The Plant Anaphase Promoting-Complex/Cyclosome

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Abstract

The Anaphase-Promoting Complex/Cyclosome (APC/C) represents a large multi-subunit E3-ubiquitin ligase complex that controls the unidirectional progression through the cell cycle by the ubiquitination of specific target proteins, marking them for proteasomal destruction. Although the APC/C's role is largely conserved among eukaryotes, its subunit composition and target spectrum appear to be species specific. In this review, we focus on the plant APC/C complex, whose activity correlates with different developmental processes, including polyploidization and gametogenesis. After an introduction into proteolytic control by ubiquitination, we discuss the composition of the plant APC/C and the essential nature of its core subunits for plant development. Subsequently, we describe the APC/C activator subunits and interactors, most being plant specific. Finally, we provide a comprehensive listing of confirmed and suspected plant APC/C target proteins. Identification of growth-related targets might offer opportunities to increase crop yield and resilience of plants to climate change by manipulating APC/C activity.

1. INTRODUCTION

The controlled breakdown of specific proteins in a swiftly and timely manner represents an efficient and essential strategy to respond quickly to developmental or environmental cues. One way to achieve this is through the ubiquitin-proteasome pathway. Ubiquitination is a specific post-translational modification in which ubiquitin moieties are attached to a substrate protein. Although ubiquitination can affect a protein's localization, activity or interaction with other proteins, probably the best-known outcome is the recognition and subsequent proteolysis of the ubiquitinated protein by the 26S proteasome (Glickman & Ciechanover 2002, Schnell & Hicke 2003). The attachment of a ubiquitin moiety involves a cascade of enzymatic reactions. First, an E1-activating enzyme covalently binds to and activates the ubiquitin in an ATP-dependent reaction, then the ubiquitin transfers to an E2-conjugating enzyme, and finally an E3 ubiquitin-ligase attaches the ubiquitin to a lysine on the target protein, repeating the process to create an ubiquitin chain that marks it for proteasomal degradation (Eme et al 2011, Hershko & Ciechanover 1998, Schrock et al 2020). A hierarchy exists in this E1-E2-E3 cascade, with only a few different E1 genes, tens of E2 genes, and more than a thousand E3-ligases present in most plant species (Chen & Hellmann 2013). One particular E3-ligase dedicated to cell cycle control is the Anaphase-Promoting Complex/Cyclosome (APC/C). As a highly conserved E3-ligase complex, it assures the unidirectional progression through the cell cycle by selectively marking specific target proteins for destruction (Heyman & De Veylder 2012, Marrocco et al 2010). This large multi-subunit complex consists of core structural subunits, catalytic subunits and different activator proteins, while additionally being controlled through association with positive or negative regulators. Here, we will review the composition of the plant APC/C, highlight its role in plant development and discuss the importance of the proteolysis of its targets.

2. COMPOSITION OF THE PLANT APC/C

The core APC/C complex is largely conserved among the various eukaryotic kingdoms and consists of three domains (**Figure 1**), each holding different subunits that in *A. thaliana* are present as single-copy genes, except *APC3*, for which two isoforms are present (**Table 1**). The complex comprises a central scaffolding platform formed by APC1, APC4 and APC5, a structural arm formed by the tetratricopeptide repeat (TPR) interaction domain-containing proteins APC3, APC6, APC7 and APC8, and a catalytic arm formed by APC2 and APC11 (Capron et al 2003a, Schreiber et al 2011, Thornton et al 2006, Van Leene et al 2010, Zhou et

al 2016). Both APC2 and APC11 are essential to perform the ubiquitin transfer reaction, with APC11 providing the interaction with the E2 ubiquitin-conjugating enzyme (**Figure 1**), but without providing any target specificity (Tang et al 2001, Zhou et al 2016). Rather, recognition and binding of target proteins depends on the creation of a binding pocket by two co-activators (**Figure 1**), being APC10 and an interchangeable subunit belonging to one of two classes of WD-40 interaction domain-containing proteins, respectively called CELL DIVISION CYCLE 20/FIZZY (CDC20/FZ) and CDC20 HOMOLOG1/FIZZY-RELATED (CDH1/FZR), the latter also known in plants as CELL CYCLE SWITCH 52 (CCS52) (da Fonseca et al 2011, Kevei et al 2011, Tarayre et al 2004). Together, these activator proteins recruit the APC/C ubiquitination targets through recognition of conserved short linear amino acid motifs known as **degrons** (Davey & Morgan 2016), such as the Destruction box (D-box, consensus motif RxxLxxxxN), the KEN- or GxEN-box, or the ABBA motif (consensus motif [ILVF]x[ILMVP][FHY]x[DE]) (Di Fiore et al 2015), usually present in the disordered regions of the substrate protein sequence (da Fonseca et al 2011, Pfleger & Kirschner 2000, Qin et al 2016). Additionally, phosphorylation of certain amino acid residues within or surrounding the degrons can positively or negatively influence substrate recognition (Davey & Morgan 2016).

Next to these APC/C elements, several small subunits are known from studies in yeast and vertebrates, i.e. APC9 and APC12 to APC16 (Eme et al 2011, Schreiber et al 2011, Schwickart et al 2004, Thornton et al 2006). To date, no plant APC9, APC14 or APC16 homologs have been identified, indicating that they might be non-essential for the function of the plant APC/C (Eme et al 2011). The *APC12* gene, also known as *CDC26*, has only recently been identified in the plant lineage and is located in the 5' untranslated region of TTM3, which encodes the metabolic enzyme *TRIPHOSPHATE TUNNEL METALLOENZYME 3*, making it an interesting example of an upstream open reading frame or **uORF** (Lorenzo-Orts et al 2019). Both genes are expressed from a bicistronic transcript, and, although they share no functional relationship, translation of *TTM3* is essential for adequate production of APC12. APC13, also known as SWM1 in yeast and BONSAI in *Arabidopsis thaliana*, promotes the stable association of APC3, APC6 and APC8 within the APC/C complex and is essential for ubiquitin ligase activity (**Figure 1**) (Schwickart et al 2004, Zhou et al 2016). Lastly, APC15, also known as Mnd2 in yeast, is a small protein shown in human cells to be part of the central platform of the APC/C, and thought to play a role in promoting the release and subsequent degradation of ubiquitinated CDC20 (Eloy et al 2015, Mansfeld et al 2011, Uzunova et al 2012). A potential APC15 homolog has been identified in several plant species, including *A. thaliana*, but to date, no

functional studies have been performed on these homologs (Eloy et al 2015, Uzunova et al 2012).

Several plant-specific proteins that interact with the APC/C to modulate its activity were additionally identified (**Figure 1, Table 1**). Of these, the homologs ULTRAVIOLET-B-INSENSITIVE 4 (UVI4)/POLYCHOME (PYM) and UVI4-Like/OMISSION OF SECOND DIVISION 1 (OSD1)/GIGAS CELL 1 (GIGAS) act as APC/C inhibitors, whereas both THREE DIVISION MUTANT1/MALE STERILE 5 (TDM1/MS5) and SAMBA represent APC/C activators (Cifuentes et al 2016, Cromer et al 2012, Eloy et al 2012, Heyman et al 2011, Iwata et al 2011).

3. THE APC/C CORE SUBUNITS ARE ESSENTIAL FOR PLANT VIABILITY AND DEVELOPMENT

Foremost, the targeting for breakdown of cell cycle proteins by the APC/C is essential for the progression through mitosis and meiosis, which is evidenced by the fact that for most of the core APC/C subunits, no homozygous loss-of-function mutants can be obtained. In case of *APC1*, *APC2*, *APC4*, *APC6* or *APC10*, a full knock-out causes problems with female gamete formation, resulting in aborted ovules (**Figure 2A**) (Capron et al 2003b, Eloy et al 2011, Kwee & Sundaresan 2003, Wang et al 2013, Wang et al 2012). On the other hand, loss of *APC8* or *APC13* leads to defects during male gamete formation (**Figures 2B and 2C**) (Xu et al 2019, Zheng et al 2011). Similarly, self-pollination of *apc3a/+ apc3b/+* heterozygous mutants causes 25% aborted ovules and a greater than expected reduction in transmission of the *apc3b* genotype, indicating that female as well as male gametes lacking both *APC3A* and *APC3B* are not viable (Pérez-Pérez et al 2008). Next to a similar reduced transmission through both the male and female parental line, and an abortion of zygote development within the first few division cycles,-loss of APC11 function causes asynchronous cell division in the multinucleate or **syncytial endosperm** (Guo et al 2018, Guo et al 2016). Mutants in *APC1* and *APC4* display the same endosperm phenotype, indicating that next to mitosis and meiosis the APC/C plays an important role in controlling the synchrony of divisions in multinucleated cells (Guo et al 2018). Lastly, an *APC12* T-DNA insertion mutant shows defective embryo development and an arrest of seed germination, but no obvious impairment in female or male gametogenesis, indicating that this subunit might not be essential for meiosis (**Figures 2D and 2E**) (Lorenzo-Orts et al 2019).

