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Authors: Bolaños-Villegas, Pablo, Xu, Wanyue, Martínez-García, Marina, Pradillo, Mónica, and Wang, Yingxiang

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Insights Into the Role of Ubiquitination in Meiosis: Fertility, Adaptation and Plant Breeding

Pablo Bolaños-Villegas[a,1], Wanyue Xu[b], Marina Martínez-García[c], Mónica Pradillo[d], and Yingxiang Wang[b]

[a] Fabio Baudrit Agricultural Research Station, University of Costa Rica, Alajuela 20102, Costa Rica
[b] State Key Laboratory of Genetic Engineering, Ministry of Education Key Laboratory of Biodiversity Sciences and Ecological Engineering, Institute of Plant Biology, School of Life Sciences, Fudan University, Shanghai, 200438, China
[c] Department of Genetics, Harvard Medical School, Boston, MA 02115, United States of America
[d] Department of Genetics, Physiology and Microbiology, Faculty of Biology, Universidad Complutense, C/ José Antonio Novais, 12, Madrid 28040, Spain
[1] Address correspondence to pablo.bolanosvillegas@ucr.ac.cr

Ubiquitination is a post-translational modification process that plays a central role in protein degradation in eukaryotic cell cell division, including meiosis. This modification affects different cellular processes on a global scale by its pleiotropic ability to modify numerous proteins. Meiosis is essential for sexual reproduction and involves two rounds of nuclear division following a single round of DNA replication to produce haploid gametes. Unlike mitosis, meiosis has a unique prophase I, which involves homologous chromosome interaction including pairing, synapsis, recombination and segregation. Over the last several decades, molecular genetic studies have identified many proteins that participate in meiotic progression. In this review, we focus on the recent advances regarding the role of ubiquitination during plant meiotic cell cycle progression and recombination, especially the role played by the Anaphase-Promoting Complex and E3 ligases in modulating crossover formation and its impact on evolution and plant breeding.

INTRODUCTION

Meiosis is one of the crucial processes during the life of flowering plants. During meiosis, the chromosome number is reduced by half, leading to the formation of haploid gametes that eventually fuse and restore ploidy in the following generation (Cromer et al., 2012). Meiosis is different from somatic cell division (mitosis) in several ways (see Table 1); for instance, unlike mitosis, which produces two diploid cells, meiosis involves two rounds of nuclear division, meiosis I and II, following a single round of DNA replication, thus producing four haploid nuclei (Wang and Copenhaver, 2018). However, meiosis II is similar to mitosis with segregating sister chromatids (see Table 1), while meiosis I is unique and involves segregation of homologous chromosomes (Wang and Copenhaver, 2018).

To accurately perform reductional division, specific meiotic steps include homolog pairing, the formation of the synaptonemal complex (SC), a tripartite protein structure, and the maturation of recombination intermediates into crossovers (COs), that are visualized as physical attachments (chiasmata) between homologs (see Fig. 1). These events ensure the proper segregation of homologs to opposite poles at anaphase I (d'Erfurth et al., 2010). In higher eukaryotes, the core cell cycle machinery is shared between meiosis and mitosis. Most notably, cell cycle entry and progression are determined by the activity of cyclin-dependent kinases (CDKs) and associated A- and B-type cyclin subunits that establish activity and specificity (Bulankova et al., 2013). In eukaryotes, CDK activity peaks during the M-phase, when chromosomes are attached to the microtubule and align at the mid-cell plate. Subsequent activation of the Anaphase-Promoting Complex (APC/Cyclosome [APC/C]) initiates proteolytic destruction of A- and B-type cyclins and allows for chromosome segregation (Bulankova et al., 2013) (see Fig. 2). The *Arabidopsis* APC/C is a conserved multisubunit cullin-based RING E3 ubiquitin ligase complex (d'Erfurth et al., 2010; Choi et al., 2014) composed of at least 11 core subunits (Bulankova et al., 2013) that plays an essential role during mitosis, meiosis and post-mitotic cell differentiation (Eloy et al., 2012). In eukaryotic organisms, E3 ubiquitin ligases act as mediators between E1 ubiquitin activation, E2 ubiquitin conjugation enzymes and degradation by the 26S proteasome (Choi et al., 2014). They also provide specificity for the substrate (Bulankova et al., 2013). Ubiquitination takes place when an E3 ligase enzyme binds to both the substrate and an E2 thioesterified with ubiquitin (Deshaies and Joazeiro, 2009), bringing them close enough so that the ubiquitin is transferred from the E2 to the substrate via a covalent E3-ubiquitin thioester intermediate (Deshaies and Joazeiro, 2009). Eukaryotes have two major types of E3 ligases with an HECT and a RING domain, respectively. RING domain ligases feature conserved cysteine and histidine residues that form an interleaved structure with two zinc coordination sites for protein interactions (Deshaies and Joazeiro, 2009).

