Interaction of CPR5 with Cell Cycle Regulators UVI4 and OSD1 in Arabidopsis

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Abstract

The impact of cell cycle on plant immunity was indicated by the enhancement of disease resistance with overexpressing OSD1 and UVI4 genes that are negative regulators of cell cycle controller APC (anaphase promoting complex). CPR5 is another gene that is implicated in cell cycle regulation and plant immunity, but its mode of action is not known. Here we report the analysis of genetic requirement for the function of UVI4 and OSD1 in cell cycle progression control and in particular the involvement of CPR5 in this regulation. We show that the APC activator CCS52A1 partially mediates the function of OSD1 and UVI4 in female gametophyte development. We found that the cpr5 mutation suppresses the endoreduplication defect in the uvi4 single mutant and partially rescued the gametophyte development defect in the osd1 uvi4 double mutant while the uvi4 mutation enhances the cpr5 defects in trichome branching and plant disease resistance. In addition, cyclin B1 genes CYCB1;1, CYCB1;2, and CYCB1;4 are upregulated in cpr5. Therefore, CPR5 has a large role in cell cycle regulation and this role has a complex interaction with that of UVI4 and OSD1. This study further indicates an intrinsic link between plant defense responses and cell cycle progression.

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Abstract

Regulation of cell cycle, in the form of meiosis, mitosis, and endoreduplication, is critical for plant growth and development [1–4]. Progression through cell division cycles is governed by activities of cyclin-dependent kinase (CDK)-cyclin complexes which are bound and activated by cyclins at specific cell cycle phases. Eight classes of CDKs including CDKA to CDKG and CDK-like kinases (CKLs) were identified in Arabidopsis, but only CDKA and CDKB were reported to be involved in the regulation of cell cycle progression [5]. During mitotic cell cycles, activities of CDKs are relatively high at G1/S and G2/M transition phases where a large number of proteins are phosphorylated to promote the onset of DNA replication and mitosis respectively. During endocycles, the activity of mitotic CDK-cyclin complex at the G2/M transition phase needs to be repressed, which could be achieved by activation of the anaphase-promoting complex/cyclosome (APC/C).

APC/C is a multi-subunit E3 ubiquitin-ligase that degrades cell cycle proteins to control cell cycle progression [6,7]. The activity and substrate specificity of APC/C activity is controlled by two types of activators: Cell division cycle 20/Fizzy (CDC20/FZY) and CDC20 homolog/Fizzy-related (CDH1/FZR). Mitotic cell cycle progression requires the function of both CDC20 and CDH1, while the onset and progression of endocycles are controlled by CDH1 only. Arabidopsis has five CDC20 homologs (CDC20.1 to CDC20.5) and three CDH1 homologs (CCS52A1, CCS52A2, and CCS52B). Both CCS52A1 and CCS52A2 are reported to regulate the onset of endoreduplication, but the function of CCS52B is largely unknown [8–11].

Two homologous genes OSD1/GIG1 (Omission of the Second Division/gigas cell 1) and its homolog UVI4 (UV Insensitive 4) are negative regulators of APC/C [12,13]. The loss of OSD1 function led to omission of the second meiotic division and a subsequent production of diploid gametes [14]. A double mutant of cya1;2 and osd1 had no chromosome segregation during male meiosis, indicating that OSD1 promotes transitions in both meiotic divisions [15]. The mutant osd1/gig1 has giant cotyledon epidermal cells with higher ploidy, indicating a role of OSD1 in endoreduplication or endomitosis in cotyledons [13]. The loss of UTH function leads to enhanced resistance to UV-B and increased ploidy level in somatic tissues, indicating that UTH inhibits endocycles [16,17].

Interactomics experiments by overexpressing core cell cycle genes in Arabidopsis suspension cell culture revealed that both OSD1 and UVI4 interact with the APC/C complex [18]. Yeast two-hybrid analyses supported an interaction of both UVI4 and OSD1 with the catalytic activator subunits of APC/C including CCS52A1, CCS52B, CDC20.1, and CDC20.5 [12,13]. It is likely that more than one of these activators mediate the function of OSD1 or UVI4. While the cc52a1 mutation largely suppressed the enhanced endoreduplication defect in uv4 [12], overexpression of CDC20.1 or CCS52B was reported to further enhance the ploidy level in osd1 and uvi4 mutants [13]. Multiple cyclin proteins are regulated by OSD1 and UVI4. Increased degradation of the CYCA2;3 protein was observed in the uv4 mutant while transient overexpression of UVI4 or OSD1 under the dexamethasone-
inducible promoter triggered higher accumulation of CYCB1;2 and CYCB1;1 proteins [12,13,19].