Due to the embryo-lethality of APC/C subunit knock-out mutants, the role of APC/C function in plant growth post-germination must be investigated using partial loss-of-function

mutants, RNA interference (RNAi) or overexpression lines. The exception to this rule is *APC3*, because it is present in two gene copies in Arabidopsis. Of these two, *APC3b* appears the most important for post-embryonic development, because, as its alternative name *HOBBIT* (*HBT*) suggests, mutant plants showed a severe dwarf phenotype (**Figures 2F and 2G**), while *APC3a* mutants do not appear to be affected in growth or development (Pérez-Pérez et al 2008, Willemsen et al 1998). The dwarf growth in *apc3b* mutants is not due to problems during embryonal development, because the embryonic root and shoot primordia form correctly (Blilou et al 2002). Perturbations occur rather postembryonal, resulting in plants with a reduced root length due to a smaller root elongation zone and with smaller leaves due to problems in cell division and expansion accompanied by a reduction in DNA **endoploidy**, highlighting a role of the APC/C in control of the endocycle and cell differentiation (Blilou et al 2002, Serralbo et al 2006). Similarly, reduction of *APC6* or *APC10* transcription levels using RNAi constructs yielded plants with smaller leaves and short inflorescences, accompanied by modifications in vain patterning and a reduction in epidermal cell size and endoploidy (Marrocco et al 2009). Furthermore, inflorescence stems show a strong reduction in elongation, often having a broom-like appearance with normal appearing flowers and siliques clustering at the end of the short stems, although fertility seemed unaffected (Marrocco et al 2009). A weak *apc8* allele identified in a mutagenesis screen showed similar defects in inflorescence development, next to distorted leaf shapes and an abnormal shoot **meristem** development (Zheng et al 2011). Using an **artificial microRNA** (amiRNA) construct to reduce *APC11* expression yielded plants with short roots and stunted leaves with multinucleate cells (**Figure 2H**), indicating problems with cytokinesis (Guo et al 2018). For *APC13/BNS*, a reduction of expression, either through hypermethylation caused by a mutation in the chromatin-remodeling gene *DECREASE IN DNA METHYLATION 1* (*DDM1*), through introduction of an RNAi construct, or through T-DNA insertion, yielded plants with disturbed phyllotaxis and short inflorescence stems, similar to what was seen for APC6 and APC10 (Saze & Kakutani 2007). In contrast to the loss-of-function phenotypes, plant growth is stimulated in APC/C subunit-overexpressing plants, as plants overaccumulating APC10 show an increased leaf size due to an enhanced cell division rate (**Figures 2I and 2J**) (Eloy et al 2011). Combined, the available data highlight a role for the APC/C in a plethora of developmental processes, polyploidy, and cytokinesis.

4. THE CDC20/FZ TYPE OF APC/C ACTIVATING SUBUNITS

Among the CDC20-class of APC/C activating subunits, six *CDC20* homologous sequences are known in *A. thaliana*, of which *CDC20.1* and *CDC20.2* appear to be the two most important

isoforms, with evidence suggesting that CDC20.3 to CDC20.6 are non-functional **pseudogenes** (Capron et al 2003a). Both *CDC20.1* and *CDC20.2* display a basal expression level throughout the cell cycle, with an increased expression starting from S-phase and peaking during M-phase (Kevei et al 2011). They also show a very similar tissue-specific expression pattern in dividing tissues in the root and rosette, while showing specialization in the inflorescence, with *CDC20.1* being specific for the flower buds, anthers, pollen grains and developing seeds, whereas *CDC20.2* is expressed in the sepals and style. Matching their expression patterns, abolition of either one of the *CDC20* genes does not lead to any obvious growth phenotype, illustrating that they are largely functionally redundant during vegetative growth (Kevei et al 2011). Additionally, *cdc20.1* but not *cdc20.2* mutant plants show a reduced fertility due to problems in male gametogenesis caused by abnormal chromosome segregation during meiosis, leading to inviable pollen (**Figures 3A to 3F**), in agreement with the fact that only *CDC20.1* is expressed in anthers (Niu et al 2015). Simultaneous knock-down of the *CDC20.1* and *CDC20.2* genes by RNAi, however, results in a dose-dependent developmental delay (**Figure 3G**), accompanied by a smaller rosette size and a shorter root length caused by a reduction in cell number, while differentiation appears to be unaffected (Kevei et al 2011). Furthermore, a consistent reduction in fertility caused by defective pollen development can be observed (**Figure 3H**).

Previous work in yeast has shown that one of the main functions of CDC20 during cell division is to mark key proteins for destruction to promote the transition from metaphase to anaphase and the separation of the sister chromatids (Holt et al 2008, Morgan 2007). Until the last chromosome has attached to the kinetochore, the **spindle assembly checkpoint** (SAC) prevents the APC/C^{CDC20} from recognizing these targets by sequestering CDC20 into the mitotic checkpoint complex (MCC), thereby assuring genomic stability (Izawa & Pines 2015). In Arabidopsis, both CDC20.1 and CDC20.2 interact with several members of the MCC, including MITOTIC ARREST DEFICIENT 2 (MAD2), BUDDING UNINHIBITED BY BENOMYL 3.1 (BUB3.1) and BUB1-RELATED 1 (BUBR1)/MAD3, indicating that the sequestration of CDC20 by the MCC has been conserved in plants (Kevei et al 2011).

5. THE CCS52/CDH1/FZR-TYPE OF APC/C ACTIVATORS

The plant homologs of the animal CDH1/FZR-types of APC/C-activators, named CCS52, were first described when isolated from the root **nodules** of *Medicago sativa*, tissues with a high degree of endoreplication (Cebolla et al 1999). In *A. thaliana*, three *CCS52* genes are present, the A-types *CCS52A1/FZR2* and *CCS52A2/FZR1*, and the B-type *CCS52B/FZR3* (Tarayre et al

2004). Their cell cycle phase-specific expression indicates that the functions of the A- and B-types diverged, as both *CCS52A* genes show a peak in expression from late M-phase to early G2-phase, while *CCS52B* expression is restricted to G2/M and M-phase (Fülöp et al 2005, Tarayre et al 2004). Although both A-type isoforms show a very similar cell cycle phase-dependent expression pattern, their tissue-specific expression patterns and mutant phenotypes indicate specialization and a partially overlapping but complementary role during plant development. This is exemplified by the fact that double mutants are not viable (Baloban et al 2013). Knock-out leaves of either of the *CCS52A* isoforms show reduced cell sizes and DNA endoploidy levels (**Figures 4A to 4C**), indicating that both genes function in the control of the onset of differentiation and endoreplication in the leaf (Baloban et al 2013, Heyman et al 2017, Lammens et al 2008, Larson-Rabin et al 2009, Liu et al 2012). The *ccs52a2* mutant, but not the *ccs52a1* mutant, additionally shows a reduction in total leaf epidermal cell number, leading to a severe dwarf growth phenotype (**Figures 4F to 4H**) (Baloban et al 2013). On the other hand, overexpression of either *CCS52A* gene leads to an increased leaf cell size (**Figures 4D and 4E**) and higher ploidy levels, but a reduction in total cell number (Baloban et al 2013). As a result, both knockout and overexpression of the *CCS52A2* isoform results in a smaller leaf size (**Figures 4H and 4J**) (Baloban et al 2013). In trichomes, only *CCS52A1* is expressed, and, accordingly, only *ccs52a1* mutants show a reduction in trichome branch number (**Figures 4K to 4M**), a characteristic strongly correlated with their DNA endoploidy levels (Kasili et al 2010, Lammens et al 2008, Larson-Rabin et al 2009). Overexpression of both *CCS52A* genes leads to a similar increase in trichome branch number (**Figures 4N and 4O**), indicating that their functional differences are dictated by their distinct expression patterns and not determined on the protein level (Baloban et al 2013). On the other hand, only *CCS52A2* is strongly expressed in the shoot apical meristem (SAM), with *ccs52a2* mutant plants showing a disturbed SAM organization, frequently leading to meristem bifurcation (Baloban et al 2013, Liu et al 2012). Accordingly, inflorescences in *ccs52a2* plants show reduced **apical dominance** and terminate early, leading to short flowering stalks with few flowers (**Figures 4W to 4B'**) (Baloban et al 2013, Liu et al 2012).

In the root, a similar functional diversification can be observed. Expression of *CCS52A1* is confined to the root transition and elongation zone, whereas that of *CCS52A2* is located more distally in the root apical meristem (RAM), including the **quiescent center** (QC), the stem cell niche, the division zone and the vasculature (**Figures 4S and 4T**) (Baloban et al 2013, Liu et al 2012, Vanstraelen et al 2009). Accordingly, *ccs52a1* mutant plants show slightly longer roots (**Figures 4P and 4Q**), accompanied by an increase in root meristem length and a reduction in

cell ploidy levels, due to a delay in the onset of differentiation, whereas *ccs52a2* mutant plants show dramatically shorter roots (**Figures 4R**), accompanied by a severely disorganized and shorter root meristem, due to a failure to restrict divisions in the QC and the stem cell niche (**Figures 4U and 4V**) (Heyman et al 2013, Vanstraelen et al 2009). As such, both genes appear to play a role in the inhibition of the mitotic cell cycle, on the one hand controlling the timing of cell cycle exit and onset of differentiation, a role mostly reserved for *CCS52A1*, and on the other hand keeping the division rate of the stem cells in the apical meristems low, a role specific for *CCS52A2*. Additionally, *CCS52A2* plays an important role during male gametogenesis, with pollen in *ccs52a2* mutant plants often being deformed (**Figure 4G'**) and many ovules remaining unfertilized and aborting following self-fertilization (**Figures 4C' to 4F'**), leading to a strong reduction in fertility, in combination with a severe lack of flowers (**Figures 4Z to 4B'**)(Baloban et al 2013, Liu et al 2012). The precise role of *CCS52A2* during male gametogenesis and meiosis, however, remains to be investigated.