Table 1. Key differences between mitosis and meiosis.

Key differences between mitosis and meiosis		
	Mitosis	Meiosis
Number of cell divisions	One round	Two rounds
Cell type formed	Somatic cells	Germ cells
Number of daughter cells	Two diploid cells	Four haploid cells
Type of genetic information in daughter cells	Identical	Recombinant, not identical
Orientation of sister chromatids	Bi-orientation	Mono-orientation during meiosis I, and bi-orientation during meiosis II
Number of times that the cohesin complex is released	Once	Twice
Interaction between homologous chromosomes	No	Yes
Chromosome segregation	Segregation of sister chromatids	Segregation of homologous chromosomes and sister chromatids

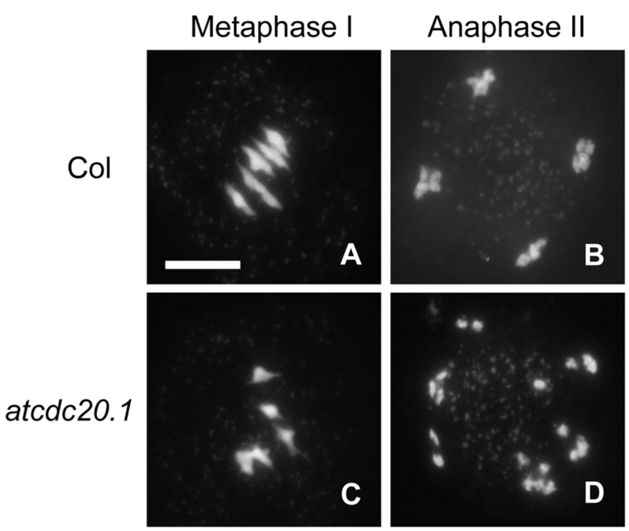

Figure 1. Meiotic chromosome morphology in Col-0 and the *atcdc20*.1 mutant as observed by DAPI staining. The five wild-type bivalents are aligned regularly at the equatorial plate during metaphase I (A), and segregate equally into four nuclei during anaphase II (B), while the five *atcdc20*.1 bivalents are not well aligned during metaphase I (C), and form multiple nuclei at anaphase II (D). Bar = 5 μm.

Plant A-type cyclins, especially *Arabidopsis* CYCA A2 (CYCA2;1: At5g25380) and CYCA2;3 proteins (At1g15570) (see Table 2 and Fig. 2), show important functional specificity during mitotic cell cycle progression and control of ploidy (Eloy et al., 2012). The meiotic plant A-type cyclin CYCA1;2/TARDY ASYNCHRONOUS MEIOSIS (TAM; At1g77390) (see Table 2 and Fig. 2) is essential for the transition between the first and second meiotic division, and defects in its function lead to exit from meiosis after prophase I (d'Erfurth et al., 2010) and the formation of diploid gametes (d'Erfurth et al., 2010).

The role of plant B-type cyclins is less clear, although 11 tentative genes were believed to exist in *Arabidopsis* at one point (Cromer et al., 2012). Functional characterization of B-type cyclin CYCB3;1 (At1g16330) (see Table 2 and Fig. 2) indicated that this protein has a role in spindle organization and cell wall formation in male meiocytes and that it cooperates with plant-specific cyclin SOLO DANCERS (SDS: At1g14750) (see Table 2 and Fig. 2), which has a role in meiotic recombination (Azumi et al., 2002). In eukaryotes, both A- and B-type cyclins are targeted for proteolysis by the APC/C via recognition of two specific amino acid motifs: 1) destruction (D) box and 2) KEN box (Eloy et al., 2012).