Intriguingly, perturbation of cell cycle progression affects plant immune responses. Overexpression of either UVI4 or OSD1 and reduction of the function of APC10 resulted in enhanced disease resistance against virulent bacterial pathogen via upregulation of disease resistance (R) genes in a CYCB1;1-dependent manner [20]. R genes encode plant immune receptors that recognize directly or indirectly effector proteins secreted from pathogens, and activation of R proteins leads to transcriptional reprogramming and often programmed cell death to inhibit the spreading of pathogens in plants. There is therefore an apparent connection between cell cycle progression and disease resistance that is often associated cell death in plants. In animal and fungal systems, cell cycle progression is tightly linked to cell survival. Cell damage is assessed by various cell cycle checkpoints and either causes cell cycle arrest for DNA repair or leads to cell death [21]. In plants, a few examples exist for the association of cell cycle arrest and disease resistance associated with cell death. In addition to OSD1 and UVI4 that affect both cell cycle and disease resistance, the Arabidopsis CPR5 gene is implicated in both processes. The loss-of-function [l-o-f] cpr5 mutant shows increased disease resistance to bacterial pathogens accompanied by high accumulation of salicylic acid and ectopic cell death [22,23]. It also has abnormal trichomes due to reduced endoreduplication and cell death [24]. The cpr5 mutant has additional mutant phenotypes including early senescence, hyper sensitivity to sucrose [25], low leaf potassium content [26], abnormal response toABA [27], and abnormal cell wall biosynthesis [28]. CPR5 is likely a component of a general biochemical or cellular process and thus has a broad impact on different processes. Little is known about the biochemical properties of CPR5 besides that it has a transmembrane segment and is localized to both cytoplasm and nucleus [29].

Here we report the analyses of genetic interactions between OSD1, UVI4, CCS32A1 and CPR5 genes in the regulation of cell cycle progression. Loss of function mutations of osd1 and uv14 individually promote endoreduplication and together lead to lethality of female gamete. We show that the lethality of osd1 uv14 could be partially suppressed by a mutation in the APC activator CCS32a1. Interestingly, the cpr5 mutation suppressed many defects of uv14 single mutant and the lethality of the osd1 uv14 double mutant while the uv14 mutation enhanced the cpr5 defect in trichome branching and disease resistance. In addition, the expression of CYCB1;1 and CYCB1;2 genes are upregulated in cpr5. These data indicate that CPR5 has a critical role in cell cycle regulation and this role has a complex interaction with those of OSD1 and UVI4. It further indicates an intrinsic connection between cell cycle regulation and plant immunity.

Materials and Methods

Plant Materials, Growth and Transformation

Seeds of SALK_083656 were obtained from Arabidopsis Biological Research Center (ABRC). Heterozygous Seeds of osd1-2 (GT21481) were obtained from cold spring harbor laboratory. The osd1-2 mutant was introgressed from heterozygous osd1-2 into Col-0 for seven times. Plants were grown under either 12 hour light/12 hour dark or constant light condition at 22 C. Plant transformation was performed as previously described [30–32].

Bacterial Growth Assay

Four-week old plants grown under 12 hour light/12 hour dark condition were inoculated by Pst DC3000 at the concentration of 1×10⁸ colony forming units (cfu)/ml (OD600 = 0.2), and bacterial growth in different genotypes was analyzed at day 3 after the inoculation [33].