Several of the molecular players involved in establishing the functional similarities and differences between CCS52A1 and CCS52A2 have been identified. Both *CCS52A* isoforms are negatively regulated in proliferating tissues by the binding of the E2FA transcriptional activator complexed with the RETINOBLASTOMA RELATED 1 (RBR1) transcriptional repressor on the E2F cis-acting elements in their respective promoters (Magyar et al 2012). Additionally, E2Fe/DP-E2F-like 1 (DEL1), an atypical E2F that acts as a repressor, specifically inhibits the expression of *CCS52A2*, but not that of *CCS52A1*, as such preventing a premature onset of endoreplication in dividing tissues (Lammens et al 2008, Vlieghe et al 2005). The Mediator complex subunit MED16 binds to the promoters of both CCS52A isoforms to repress their transcription, resulting in the suppression of endoreplication and cell growth (Liu et al 2019). MED16 depends on a direct interaction with DEL1 for association with the *CCS52A2* promoter, while the MED16 interaction partner allowing it to bind the *CCS52A1* promoter remains to be elucidated (Liu et al 2019). Expression of *CCS52A1*, and not that of *CCS52A2*, was also found to be repressed by the trihelix transcription factor GT2-like 1 (GTL1) to end ploidy-driven cell growth in trichomes, and activated by the ARABIDOPSIS RESPONSE REGULATOR2 (ARR2) transcription factor upon cytokinin signaling to initiate endoreplication during root development (Breuer et al 2012, Takahashi et al 2013).

In contrast to the CCS52A-type activators, only limited information is currently available on the role of CCS52B. The G2/M-specific expression profile of *CCS52B* indicates that it might play a role similar to that of CDC20, perhaps leading to a finer control of the APC/C-dependent regulation of mitotic target proteins (Tarayre et al 2004). Both CDC20 and CCS52B have been

found to be regulated by nuclear sequestration, as mRNA of both *CDC20* and *CCS52B* is kept in the nucleus until the nuclear envelope breaks down at the prometaphase, after which the transcripts move to the cytosol for translation, in such manner preventing the premature targeting of mitotic cyclins by the APC/C (Yang et al 2017). Accordingly, overexpressing the *Medicago trunculata CCS52B* in tobacco cells leads to a depletion of cells with 2C DNA ploidy content and a dramatic increase in cells with 4C, suggesting that cells become blocked in mitosis and fail to re-enter the G1-phase (Tarayre et al 2004). In rice, plants lacking *OsCCS52B* show a dwarf phenotype caused by a reduction in cell size, with the nuclear size and ploidy levels remaining unaffected, indicating that OsCCS52B plays a role in cell expansion independently of endocycle control (Su'udi et al 2012). Further investigation is needed to ascertain the exact role of CCS52B in plant development.

5. REGULATION OF APC/C FUNCTIONALITY BY INTERACTORS

In every species, the activity of the APC/C is regulated by its physical interaction with inhibiting and activating proteins. For example, the vertebrate EARLY MITOTIC INHIBITOR 1 (Emi1), also known as REGULATOR OF CYCLIN A1 (Rca1) in *Drosophila Melanogaster*, inhibits the APC/C^{CDH1} during the mitotic cell cycle by acting like a pseudo-substrate (Di Fiore & Pines 2007). To date, no sequence homolog of EMI1 has been discovered in plants, however, the plant-specific protein POLYCHOME (PYM)/UV-B INSENSITIVE 4 (UVI4), identified in an APC/C interactome study, shows a similar domain organization and has been proposed to be a functional EMI1 homolog (Hase et al 2006, Heyman et al 2011, Perazza et al 1999, Van Leene et al 2010). The *pym*/*uvi4* mutations were originally discovered in plants showing supernumerary trichome branches (**Figures 5A and 5B**) and increased resistance to UV-B light (**Figures 5C and 5D**), both correlating with increased endoploidy levels (Hase et al 2006, Perazza et al 1999). Accordingly, UVI4 interacts with CCS52A1 to inhibit its function as an APC/C activator during the G1-to-S transition (Heyman et al 2011). Mutation of *UVI4* leads to an increased activity of the APC/$C^{CCS52A1}$, triggering a premature onset of the endocycle (**Figures 5E and 5F**) (Boudolf et al 2009, Heyman et al 2011, Imai et al 2006). UVI4 interacts as well with the UBIQUITIN-SPECIFIC PROTEASE 14 (UBP14), a deubiquitinating enzyme that is able to remove ubiquitin moieties from target proteins, and both synergistically influence endoreplication levels (Xu et al 2016). This points towards another layer of control over APC/$C^{CCS52A1}$ activity, possibly by UVI4 bringing UBP14 closer to ubiquitinated APC/C target

proteins or regulators, after which it could deubiquitinate them and prevent their breakdown by the proteasome.

Emi1 has a meiosis-specific homolog in vertebrates, *Erp1/Emi2*, which functions in regulating the transition from meiosis I to meiosis II (Ohe et al 2007, Tung et al 2005). Similarly, the Arabidopsis genome encodes the *UVI4* homolog *UVI4-LIKE/OMISSION-OF-SECOND-DIVISION 1 (OSD1)/GIGAS CELL 1 (GIG1)* that plays a role in the second mitotic division during meiosis, with *osd1* mutants having diploid male and female gametes and tetraploid progeny (**Figures 5G and 5H**) (Cromer et al 2012, d'Erfurth et al 2010, d'Erfurth et al 2009). However, plants lacking OSD1 function also show abnormal cells in cotyledons, including large guard cells and round cells with enlarged nuclei, and higher DNA ploidy levels as a result of endomitosis (**Figures 5I and 5J**), indicating that OSD1 plays a role during the mitotic cell cycle as well (Iwata et al 2011). OSD1 is hypothesized to bind and inhibit APC/C^{CDC20} during mitosis to prevent endomitosis to occur, correlating with its G2-to-M phase peak expression pattern (Heyman & De Veylder 2012, Iwata et al 2011). Although UVI4 and OSD1 likely have complementary roles, being sequentially active during G1-S and G2-M, respectively, and targeting different APC/C complexes, some redundancy is expected as well, since *uvi4 osd1* double mutants are difficult to obtain and show severe developmental defects including female gamete lethality (Cromer et al 2012, Iwata et al 2011).

Another APC/C regulator, called SAMBA, is a plant-specific protein that was discovered in an APC/C interactome study (Van Leene et al 2010). *A. thaliana SAMBA* is expressed in developing embryos and during early growth (Eloy et al 2012). Accordingly, *A. thaliana samba* mutants show enlarged organs, including larger seeds, embryos, and meristems (Figures 5K to 5N), due to an increased number of cells established pre-germination (Eloy et al 2012). Similarly, maize *samba* mutants show an increased cell production owing to a faster cell cycle, although plants are dwarfed due to a reduced cell elongation (Gong et al 2021). At later plant growth stages, *SAMBA* is expressed in pollen grains, where it is important for the mitotic divisions during male gametogenesis, with *samba* mutants showing a strongly reduced male fertility (Figures 5O to 5Q) (Eloy et al 2012). SAMBA interacts with the CCS52-activated APC/C complex and several A-type cyclins, whereas the *samba* mutant shows increased levels of CYCA2;3 protein, potentially explaining the increased cell number phenotype (Eloy et al 2012, Gong et al 2021). This makes SAMBA a probable APC/C activator, potentially aiding in targeting A-type cyclins for APC/C-mediated destruction by the proteasome.

Finally, THREE DIVISION MUTANT1/MALE STERILE 5 (TDM1/MS5) is a protein essential for male meiotic exit, as *tdm1* mutants fail to terminate meiosis after meiosis II,

leading to an aberrant third meiotic division (Figures 5R to 5T), loss of pollen viability and infertility (Figure 5U) (Cromer et al 2012, Glover et al 1998, Ross et al 1997). The exact function of TDM1 during meiosis is still unknown, but the protein shows limited sequence similarity to APC6, containing similar TPR domains (Cromer et al 2012), indicating that it might be part of the meiotic APC/C complex (Cifuentes et al 2016). TDM1 activity is negatively controlled through phosphorylation by the cyclin-dependent kinase CDKA;1 in complex with the cyclin CYCA1;2/TAM (Cifuentes et al 2016). Through suppression of TDM1 function and working synergistically with OSD1, CYCA1;2-CDKA;1 restricts APC/C activity during meiosis I, ensuring the transition from meiosis I to meiosis II, as *cyca1;2* loss-of-function mutants show a premature exit of meiosis, while non-destructible CYCA1;2 gain-of-function mutants demonstrate a third meiotic division similar to *tdm1* mutants (Cifuentes et al 2016, Cromer et al 2012, d'Erfurth et al 2010). As *CYCA1;2* is not expressed during meiosis II, TDM1 proteins are not phosphorylated and able to promote APC/C activity to remove meiotic CYC-CDK complexes and induce an exit from meiosis II.

