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Programmed induction of endoreduplication by DNA double-strand breaks in *Arabidopsis*

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Abstract

Genome integrity is continuously threatened by external stresses and by endogenous hazards such as DNA replication errors and reactive oxygen species. The DNA damage checkpoint in metazoans ensures genome integrity, by delaying cell cycle progression to repair damaged DNA or by inducing apoptosis. ATM and ATR (ataxia-telangiectasia-mutated and -Rad3-related) are sensor kinases that relay the damage signal to transducer kinases Chk1 and Chk2, and to downstream cell cycle regulators. Plants also possess ATM and ATR orthologues, but lack obvious counterparts of downstream regulators. Instead, the plant-specific transcription factor SOG1 (suppressor of gamma response 1) plays a central role in the transmission of signals from both ATM and ATR kinases. Here we show that, in *Arabidopsis,* endoreduplication is induced by DNA double-strand breaks (DSBs), but not directly by DNA replication stress. When root or sepal cells, or undifferentiated suspension cells, were treated with DSB inducers, they displayed increased cell size and DNA ploidy. We found that the ATM–SOG1 and ATR–SOG1 pathways both transmit DSB-derived signals, and that either one suffices for endocycle induction. These signaling pathways govern the expression of distinct sets of cell cycle regulators, such as cyclin-dependent kinases (CDKs) and their suppressors. Our results demonstrate that *Arabidopsis* undergoes a programmed endoreduplicative response to DSBs, suggesting that plants have evolved a distinct strategy to sustain growth under genotoxic stress.

Introduction

Damaged DNA needs to be repaired to prevent loss or incorrect transmission of genetic information. Eukaryotic DNA damage checkpoints delay or arrest the cell cycle to provide time for DNA repair before the cell enters a new round of DNA replication or mitosis (1). In metazoans, ATM and ATR (ataxia-telangiectasia-mutated and -Rad3-related) are sensor kinases that play a crucial role in the checkpoint system. ATM specifically responds to DNA double-strand breaks (DSBs), and ATR primarily senses replication stress caused by a persistent block of replication fork progression. ATM deficiency confers hypersensitivity to ionizing radiation (2), whereas ATR knockout mutation is lethal (3, 4) and dominant-negative cell lines display hypersensitivity to UV-B light, gamma radiation, hydroxyurea (HU) and aphidicolin (5, 6). ATM and ATR relay the damage signal to transducer kinases Chk2 and Chk1, respectively, which then amplify the signal and regulate an overlapping set of substrates that trigger cell cycle arrest and DNA repair (1). The transcription factor p53, cyclin-dependent kinase (CDK) inhibitor p21 and Cdc25 phosphatase are downstream regulators that control cell cycle arrest in response to DNA damage.

Comparative sequence analyses among plants, yeast and animals indicate that some of the factors involved in DNA damage checkpoint and DSB repair systems are conserved between vertebrates and plants (7). Plants also possess ATM and ATR orthologues, and knockout mutants show similar phenotypes to those of their mammalian counterparts. *Arabidopsis atm* mutants are sensitive to gamma radiation and are defective in transcriptional induction of repair genes in response to irradiation (8). *atr* mutants are sensitive to replication-blocking agents including HU and aphidicolin and to UV-B light (9). It is noteworthy, however, that, unlike mammalian ATR knockouts, *Arabidopsis atr* mutants are viable (9), suggesting that regulatory

mechanisms underlying the DNA damage response are diverged between plants and animals. This is not surprising because plants are continuously exposed to environmental stresses, and thus need to deploy a robust checkpoint system to cope with genotoxic stress. Plants lack obvious counterparts of Cdc25, Chk1, Chk2, p21 and p53, while a recent report has identified a plant-specific transcription factor, SOG1 (suppressor of gamma response 1), that participates in pathways governed by both ATM and ATR kinases (10). In this study, we found that *Arabidopsis* cells treated with DSB inducers displayed increased cell size and DNA ploidy without a concomitant change in chromosome number. This endoreduplicative response required ATM, ATR and SOG1, indicating that it is a unique programmed mechanism for plants to survive under genotoxic stress.

Results

DSBs cause endoreduplication in *Arabidopsis* root tips and sepals

To identify plant-specific mechanisms underlying the genotoxic stress response, we first observed *Arabidopsis* roots that had been exposed to the radiomimetic reagent zeocin (11, 12). When seedlings were transferred to 10 μM zeocin plates, root growth was arrested and cyclin B1 expression (indicative of progression into G2) was reduced (Fig. S1 *A* and *B*). *AtGR1* and *RAD51* transcripts were both strongly induced as early as 8 h after transfer to zeocin, consistent with activation of the known plant transcriptional response to DSBs (Fig. S1*C*). Interestingly, the epidermal cells of the root tip were enlarged by this treatment (Fig. 1*A*). A plot of epidermal cell area against distance from the quiescent center (QC) at 24 h revealed that zeocin-induced cell expansion became pronounced in cells at distances over 150 μm (Fig. 1*B*); for instance, a significant increase in cell area was observed for cells at distances between

160 μm and 190 μm from the QC (Table S1). Comparable cell enlargement was observed in cortex cells (Fig. S1 *D* and *E*, and Table S1).

To examine whether zeocin also causes cell expansion in other tissues, we observed epidermal cells of *Arabidopsis* sepals. The abaxial side of the sepal epidermis contains elongated giant cells whose length is ~20% that of the sepal, interspersed with much smaller (~1% of sepal length) cells (13) (Fig. S2*A*). During flower development, early onset of endoreduplication produces these giant cells with a concomitant increase of DNA content and cell area, while the surrounding cells continue to divide and remain small (13). However, when flower buds at an early stage were treated with 20 μM zeocin for 43 h, not only the giant cells but also many of the neighboring cells became enlarged (Fig. S2). This indicates that zeocin induced cell expansion in the smaller dividing cells.