Ploidy Measurement

The first and second true leaves from two plants of 4-week old were collected and chopped in 1” buffer containing 97.5% MgSO4 (0.246% MgSO4, 7H2O, 0.37% KCl and 0.12% Heps), 0.1% DTT and 2.5% Triton X-100 [34]. 10 μl of PI (propidium iodide) stock solution (5 mg/ml) and 5 μl RNase stock solution (10 mg/ml) were added into each sample of approximately 600 μl. Beckman-Coulter Epics XL-MCL flow cytometer was used to measure ploidy with rice and maize leaf samples as controls. Three replicates were analyzed for each sample. Ploidy index (PI) was calculated by the formula: PI = (%2C nuclei×1) + (%4C nuclei×2) + (%8C nuclei×3) + (%16C nuclei×4) + (%32C nuclei×5). Independent experiments were conducted at least twice. The representative data were shown in figures.

Confocal Microscopy

The development of ovules was analyzed by using Leica TCS SP2 confocal microscope according to protocols previously described [35,36].

Quantitative RT-PCR

qRT-PCR was conducted by using FastStart universal SYBR Green Master mix (Roche). All primers are listed in Table S1 [37].

Results

Overexpression of OSD1 and UVI4 Affects Endoreduplication in Leaves

OSD1 has an essential role of cell cycle regulation in gametophyte development and cotyledon development. To determine whether or not OSD1 also has a role in vegetative growth, we analyzed ploidy levels of leaf cells in both loss of function mutants and overexpression transgenic lines of OSD1. Because homozygous osd1-2 plants produce diploid male and female gametes, and thus tetraploid progenies, we selected homozygous osd1-2 plants (a transposon mutant GT21481) in the Landsberg erecta (Ler) background for ploidy analysis from progenies of heterozygous rather than homozygous osd1-2 plants. The control was an uv14-2 allele in Ler (Landsberg erecta) previously named pjm [17]. In the first pair of leaves of 4-week old seedlings, uv14-2 had an increase of higher-ploidy cells (32C and 16C) as analyzed by flow cytometry (Figure 1A), which is consistent with the previous finding for the uv14-1 mutant in Col-0. The osd1-2 mutant in Ler also had a significant increase of the number of 16C and 32C cells compared to wild-type Ler (Figure 1A). Ploidy indices were calculated as 2.80 in Ler-0, 3.15 in uv14-2 and 3.24 in osd1-2 (Figure 1A). Both uv14-2 and osd1-2 mutants had significantly higher ploidy indices than Ler-0. Therefore, OSD1 also has a role in the development of true leaves by inhibiting endoreduplication.

The effect of OSD1 overexpression on cell cycle progression was examined in the OSD1 overexpression allele osd1-4 resulting from activation tagging mutagenesis [20]. This osd1-4 mutation enhanced defense responses of bon1-2, a l-o-f mutant of an immunity negative regulator BON2/411 (BON1) in theWs ecotype [20]. A reduced ploidy level in leaf cells was observed in the osd1-4 mutant compared to the wild type, especially when plants were grown under weaker light illumination (Figure 1B). The osd1-4 plant had more cells with 2C, 4C, and 8C at the expense of cells with 16C and 32C compared to the wild-typeWs plant (Figure 1B).
The bon1-2 mutation did not alter ploidy distribution in leaf cells, and the bon1-2 osd1-4 mutant had reduced endoreduplication similarly to osd1-4. Ploidy indices were calculated as 3.48 in Ws, 3.47 in bon1-2, 3.18 in osd1-4 and 3.15 in bon1 osd1-4 (Figure 1B). Both osd1-4 and bon1-2 osd1-4 had significantly lower ploidy index than Ws and bon1-2 while no significant difference was observed between osd1-4 and bon1-2 osd1-4, indicating that overexpression of OSD1 triggers the reduction of endoreduplication. Thus, the level of OSD1 is critical in controlling ploidy levels in leaf cells, most likely through regulating endoreduplication.

UVI4 regulates endoreduplication and the loss of UVI4 function results in higher ploidy level in leaf cells [16]. We tested whether or not a higher expression of UVI4 can affect cell cycle progression. Overexpression of a UVI4 and GFP fusion as in UVI4-OE transgenic plants confers a dwarf phenotype with multiple shoots in both the wild-type Col-0 and the uvi4-1 mutant in Col-0 (referred to as uvi4) [20]. Due to the low germination efficiency, the UVI4-OE plants were grown on KMS medium for one and half week before being transferred to soil and grown under 12 h light/12 h dark condition for 2 weeks. The fourth leaves of the overexpression lines were used to analyze the trichome phenotype.