Activation of the *Drosophila* APC/C requires the activity of Cdc20/Fizzy and Cdh1/Fizzy-related proteins, known in plants as CELL CYCLE SWITCH 52 (CCS52A1: At4g22910, CCS52A2: At4g11920, and CCS52B: At5g13840) (Heyman et al., 2011) (see Table 2). CELL DIVISION CYCLE 20 (CDC20) is activated from late G2 phase onward, but anaphase to early S phase activation depends on CADHERIN 1 PRECURSOR (CDH1) (Heyman and De Veylder, 2012). Nonetheless, how *Arabidopsis* CCS52A1, CCS52A2, and CCS52 operate during plant meiosis is entirely unclear (Heyman et al., 2011).

The *Arabidopsis* APC/C itself is negatively regulated by several proteins, including the regulator of male gametogenesis and A-type cyclin stabilizer SAMBA (At1g32310) (Eloy et al., 2012) as well as the meiotic regulator OMISSION OF SECOND DIVISION 1 (OSD1: At3g57860) (d'Erfurth et al., 2010) (see Table 2 and Fig. 2), the presumed homolog of protein Mes1 in fission yeast and Emi2/Erp1 in metazoans (Cifuentes et al., 2016). The OSD1 amino acid sequence shares with Mes1 having two putative APC/C degradation motifs, a D-box (residues 104-110) and a KEN-box (residues 80-83), which have been shown to determine Mes1 function (Iwata et al., 2011). The primary function of Emi1 is to inhibit the APC/C-CDH1/FZR complex, terminating DNA replication and enabling transition from the G2 to M phase (Iwata et al., 2011), whereas Emi2 inhibits the APC/C-CDC20 complex, enabling progression through meiosis (Iwata et al., 2011).