6. PLANT TARGETS OF THE APC/C

Although the targets of the APC/C have been extensively studied in animals and yeast, the list of known confirmed targets in plants remains limited (summarized in **Table 2**). Mitotic cyclins were the first demonstrated group of APC/C targets in yeast and also represent likely targets in the plant kingdom (Peters 2002). The Arabidopsis genome contains 21 mitotic cyclins, 10 A-types and 11 B-types, each subdivided into three classes (Vandepoele et al 2002). Of these, all A-type cyclins, except CYCA3;3, and all B1-type cyclins, contain a full RxxLxxxxN D-box APC/C recognition motif, strongly indicating that all of them act as putative substrates of the APC/C. Indeed, direct interactions between the CCS52 isoforms and CYCA1;1, CYCA1;2, CYCA3;4, CYCB1;1 and CYCB1;2 have been shown by pull-down experiments (Fülöp et al 2005). A strong interaction between the two main CDC20 isoforms and CYCB2;1 and CYCB2;2 was shown in yeast-2-hybrid (Y2H) experiments, whereas CCS52A2 and CCS52B were shown to interact with CYCB2;3 in a bimolecular fluorescence complementation (BiFC) experiment (Boruc et al 2010, Kevei et al 2011). Aside from direct interaction evidence with APC/C activator subunits, protein levels of several cyclins were found to be dependent upon APC/C activity. As such, CYCA2;3 was observed to accumulate in *ccs52a1* and *samba* mutant plants, but to be degraded in plants lacking the APC/C inhibitor UVI4 or the UVI4 interactor UBP14 (Boudolf et al 2009, Eloy et al 2012, Heyman et al 2011, Xu et al 2016). CYCA3;1 was seen to accumulate in *apc2* mutant female gametophytes (Capron et al 2003b), while a tomato

CYCA3 isoform was found to interact with CCS52A and to accumulate in *CCS52A*-silenced protoplasts (Mathieu-Rivet et al 2010). Additionally, CYCA3;4, which is normally broken down after the prophase, was found to remain present throughout the cell cycle in plants lacking CCS52A2, while the severity of *ccs52a2* mutant phenotypes was found to correlate with CYCA3;4 protein levels (Willems et al 2020). Similarly, CYCB1;1 accumulates in *apc1*, *apc4*, *apc6* and *apc11* mutant female gametophytes and *apc8* mutant male gametophytes (Guo et al 2018, Guo et al 2016, Kwee & Sundaresan 2003, Wang et al 2013, Wang et al 2012, Zheng et al 2011), while degradation of CYCB1;1 was observed in APC10-overproducing leaves and *in vitro* when co-incubated with APC11 (Eloy et al 2011, Guo et al 2016). Lastly, accumulation of CYCB1;2 was demonstrated in plants overexpressing the APC/C inhibitor *OSD1* (Iwata et al 2011). The change in protein abundance of these cyclins is a good indication that they are direct targets for APC/C-mediated proteolysis, although it cannot be excluded that their accumulation represents an indirect effect of induced cell cycle alterations by the APC/C subunit mutants. Stronger evidence from experimental data showing direct ubiquitination of the target is lacking in most cases. To date, ubiquitination has only been shown *in vitro* for CYCB1;1 after co-incubation with APC3a or APC11 (Guo et al 2016, Rojas et al 2009).

Aside from cyclins, other core cell division components have been identified as potential plant APC/C targets. The protein level of the plant-specific CDKB1;1, one of the cyclin binding partners, increases upon treatment of plants with the proteasome inhibitor MG-132 and is reduced in a *ubp14* mutant background in which UVI4-mediated inhibition of APC/C function is reduced (Xu et al 2016). The cell wall biosynthesis enzyme CELLULOSE SYNTHASE LIKE-D5 (CSLD5), important for cellulose deposition at the growing cell wall during cell division, shows a cell cycle-dependent accumulation and is rapidly ubiquitinated and marked for breakdown by the APC/$C^{CCS52A2}$ upon completion of mitosis (Gu et al 2016). In rice, ROOT ARCHITECTURE ASSOCIATED 1 (OsRAA1), a small GTP-binding protein interacting with the spindle and representing a homolog of the Arabidopsis FLOWERING-PROMOTING FACTOR 1 (FPF1), was shown to act as an inhibitor of root growth and the metaphase-to-anaphase transition (Ge et al 2004, Han et al 2008, Xu et al 2010). OsRAA1 interacts with a component of the 26S proteasome and is degraded in a D-box-dependent manner, making it a likely APC/C target (Han et al 2008). Some APC/C subunits might also be APC/C targets themselves. Like in animal cells, the APC/C activating subunit CDC20 is thought to be targeted for destruction by APC/C^{CCS52}, and indeed both active Arabidopsis CDC20 isoforms contain a D-box sequence and bind all three CCS52 isoforms, while CDC20 protein levels, in contrast to those of CCS52B, are stabilized upon treatment with MG-132 (Kevei et al 2011, Yang et al

2017). Likewise, the APC/C catalytic subunit APC11 has been found to ubiquitinate itself, probably resulting in the deactivation of the APC/C complex through removal of APC11 (Guo et al 2016).

One of the important roles of the APC/C during cell division is to promote the separation of sister chromatids during anaphase. Research in yeast and vertebrates has shown that the chromatid cohesion proteins Shugoshin and Securin are both targeted for destruction by the APC/C^{CDC20} to achieve this goal (Holt et al 2008, Jonak et al 2017, Sullivan & Morgan 2007). The *A. thaliana* Shugoshin homologs SGO1 and SGO2 have not yet been shown to be targeted by the APC/C, although they fulfil similar roles during meiosis as described in vertebrates, preventing premature loss of sister chromatid cohesion, and they contain the KEN- and D-box motifs for APC/C recognition, making them likely APC/C^{CDC20} targets in plants as well (Cromer et al 2013, Zamariola et al 2014). Although Shugoshin has been shown to be associated with mitotic chromosomes in rice, no vegetative phenotypes were found in *sgo* mutants in rice and *A. thaliana* (Wang et al 2011, Zamariola et al 2014), indicating other factors might complement them during mitosis. Recently, the PATRONUS/COPPER MODIFIED RESISTANCE (PANS1/CMR1) and PANS2 proteins have been revealed to be the *A. thaliana* homologs of securin, despite showing only limited sequence homology with its vertebrate counterpart (Cromer et al 2019). Accordingly, PANS1 interacts with the APC/C complex in a D- and DEN-box (a variant of the KEN-box)-dependent manner, whereas an indestructible form of PANS1 lacking the D-box prevents sister chromatid separation (Cromer et al 2013, Cromer et al 2019). Furthermore, *PANS1* shows a spatial and temporal expression pattern being very similar to that of *CDC20.1*, while overexpression of *PANS1* was shown to increase protein abundance of the APC/C^{CDC20} target CYCB1;2, possibly due to excess PANS1 binding to and titrating out free CDC20.1 (Juraniec et al 2016). Likewise, the rice PANS1 homolog RICE SALT SENSITIVE 1 (RSS1) also interacts with CDC20 in a D-box-dependent manner and its protein stability was shown to be cell cycle phase-dependent, marking it as a likely APC/C target (Ogawa et al 2011).

Aside from cell cycle components, other proteins have been proposed to be APC/C targets. DSRNA-BINDING PROTEIN 4 (DRB4), involved in RNA silencing, interacts with the APC10 subunit, whereas silencing of either *APC6* or *APC10* results in DRB4 accumulation (Marrocco et al 2012). The ETHYLENE RESPONSE FACTOR 115 (ERF115) is a transcription factor with a key role in controlling divisions in the root QC (Heyman et al 2013). It was first identified as an interactor of CCS52A2 in APC/C **tandem-affinity purification** experiments, after which its stability was shown to be influenced by presence of CCS52A2, revealing it as a target of

APC/$C^{CCS52A2}$ that partially explains the *ccs52a2* mutant's disturbed stem cell niche phenotype (Heyman et al 2013, Van Leene et al 2010). In rice, two GRAS-type transcription factors involved in shoot branching and root growth, i.e. the homologs of Arabidopsis SCARECROW-LIKE 18/LATERAL SUPPRESSOR (SCL18/LAS) called MONOCULM 1 (MOC1) and of SHORT ROOT (SHR), respectively interact with the rice CCS52 homolog TILLERING ENHANCER/TILLERING AND DWARF 1 (TE/TAD1) in a D-box-dependent manner and are broken down in an APC/C^{CCS52}-dependent manner (Lin et al 2012, Lin et al 2020, Xu et al 2012). Additionally, eight of the ten rice PYR/PYL/RCAR **abscisic acid** receptors contain the D-box motif and are targeted for proteasomal degradation in a CCS52-dependent manner, (Lin et al 2015). More recently, the APC/C has been demonstrated to control silencing of transposable elements by ubiquitinating DEFECTIVE IN MERISTEM SILENCING 3 (DMS3) in a D-box-dependent manner, a protein involved in recruiting DNA polymerase V during RNA-directed DNA methylation (Zhong et al 2019).