UVI4-OE generated in either Col-0 and uvi4 had decreased branching trichome number compared to Col-0 or uvi4 (Figure 1C). While trichome branch numbers in wild-type Col-0 and uvi4 are more than two, around 2.5% of the trichomes in lines 5 and 17 of UVI4-OE in Col-0, and 1.5% and 2.4% of the trichomes in lines 3 and 5 of UVI4-OE in uvi4, respectively, had only two branches (Figure 1C). Statistical analysis by chi-square test indicates that the distribution of trichome branch numbers in lines 5 and 17 in Col-0 are significantly reduced compared to that in wild type Col-0. Lines 3 and 5 of UVI4-OE in uvi4 had significantly reduced trichome branching numbers compared to uvi4 but were similar to the wild-type Col-0. These data indicate that overexpression of UVI4 reduces the level of cell ploidy and the expression level of UVI4, like that of OSD1, regulates endoreduplication.

The ccs52a1 Mutation Leads to a Partial Suppression of the Lethality of osd1 uvi4

We constructed double mutant of osd1 and uvi4 and found that it is lethal during both female gametogenesis and embryogenesis.
between CPR5UVI4 mutant, which affects the differentiation of trichomes. In parallel, enhancement of CPR5 mutant had even fewer trichome branches than CPR5 mutant, which was crossed to the uvi4 mutant which is also in the Col-0 background. No homozygous osd1 uvi4 mutant seedlings could be identified from progenies from a double heterozygous plant osd1/OSD1 uvi4/UVI4 OSU4/UVI4 (Figure 2A) or from osd1/OSD1 uvi4/uv4 plants (Figure 2B). Progeny testing from reciprocal crosses between osd1/OSD1 uvi4/uv4 and wild-type Col-0 reveals that the transmission rate of the osd1 uvi4 female gametophytes was approximately 20% (17/83) of that of the OSD1 uvi4 female gametophytes while the that of the osd1 uvi4 male gametophytes was not drastically reduced relative to that of the OSD1 uvi4 male gametophytes (Figure 2C). Similar to previous report [38], we found that the female gametophyte development in osd1 uvi4 is arrested at FG1 stage and the megaspore cannot complete mitosis to develop into a functional female gametophyte (Figure S1). Even with some transmission of the osd1 uvi4 female gametophytes, the osd1/OSD1 uvi4/uv4 zygote could not be found in progenies of osd1/OSD1 uvi4/UVI4 or osd1/OSD1 uvi4/uv4 (Figure 2A, B), indicating the OSD1 and UVI4 together are essential for zygote development as well. Indeed, undeveloped ovules and arrested embryos were observed in siliques of the osd1/OSD1 uvi4/uv4 plant (Figure 2D).

In contrast to OSD1 and UVI4, CCS25A1 positively regulates the activity of APC/C [10], and ccs25a1 suppressed the endoreduplication phenotype of uv4 [12]. To determine whether or not CCS25A1 activity is also responsible for the lethality of osd1 uv4, we crossed a loss-of-function mutation of CCS25A1 (SALK_083656) with uv4/uv4 osd1/OSD1. Plants with the osd1/OSD1 uv4/uv4 ccs25a1/ccs25a1 genotype were isolated in the F2 population and their progenies were analyzed. Among 96 progenies analyzed, 2 plants were identified as osd1 uv4 ccs25a1 homozygous triple mutants, 41 plants as OSD1/OSD1 uv4/uv4 ccs25a1/ccs25a1, and 55 as osd1/OSD1 uv4/uv4 ccs25a1/ccs25a1 (Figure 2E). Thus the lethality of female gametophyte and zygote defects of uv4 osd1 is partially dependent on the activities of CCS25A1. The triple mutant of osd1 uv4 ccs25a1 was much smaller than wild-type Col-0 or ccs25a1 (Figure 2F), suggesting that other CCS25 genes might mediate the function of OSD1 and UVI4 in zygote development.