Cell size usually correlates with nuclear DNA content (14); indeed, the percentage of 2C cells in zeocin-treated root tips was reduced, while that of 16C cells was increased (Fig. 1*C*). A similar but less pronounced effect of zeocin has also been noted in leaves (15). In root tips, the content of histone H2B-labeled DNA was elevated, especially in cells more than 150 μm from the QC (Fig. S3*A*), indicating that zeocin-induced cell enlargement is accompanied by an increase in DNA content, as observed during normal cell differentiation processes of *Arabidopsis*. The number of kinetochores, counted by marking the centromeric histone H3, was unaffected by zeocin (Fig. S3*B*), suggesting that chromosome number did not change. These results indicate that zeocin treatment induced endoreduplication rather than endomitosis (in which cells enter but do not complete mitosis (16)). Since endoreduplication rarely occurs in cells located less than 200 μm from the untreated QC (17), our results demonstrate that zeocin induces an early onset of endoreduplication in the root

meristem.

Gamma irradiation also enlarged *Arabidopsis* root cells (Fig. 1*D*). Since both zeocin and gamma rays induce DSBs, we then tested other genotoxic agents that induce replication blocks: HU, methyl methanesulfonate (MMS), cisplatin, and UV irradiation. Under conditions where root growth was retarded but not arrested (Fig. 1*E*), none of these agents induced cell expansion to the degree observed with zeocin and gamma radiation (Fig. 1*D*). Simultaneous application of zeocin and HU, but not zeocin and cisplatin, caused a partial inhibition ($P = 0.017$; Student's *t*-test) of cell expansion relative to zeocin treatment alone (Fig. S4*A*). Nevertheless, a significant cell enlargement still occurred with zeocin + HU relative to the non-treated control (Table S1), indicating that cells treated with HU retain their potential to undergo endoreduplication; the lack of significant difference in cell size between HU-treated and non-treated cells (Fig. 1*D* and Table S1) was thus attributable mainly to an inability of HU to induce cell expansion rather than to its inhibitory effect on DNA synthesis. These results indicate both that zeocin and gamma irradiation indeed caused DSBs and induced endoreduplication, and also that cell enlargement is not an inevitable result of root growth inhibition. The enlarged cells observed in cisplatin- or MMS-treated root tips (Table S1) may be due to DSBs arising from a drug-induced replication block.

DSB-induced endoreduplication requires ATM, ATR and SOG1

To identify regulatory factors that are engaged in DSB-induced endoreduplication, we observed zeocin responses in several *Arabidopsis* mutants. We found that zeocin-induced cell expansion occurred in *atm-2* or *atr-2*, but was significantly suppressed in the *atm-2 atr-2* double mutant or in *sog1-1* (Fig. 2 *A* and *B*, and Table S1). This

demonstrates that the ATM–SOG1 and ATR–SOG1 pathways both transmit DSB-derived signals, and that either one suffices for endocycle induction. While *sog1-1* is more resistant to zeocin than wild-type, 24 h treatment with 10 μM zeocin almost abolished root growth (Fig. 2*C*) but did not increase cell area. This again indicates that DSB-induced endoreduplication is not a passive outcome of a block of mitosis; rather, it is a programmed process mediated by ATM/ATR–SOG1.

A previous report showed that *WEE1*, which encodes an inhibitory kinase of CDKs, was induced by genotoxic stress and that *wee1* mutants were hypersensitive to HU, indicating a function in the DNA damage checkpoint (18). However, we found that root growth and root cell size in *wee1* seedlings were comparable to those in wild-type, regardless of zeocin treatment (Fig. 2 *A* and *C*). Previous studies have shown that CCS52A, an activator of the anaphase-promoting complex/cyclosome, promotes transition to the endocycle during development (19, 20). However, neither the *ccs52a1* nor the *ccs52a2* mutation inhibited zeocin-induced endoreduplication (Fig. S4 *B* and *C*, and Table S1). WEE1 and CCS52 are thus nonessential for DSB-induced endoreduplication, although they may function with other cell cycle regulators as described below.

DSBs inhibit mitotic entry and produce endoreduplicated cells in cell culture

To survey the expression profiles of cell cycle regulators in response to DSBs, we used an *Arabidopsis* cell culture, MM2d. MM2d cells usually have a basal ploidy of 6C (21), but after 72 h of 50 μM zeocin treatment a 24C population appeared (Fig. 3*A*); cell and nuclear sizes increased, while the kinetochore number remained constant at around 30 or 60 (Fig. 3 *B* and *C*). We then monitored cell cycle progression by subculturing seven-day-old MM2d cells, which are partially arrested at G1 phase, into

new medium. This procedure excludes any possible effect of replication stress that might arise from synchronization with S-phase blockers. While the cell cycle duration was around 24 h, zeocin treatment delayed cell cycle progression by about 6 h (Fig. 3*D*; compare ploidy distribution for 12 h without zeocin and 18 h with zeocin), and the proportion of 6C cells did not increase after 18 h. Instead, a fraction of endoreduplicated 24C cells appeared when the cells had been exposed to zeocin for 48 h, and the 24C peak became prominent after 72 h (Fig. 3*D*).

