These observations suggest that the SOG1-mediated mechanism might also underlies programmed response to other stresses in controlling plant root growth.

In this study, I found that in the wild-type, the primary roots growth is more tolerant to DNA damage than the LR formation. Under 2.5 μM zeocin treatment, LR density was severely reduced (about 47% reduction), while primary roots is relatively less inhibited (15% reduction) by the zeocin treatment (Figure 3-1 B, D). I also found that zeocin treatment severely perturbed LR formation in *sog1-1* mutant, whereas primary roots grew rather faster in the mutant than in the wild-type after zeocin treatment. It is likely that DNA damage was highly accumulated both in primary roots and LRP; therefore, dividing cells in the primary root meristem are more tolerant to DNA damage as compared to cells constituting LRP. Dividing cells in the primary root tip are already organized in a well-established root apical meristem. In contrast, developing LRP cells are still in a process to establish a functional LR meristem. Furthermore, primary root meristem is known to possess a stable auxin with its maximum in the QC and young columella cells (Sabatini et al. 1999), In contrast, in a developing LRP, an auxin maximum is still being established gradually toward the tip of that newly formed LRP (Benková et al. 2003). Recently, Takahashi et al. (unpublished data) found that auxin plays a role in the genome maintenance under genotoxic stress condition. Although zeocin causes DNA damage, exogenous auxin application suppresses the accumulation of DNA damage and cell death upon zeocin treatment (Takahashi et al. unpublished data).

Furthermore, auxin signaling mutant *plt1* exhibits severe cell death in the root meristem upon DNA damage, while *iaa5 iaa29* mutant which is defective in the inhibition of auxin signaling displays tolerance to DNA damage (Takahashi et al. unpublished data). In agreement with this notion, I found that auxin receptor mutant *tir1* showed hypersensitivity in LR formation (Figure 3-5). Therefore, high accumulation of auxin in the primary root meristem might suppress DNA damage effects more effectively than the one in the LRP. This difference in auxin accumulation in LR and primary root meristem may explain the differential sensitivity of the two tissues to DNA damage. It has been reported that osmotic stress, which is known to cause DNA damage (Balestrazzi *et al.* 2011), also severely inhibits LR formation, while primary root growth is not retarded (Deak & Malamy 2005). This result indicates that stress-induced DNA damage signals are interpreted differentially between primary roots and LRs, allowing changes in root architecture. It is interesting to study the differences in DNA damage signaling between the two root tissues and their physiological roles in survival under changing environmental conditions.

ATM is activated by DSBs, whereas ATR responds to SSBs and DNA replication stress (Garcia et al. 2003; Culligan et al. 2004). Here, I demonstrated that zeocin-induced DSBs, but not HU-triggered DNA replication stress, inhibit LR formation. Moreover, I showed that compared with the wild type, *atm-2* and *sog1-1*, but not *atr-2*, exhibited hypersensitivity to zeocin in terms of LRP development. These results suggest that DSBs, which can represent fatal damage accompanied by a loss of chromosome arms, have a higher impact on accumulating DNA damage as compared with replication stress, and the

ATM–SOG1 pathway, rather than the ATR–SOG1 pathway, plays a major role in provoking DNA repair in LRP. Previous microarray data indicate that the expression of several DNA repair genes is upregulated by gamma ray irradiation in the *atr* mutant, but not in the *atm* or *sog1* mutants, at a level comparable to that in the wild-type (Table 4-1) (Culligan et al. 2006; Yoshiyama et al. 2009), supporting the idea that ATM–SOG1 mainly participates in repairing damaged DNA. Recently, it has been shown that high aluminum stress specifically activates the ATR–SOG1 pathway and inhibits root growth (Sjogren et al. 2015); therefore, ATM and ATR may respond differentially to external cues and control primary and LR growth.

It has been shown that exogenous cytokinins inhibit the auxin-induced expression of *PIN* genes during LR development, indicating that cytokinins prevent the PIN-dependent establishment of the auxin gradient required for LR initiation (Laplaze et al. 2007). In addition to the transcriptional repression of *PIN*s, cytokinin promotes protein degradation of PIN1 in LRP at stage I, leading to inhibition of LRP development (Marhavý et al. 2011). In this study, I demonstrated that zeocin treatment increases the expression of cytokinin biosynthesis genes and arrests LRP development at a very early stage. Therefore, DSBs likely inhibit LR formation by increasing cytokinin levels, which in turn down-regulating *PIN* gene expression and protein accumulation.

In the transition zone of primary roots, cytokinins induce the expression of *SHY2/IAA3*, one of the Aux/IAA auxin signaling repressors, and inhibit *PIN* expression. This promotes the transition from cell division to cell differentiation, restricting the size of the root meristem (Dello Ioio et al. 2008). Since multiple Aux/IAAs-ARFs auxin

response modules play a central role during LR development, the DNA-damage induced cytokinin biosynthesis may also promote expression of the relevant *Aux/IAA* genes, thereby repressing auxin signaling and inhibiting LR development.

We previously reported that DSBs promote an early transition from cell division to cell differentiation in the root meristem (Adachi et al. 2011). Furthermore, we recently found that DSBs upregulate the level of bioactive cytokinin iP in root transition zone (Supplemental Figure 3-1). In addition, *de novo* cytokinin synthesis inhibits the expression of *PINs*, thereby disturbing downward auxin flow (Takahashi et al. unpublished data). These data indicate that cytokinin-mediated inhibition of cell division is commonly used mechanism for stress response in roots.