The cpr5 Mutation Largely Suppresses the Endoreduplication Defects in uv4 but not the Meiotic Defects in osd1 Mutant

The fact that overexpression of OSD1 or UVI4 confers enhanced disease resistance and reduced endoreduplication prompted us to look at genetic interaction of OSD1 and UVI4 with the CPR5 gene that is also involved in these two processes [22-25]. The double l-o-s mutant was generated between cpr5-2 [22] (referred as cpr5 from now on) and uv4 in Col-0, and analyzed for trichome branching numbers indicative for endoreduplication and ploidy levels [24]. On the adaxial side of the fourth leaf in the three-week-old plants grown under constant light, wild type Col-0 typically had trichomes with three and four branches, the cpr5 mutant had one or two branches, and uv4 had mostly three to five branches (Figure 3A). The cpr5 uv4 double mutant had fewer branches than uv4 (Figure 3A), indicating that cpr5 suppressed uv4 defects in trichome branching regulation. Intriguingly, the uv4 cpr5 double mutant had even fewer trichome branches than cpr5. This enhancement of cpr5 by uv4 suggests a complex interaction between UVI4 and CPR5 in trichome development. Perhaps the developmental programs are further altered in the cpr5 uv4 mutant, which affects the differentiation of trichomes. In parallel, nuclear DNA content in the first pair of leaves was analyzed by flow cytometry. Ploidy indices were calculated as 2.69 in Col-0, 2.56 in cpr5, 2.96 in uv4 and 2.70 in uv4 cpr5 (Figure 3B). The cpr5 and uv4 mutant had significantly lower and higher ploidy index compared to Col-0, respectively, while the cpr5 uv4 double mutant had a similar ploidy index to the wild type Col-0 (Figure 3B), indicating a suppression or compensation of endoreduplication defects of uv4 by cpr5 in leaf cells.

We analyzed the genetic interaction between osd1 and cpr5 as well. OSD1 is essential for the second meiotic division, and loss of function of OSD1 leads to diploid gametes and tetraploid progenies [14]. The cpr5 mutant was crossed with the heterozygous osd1, and the cpr5 osd1 double mutant was isolated from the F2 population. Ploidy levels in progenies of these cpr5 osd1 homozygous F2 plants were measured. As expected, no 2C cells were detected in the progenies of the homozygous osd1 plants named osd1 (BC7F3) (Figure 3C), because the genome was duplicated due to the production of diploid gametes. No 2C cells were detected in the cpr5 osd1 F3 plants either (Figure 3C), indicating that cpr5 mutation does not suppress the meiotic defect in the osd1 mutant. Ploidy indices were 2.86 in Col-0, 2.53 in cpr5, 3.54 in osd1 BC7F3 plants and 3.24 in cpr5 F3 plants (Figure 3C). The cpr5 osd1 F3 plants had significantly lower ploidy index than osd1 BC7F3, similar to the significant reduction of index in cpr5 compared to Col-0. Thus, a cpr5 defect exists even in the background of the osd1 tetraploid.

The cpr5 Mutation Partially Suppressed the Lethality of the uv4 and osd1 Double Mutant

To determine whether or not cpr5 mutation affects gametophyte development, we first carried out reciprocal test crosses between heterozygous cpr5/CPR5 and the wild type Col-0 and genotyped their F1 progenies. The female transmission rate of cpr5 was 108% (27/25) relative to the wild type, and the male transmission rate of cpr5 was 169% (61/36) relative to the wild type (Figure 4A). This indicates that cpr5 mutation enhances transmission of male gametophytes.

We subsequently analyzed the interaction between cpr5 and the osd1 uv4 double mutant in gametophyte development. The osd1/OSD1 cpr5/cpr5 was crossed to uv4/uv4 cpr5/cpr5, and the osd1/OSD1 uv4/UVI4 cpr5/cpr5 plants were selected among the F1 progenies. Analysis of their progenies revealed a reduced transmission of osd1 gamete relative to the wild-type OSD1 gamete in the cpr5 background, and this reduction did not occur in the wild type background (Figure 2A). In progenies of osd1/OSD1 uv4/uv4 cpr5/cpr5, plants with the osd1/OSD1 cpr5/cpr5 genotype relative to those with the OSD1/OSD1 cpr5/cpr5 genotype were about 50% instead of the expected 200%: 12 versus 23 with UVI4/UVI4, 18 versus 35 with uv4/UVI4, and 6 versus 11 with uv4/uv4 (Figure 4B). This indicates that the osd1 cpr5 gamete has a lower transmission rate than OSD1 cpr5. It is likely that osd1 has a lower transmission rate but goes undetected as its transmission activity nevertheless reaches a threshold. But this activity becomes lower than threshold when cpr5 confers a higher threshold.