Plants have two types of CDKs that directly control the cell cycle: CDKA, an orthologue of yeast Cdc2/Cdc28, and CDKB, a plant-specific CDK. The latter includes two subtypes, CDKB1 and CDKB2, which are expressed from late S-to-M phase and from G2-to-M phase, respectively (22). In zeocin-treated MM2d cells, transcript and protein accumulation for CDKA was almost the same as that in non-treated cells until 96 h, while their accumulation for CDKB1 was delayed (probably due to the slower cell cycle progression described above) (Fig. 4 *A* and *B*). For CDKB2, transcripts and protein started to accumulate after 8 h in the absence of zeocin, but did not accumulate in zeocin-treated cells (Fig. 4 *A* and *B*). This suggests that the cell cycle was arrested before the onset of *CDKB2* expression, and that cells then switched to the endocycle, which consists of G and S phases without mitosis (16). These results show that zeocin induces a transition to the endocycle in undifferentiated cultured cells, indicating that DSBs cause endoreduplication irrespective of the cell's differentiation state.

DSBs control coordinated expression of cell cycle-related genes

We conducted a microarray analysis using samples from partially synchronized MM2d cells at different time points. Among 23,338 genes whose expression passed a

quality control filter with replicated samples, we identified 3,678 that showed a significant change at the mRNA level. Among cell cycle-related genes, we found that many genes for cyclins A (CYCA) and B (CYCB) were downregulated upon zeocin treatment. Transcripts of mitotic cyclins normally accumulate as cells enter the G2 phase (within 12 h after subculturing) (23), but they displayed no such increase in the presence of zeocin (Fig. S5*A* and Table S2). Whether their downregulation is a cause or a consequence of cell cycle arrest by DSBs remains to be clarified.

On the other hand, zeocin upregulated the expression of several cell cycle-related genes: *CYCB1;1*, *CCS52A1*, *WEE1*, and the CDK inhibitors *SIM*, *SMR1* and *SMR5* (Fig. S5*B* and Table S2). *CYCB1;1* is a unique *CYCB* that is rapidly upregulated in response to ionizing radiation (24). The other five factors are all negative regulators of CDK activity, and are thus likely to contribute to DSB-induced cell cycle arrest. In fact, overexpression of *CCS52A1*, or of the CDK inhibitor *SIM*, is known to promote endoreduplication (20, 25). In a previous analysis of global transcription, transcripts of these CDK suppressors were significantly elevated by gamma irradiation (24); since this response was cancelled in the *atm* mutant, but not in *atr*, it is inferred that DSB signals upregulate these genes via the ATM pathway. A SOG1-dependent response is also noted for *SMR5* and *WEE1* in previous microarray data (10). (For *SIM*, *SMR1* and *CCS52A1*, transcriptional induction by short-term gamma irradiation was not highly significant (10, 24), making it difficult to assess the effect of the *sog1* mutation.)

DSBs induce CDKB2 protein degradation via the ATR–SOG1 pathway

As described above, both ATM and ATR, and also SOG1, are involved in DSB-induced endoreduplication, but all the CDK suppressors mentioned above are

controlled via the ATM pathway. This clearly implies that other cell cycle regulator(s) are under the control of the ATR–SOG1 pathway. We studied CDK expression in *Arabidopsis* root tips and found that expression of *CDKB2;1*, but not *CDKA;1* or *CDKB1;1*, was suppressed by zeocin treatment, as observed in MM2d cells (Fig. S6*A*). To compare *CDKB2;1* expression at the RNA and protein levels, we used *ProCDKB2;1:GUS* (a fusion of the *CDKB2;1* promoter and the GUS gene, to monitor the promoter's activity) and *ProCDKB2;1:NT-GUS* (the same promoter and the first *CDKB2;1* exon (*NT*) fused in-frame to GUS, to monitor protein-level expression of CDKB2;1) (26). GUS expression in the *ProCDKB2;1:GUS* lines decreased gradually in the presence of 10 μM zeocin (Fig. S6*B*), probably reflecting the stability of GUS protein. By contrast, accumulation of the NT-GUS fusion protein was drastically reduced after 8 h, and this reduction was suppressed when a proteasome inhibitor, MG132, was applied together with zeocin (Fig. 4*C* and Fig. S6*B*). It is noteworthy that, in the presence of zeocin, CDKB2;1 was stabilized by the *sog1* mutation in the *xpf-2* background, where the *sog1-1* mutant was originally isolated as a suppressor of the growth arrest induced by gamma irradiation (27) (Fig. 4*D*). CDKB2;1 degradation was also suppressed in *atr-2* (Fig. 4*E*). Although we could not reproducibly observe a significant decrease in *NT-GUS* expression in *atm-2*, for unknown reasons, our data with the *atr-2* mutant indicate that the ATR–SOG1 pathway plays a major role in CDKB2;1 degradation via the ubiquitin-proteasome pathway.

Discussion

Two recent reports have shown that *Arabidopsis* root and shoot stem cells and their descendants undergo cell death upon DNA damage (12, 28). In the stem cell niche, programmed cell death may be a viable way to remove DNA-damaged cells, but here

we found that DSBs induced early onset of endoreduplication in the transition zone of roots and the sepal epidermis. A previous study suggested that the loss of a component of the replisome complex increased cell size and DNA ploidy (29). These changes might instead have been triggered by DSBs arising from a block in replication, however, because our results indicate that DNA replication stress does not itself induce endoreduplication. DSBs are so toxic because, if they are not repaired prior to cell division, daughter cell(s) will lose part of an arm of an affected chromosome. Plant cells afflicted by ionizing radiation are therefore faced with the choice of either delaying cell division to repair the damage or inducing cell death. However, our findings indicate that a third option is available, namely cell expansion by repeating DNA replication without cell division. A likely benefit of this choice is that entry into endoreduplication prevents DNA-damaged cells from proliferating and also from dying. Because plant cells, unlike animal cells, cannot migrate within tissues, cell death usually leaves behind an opening in the local tissue structure (e.g., xylem in vascular tissue). *Arabidopsis* may therefore have acquired the strategy of actively inducing endoreduplication to prevent such gaps from arising in damaged tissue, and thus to guarantee uninterrupted development during the life cycle.