In contrast to the increase of cytokinin content in root, we recently find that cytokinin contents are decreased in shoots under DNA damage (Takahashi et al. unpublished data). Furthermore, it has been reported that the content of bioactive cytokinins is reduced in shoot in response to other stresses, such as drought and salt stresses (Nishiyama et al. 2011). Therefore, it is likely that cytokinin biosynthesis is downregulated in shoots under stress conditions. Cytokinins are known to promote cell proliferation in shoots, while in root, it restricts the meristem zone where mitotic cell division occur (Schaller et al. 2014; Dello Ioio et al. 2008; Moubayidin et al. 2010). Thus, plants may respond to stress by reducing endogenous cytokinin contents to retard shoot growth, although cytokinin biosynthesis is activated in roots. This implies that stress signals converge on the regulatory mechanisms of cytokinin biosynthesis and suppress overall growth, thus it is important to uncover the molecular mechanisms of how

cytokinin biosynthesis is differentially regulated in shoots and roots of plants.

SOG1 govern the plant DNA damage response through the transcriptional induction of hundreds of genes involved in DNA repair, cell cycle arrest, early onset of endoreplication, and stem cell death in response to DNA damage (Mannuss et al., 2012; Yoshiyama et al., 2009; Adachi, et al., 2011; Furukawa, et al., 2010). Previous study revealed that SOG1 directly induces the expression of CDK inhibitor genes *SIAMESE-RELATED 5* (*SMR5*) and *7* (*SMR7*) *in vivo* (Yi, et al., 2014). It has been reported that cytokinins reduce the expression of mitotic cyclins (*e.g. CYCB1;1, CYCB2;1, CYCB2;3,* and *CYCA2;1*) and CDKs (*CDKA;1, CDKB1;1, and CDKB2;2*) genes during LR initiation to inhibit cell division, although the expression of G1 to S transition and S phase-specific cyclin genes is not affected by exogenous cytokinin application (Li et al., 2006). This result suggests that in addition to upregulation of CDK inhibitory factors, SOG1-activated cytokinin biosynthesis differentially inhibits cell division during LR initiation through the reduction of mitotic genes under DNA damage, resulting in reduction of CDK activity. Since DNA repair mechanism is activated in LRPs upon DNA damage, the decrease in CDK activity would be important for the maintenance of genome integrity in LRP.

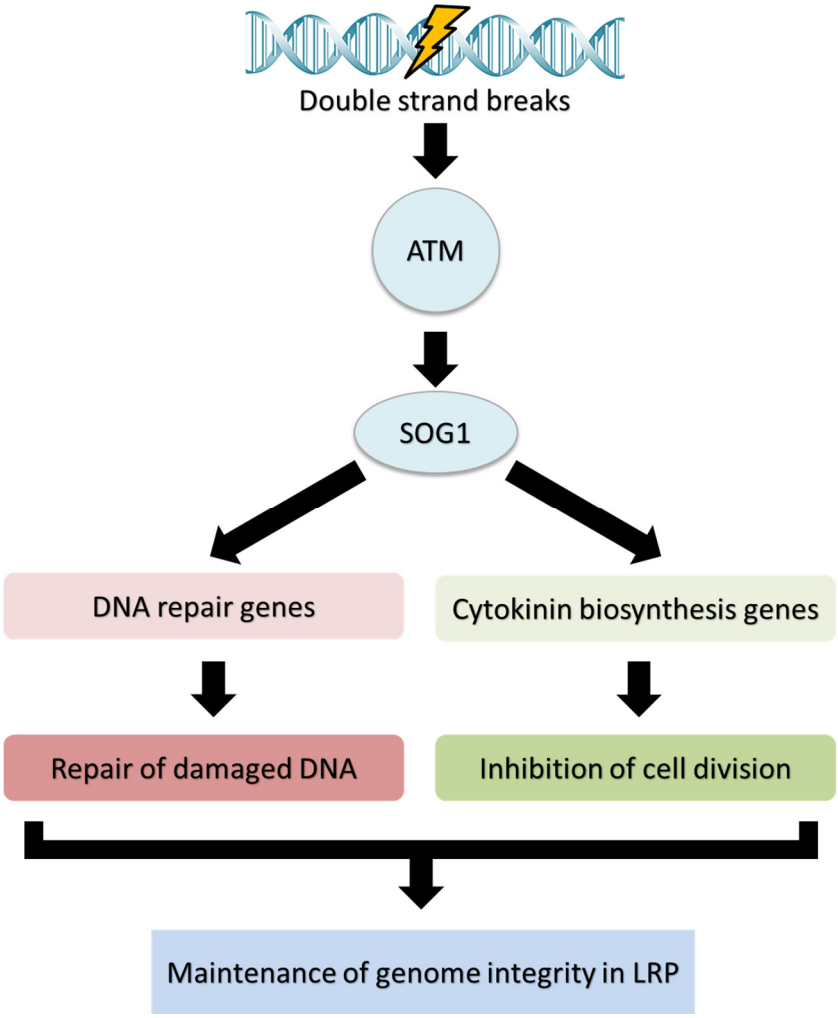



**Figure 4-1.** Model for ATM/SOG1-mediated maintenance of genome integrity in LRP.

DSBs activate the ATM sensor kinase, which then phosphorylates and activates SOG1. Activated SOG1 differentially induces the expression of DNA repair genes and cytokinin biosynthesis genes, thereby repairing damaged DNA and inhibiting cell division in LRPs. DSBs, DNA double-strand breaks; LRPs, lateral root primordium.