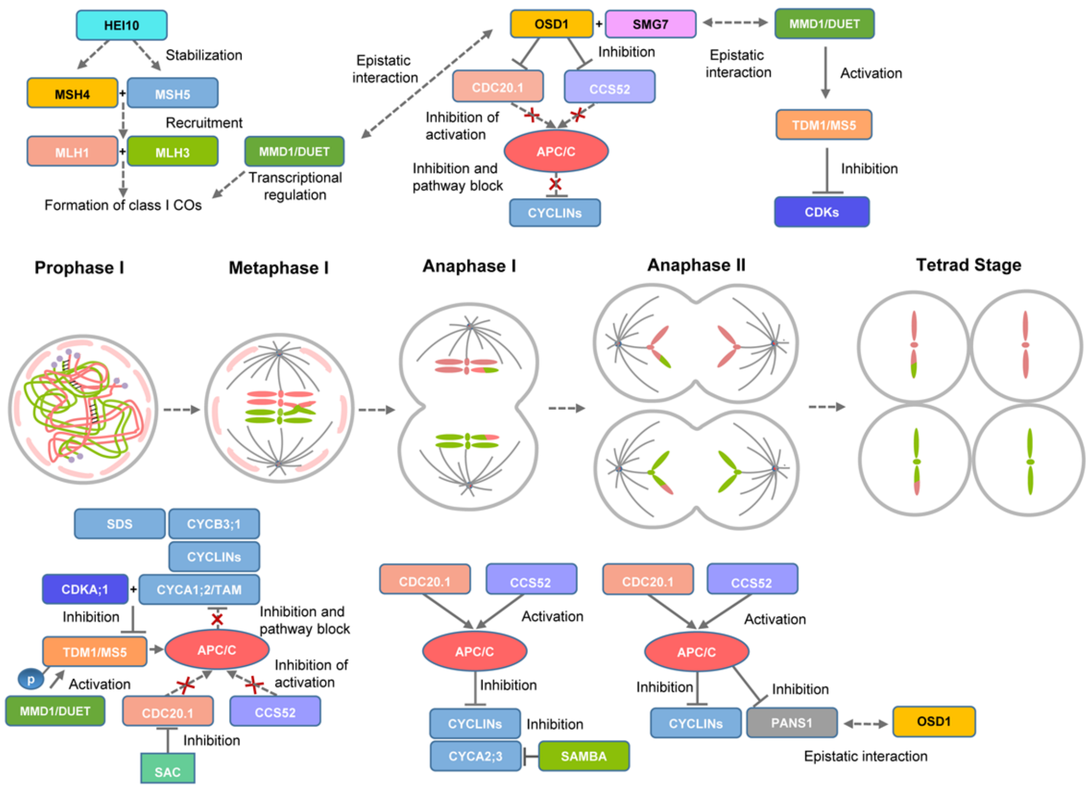

Figure 2. Schematic representation of protein interactions between ubiquitination regulators during meiosis in Arabidopsis thaliana. Abbreviations and notes: spindle assembly checkpoint (SAC). SDS, CYCA and CYCB are plant cyclins. CDC20.1 and CCS52 are APC/C activators. Notes: SAC means Spindle Assembly Checkpoint. Red crosses indicate that the corresponding processes or pathways are blocked. Arrowheads indicate activation of processes. Inhibition means that targeting induces either proteasomal degradation, phosphorylation or downregulation of activity.

The APC/C is also believed to interact directly with protein THREE DIVISION MUTANT1/MALE STERILE 5 (TDM1/MS5: At4g20900) (see Table 2 and Fig. 2), a homodimer that may regulate termination of meiosis at the end of the second meiotic division and whose activity during the first division may be inhibited by CDKA;1-CYCA1.2/TAM-mediated phosphorylation at T16 (Cifuentes et al., 2016). TDM1 is believed to share limited structural similarity with the APC/C subunit CDC16/Cut9/APC6 and contains a tetratricopeptide repeat (TPR) domain known to mediate protein–protein interactions (Cifuentes et al., 2016).

MULTIPLE ROLES OF CDKA;1 IN *ARABIDOPSIS*

In yeast and mammals, CDK activity by Cdc2/Cdc28 and Cdk1 is indispensable for cell cycle progression (Nowack et al., 2012). The *Arabidopsis* functional homolog of Cdc2/CDC28 and Cdk1 is CDKA;1 (At3g48750) (see Table 2 and Fig. 2), and it can complement yeast *cdc2* and *cdc28* mutants (Nowack et al., 2012). *Arabidopsis cdka;1*/- and *CDKA;1*-overexpression mutants show defects in pollen mitosis I, defective embryogenesis, reduced root stem growth, underbranched trichomes, and cell cycle arrest at the G2 phase (Nowack et al., 2012), which suggest control of the M and S phases. CDKA;1 protein is also believed to form a complex with CYCA1.2/TAM that phosphorylates TDM1 at residue T16 and leads to exit from the first meiotic division (Cifuentes et al., 2016). TAM is also involved in exit from the second division and organization of the meiotic spindle formation and chromosome condensation and may be the actual *Arabidopsis* homolog of the yeast APC/C CDC16/Cut9/APC6 subunit (Cifuentes et al., 2016).

THE ROLE OF OSD1 DURING MALE MEIOSIS

Arabidopsis OMISSION OF SECOND DIVISION (*OSD1*, At3g57860, also known as *GIGAS* and *UVI4-Like*) is a regulator of the APC/C and presumably operates by controlling the degradation of cyclins to influence exit from mitosis or meiosis (Crismani et al., 2013; D'Erfurth et al., 2009) (see Fig. 2). *OSD1* has a role in regulating endomitotic proliferation in vegetative tissues and female gametophyte development (d'Erfurth et al., 2010). However, characterization of both *osd1-1* and *osd1-2* progenies indicated that they are all tetraploid and triploid, indicating that *OSD1* functions to control the transition from first to second meiotic division (D'Erfurth et al., 2009). Alignment of amino acid sequences indicated that OSD1 shares limited homology with the APC/C inhibitor Mes1 from *Schizosaccharomyces pombe* (d'Erfurth et al., 2010), including a D-box (residues 104-110, RxxLxx[LIVM]) and a GxEN/KEN-box (residues 80-83), which have been shown to determine Mes1 function. Moreover, OSD1

Table 2. *Arabidopsis thaliana* genes presumed to be involved in meiotic ubiquitination of proteins.