Our results showed that both the ATM–SOG1 and ATR–SOG1 pathways are associated with DSB-induced endoreduplication (Fig. S7). Single mutants for *ATM* or *ATR* exhibited endoreduplication to an extent similar to that seen in wild-type plants. This result was unexpected because most of the genes induced by gamma irradiation are under the control of ATM (24). However, while ATM responds to DSBs induced directly by ionizing radiation, ATR is also associated with long-term responses through its sensing of replication blocks which occur in lesions during G1-to-S progression (24). Indeed, a radioresistant-growth phenotype in *Arabidopsis* seedlings

was observed in *xpf-2 atr-4* but not in *xpf-2 atm-2* mutants, suggesting that ATR is required to maintain cell cycle arrest (10). These results are consistent with our observation that ATR, together with ATM, is associated with inhibition of entry to M phase and endocycle induction. It is interesting that, in root stem cells, UVB- and gamma-induced programmed cell death is activated by SOG1 and by either ATM or ATR (12, 28). Although it remains an open question how DNA damage kills *Arabidopsis* stem cells, G2 arrest may be provoked prior to cell death by overlapping mechanisms with those involved in DSB-induced endoreduplication.

We showed that *wee1* seedlings responded normally to zeocin. This result, together with the previously observed hypersensitivity of *wee1* mutants to aphidicolin and HU (18), indicates that WEE1 is essential to the replication stress response, but that its function is not critical for the DSB response. *CCS52A1* is expressed in the elongation zone of *Arabidopsis* roots, and its overexpression stimulates endoreduplication and mitotic exit (20, 30). However, we could find DSB-induced cell expansion in *ccs52a1* mutants, indicating that CCS52A1 is not essential for DSB-induced endoreduplication. These observations are consistent with the finding that endoreduplication occurred in the *atm-2* mutant, in which *WEE1* and *CCS52A1* are not induced by DSBs (24). Previous reports indicated that *CDKB2* knockdown lines have significantly higher DNA content (31). However, in the *atr-2* mutant, zeocin did not decrease the protein level of CDKB2, but endoreduplication occurred normally. We therefore propose that DSB signals affect the expression of distinct sets of cell cycle regulators, such as CDK suppressors and CDKB2, through each of the ATM- and ATR-dependent pathways, and switch the mitotic cell cycle to the endocycle (Fig. S7). Our observations also indicate that, although SOG1 plays a central role in the transmission of signals from both ATM and ATR, its function diverges along the

ATM- and ATR-dependent pathways. Further studies will reveal how DSB signals control entry into endoreduplication by modulating the SOG1 function.

Materials and Methods

Plant materials and growth conditions. *atm-2*, *atr-2*, *wee1-3*, *ccs52a1-1*/*fzr2-1* and *ccs52a2-1* are in the Col background, while *xpf*-2 was isolated in the Landsberg *erecta* (L*er*) background, and *sog1-1* is a hybrid of Col and L*er* (27). Origins of mutant lines and growth conditions for plants and cell culture are described in *SI Materials and Methods*.

Propidium iodide staining and GUS staining. Cells in root tips and sepals were stained with propidium iodide according to the method described by Truernit *et al.* (32) with some modifications. Stained samples were observed with a confocal laser scanning microscope (FV1000, Olympus), and cell area and distance from QC were measured with MBF ImageJ software by tracing the contours of cells. For a detailed description of propidium iodide staining, see *SI Materials and Methods*. GUS staining was performed as described previously (26); samples were incubated at 37 °C for 24 h except for *ProCDKA;1:CDKA;1-GUS* (10 min), *ProCDKB1;1:CDKB1;1-GUS* (30 min), and *ProCDKB2;1:CDKB2;1-GUS* (50 min).

Kinetochore counting and DNA ploidy measurements. To determine kinetochore number, fluorescent signals of anti-HTR12 antibodies in MM2d cells and tdTomato in roots of *Pro35S:tdTomato-HTR12* were counted using the Cell Counter plug-in of ImageJ. For a detailed description of kinetochore counting, see *SI Materials and Methods*. To measure the DNA ploidy in each cell, roots of *ProRPS5A:H2B-*

tdTomato were observed under a fluorescence microscope (IX-81; Olympus) in combination with the 4D viewer /3D measurement module of Metamorph ver. 7.5 (Molecular Devices). Ploidy distribution in roots and MM2d cells was measured with a ploidy analyzer PA (Partec) and CyStain UV precise P (Partec).

RT-PCR and immunoblotting. Total RNA was extracted from 0.5-cm root tips of 5-day-old seedlings or MM2d cells. Immunoblotting was conducted with specific antisera raised against *Arabidopsis* CDKs as the primary antibody and horseradish peroxidase-conjugated goat anti-rabbit IgG (GE Healthcare) as the secondary antibody. For detailed descriptions of RT-PCR and immunoblotting, see *SI Materials and Methods*.

Microarray analysis. The analysis was performed with the Agilent *Arabidopsis* 3 Oligo Microarray for 44K Microarray analysis (Agilent Technologies). Samples of MM2d cells were biologically duplicated, and each RNA sample was labeled for Cy3-cRNA probes, according to the instructions for a one-color experiment. The hybridized and washed material on each glass slide was scanned by an Agilent DNA microarray scanner G2505B (Agilent Technologies). For a detailed description of microarray analysis, see *SI Materials and Methods*. The microarray data have been deposited at http://cibex.nig.ac.jp/index.jsp under the accession number CBX148.