7. CONCLUDING REMARKS

In conclusion, the APC/C represents an important factor for plant growth and development, and while much research has been conducted in recent years, especially regarding the function of its subunits, much remains to be discovered about its targets and their roles in the cell cycle. As research into the different APC/C subunits in plants has shown, the APC/C is essential for cell cycle progression, both during regular mitotic cell cycle progression, as well as during variants like meiosis or the endocycle. Several plant APC/C ubiquitination targets have already been identified and well described, but much remains to be investigated. Cyclins represent an obvious group of proteins for further investigation, as evidence points to many of them being targeted for ubiquitination by the APC/C (Table 2). However, in-depth functional research into the role of their proteolytic control by the APC/C during cell cycle progression and plant development is lacking, leaving much room for further research. The list of possible ubiquitination targets is long, with many proteins containing one or several degrons in their amino acid sequence. Forming a connection to many different developmental pathways, the APC/C represents a good target for plant growth modification. However, the modulation of APC/C activity through targeting of its core subunits yields many pleiotropic effects. Therefore, the identification of downstream APC/C targets may allow for a more specific modification of cell division and differentiation. Modulation of specific targets may allow to precisely control plant growth without introducing the growth penalties observed when manipulating the

APC/C's core subunits and regulators, in such manner potentially contributing to an increase in crop yield or resilience of plants to climate change.

SUMMARY POINTS

- The Anaphase-Promoting Complex/Cyclosome (APC/C) is an E3-ubiquitin ligase conserved throughout eukaryotes that marks key proteins for proteasomal destruction to allow unidirectional progression of the cell cycle.
- The APC/C is a large complex of at least 14 different subunits, most being essential for plant viability.
- The APC/C contains an interchangeable activator subunit that determines its substrate specificity, belonging to either the CELL DIVISION CYCLE 20/FIZZY (CDC20/FZ) or the CDC20 HOMOLOG1/FIZZY-RELATED (CDH1/FZR)/CELL CYCLE SWITCH 52 (CCS52) class. Plants most often contain a larger number of APC/C activator subunit homologs compared to other eukaryotes, probably allowing for a more specific control of cell cycle progression in response to environmental and developmental cues.
- Several plant-specific APC/C-interacting proteins have been discovered in recent years, such as POLYCHOME (PYM)/UV-B INSENSITIVE 4 (UVI4), UVI4-LIKE/OMISSION-OF-SECOND-DIVISION 1 (OSD1)/GIGAS CELL 1 (GIG1), SAMBA and THREE DIVISION MUTANT1/MALE STERILE 5 (TDM1/MS5), providing additional control over APC/C function.
- Although many proteins are suspected to be APC/C ubiquitination targets, the list of confirmed targets in plants remains relatively short.
- The APC/C does not only target proteins with a direct role in cell cycle progression, such as cyclins or securin, but also a plethora of other types of proteins, such as transcription factors and hormone receptors, potentially linking cell cycle progression with environmental signaling.
- The APC/C, being a key regulator of cell cycle progression and thus plant growth, forms an interesting target for crop breeders through modification of the activity of its regulators or targets.

TERMS AND DEFINITIONS

Abscisic acid: a phytohormone involved in the response to environmental stresses, seed dormancy, the control of organ size and stomatal closure.**Apical dominance:** suppression of shoot branching by the main stem

Degron: a structural element of a protein, most frequently an amino acid motif that is involved in the regulation of its degradation rate.

uORF: an upstream open reading frame (ORF) found within the 5' untranslated region of an mRNA.

Meristems: focal regions where cell divisions take place

Syncytial endosperm: multi-nuclear endosperm tissue arising due to nuclear divisions in absence of cell division.

Endoploidy: DNA replication in the absence of mitosis and cyctokinesis, insulting in an increase of the DNA ploidy level.

Artificial microRNA: tool to silence the expression of a target gene by using modified endogenous microRNA precursors.

Pseudogene: DNA segment resembling a functional gene but that has become non-functional during evolution

Spindle assembly checkpoint: cell cycle surveillance mechanisms controlling proper attachment of the chromosomes' kinetochores to the bipolar spindle and proper alignment of the chromosomes in the dividing cell, controlling the anaphase-to-metaphase transition.

Nodule: nitrogen-fixing organelles found on legume roots, formed in response to interaction with symbiotic bacteria.

Quiescent center: pool of rarely dividing stem cells within the center of the root stem cell niche, suggested to act as a stem cell reservoir that is called upon when stem cells need replacement.

Tapetum: specialized layer of cells within anthers, important for nutrition and development of pollen grains.

Tandem-affinity purification: an affinity tag-based purification technique for studying protein–protein interactions under native conditions

DISCLOSURE STATEMENT

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FIGURES AND FIGURE LEGENDS

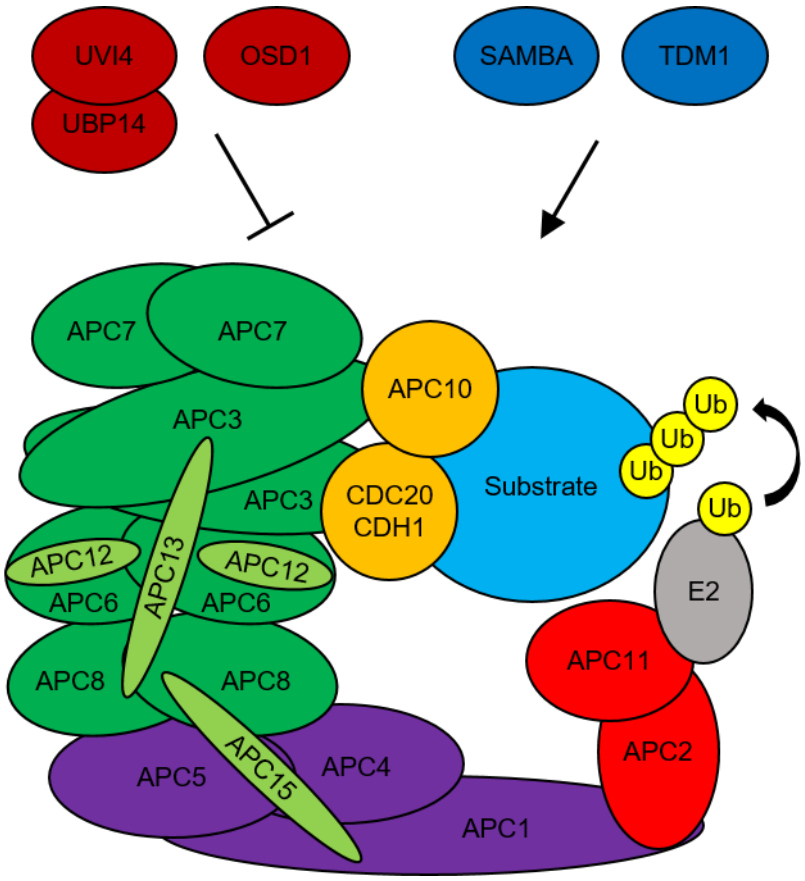

Figure 1: The structure of the APC/C complex

The APC/C complex can be divided in three domains. The central scaffolding platform consists of APC1, APC4 and APC5 (shown in purple). The catalytic arm is formed by APC2 and APC11 (in red), with APC11 providing the interaction with the E2-conjugating enzyme (in gray) for transfer of ubiquitin moieties (in yellow) to the target substrate (in blue). The structural arm is formed by the TPR domain-containing proteins APC8, APC6, APC3 and APC7 (in green), all present as dimers. Through the two APC3 subunits the arm binds the co-activators APC10 and one of the CDC20 or CDH1-type subunits (in orange), which recognize the degron-containing substrate protein and correctly position it for ubiquitination by the catalytic arm. The smaller subunits APC12 and APC13 are thought to stabilize the TPR domain-containing arm, with APC12 present as a dimer and binding with APC6, and APC13 binding with APC8, APC6 and APC3; APC15 forms part of the central platform interacting with APC1, APC4, APC5 and APC8. UVI4, working in concert with UBP14, and OSD1 are thought to be APC/C inhibitors (dark red), while SAMBA and TDM1 (dark blue) are likely APC/C activators.