**Table 4-1.** Overview of the transcriptionally induced core DNA repair genes in wild-type, *atm-2, atr-2,* and *sog1-1* after gamma ray irradiation

| | | Culligan et al. (2006) | | | Yoshiyama et al. (2009) | |
|---|---|---|---|---|---|---|
| AGI | Description | WT | *atr-2* | *atm-2* | WT | *sog1-1* |
| AT4G21070 | *AtBRCA1* | 250.79 | 226.01 | 3.16 | 57.79 | 0.97 |
| AT5G20850 | *AtRAD51* | 58.10 | 54.70 | 1.57 | 31.20 | 1.38 |
| AT3G19210 | *AtRAD54* | 2.12 | 2.29 | 0.95 | 2.36 | 1.20 |
| AT5G40840 | *SYN2* | 56.90 | 62.41 | 1.63 | 27.47 | 1.06 |
| AT5G24280 | *GMI1* | 58.51 | 47.33 | 1.18 | 42.83 | 1.18 |
| AT2G31320 | *AtPARP1* | 24.60 | 22.69 | 1.16 | 9.97 | 1.07 |
| AT4G02390 | *AtPARP2* | 70.35 | 55.26 | 1.44 | 59.32 | 1.52 |

Five-day-old seedlings were irradiated at 100 Gy and harvested 1.5 h after the end of the irradiation period. The numbers shown are normalized fold changes with respect to unirradiated plants. Data were obtained from Culligan et al. (2006) and Yoshiyama et al. (2009).

## Acknowledgements

In the name of The Almighty God, The Most Gracious, The Most Merciful. All praises and thanks be to Him, for only with His blessings and graces, I can finally finish this work. First and foremost, I would like to convey my sincere gratitude to my beloved parents, Ibunda Juharni and Ayah L. M. Davis; my dear wife, Nia Dahniar and my son, anakda La Ode Hamzah Abdurrahman Davis, for their love, support, and patience for me all the time.

I would like to express my respect and gratitude to my supervisor, Prof. Masaaki Umeda, for his supervision, support, and valuable guidance throughout the course my study. Truly, I am forever in debt to him. My special thanks goes to Dr. Naoki Takahashi, for his guidance and continuous supports during my doctoral course in NAIST. Furthermore, I would like to thank my advisor committee, Prof. Seiji Takayama and Assoc. Prof. Mitsuhiro Aida for their valuable advices and discussions for the betterment of this research. I would like to extend my gratitude to Dr. Hitoshi Sakakibara (RIKEN) for the root's cytokinin content data; Nobuo Ogita (NAIST), Dr. Anne Britt (UC Davis), and Dr. Tatsuo Kakimoto (Osaka University) for providing plant materials used in this research. I also would like to thank the current and former members of the laboratory of Plant Growth Regulation (Umeda lab), for the conducive and cheerful atmosphere they bring in the lab. I will always remember all the great time we had experienced together.

I would like to thank the Ministry of Education, Culture, Sports, Science & Technology (MEXT) in Japan for this education opportunity and financial support

through NAIST Global COE program and MEXT Scholarship "Top Global University Project".

I would like to say my sincere gratitude to Mrs. Sachiko Iida for providing me housing accommodation during the final year of my study. I would also like to thank the Muslim and the Indonesian communities in NAIST for their continuous help and supports in my daily life while pursuing the doctoral course in NAIST.

# References


Abe, K., Osakabe, K., Nakayama, S., Endo, M., Tagiri, A., Todoriki, S., Ichikawa, H., Toki, S. (2005) Arabidopsis *RAD51C* gene is important for homologous recombination in meiosis and mitosis. *Plant Physiol.* **139**, 896–908.

Adachi, S., Minamisawa, K., Okushima, Y., Inagaki, S., Yoshiyama, K., Kondou, Y., Kaminuma, E., Kawashima, M., Toyoda, T., Matsui, M., Kurihara, D., Matsunaga, S., Umeda, M. (2011) Programmed induction of endoreduplication by DNA double-strand breaks in *Arabidopsis*. *Proc. Natl. Acad. Sci. USA* **108**, 10004–10009.

Aguilera, A., Garcia-Muse, T. (2013) Causes of genome instability. *Annu Rev Genet*. **47**, 1–32.

Alizadeh, E., Orlando, T.M., Sanche, L. (2015) Biomolecular damage induced by ionizing radiation: the direct and indirect effects of low-energy electrons on DNA. *Annu Rev Phys Chem.* **66**, 379-398.

Apel, K., Hirt, H. (2004). Reactive oxygen species: metabolism, oxidative stress, and signal transduction. *Annu. Rev. Plant Biol.* **55**, 373–399.

Balestrazzi, A., Confalonieri, M., Macovei, A., Carbonera, D. (2010) Seed imbibition in *Medicago truncatula* Gaertn.: Expression profiles of DNA repair genes in relation to PEG-mediated stress. *J. Plant Physiol.* **168**, 706–713.

Benková, E., Bielach, A. (2010) Lateral root organogenesis - from cell to organ. *Curr Opin Plant Biol*. **13**, 677-683.

Benková, E., Michniewicz, M., Sauer, M., Teichmann, T., Seifertová, D., Jürgens, G.,

Friml, J. (2003) Local, efflux-dependent auxin gradients as a common module for plant organ formation. *Cell* **115**, 591–602.

Beranek, D.T. (1990) Distribution of methyl and ethyl adducts following alkylation with monofunctional alkylating agents. *Mutat. Res.* **231**, 11–30.

Bielach, A., Podlesáková, K., Marhavy, P., Duclercq, J., Cuesta, C., Müller, B., Grunewald, W., Tarkowski, P., Benková, E. (2012) Spatiotemporal regulation of lateral root organogenesis in Arabidopsis by cytokinin. *Plant Cell* **24**, 3967-3981.

Block-Schmidt, A.S., Dukowic-Schulze, S., Wanieck, K., Reidt, W., Puchta, H. (2011) BRCC36A is epistatic to BRCA1 in DNA crosslink repair and homologous recombination in Arabidopsis thaliana. *Nucleic Acids Res*. **39**, 146-154.