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ProCYCB1;2:CYCB1;2-GUS line. We also thank the ABRC at Ohio State University for providing seeds of T-DNA insertion mutants. This work was supported by Grants-in-Aid for Scientific Research on Innovative Areas (Grant No. 22119009) from the Ministry of Education, Culture, Sports, Science and Technology of Japan, and by the Program for Promotion of Basic Research Activities for Innovative Biosciences. Imaging analyses were supported by BIRD, Japan Science and Technology Agency. S.A. and D.K. were supported by Research Fellowships of the Japan Society for the Promotion of Science for Young Scientists.

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Figure Legends

Fig. 1. DSB-induced cell enlargement in root tips. (*A*) Propidium iodide-stained root tips. Five-day-old seedlings were treated with 0 or 10 μM zeocin for 24 h. (*B*) Measurement of root cell size. Five-day-old seedlings (n = 5) were treated with 0 or 10 μM zeocin, and epidermal cell area was measured after 24 h. Regression lines are included; R^2 = 0.61 (control) and 0.81 (+zeocin). The statistical significance of regression was estimated from the F-test; Significance $F < 0.001$ for both control and +zeocin. (*C*) DNA ploidy distribution in root tips. Seven-day-old seedlings were treated with 10 μM zeocin, and nuclear ploidy in 0.5-cm root tips was measured with a ploidy analyzer at the indicated time points. (*D, E*) Measurement of cell size and root growth upon genotoxic stress. Five-day-old seedlings ($n \geq 4$ for *D*, $n \geq 11$ for *E*) were irradiated with 150 Gy gamma or 1 kJ m^{-2} UV rays, or treated with 10 mM HU, 50 μM cisplatin (CP), or 100 ppm MMS. Epidermal cell area (*D*) and root growth (*E*) were measured after 24 h. Regression lines are included in *D*; R^2 = 0.21 (-gamma rays), 0.64 (+gamma rays), 0.62 (-UV), 0.70 (+UV), 0.26 (-HU), 0.56 (+HU), 0.38 (-CP and +CP), 0.37 (-MMS), and 0.52 (+MMS); Significance $F < 0.001$ for all regression analyses. The error bars in *E* represent SD.

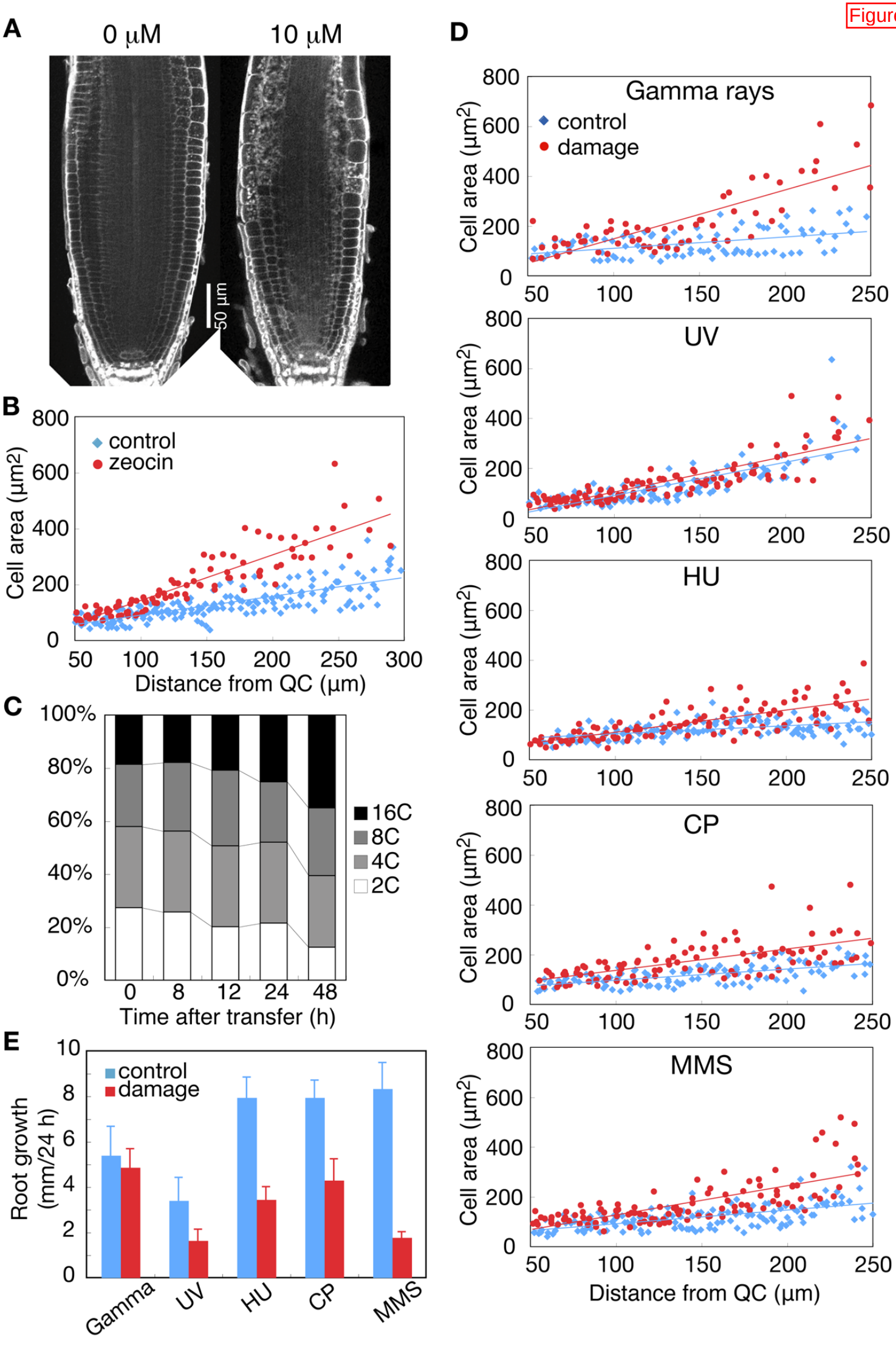
Fig. 2. DSB-induced endoreduplication via the ATM/ATR–SOG1 pathway. (*A, B*) Five-day-old wild-type and mutant seedlings (n = 5) were treated with 0 or 10 μM zeocin, and epidermal cell area was measured after 24 h. Regression lines are included; R^2 = 0.61 (Col, control), 0.81 (Col, +zeocin), 0.62 (*atm-2*, control), 0.64 (*atm-2*, +zeocin), 0.49 (*atr-2*, control), 0.68 (*atr-2*, +zeocin), 0.60 (*atm-2 atr-2*, control), 0.63 (*atm-2 atr-2*, +zeocin), 0.49 (*wee1-3*, control), 0.67 (*wee1-3*, +zeocin), 0.42 (Col/L*er*, control), 0.62 (Col/L*er*, +zeocin), 0.51 (*sog1-1*, control), and 0.45