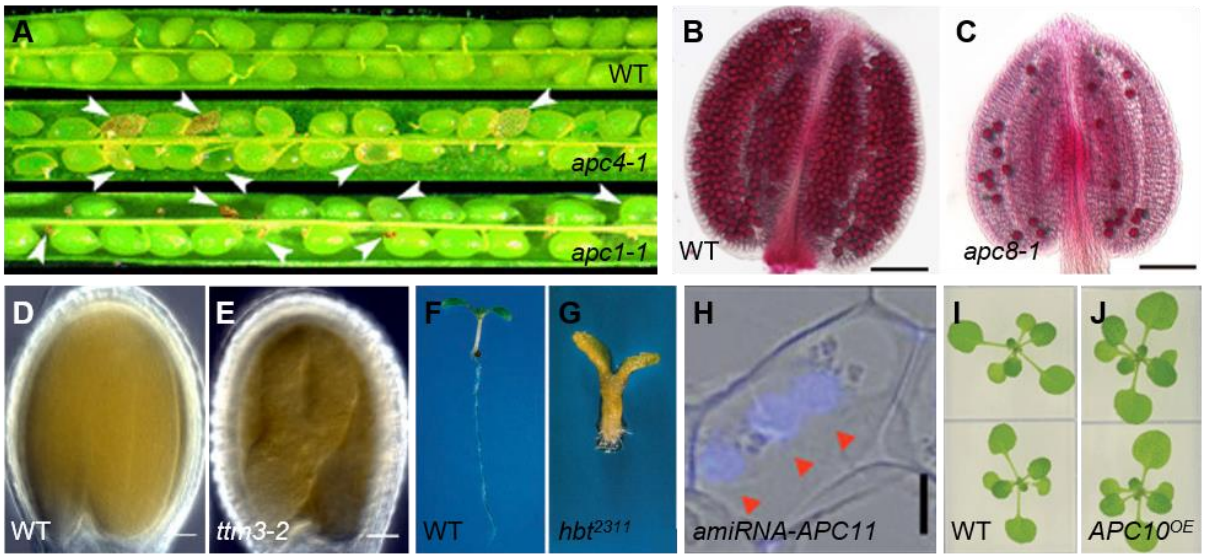

Figure 2: Phenotypes of core APC/C subunit mutants.

A: Loss of *APC1* or *APC4* causes problems during female gametogenesis and embryogenesis, leading to aborted ovules and seeds arrested in different stages of development (white arrowheads). Siliques from wild-type (WT), apc4-1/+ or apc1-1/+ mother plants are shown. Picture adapted from Wang et al. (2013) with permission from John Wiley and Sons.

B-C: Loss of *APC8* causes defects during male gametogenesis, as visualized by a pollen viability assay using Alexander staining on WT (B) and *apc8-1* (C) anthers. Viable and unviable pollen are colored red and green, respectively. Scale bars represent 100 µm. Picture adapted from Xu et al., 2019.

D-E: Seeds from *ttm3-2* mutant plants, which harbor a T-DNA insertion in the coding sequence of *APC12*, do not germinate due to defective embryo development. Pictures show mature seeds of WT (D), with the embryo fully developed, and *ttm3-2* (E), with the embryo stalled in the torpedo stage. Scale bars represent 20 µm. Picture adapted from Lorenzo-Orts et al. (2019) with permission from Springer Nature.

F-G: Plants homozygous for the *APC3b* mutant allele hbt^{2311} (G) show severely inhibited seedling growth compared to wild type (F). Mutant seedlings are shown at four times the magnification of the wild-type seedling. Picture adapted from Willemsen et al., 1998.

H: Plants with reduced expression of *APC11* show multinucleated cells, as seen in cotyledon epidermal cells of plants expressing the *amiRNA-APC11* construct. Nuclei (red arrows) visualized through 4′,6-diamidino-2-phenylindole (DAPI) staining are shown in blue. Scale bar represents 10 µm. Picture adapted from Guo et al. (2018) with permission from John Wiley and Sons.

I-J: Plants ectopically expressing *APC10* (J) show a larger leaf size than control plants (I). Picture adapted from Eloy et al. (2011) with permission from John Wiley and Sons.

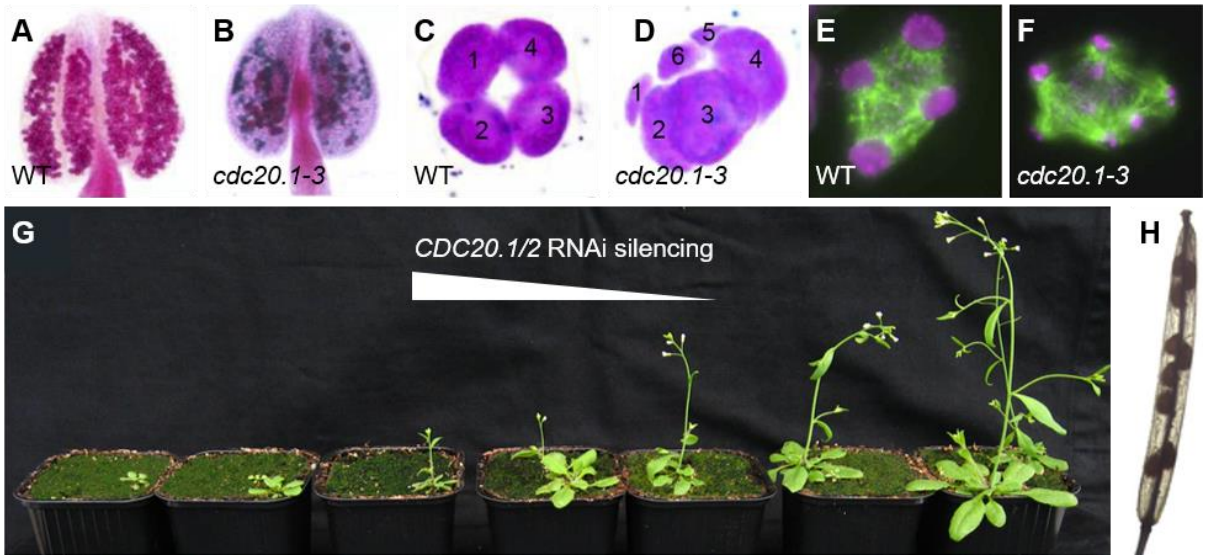

Figure 3: Phenotypes of the CDC20 type of APC/C activator subunit mutants.

A-F: *CDC20.1* mutants (B, D and F) show severely reduced male fertility compared to wild-type plants (A, C and E). Anthers from wild type (A) and *cdc20.1-3* (B) colored by Alexander staining, showing viable and inviable pollen in red and green, respectively. Tetrad stage microspores of wild-type plants (C) have four equally sized members, whereas in the mutant (D) more than four microspores of unequal size can be seen (numbered). Visualization of spindle morphology in wild-type (E) and *cdc20.1-3* (F) meiocytes at telophase II, with spindle microtubuli shown in green and chromosomes shown in magenta. Pictures adapted from Niu et al., 2015.

G-H: Simultaneous knock-down of *CDC20.1* and *CDC20.2* through RNAi causes a dose-dependent growth delay (G), while also leading to seed abortion in the siliques (H). Pictures adapted from Kevei et al., 2011.

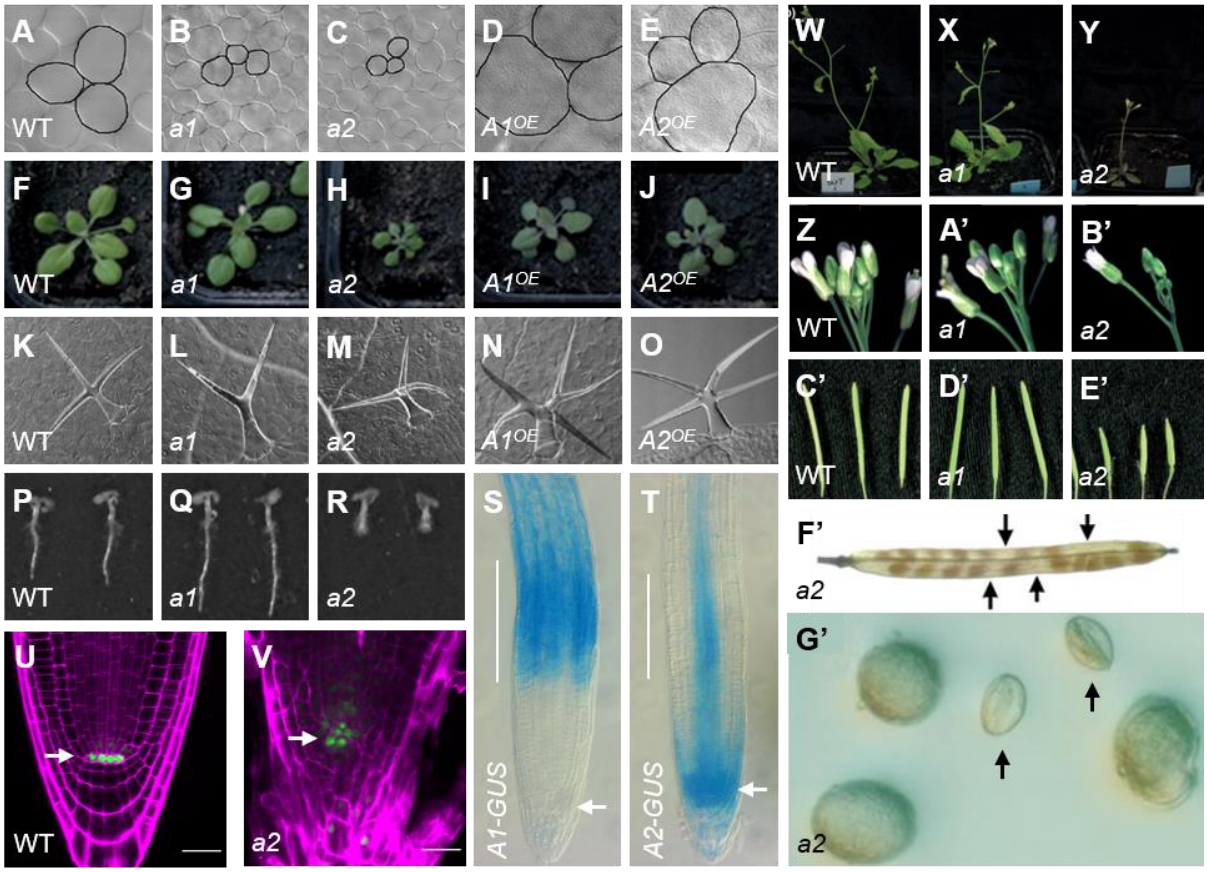

Figure 4: Phenotypes of the CCS52A-type APC/C activator subunit mutants.