Britt, A.B. (1996) DNA damage and repair in plants. *Annu. Rev. Plant Biol.* **47**, 75-100.

Casimiro, I., Beeckman, T., Graham, N., Bhalerao, R., Zhang, H., Casero, P., Sandberg, G., Bennett, M.J. (2003) Dissecting *Arabidopsis* lateral root development. *Trends Plant Sci.* **8**, 165–171.

Casimiro, I., Marchant, A., Bhalerao, R.P., Beeckman, T., Dhooge, S., Swarup, R., Graham, N., Inzé, D., Sandberg, G., Casero, P.J., Bennett, M. (2001) Auxin transport promotes Arabidopsis lateral root initiation. *Plant Cell*. **13**, 843-852.

Chang, L., Ramireddy, E., Schmülling, T. (2015) Cytokinin as a positional cue regulating lateral root spacing in Arabidopsis. *J Exp Bot.* **66**, 4759-4768.

Chapman, J.R., Taylor, M.R., Boulton, S.J. (2012) Playing the end game: DNA double-strand break repair pathway choice. *Mol Cell*. **47**, 497-510.

Chowdhury, D., Choi, Y.E., Brault, M.E. (2013) Charity begins at home: non-coding

RNA functions in DNA repair. *Nat Rev Mol Cell Biol*. **14**, 181-189.

Ciccia, A., Elledge, S.J. (2010) The DNA damage response: making it safe to play with knives. *Mol Cell*. **40**, 179-204.

Clough, S.J., Bent, A.F. (1998) Floral dip: a simplified method for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana*. *Plant J.* **16**, 735–743.

Culligan, K.M., Robertson, C.E., Foreman, J., Doerner, P., Britt, A.B. (2006) ATR and ATM play both distinct and additive roles in response to ionizing radiation. *Plant J.* **48**, 947–961.

Culligan, K.M., Tissier, A., Britt, A. (2004) ATR regulates a G2-phase cell-cycle checkpoint in *Arabidopsis thaliana*. *Plant Cell* **16**, 1091–1104.

D'Agostino, I.B., Deruère, J., Kieber, J.J. (2000) Characterization of the response of the Arabidopsis response regulator gene family to cytokinin. *Plant Physiol.* **124**, 1706–1717.

Davis, A.J., Chen, D.J. (2013) DNA double strand break repair via non-homologous end-joining. *Transl Cancer Res*. **2**, 130-143.

Deak, K.I., Malamy, J. (2005) Osmotic regulation of root system architecture. *Plant J.* **43**, 17–28.

Deans, A.J., West, S.C. (2011) DNA interstrand crosslink repair and cancer. *Nat Rev Cancer.* **11**, 467-480.

Dello Ioio, R., Nakamura, K., Moubayidin, L., Perilli, S., Taniguchi, M., Morita, M.T., Aoyama, T., Costantino, P., Sabatini, S. (2008) A genetic framework for the control of cell division and differentiation in the root meristem. *Science* **322**, 1380–1384.

De Rybel, B., Vassileva, V., Parizot, B., Demeulenaere, M., Grunewald, W., Audenaert, D., Van Campenhout, J., Overvoorde, P., Jansen, L., Vanneste, S., Möller, B., Wilson, M., Holman, T., Van Isterdael, G., Brunoud, G., Vuylsteke, M., Vernoux, T., De Veylder, L., Inzé, D., Weijers, D., Bennett, MJ., Beeckman, T. (2010) A novel aux/IAA28 signaling cascade activates GATA23-dependent specification of lateral root founder cell identity. *Curr Biol*. **20**, 1697-1706.

De Smet, I. (2012) Lateral root initiation: one step at a time. *New Phytol*. **193**, 867-873.

De Smet, I., Lau, S., Voss, U., Vanneste, S., Benjamins, R., Rademacher, E.H., Schlereth, A., De Rybel, B., Vassileva, V., Grunewald, W., Naudts, M., Levesque, M.P., Ehrismann, J.S., Inzé, D., Luschnig, C., Benfey, P.N., Weijers, D., Van Montagu, M.C., Bennett, M.J., Jürgens, G., Beeckman, T. (2010) Bimodular auxin response controls organogenesis in Arabidopsis. *Proc Natl Acad Sci U S A*. **107**, 2705-2710.

De Smet, I., Vanneste, S., Inzé, D., Beeckman, T. (2006) Lateral root initiation or the birth of a new meristem. *Plant Mol Biol*. **60**, 871-887.

De Smet, I., Vassileva, V., De Rybel, B., Levesque, M.P., Grunewald, W., Van Damme, D., Van Noorden, G., Naudts, M., Van Isterdael, G., De Clercq, R., Wang, J.Y., Meuli, N., Vanneste, S., Friml, J., Hilson, P., Jürgens, G., Ingram, G.C., Inzé, D., Benfey, P.N., Beeckman T. (2008) Receptor-like kinase ACR4 restricts formative cell divisions in the Arabidopsis root. *Science*. **322**, 594-597.

Duan, L., Dietrich, D., Ng, C.H., Chan, P. (2013) Endodermal ABA signaling promotes lateral root quiescence during salt stress in *Arabidopsis* seedlings. *Plant Cell* **25**, 324–341.

Dubrovsky, J.G., Sauer, M., Napsucialy-Mendivil, S., Ivanchenko, M.G., Friml, J., Shishkova, S., Celenza, J., Benková, E. (2008) Auxin acts as a local morphogenetic trigger to specify lateral root founder cells. *Proc. Natl. Acad. Sci. USA.* **105**, 8790–8794.

Eastman, A. (1985) Interstrand cross-links and sequence specificity in the reaction of *cis*-dichloro(ethylenediamine)platinum(II) with DNA. *Biochemistry* **24**, 5027–5032.