(*sog1-1*, +zeocin); significance $F < 0.001$ for all regression analyses. (*C*) Five-day-old seedlings of wild-type, *wee1-3* and *sog1-1* were treated with 0 μM (blue), 2 μM (yellow) or 10 μM (red) zeocin, and root growth was measured. The error bars represent SD ($n \geq 23$). Hybrids of Columbia and Landsberg *erecta* (Col/L*er*) were used as a control for *sog1-1*.

Fig. 3. DSB-induced endoreduplication in *Arabidopsis* cultured cells. (*A*) DNA ploidy distribution in MM2d cells treated with or without 50 μM zeocin for 72 h. (*B*) DAPI-stained MM2d cells treated with or without 50 μM zeocin for 48 h. (*C*) Kinetochore number and nuclear area in MM2d cells treated with or without 50 μM zeocin for 72 h. (*D*) DNA ploidy analysis of partially synchronized MM2d cells. A seven-day-old culture was subcultured into medium with or without 50 μM zeocin and cultured for the indicated times. Positions of 6C, 12C and 24C peaks are shown with asterisks, double asterisks and open triangles, respectively.

Fig. 4. Expression profiles of CDKs in response to DSBs. MM2d cells were treated with or without 50 μM zeocin as shown in Fig. 3*D*. (*A*) Transcript accumulation of CDKs. Total RNA was subjected to real-time RT-PCR. Expression levels were normalized to *ACT8* and are indicated as relative values, with those for 0 h set to 1. The error bars represent the SD of three replicates. (*B*) Protein accumulation of CDKs. One hundred micrograms of protein extract were used for immunoblotting with specific antibodies against the indicated *Arabidopsis* CDKs. (*C* to *E*) Degradation of CDKB2 protein via the ATR–SOG1 pathway. Seven-day-old *ProCDKB2;1:NT-GUS* seedlings were treated with 10 μM zeocin, or 10 μM zeocin and 100 μM MG132, for 8 h (*C*). *ProCDKB2;1:NT-GUS* was introduced into *xpf-2*, *xpf-2 sog1-1* (*D*), or *atr-2*

(*E*), and GUS expression was observed after treatment with or without 10 μM zeocin for 24 h.


A
0 μM
10 μM
50 μm
B
control
zeocin
Cell area (μm2)
Distance from QC (μm)
C
100%
80%
60%
40%
20%
0%
16C
8C
4C
2C
0
8
12
24
48
Time after transfer (h)
D
Gamma rays
control
damage
UV
HU
CP
MMS
Cell area (μm2)
Distance from QC (μm)
E
control
damage
Root growth (mm/24 h)
Gamma
UV
HU
CP
MMS