A-O: CCS52A1 and CCS52A2 have both overlapping and distinct functions during leaf development. Pictures showing mature mesophyll cells (A-E), whole rosettes (F-J) and mature trichomes (K-O) of wild-type (WT) plants (A, F and K), of *ccs52a1* (B, G and L) and *ccs52a2* (C, H and M) mutant plants, and of *CCS52A1* (D, I and N) and *CCS52A2* (E, J and O) overexpressing plants. Pictures adapted from Baloban et al. (2013) with permission from John Wiley and Sons.

P-R: The two CCS52A isoforms differently affect root growth. Pictures showing representative WT (P), *ccs52a1* (Q) and *ccs52a2* (R) seedlings at 4 days after stratification (DAS). Pictures adapted from Vanstraelen et al., 2009.

S-T: CCS52A1 and CCS52A2 are present in different parts of the root tip. Pictures showing a histochemical GUS staining in root tips of seedlings carrying the *CCS52A1pro:CCS52A1-GU*S (S) or *CCS52A2pro:CCS52A2-GUS* (T) translational reporter. The transition zone and the quiescent center (QC) are indicated by lines and arrows, respectively.

U-V: The root meristem organization is disturbed in *ccs52a2* mutant plants, correlating with a loss of cell division control in the QC. Representative confocal images of WT (U) and *ccs52a2-1* (V) root tips at 5 DAS carrying the *WOX5pro:GFP-GUS* QC cell-specific reporter construct.

The GFP signal is shown in green, while cell walls are visualized through propidium iodide staining (magenta). Arrows indicate the position of the QC. Scale bars represent 25 µm.
W-G': Lack of CCS52A2, but not of CCS52A1, leads to a reduction in flowering and fertility. Pictures showing flowering stalks (W-Y), inflorescences (Z-B'), full-grown siliques (C'-F') and pollen (G') of WT (W, Z and C'), *ccs52a1* (X, A' and D') and *ccs52a2* (Y, B', E', F' and G') plants. Arrows indicate aborted seeds (F') and abnormally shaped pollen grains (G'). Pictures adapted from Baloban et al. (2013) with permission from John Wiley and Sons.

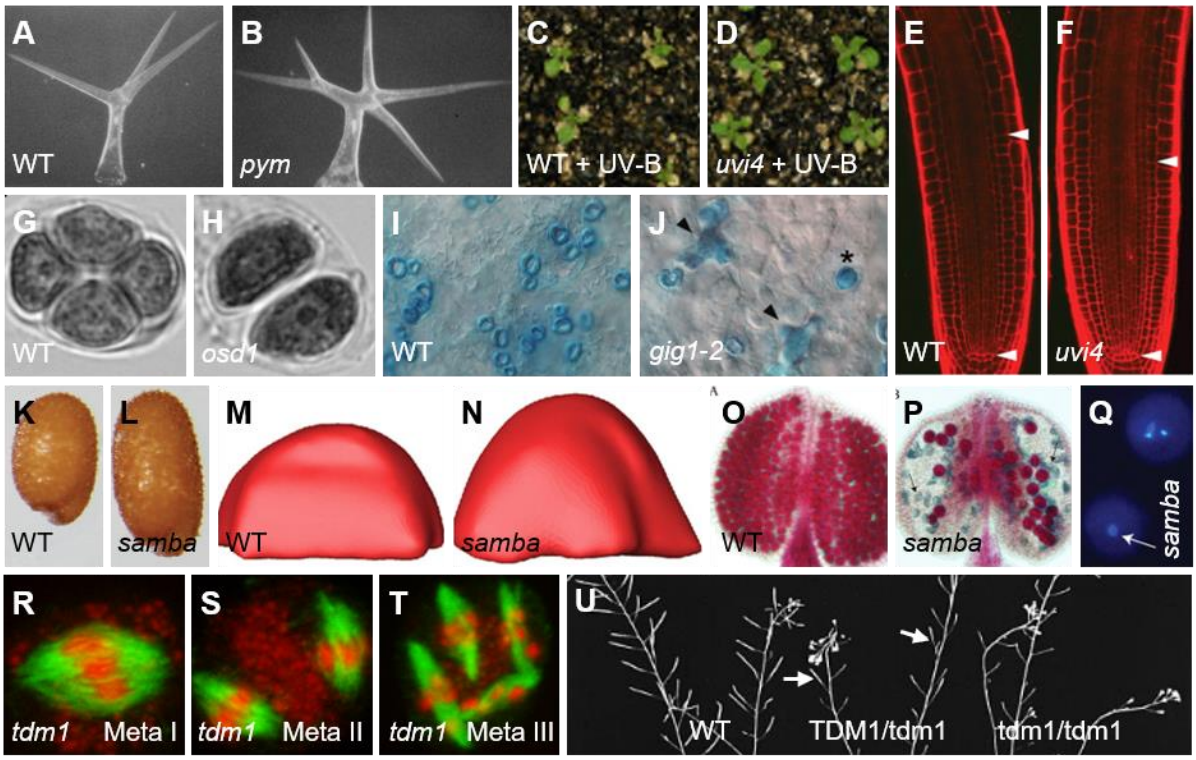

Figure 5: Phenotypes of APC/C interactor mutants

A-B: Compared to the wild type (WT, A), the pym mutant (B) shows supernumerary trichome branching. Pictures adapted from Perazza et al. (1999) with permission from Oxford University Press.

C-D: Compared to WT plants (C), uvi4 plants (D) show a greater tolerance for treatment with UV-B light. Pictures adapted from Hase et al. (2006) with permission from John Wiley and Sons.

E-F: The uvi4 mutant (F) shows a shorter root meristem compared to the WT (E) due to an earlier onset of the endocycle. Arrows indicate the start and end of the meristem. Pictures adapted from Heyman et al., 2011.

G-H: The osd1 mutant skips the second meiotic division, resulting in diploid gametes. Pictures showing normal WT tetrad (G) and osd1 dyad (H) male meiotic products. Pictures adapted from d'Erfurth et al., 2010.

I-J: Abnormal guard cells could be seen in the gig1-2 mutant background (J) compared to the WT (I). Arrowheads show the abnormally large guard cell, while a round cell is indicated with an asterisk. Plants carry the mature guard cell-specific marker KAT1:GUS (blue). Pictures adapted from Iwata et al. (2011) with permission from Oxford University Press.

K-N: samba mutants (L and N) show enlarged tissues compared to the WT (K and M). Mature seeds (K-L) and reconstructed shoot apical meristems are shown (M-N). Pictures adapted from Eloy et al., 2012.

O-P: The samba mutant (P) shows a decreased male fertility compared to the WT (O). Pictures showing Alexander stained anthers, with viable and inviable pollen colored red and green, respectively. Pictures adapted from Eloy et al., 2012.

Q: samba pollen frequently contain only a vegetative nucleus (arrow), whereas normal pollen contain a vegetative nucleus and two densely stained sperm nuclei. Nuclei were stained using DAPI. Pictures adapted from Eloy et al., 2012.

R-T: Male gametogenesis in tdm1 mutant plants is carried out as normal through metaphase I and metaphase II, but afterwards a third separation of chromosomes is initiated, leading to 8 nuclei with an aberrant number of chromosomes. Pictures showing immunolocalization of tubulin during the consequent meiosis metaphases, with DNA appearing in red and tubulin in green. Pictures adapted from Cromer et al., 2012.

U: Loss of TDM1 function leads to infertility. A WT plant (left) with normally sized siliques, a heterozygous plant (middle) with only few seed-containing siliques (arrows) and a homozygous mutant plant (right) with small empty siliques are shown. Pictures adapted from Glover et al. (1998) with permission from John Wiley and Sons.