Fu, D., Calvo, J.A., Samson, L.D. (2012) Balancing repair and tolerance of DNA damage caused by alkylating agents. *Nat Rev Cancer.* **12**, 104-120.

Fukaki, H., Nakao, Y., Okushima, Y., Theologis, A., Tasaka, M. (2005) Tissue-specific expression of stabilized SOLITARY-ROOT/IAA14 alters lateral root development in Arabidopsis. *Plant J*. 2005. **44**, 382-395.

Fukaki, H., Tameda, S., Masuda, H., Tasaka, M. (2002) Lateral root formation is blocked by a gain-of-function mutation in the SOLITARY-ROOT/IAA14 gene of Arabidopsis. *Plant J*. **29**, 153-168.

Furukawa, T., Curtis, M.J., Tominey, C.M., Duong, Y.H., Wilcox, B.W., Aggoune, D., Hays, J.B., Britt, A.B. (2010) A shared DNA-damage-response pathway for induction of stem-cell death by UVB and by gamma irradiation. *DNA Repair (Amst)*. **9**, 940-948.

Garcia, V., Bruchet, H., Camescasse, D., Granier, F., Bouchez, D., Tissier, A. (2003) AtATM is essential for meiosis and the somatic response to DNA damage in plants. *Plant Cell* **15**, 119–132.

Goh, T., Joi, S., Mimura, T., Fukaki, H. (2012) The establishment of asymmetry in

Arabidopsis lateral root founder cells is regulated by LBD16/ASL18 and related LBD/ASL proteins. *Development* **139**, 883-893.

Heitzeberg, F., Chen, I.P., Hartung, F., Orel, N., Angelis, K.J., Puchta, H. (2004) The Rad17 homologue of Arabidopsis is involved in the regulation of DNA damage repair and homologous recombination. *Plant J*. **38**, 954-968.

Heyer, W.D., Ehmsen, K.T., Liu, J. (2010) Regulation of homologous recombination in eukaryotes. *Annu Rev Genet*. **44**, 113-139.

Hill, K., Mathews, D.E., Kim, H.J., Street, I.H., Wildes, S.L., Chiang, Y.H., Mason, M.G., Alonso, J.M., Ecker, J.R., Kieber, J.J., Schaller, G.E. (2013) Functional characterization of type-B response regulators in the Arabidopsis cytokinin response. *Plant Physiol.* **162**, 212–224.

Hirota, A., Kato, T., Fukaki, H., Aida, M., Tasaka, M. (2007) The auxin-regulated AP2/EREBP gene PUCHI is required for morphogenesis in the early lateral root primordium of Arabidopsis. *Plant Cell* **19**, 2156-2168.

Hu, Z., Cools, T., De Veylder, L. (2016) Mechanisms used by plants to cope with DNA Damage. *Annu. Rev. Plant Biol.* **67**, 439–462.

Huang, C.H., Mirabelli, C.K., Jan, Y., Crooke, S.T. (1981) Single-strand and double-strand deoxyribonucleic acid breaks produced by several bleomycin analogues. *Biochemistry* **20**, 233–238.

Hwang, I., Sheen, J., Müller, B. (2012) Cytokinin signaling networks. *Annu. Rev. Plant Biol.* **63**, 353–380.

Jain, A., Sinilal, B., Dhandapani, G., Meagher, R.B., Sahi, S.V. (2013) Effects of

deficiency and excess of zinc on morphophysiological traits and spatiotemporal regulation of zinc-responsive genes reveal incidence of cross talk between micro- and macronutrients. *Environ Sci Technol*. **47**, 5327-5335.

Jia, Q., den Dulk-Ras, A., Shen, H., Hooykaas, P.J., de Pater, S. (2013) Poly(ADP-ribose)polymerases are involved in microhomology mediated back-up non-homologous end joining in Arabidopsis thaliana. *Plant Mol Biol*. **82**, 339-351.

Jung Y., Lippard, S.J. (2007) Direct cellular responses to platinum-induced DNA damage. *Chem Rev*. **107**,1387-1407.

Kakimoto, T. (2001) Identification of plant cytokinin biosynthetic enzymes as dimethylallyl diphosphate: ATP/ADP isopentenyltransferases. *Plant Cell Physiol.* **42**, 677–685.

Kartalou, M., Essigmann, J.M. (2001) Mechanisms of resistance to cisplatin. *Mutat Res*. **478**, 23-43.

Kieber, J.J., Schaller, G.E. (2014) Cytokinins. *Arabidopsis Book*. **12**, e0168.

Kochian, L.V., Piñeros, M.A., Liu, J., Magalhaes, J.V. (2015) Plant Adaptation to Acid Soils: The Molecular Basis for Crop Aluminum Resistance. *Annu Rev Plant Biol.* **66**, 571-598.

Kurakawa, T., Ueda, N., Maekawa, M., Kobayashi, K., Kojima, M., Nagato, Y., Sakakibara, H., Kyozuka, J. (2007) Direct control of shoot meristem activity by a cytokinin-activating enzyme. *Nature* **445**, 652–655.

Kuroha, T., Tokunaga, H., Kojima, M., Ueda, N., Ishida, T., Nagawa, S., Fukuda, H., Sugimoto, K., Sakakibara, H. (2009) Functional analyses of *LONELY GUY*

cytokinin-activating enzymes reveal the importance of the direct activation pathway in *Arabidopsis*. *Plant Cell* **21**, 3152–3169.

Kurowska, M., Daszkowska-Golec, A., Gruszka, D., Marzec, M., Szurman, M., Szarejko, I., Maluszynski, M. (2011) TILLING: a shortcut in functional genomics. *J Appl Genet*. **52**, 371-390.