Significantly, we identified osd1/OSD1 uv4/uv4 cpr5/cpr5 plants (3 out of 118) from the progenies of osd1/OSD1 uv4/UVI4 cpr5/cpr5 (Figure 4B). In addition, when progenies of osd1/OSD1 uv4/uv4 cpr5/cpr5 were analyzed, 8 out of 64 plants were genotyped as osd1/OSD1 uv4/uv4 cpr5/cpr5 were analyzed, 8 out of 64 plants were genotyped as osd1/OSD1 uv4/uv4 cpr5/cpr5 (Figure 4C). Therefore, cpr5 rescued the embryo lethal defect of osd1/OSD1 uv4/uv4 zygotes and likely increased the survival and transmission of female gametophytes of osd1 uv4. While viable triple mutant of osd1 uv4 cpr5 was obtained, these plants had more compact rosette leaves and were much smaller than cpr5 (Figure 4D). We further determined the gamete transmission rates by crossing osd1/OSD1 uv4/uv4 cpr5/CPR5 to Col-0 and uv4 respectively. Progeny genotyping show that the
transmission rate of osd1 uvi4 female gametophytes was about 40% (20/50) and 45% (15/33) of that of OSD1 uvi4 when crossed to Col-0 and uvi4 respectively. The transmission rate of osd1 uvi4 cpr5 female gametophytes was increased to 73% (29/40) and 108% (41/38) in crosses to Col-0 and uvi4 respectively (Figure 4E). Thus, cpr5 suppressed the lethality in the female gamete of osd1 uvi4. The difference of apparent rescue extent of the female gametophyte in crosses to the wild type and uvi4 might result from a different survival rate at the zygote stage.

The cpr5 Mutation Activates the Expression of Cell Cycle Genes

Endoreduplication is inhibited by the loss of CPR5 function. We further investigated how cell cycle progression is affected in cpr5 by analyzing expression of genes specific to distinct phase of the cell cycle. These include G1-phase CYCD3;3, S-phase histone H3.1, G2-phase CYCA2;1 and G2 to M transition CYCB1;1 [37,39]. CYCB1;1, but not the other two genes, was found to have an altered expression in cpr5 compared to wild-type Col-0 by RT-PCR. The CYCB1;1 expression was two times more in cpr5 than in the wild type when the first pair of leaves or the whole seedling were sampled (Figure 5, S2). We subsequently looked at expression of other members of the CYCB1 family by qRT-PCR and found that the expression levels of CYCB1;2 and CYCB1;4 in cpr5 were 1.5 and 1.7-fold respectively of that in Col-0 (Figure 5). These data indicate that cpr5 affects the expression of B1-type cyclin genes which might be involved in endoreduplication regulation [40].

Both uvi4 and osd1 Mutations Enhance the Disease Resistance of cpr5

To determine how the CPR5 might interact with UVI4 and OSD1 to affect defense responses, we analyzed diseases resistance
phenotypes of cpr5 mutant combinations with osd1 or uvi4. The growth of virulent bacterial pathogen Pseudomonas syringae pv. tomato DC3000 (Pst DC3000) were analyzed in cpr5 uvi4 and cpr5 osd1 double mutants. To avoid genome duplication effects from the osd1 mutation, we isolated cpr5 osd1 from progenies of cpr5/cpr5 OSD1/osd1 plants. Four-week old plants were spray inoculated with the bacterial pathogen. At day 3 after inoculation, both the cpr5 and osd1 single both supported 3 times less pathogen growth than the wild type, while uvi4 supported the same amount of growth as the wild type (Figure 6). The double mutants cpr5 uvi4 and cpr5 osd1 supported even less growth than the cpr5 single mutant, with a 4-fold and 10-fold reduction compared to cpr5, respectively (Figure 6). This indicates that the cpr5 mutation has a synergistic effect on disease resistance with the uvi4 and osd1 mutations.