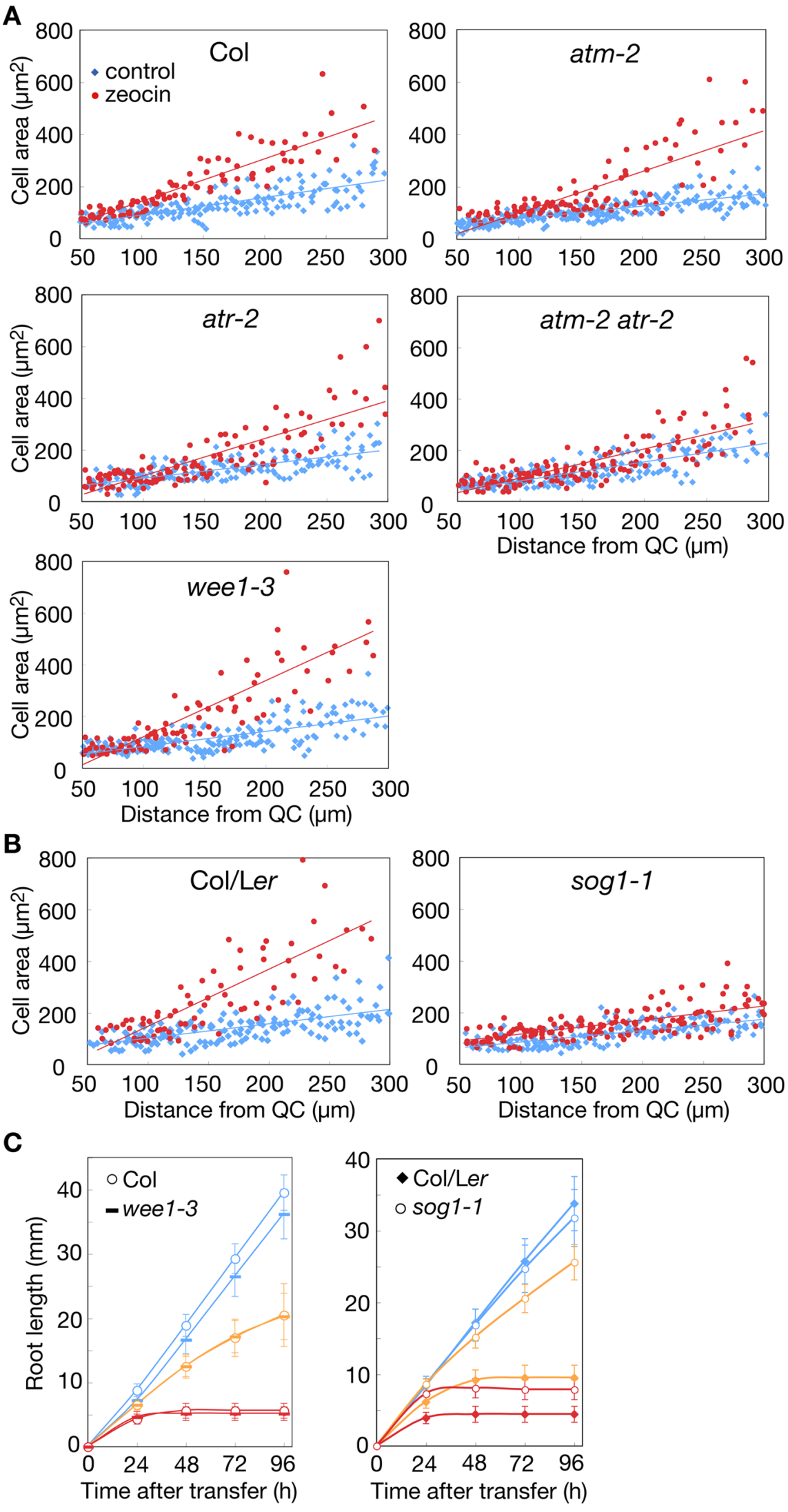

A
Col
control
zeocin
atm-2
atr-2
atm-2 atr-2
wee1-3
Cell area (μm2)
Distance from QC (μm)
B
Col/Ler
sog1-1
C
Col
wee1-3
Col/Ler
sog1-1
Root length (mm)
Time after transfer (h)