Laplaze, L., Benkova, E., Casimiro, I., Maes, L., Vanneste, S., Swarup, R., Weijers. D., Calvo, V., Parizot, B., Herrera-Rodriguez, M.B., Offringa, R., Graham, N., Doumas, P., Friml, J., Bogusz, D., Beeckman, T., Bennett, M. (2007) Cytokinins act directly on lateral root founder cells to inhibit root initiation. *Plant Cell* **19**, 3889–3900.

Larsen, P.B., Kochian, L.V., Howell, S.H. (1997) Al inhibits both shoot development and root growth in *als3*, an Al-sensitive Arabidopsis mutant. *Plant Physiol.* **114**, 1207-1214.

Laskowski, M., Grieneisen, V.A., Hofhuis, H., Hove, C.A., Hogeweg, P., Marée, A.F., Scheres, B. (2008) Root system architecture from coupling cell shape to auxin transport. *PLoS Biol*. **6**, e307.

Lavenus, J., Goh, T., Roberts, I., Guyomarc'h, S., Lucas, M., De Smet, I., Fukaki, H., Beeckman, T., Bennett, M., Laplaze, L. (2013) Lateral root development in Arabidopsis: fifty shades of auxin. *Trends Plant Sci*. **18**, 450-458.

Li, G., Song, H., Li, B., Kronzucker, H.J., Shi, W. (2015) Auxin Resistant1 and PIN-FORMED2 Protect Lateral Root Formation in Arabidopsis under Iron Stress. *Plant Physiol*. **169**, 2608-2623.

Lieber, M.R. (2010) The mechanism of double-strand DNA break repair by the

nonhomologous DNA end-joining pathway. *Annu Rev Biochem.* **79**, 181-211.

Li, X., Mo, X., Shou, H., Wu, P. (2006) Cytokinin-mediated cell cycling arrest of pericycle founder cells in lateral root initiation of Arabidopsis. *Plant Cell Physiol*. **47**, 1112–1123.

Llorente, B., Smith, C.E., Symington, L.S. (2008) Break-induced replication: what is it and what is it for? *Cell Cycle* **7**, 859–864.

Malamy, J.E. (2005) Intrinsic and environmental response pathways that regulate root system architecture. *Plant Cell Environ.* **28**, 67–77.

Malamy, J.E., Benfey, P.N. (1997) Organization and cell differentiation in lateral roots of *Arabidopsis thaliana*. *Development* **124**, 33–44.

Mannuss, A., Trapp, O., Puchta, H. (2012) Gene regulation in response to DNA damage. *Biochim Biophys Acta*. **1819**, 154-165.

Manova, V., Gruszka, D. (2015) DNA damage and repair in plants - from models to crops. *Front Plant Sci*. **6**, 885.

Marchant, A., Bhalerao, R., Casimiro, I., Eklöf, J., Casero, P.J., Bennett, M., Sandberg, G. (2002) AUX1 promotes lateral root formation by facilitating indole-3-acetic acid distribution between sink and source tissues in the Arabidopsis seedling. *Plant Cell* **14**, 589-597.

Marhavý, P., Bielach, A., Abas, L., Abuzeineh, A., Duclercq, J., Tanaka, H., Pařezová, M., Petrášek, J., Friml, J., Kleine-Vehn, J., Benková, E. (2011) Cytokinin modulates endocytic trafficking of PIN1 auxin efflux carrier to control plant organogenesis. *Dev. Cell* **21**, 796–804.

Mason, M.G., Mathews, D.E., Argyros, D.A., Maxwell, B.B., Kieber, J.J., Alonso, J.M., Ecker, J.R., Schaller, G.E. (2005) Multiple type-B response regulators mediate cytokinin signal transduction in *Arabidopsis*. *Plant Cell* **17**, 3007–3018.

Mittler, R. (2002) Oxidative stress, antioxidants and stress tolerance. *Trends Plant Sci.* **7**, 405–410.

Miyawaki, K., Tarkowski, P., Matsumoto-Kitano, M., Kato, T., Sato, S., Tarkowska, D., Tabata, S., Sandberg, G., Kakimoto, T. (2006) Roles of *Arabidopsis* ATP/ADP isopentenyltransferases and tRNA isopentenyltransferases in cytokinin biosynthesis. *Proc. Natl. Acad. Sci. USA.* **103**, 16598–16603.

Møller, I.M., Jensen, P.E., Hansson, A. (2007) Oxidative modifications to cellular components in plants. *Annu. Rev. Plant Biol.* **58**, 459–481.

Moreno-Risueno, M.A., Van Norman, J.M., Moreno, A., Zhang, J., Ahnert, S.E., Benfey, P.N. (2010) Oscillating gene expression determines competence for periodic Arabidopsis root branching. *Science*. **329**, 1306-1311.

Moubayidin, L., Perilli, S., Dello Ioio, R., Di Mambro, R., Costantino, P., Sabatini, S. (2010) The rate of cell differentiation controls the Arabidopsis root meristem growth phase. *Curr. Biol.* **20**, 1138-1143.

Nakagawa, T., Ishiguro, S., Kimura, T. (2009) Gateway vectors for plant transformation. *Plant Biotechnol.* **26**, 275–284.

Nibau, C., Gibbs, D.J., Coates, J.C. (2008) Branching out in new directions: the control of root architecture by lateral root formation. *New Phytol.* **179**, 595–614.

Nishiyama, R., Watanabe, Y., Fujita, Y., Le, D.T., Kojima, M., Werner, T., Vankova, R.,

Yamaguchi-Shinozaki, K., Shinozaki, K., Kakimoto, T., Sakakibara, H., Schmülling, T., Tran, L.S. (2011) Analysis of cytokinin mutants and regulation of cytokinin metabolic genes reveals important regulatory roles of cytokinins in drought, salt and abscisic acid responses, and abscisic acid biosynthesis. *Plant Cell* **23**, 2169-2183.