Discussion

Previous studies on the loss of function mutants show that OSD1 and UVI4 inhibit APC/C activity and thus regulate cell cycle progression in meiosis, endoreduplication, and gametophyte development. Through analyzing their overexpression phenotypes, we further demonstrate that the level of OSD1 and UVI4 is a critical determinant for cells to enter regular mitosis or endoreduplication. This study also establishes that CCS52A1 mediates the overlapping function of OSD1 and UVI4 in gametophyte development. OSD1 and UVI4 were postulated to inhibit members of the CCS52 protein family, but the particular member that they inhibit is not clear in each of the diverse processes they regulate. The loss of ccs52a1 mutation was previously shown to completely rescue the uvi4 defect in endoreduplication [12]. Here we found that the ccs52a1 mutation partially rescued the lethality of osd1 uvi4 (Figure 2). This partial suppression may be due to the functional redundancy of CCS52A1 with two other homologs, CCS52A2 and CCS52B. Both CCS52A1 and CCS52A2 control the onset and progression of endoreduplication [8,10], and overexpression of CCS52B enhances the endoreduplication defects in uvi4 [13]. Thus, loss of both OSD1 and UVI4 function could release the inhibition on multiple CCS52 proteins (Figure 7).

Furthermore, this study reveals CPR5 as an additional player in cell cycle regulation and this function largely antagonizes that of UVI4 and OSD1 (Figure 7). The cpr5 mutation suppressed multiple defects in uvi4 single and uvi4 osd1 double mutants, including the endoreduplication phenotype of uvi4 and lethality of osd1 uvi4 double mutant (Figure 3, 4). Therefore, CPR5 functions either in parallel to or downstream of UVI4 and OSD1 complex. The cpr5 defect in trichome branching was enhanced by uvi4 although the uvi4 single mutant had an opposite phenotype to cpr5 (Figure 3A). The enhancement of cpr5 phenotype by uvi4 was also seen in the disease resistance (Figure 6B). This unusual interaction might be due to an overlapping function between OSD1 and UVI4. The loss of UVI4
might be compensated by OSD1 in a tissue and developmental stage dependent manner. For instance, the cpr5 phenotype can be enhanced by uvi4 due to the higher expression of OSD1 in trichome cells but not necessarily other cells in the leaf.

The converging point of regulation by CPR5 and UVI4 might be on the cyclins. OSD1 and UVI4 are shown to regulate cyclins mainly at the protein level through the E3 ligase complex APC/C [12,13], and a transcriptional regulation of CYCB1;1 by APC/C also takes place [41]. CPR5 directly or indirectly affects transcript level of cyclins although the mechanism is unknown. An upregulation of CYCB1;1, CYCB1;2, and CYCB1;4 is observed in the leaves of the cpr5 mutant, which may account for the reduced endoreduplication in mutant leaves. The cpr5 mutation itself promoted male gametophyte transmission (Figure 4A). Upregulation of CYCB1;1 or other cyclins might occur during gametophyte development in cpr5 leading to accelerated development and higher transmission of male gametophytes. Therefore, the cpr5 mutation may compensate the lower amount of cyclin proteins in the uvi4 mutant by upregulating the transcript of cyclins and thus suppresses the uvi4 defects (Figure 7). It is yet to determine whether or not the cyclin genes are the converging regulatory nodes of OSD1/UVI4 and CPR5 and, if so, what cyclins they are.

This study further supports a connection between defense responses and cell cycle progression. CPR5, UVI4, and OSD1 are all implicated in cell cycle regulation as well as defense response regulation. It is possible that cell cycles are often manipulated by pathogens. It has been observed that powdery mildew infection triggered endoreduplication at the infection site [42]. Recent study...
reported that an APC/C component, APC8, is one of the 5 significant hubs targeted by pathogen effectors [43]. Therefore, cell cycle machinery and consequently cell cycle progression are likely manipulated by various bacterial and oomycete pathogens. This manipulation, without countering effects from plants, could be beneficial to pathogen. However, these manipulations by pathogens might become ‘guarded’ by plant R proteins to trigger defense responses. No R proteins are known to interact with APC8 [43], however expression of R genes could be affected by cell cycle progression. For instance, the R gene SNC1 has an increased transcript level in mutants of several APC components including APC8, APC13 and APC10 as well as overexpression of OSD1 [20]. This change of R gene expression is dependent on cyclins as SNC1