Figure 3

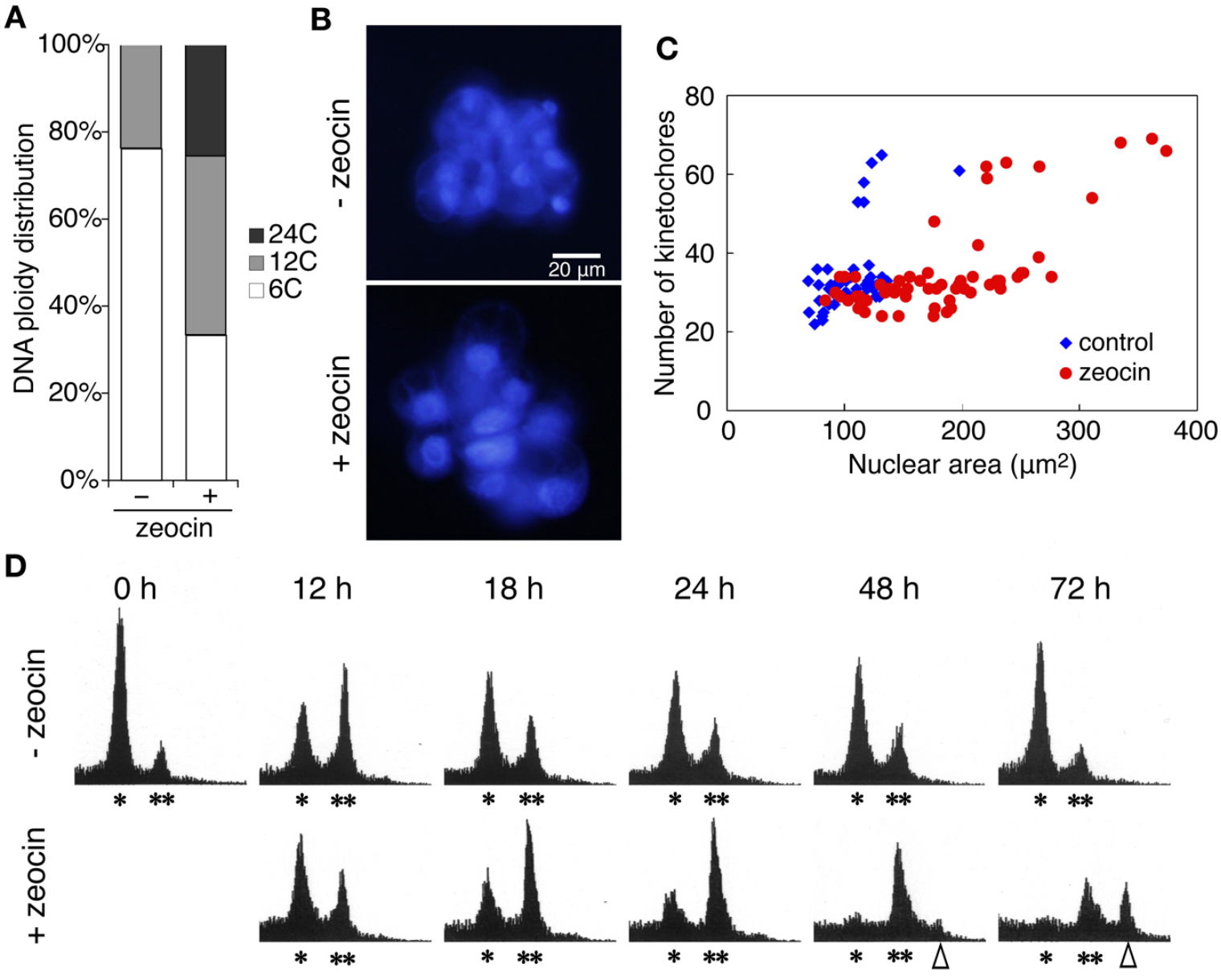
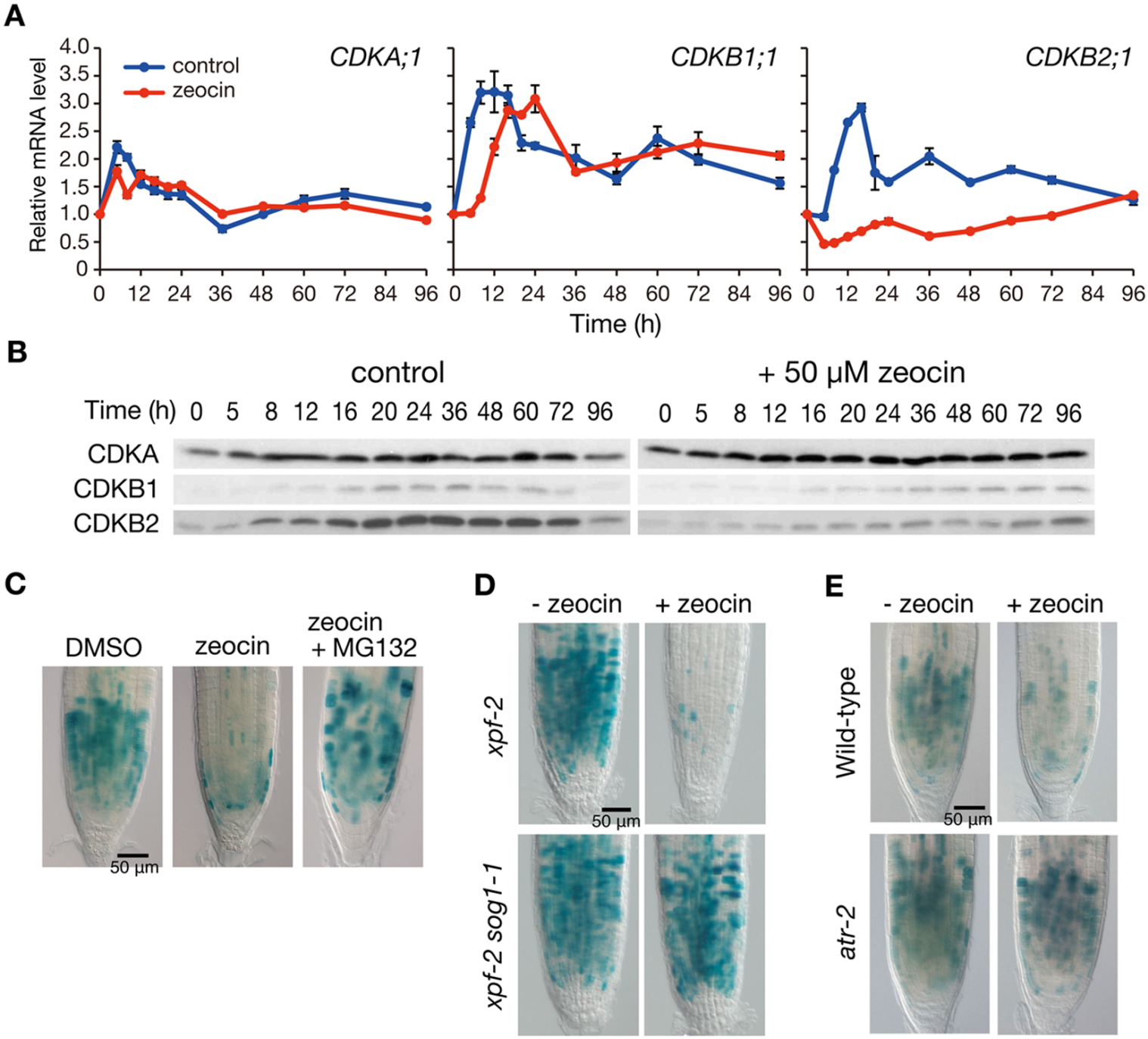

A
100%
80%
60%
40%
20%
0%
DNA ploidy distribution
24C
12C
6C
–
+
zeocin
B
- zeocin
20 µm
+ zeocin
C
Number of kinetochores
80
60
40
20
0
0
100
200
300
400
Nuclear area (µm²)
control
zeocin
D
0 h
12 h
18 h
24 h
48 h
72 h
- zeocin
+ zeocin
*
**
Δ

Figure 4


A
Relative mRNA level
control
zeocin
CDKA;1
CDKB1;1
CDKB2;1
Time (h)
B
control
+ 50 µM zeocin
Time (h) 0 5 8 12 16 20 24 36 48 60 72 96
CDKA
CDKB1
CDKB2
C
DMSO
zeocin
zeocin + MG132
50 µm
D
- zeocin
+ zeocin
xpf-2
xpf-2 sog1-1
50 µm
E
- zeocin
+ zeocin
Wild-type
atr-2
50 µm