Noll, D.M, Mason, T.M., Miller, P.S. (2006) Formation and repair of interstrand cross-links in DNA. *Chem Rev*. **106**, 277-301.

Okushima, Y., Fukaki, H., Onoda, M., Theologis, A., Tasaka, M. (2007) ARF7 and ARF19 regulate lateral root formation via direct activation of LBD/ASL genes in Arabidopsis. *Plant Cell* **19**, 118-130.

Osakabe, K., Yoshioka, T., Ichikawa, H., Toki, S. (2002) Molecular cloning and characterization of RAD51-like genes from *Arabidopsis thaliana*. *Plant Mol. Biol.* **50**, 71–81.

Péret, B., De Rybel, B., Casimiro, I., Benková, E., Swarup, R., Laplaze, L., Beeckman, T., Bennett, M.J. (2009) *Arabidopsis* lateral root development: an emerging story. *Trends Plant Sci.* **14**, 399–408.

Péret, B., Middleton, A.M., French, A.P., Larrieu, A., Bishopp, A., Njo, M., Wells, D.M., Porco, S., Mellor, N., Band, L.R., Casimiro, I., Kleine-Vehn, J., Vanneste, S., Sairanen, I., Mallet, R., Sandberg, G., Ljung, K., Beeckman, T., Benkova, E., Friml, J., Kramer, E., King, J.R., De Smet, I., Pridmore, T., Owen, M., Bennett, M.J. (2013) Sequential induction of auxin efflux and influx carriers regulates lateral root emergence. *Mol Syst Biol.* **9**, 699.

Pernisová, M., Klíma, P., Horák, J., Válková, M., Malbeck, J., Soucek, P., Reichman, P.,

Hoyerová, K., Dubová, J., Friml, J., Zazímalová, E., Hejátko, J. (2009) Cytokinins modulate auxin-induced organogenesis in plants via regulation of the auxin efflux. *Proc. Natl. Acad. Sci. USA*. **106**, 3609–3614.

Petricka, J.J., Winter, C.M., Benfey, P.N. (2012) Control of Arabidopsis root development. *Annu Rev Plant Biol*. **63**, 563-590.

Povirk, L.F. (1996) DNA damage and mutagenesis by radiomimetic DNA-cleaving agents: bleomycin, neocarzinostatin and other enediynes. *Mutat. Res.* **355**, 71–89.

Povirk, L.F. (2012) Processing of damaged DNA ends for double-strand break repair in mammalian cells. *ISRN Mol Biol*. doi: 10.5402/2012/345805.

Puchta, H. (2005) The repair of double-strand breaks in plants: mechanisms and consequences for genome evolution. *J Exp Bot*. **56**, 1-14.

Puchta, H., Fauser, F. (2014) Synthetic nucleases for genome engineering in plants: prospects for a bright future. *Plant J*. **78**, 727-741.

Rabik, C.A., Dolan, M.E. (2007) Molecular mechanisms of resistance and toxicity associated with platinating agents. *Cancer Treat Rev*. **33**, 9-23.

Richard, O., Pineau, C., Loubet, S., Chalies, C., Vile, D., Marquès, L., Berthomieu, P. (2011) Diversity analysis of the response to Zn within the Arabidopsis thaliana species revealed a low contribution of Zn translocation to Zn tolerance and a new role for Zn in lateral root development. *Plant Cell Environ*. **34**, 1065-1078.

Rink, S.M., Lipman, R., Alley, S.C., Hopkins, P.B., Tomasz, M. (1996) Bending of DNA by the mitomycin C-induced, GpG intrastrand cross-link. *Chem. Res. Toxicol.* **9**, 382–389.

Ruegger, M., Dewey, E., Gray, W.M., Hobbie, L., Turner, J., Estelle, M. (1998) The TIR1 protein of Arabidopsis functions in auxin response and is related to human SKP2 and yeast grr1p. *Genes Dev.* **12**, 198–207.

Ruzicka, K., Simásková, M., Duclercq, J., Petrásek, J., Zazímalová, E., Simon, S., Friml, J., Van Montagu, M.C., Benková, E. (2009) Cytokinin regulates root meristem activity via modulation of the polar auxin transport. *Proc. Natl. Acad. Sci. USA*. **106**, 4284–4289.

Saban, N., Bujak, M. (2009) Hydroxyurea and hydroxamic acid derivatives as antitumor drugs. *Cancer Chemother. Pharmacol.* **64**, 213–221.

Sabatini, S., Beis, D., Wolkenfelt, H., Murfett, J., Guilfoyle, T., Malamy, J., Benfey, P., Leyser, O., Bechtold, N., Weisbeek, P., Scheres, B. (1999) An auxin-dependent distal organizer of pattern and polarity in the Arabidopsis root. *Cell* **24**, 463-472.

Schaller, G.E., Street, I.H., Kieber, J.J. (2014) Cytokinin and the cell cycle. *Curr Opin Plant Biol*. **21**, 7-15.

Sakakibara, H. (2006) Cytokinins: activity, biosynthesis, and translocation. *Annu. Rev. Plant Biol.* **57**, 431–449.

Sakakibara, H., Kasahara, H., Ueda, N., Kojima, M., Takei, K., Hishiyama, S., Asami, T., Okada, K., Kamiya, Y., Yamaya, T., Yamaguchi, S. (2005) Agrobacterium tumefaciens increases cytokinin production in plastids by modifying the biosynthetic pathway in the host plant. *Proc Natl Acad Sci USA*. **102**, 9972-9977.