Figure 5. Expression of cell cycle genes in cpr5 mutant. Expression levels of AtCYCB1;1 in both the first pair of leaves and whole seedlings of 2-week-old plants, and AtCYCB1;2 and AtCYCB1;4 in whole seedlings analyzed by quantitative real time RT-PCR. Error bars indicate standard deviations. doi:10.1371/journal.pone.0100347.g005

Figure 6. Both uvi4 and osd1 mutations enhance defense responses in cpr5. Bacterial growth assay in Col-0, uvi4, osd1, cpr5, cpr5 uvi4 and cpr5 osd1 (all diploid plants) inoculated by spray inoculation of Pst DC3000. Error bars indicate standard deviations. Letters a, b and c indicate the statistical significance determined by student t-test. doi:10.1371/journal.pone.0100347.g006

Figure 7. Roles of the OSD1, UVI4 and CPR5 genes. Both UVI4 and OSD1 negatively regulate APC/C activities partially through their interaction with CCS52A1. APC/C inhibits activities of various CDK-cyclin complexes (indicated by shaded circles) each of which regulates processes including meiosis, female gametogenesis, endoreduplication and defense response. CPR5 negatively regulates the transcript levels of some cyclin genes and thus the activities some CDK-cyclin complexes (indicated by darker shades), and affects female gametogenesis, endoreduplication and defense responses. The exact action point of CPR5 is yet to be determined. doi:10.1371/journal.pone.0100347.g007
upregulation in the OSD1 overexpression line is abolished by the cycb1;1 mutation [20]. The cpr5 has a higher expression of CYCB1;1 and CYCB1;2 which potentially can lead to upregulation of R genes as well. Therefore, upregulation of R gene transcript might be a new mechanism in monitoring effectors in addition to the up-regulation of R proteins. Cell cycle has been shown to affect gene expression [44–46] and the expression of certain R genes might be susceptible to perturbation of cell cycle and therefore form the basis of pathogen recognition.

In sum, we uncovered a role of CPR5 that antagonizes with that of UV14 and OSD1 in cell cycle regulation (Figure 7). Both UV14 and OSD1 inhibit the activity of APC/C through the interaction with APC/C activator CCS22 proteins. APC/C degrades cyclins and inhibits activities of CDK-cyclin complex which are critical for spindle assembly and cytokinesis. APC/C activator CCS22 proteins, and OSD1 inhibit the activity of APC/C through the interaction with APC/C activator CCS22 proteins. APC/C degrades cyclins and inhibits activities of CDK-cyclin complex which are critical for spindle assembly and cytokinesis. Therefore, upregulation of CPR5 might negatively regulate cell cycle components such as cyclins at the transcript level. The critical role of immune regulator CPR5 in cell cycle regulation further supports a tight connection between defense responses and the regulation of cell cycle progression.

Supporting Information

Figure S1 Female gametophyte development of osd1uvi4. (A) Confocal laser scanning microscopy images of the terminal female gametophyte in an osd1/OSD1 uvi4/+ pistil which contained wild-type female gametophyte at FG7 (left panel) and abnormal female gametophyte arrested at FG1 (right panel). SEN, secondary endosperm nucleus; CV, central vacuole; EN, egg nucleus; SN, synergid nucleus; N, nucleus; V, vacuole. (B) Confocal laser scanning microscopy of female gametophytes at early developmental stages in the osd1+/ uvi4 pistil. All gametum showed either wild-type FG1 (left panel) or FG2 (right panel) features at this stage. FM, functional megaspore. N, uninucleate. DM, degenerating megaspore. MN, micropylar nucleus. CN, chalazal nucleus. Scale bar = 10 μm. (TIF)

Figure S2 Gene expression of cell cycle marker genes in cpr5 mutants. Analysis of cell cycle marker genes in the first pair of leaves and the whole seedlings of two-week old plants by RT-PCR. AtGAPC1 was used as a control. (TIF)

Table S1 List of primers for qRT-PCR analysis. (DOCX)

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Author Contributions

Conceived and designed the experiments: ZB JH. Performed the experiments: ZB. Analyzed the data: ZB JH. Contributed to the writing of the manuscript: ZB JH.

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