Table 1: The APC/C subunits in *Arabidopsis thaliana*

Locus	Name	Subunit type	Cell cycle phase	Loss-of-function phenotype	References
AT5G05560	APC1/EMB2771	Core - structural	Constitutive	Defective female gametogenesis	(Wang et al 2013)
AT2G04660	APC2	Core - catalytic	Constitutive	Defective female gametogenesis	(Capron et al 2003b)
AT3G16320	APC3a/CDC27a	Core - structural	S-G2	No phenotype	(Pérez-Pérez et al 2008)
AT2G20000	APC3b/CDC27b/HOBBIT	Core - structural	S, G2 - M	Dwarf growth	(Pérez-Pérez et al 2008)
AT4G21530	APC4	Core - structural	Constitutive	Defective female gametogenesis	(Wang et al 2012)
AT1G06590	APC5	Core - structural	Constitutive		
AT1G78770	APC6/CDC16/NOMEGA	Core - structural	Constitutive	Defective female gametogenesis	(Kwee & Sundaresan 2003)
AT2G39090	APC7	Core - structural	Constitutive		
AT3G48150	APC8/CDC23	Core - structural	Constitutive	Defective male gametogenesis	(Xu et al 2019, Zheng et al 2011)
Not found	APC9	Non-core			
AT2G18290	APC10/DOC1/EMB2783	Core - Co-	Constitutive	Defective female gametogenesis	(Eloy et al 2011)
AT3G05870	APC11/ZYG1	Core - catalytic	Constitutive	Zygote cell division arrest	(Guo et al 2018, Guo et al 2016)
AT2G11890	APC12/CDC26	Non-core		Defective embryo development	(Lorenzo-Orts et al 2019)
AT1G73177	APC13/SWM1/BONSAI	Non-core		Defective male gametogenesis	(Zheng et al 2011)
AT5G63135	APC15	Non-core			(Uzunova et al 2012)
AT4G33270	CDC20.1	Core - activator	Early G2 - Late M	Simultaneous knockdown: dwarf growth, defective male gametogenesis	(Kevei et al 2011)
AT4G33260	CDC20.2	Core - activator	Early G2 - Late M		
AT5G27080	CDC20.3	Core - activator	-	No phenotype	(Kevei et al 2011)
AT5G26900	CDC20.4	Core - activator	-	No phenotype	(Kevei et al 2011)
AT5G27570	CDC20.5	Core - activator	-	No phenotype	(Kevei et al 2011)
AT5G27945	CDC20.6	Core - activator	-		(Kevei et al 2011)
AT4G22910	CCS52A1/FZR2	Core - activator	Late M - Early G2	Delayed endocycle onset, reduced trichome branching	(Baloban et al 2013, Vanstraelen et al 2009)
AT4G11920	CCS52A2/FZR1	Core - activator	Late M - Early G2	Delayed endocycle onset, dwarf growth, disturbed meristems	(Baloban et al 2013, Liu et al 2012, Vanstraelen et al 2009)
AT5G13840	CCS52B/FZR3	Core - activator	Early G2 - Late M	Reduced cell expansion	(Su'udi et al 2012)
AT2G42260	UVI4	Inhibitor	G1-S	Premature endocycle onset	(Heyman et al 2011)
AT3G57860	OSD1/UVI4-like/GIGAS-CELL1	Inhibitor	G2-M	Diploid gametes, endomitosis	(Cromer et al 2012, Iwata et al 2011)
AT1G32310	SAMBA	Activator		Increased organ size	(Eloy et al 2012)
AT4G20900	TDM1/MS5	Activator	Meiosis	Defective male gametogenesis	(Bulankova et al 2010, Cifuentes et al 2016)

Table 2: APC/C target proteins in plants (cyclins)

Species	Accession	Gene Name	Function	Evidence	Reference
A. thaliana	AT1G44110	CYCA1;1	G2-M specific cyclin	Interaction with all CCS52 isoforms (Pull-down)	(Fülöp et al 2005)
A. thaliana	AT1G77390	CYCA1;2	G1-S specific cyclin	Interaction with CCS52A1 and CCS52A2 (Pull-	(Fülöp et al 2005)
				Interaction with CDC20.1 and CDC20.2 (Y2H)	(Kevei et al 2011)
A. thaliana	AT1G15570	CYCA2;3	Regulation of G2-M	CYCA2;3-GFP accumulation in *ccs52a1*	(Boudolf et al 2009)
				CYCA2;3-GFP degradation in *uvi4*	(Heyman et al 2011)
				CYCA2;3-GFP degradation in *ubp14*	(Xu et al 2016)
				CYCA2;3-HA accumulation in *samba*	(Eloy et al 2012)
S. lycopersicum	SL10g04936	CYCA3	Endoreplication in fruits	Interaction with CCS52A (Y2H) and CYCA3-LUC	(Mathieu-Rivet et al 2010)
A. thaliana	AT5G43080	CYCA3;1	G1-S specific cyclin	CYCA3;1-D-box-GUS accumulation in *apc2*	(Capron et al 2003b)
A. thaliana	AT4G37490	CYCB1;1	G2-M specific cyclin	Interaction with all CCS52 isoforms (Pull-down)	(Fülöp et al 2005)
				CYCB1;1-Dbox-GUS accumulation in *apc1*	(Wang et al 2013)
				CYCB1;1-Dbox-GUS accumulation in *apc4*	(Wang et al 2012)
				CYCB1;1-Dbox-GUS accumulation in *apc6*	(Kwee & Sundaresan 2003)
				CYCB1;1-YFP accumulation in *apc8*	(Zheng et al 2011)
				CYCB1;1-Dbox-GUS degradation in $APC10^{OE}$	(Eloy et al 2011)
				CYCB1;1-His in vitro ubiquitination with APC3a	(Rojas et al 2009)
				His-CYCB1;1-Myc in vitro ubiquitination with	(Guo et al 2016)
A. thaliana	AT5G06150	CYCB1;2	G2-M specific cyclin	Interaction with all CCS52 isoforms (Pull-down)	(Fülöp et al 2005)
				CYCB1;2-Dbox-YFP accumulation upon OSD1 OE	(Iwata et al 2011)
A. thaliana	AT2G17620	CYCB2;1	M-G1 specific cyclin	Interaction with CDC20.1 and CDC20.2 (Y2H)	(Kevei et al 2011)
A. thaliana	AT4G35620	CYCB2;2	M-G1 specific cyclin	Interaction with CDC20.1 and CDC20.2 (Y2H)	(Kevei et al 2011)
A. thaliana	AT1G20610	CYCB2;3		Interaction with CCS52A2 and CCS52B (BiFC)	(Boruc et al 2010)

Table 2 (continued): APC/C target proteins in plants (non-cyclins)

Species	Accession Nr	Gene Name	Function	Evidence	Reference
A. thaliana	AT3G54180	CDKB1;1	G2-M specific CDK	CDKB1;1-GFP stabilization by MG-132 and degradation in *ubp14*	(Xu et al 2016)
A. thaliana	AT1G02730	CSLD5	Cellulose synthesis	Ubiquitinated and marked for breakdown by CCS52	(Gu et al 2016)
O. sativa	Os01g15340	OsRAA1 OsFPF1	Inhibitor of metaphase-to-anaphase transition	Interaction with 26S proteasome subunit OsRTP4	(Han et al 2008)
				Degraded in a D-box-dependent manner	
A. thaliana	AT4G33270 / AT4G33260	CDC20.1 / CDC20.2	APC/C activator subunits	Interaction with all CCS52 isoforms (Y2H)	(Kevei et al 2011)
				GFP-CDC20.1 stabilization by MG-132	(Yang et al 2017)
A. thaliana	AT3G05870	APC11	APC/C catalytic subunit	APC11 is able to self-ubiquitinate	(Guo et al 2016)
A. thaliana	AT3G10440 / AT5G04320	SGO1 / SGO2	Sister chromatid cohesion	Targeted by APC/C^{CDC20} in yeast	(Jonak et al 2017)
				Contain conserved KEN-box and D-box	(Cromer et al 2013)
A. thaliana	AT3G14190 / AT5G12360	PANS1 / PANS2	Plant securin Sister chromatid cohesion	Interaction with CDC20 in a D-box-dependent manner	(Cromer et al 2013)
				D-box mutant prevents sister chromatid separation	(Cromer et al 2019)
O. sativa	Os02g39390	RSS1		Interaction with CDC20 in a D-box-dependent manner	(Ogawa et al 2011)
				Protein stability determined by cell cycle phase	
A. thaliana	AT3G62800	DRB4	RNA silencing	Accumulated in *apc6* or *apc10*, interacts with APC10	(Marrocco et al 2012)
A. thaliana	AT5G07310	ERF115	TF involved in QC	Targeted by CCS52A2 in a D-box-dependent manner	(Heyman et al 2013)
O. sativa	Os06g40780	MOC1 / OsSCL18	TF involved in shoot branching	Interaction with CCS52 in a D-box-dependent manner	(Lin et al 2012, Xu et al 2012)
				Ubiquitinated and marked for breakdown by CCS52	
O. sativa	Os03g31880	OsSHR1	TF involved in root growth	Interaction with CCS52 in a D-box-dependent manner	(Lin et al 2020)
				Ubiquitinated and broken down by CCS52	
O. sativa	Os10g42280	RCAR10	ABA receptor	Degraded in an APC/C^{TE}-dependent manner	(Lin et al 2015)
A. thaliana	AT3G49250	DMS3	DNA methylation	Interaction with APC10 and ubiquitinated and degraded in a D-box-dependent manner	(Zhong et al 2019)