Shrivastav, N., Li, D., Essigmann, J.M. (2010) Chemical biology of mutagenesis and DNA repair: cellular responses to DNA alkylation. *Carcinogenesis*. **31**, 59-70.

Sikora, P., Chawade, A., Larsson, M., Olsson, J., Olsson, O. (2011) Mutagenesis as a tool in plant genetics, functional genomics, and breeding. *Int J Plant Genomics*. **2011**, 314829.

Sjogren, C.A., Bolaris, S.C., Larsen, P.B. (2015) 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.

Sun, J., Niu, Q.W., Tarkowski, P., Zheng, B., Tarkowska, D., Sandberg, G., Chua, N.H., Zuo, J. (2003) The Arabidopsis AtIPT8/PGA22 gene encodes an isopentenyl transferase that is involved in de novo cytokinin biosynthesis. *Plant Physiol*. **131**, 167-176.

Swarup, K., Benková, E., Swarup, R., Casimiro, I., Péret, B., Yang, Y., Parry, G., Nielsen, E., De Smet, I., Vanneste, S., Levesque, M.P., Carrier, D., James, N., Calvo, V., Ljung, K., Kramer, E., Roberts, R., Graham, N., Marillonnet, S., Patel, K., Jones, J.D., Taylor, C.G., Schachtman, D.P., May, S., Sandberg, G., Benfey, P., Friml, J., Kerr, I., Beeckman, T., Laplaze, L., Bennett, M.J. (2008) The auxin influx carrier LAX3 promotes lateral root emergence. *Nat. Cell Biol.* **10**, 946–954.

Symington, L.S. (2014) End resection at double-strand breaks: mechanism and regulation. *Cold Spring Harb Perspect Biol*. **6**, pii: a016436.

Takei, K., Sakakibara, H., Sugiyama, T. (2001) Identification of genes encoding adenylate isopentenyltransferase, a cytokinin biosynthesis enzyme, in *Arabidopsis thaliana*. *J. Biol. Chem.* **276**, 26405–26410.

Tripathy, B.C., Oelmüller, R. (2012) Reactive oxygen species generation and signaling

in plants. *Plant Signal Behav.* **7**, 1621–1633.

Tuteja, N., Singh, M.B., Misra, M.K., Bhalla. P.L., Tuteja, R. (2001) Molecular mechanisms of DNA damage and repair: progress in plants. *Crit Rev Biochem Mol Biol*. **36**, 337-397.

Ulmasov, T., Murfett, J., Hagen, G., Guilfoyle, T.J. (1997) Aux/IAA proteins repress expression of reporter genes containing natural and highly active synthetic auxin response elements. *Plant Cell* **9**, 1963-1971.

Van der Weele, C.M., Spollen, W.G., Sharp, R.E., Baskin, T.I. (2000) Growth of *Arabidopsis thaliana* seedlings under water deficit studied by control of water potential in nutrient-agar media. *J. Exp. Bot.* **51**, 1555–1562.

Vermeer, J.E., von Wangenheim, D., Barberon, M., Lee, Y., Stelzer, E.H., Maizel, A., Geldner, N. (2014) A spatial accommodation by neighboring cells is required for organ initiation in Arabidopsis. *Science*. **343**, 178-183.

Wang, C., Liu, Z. (2006) *Arabidopsis* ribonucleotide reductases are critical for cell cycle progression, DNA damage repair, and plant development. *Plant Cell* **18**, 350–365.

Wang, R., Estelle, M. (2014) Diversity and specificity: auxin perception and signaling through the TIR1/AFB pathway. *Curr Opin Plant Biol*. **21**, 51–58.

Wang, Q., Goldstein, M., Alexander, P., Wakeman, T.P., Sun, T., Feng, J., Lou, Z., Kastan, M.B., Wang, X.F. (2014) Rad17 recruits the MRE11-RAD50-NBS1 complex to regulate the cellular response to DNA double-strand breaks. *EMBO J.* **33**, 862-877.

Werner, T., Motyka, V., Laucou, V., Smets, R., Van Onckelen, H., Schmülling, T. (2003) Cytokinin-deficient transgenic Arabidopsis plants show multiple developmental

alterations indicating opposite functions of cytokinins in the regulation of shoot and root meristem activity. *Plant Cell* **15**, 2532–2550.

Yi, D., Kamei, C.L.A., Cools, T., Vanderauwera, S., Takahashi, N., Okushima, Y., Eekhout, T., Yoshiyama, K.O., Larkin, J., Van den Daele, H., Conklin, P., Britt, A., Umeda, M., De Veylder, L. (2014) 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.

Yoshiyama, K., Conklin, P.A., Huefner, N.D., Britt, A.B. (2009) Suppressor of gamma response 1 (SOG1) encodes a putative transcription factor governing multiple responses to DNA damage. *Proc. Natl. Acad. Sci. USA* **106**, 12843–12848.

Yoshiyama, K.O., Kobayashi, J., Ogita, N., Ueda, M., Kimura, S., Maki, H., Umeda, M. (2013) ATM-mediated phosphorylation of SOG1 is essential for the DNA damage response in *Arabidopsis*. *EMBO Rep.* **14**, 817–822.

Zeman, M.K., Cimprich, K.A. (2014) Causes and consequences of replication stress. *Nat Cell Biol.* **16**, 2-9.

Zhang, W., To, J.P., Cheng, C.Y., Schaller, G.E. Kieber, J.J. (2011) Type-A response regulators are required for proper root apical meristem function through the post-transcriptional regulation of PIN auxin efflux carriers. *Plant J*. **68**, 1–10.

Zhao, Y. (2012) Auxin biosynthesis: a simple two-step pathway converts tryptophan to indole-3-acetic acid in plants. *Mol Plant*. **5**, 334-338.