Gene Name	AGI Code	Function	Reference
AUXIN RESISTANT1 (AXR1)	At1g05180	May target Cullin RING Ligase 4 (CRL4, At1g60986) for activation. Required for SC formation and bivalent formation	Jahns et al., 2014
CDC20.1	At4g33270	APC/C subunit. Required for the proper alignment and segregation of chromosomes at metaphase I and anaphase I and II. Required for proper localization of protein kinase Aurora 1 and attachment of kinetochores to the meiotic spindle	Niu et al., 2015
CDKA;1	At3g48750	Arabidopsis functional homolog of the yeast and mammal Cdc2/CDC28 and Cdk1, possible activator of the Arabidopsis APC/C, regulation of exit from the first meiotic division, may interact with CYCA1.2/TAM	Cifuentes et al., 2016; Crismani et al., 2013; D'Erfurth et al., 2009
CELL CYCLE SWITCH 52 (CCS52A1, CCS52A2, and *CCS52B)*	At4g22910, At4g11920, At5g13840	Activation of the Arabidopsis Anaphase Promoting Complex/ Cyclosome (APC/C) mechanism is unknown. May interact with OSD1	Heyman et al., 2011
CYCA1;2/TARDY ASYNCHRONOUS MEIOSIS (TAM)	At1g77390	Meiotic plant A cyclin essential for the transition between the first and second meiotic division. Defects in its function lead to the formation of diploid gametes. TAM is also involved in exit from the second division, organization of the meiotic spindle formation and chromosome condensation. It may be the Arabidopsis homolog of the yeast APC/C CDC16/Cut9/APC6 subunit	d'Erfurth et al., 2010
CYCA2;1	At5g25380	Plant A cyclin, regulation of mitotic cell cycle progression and control of ploidy	Eloy et al., 2012
CYCA 2;3	At1g15570	Plant A cyclin, regulation of mitotic cell cycle progression and control of ploidy	Eloy et al., 2012
CYCB3;1	At1g16330	Plant B-type cyclin, has a role in spindle organization and cell wall formation in male meiocytes, cooperates with cyclin gene *SOLO DANCERS*	Bulankova et al., 2013
MALE MEIOCYTE DEATH1/DUET (*MMD1/DUET*)	At1g66170	It is involved in microtubule organization, homologous recombination and chromosome condensation at prophase and metaphase I. May activate transcription of APC/C-interacting protein TDM1, and regulate expression of *OSD1* and condensin genes	Reddy et at., 2003; Yang et al., 2003; Andreuzza et al., 2015; Wang et al., 2016
MSH2	At3g18524	Mismatch repair protein, MutS-homologue. Suppresses recombination between homologues from different ecotypes during meiosis	Serra et al., 2018b; Emmanuel et al., 2006
Fanconi anemia D2 (FANCD2)	At1g48360	FANCD2 promotes the formation of meiotic noninterfering COs independently from MUS81 and MSH4	Kurzbauer et al., 2018
Fanconi anemia complementation group M (FANCM)	At1g35530	DNA helicase, has meiotic anticrossover activity, FANCM may process meiotic D loops to form non-cross overs (NCOs)	Crismani et al., 2012; Serra et al., 2018b
FIDGETIN-LIKE1 (FIGL1)	At3g27120	AAA-ATPase, may counteract DMC1/RAD51-mediated inter-homologue strand invasion to limit CO formation	Girard et al., 2015; Serra et al., 2018b
HUMAN ENHANCER OF INVASION CLONE 10 (HEI10)	At1g53490	E3 ligase implicated in the formation of interference-sensitive COs, suspected to target MutS-related meiotic protein MSH5. Shows dosage activity	Chelysheva et al., 2012 and Ziolkowski et al., 2017
OMISSION OF SECOND DIVISION 1 (OSD1), also known as *GIGAS* and *UVI4-Like*	At3g57860, Os02g37850	Negative regulator of the Arabidopsis APC/C during meiosis, presumed homolog of protein Mes1 in fission yeast and Emi2/ Erp1 in metazoans. May operate by controlling the degradation of cyclins to influence exit from meiosis. OSD1 interacts directly with activator CDC20.1 (At4g33270)	Cifuentes et al., 2016; d'Erfurth et al., 2010

(continued on next page)

Table 2. *(continued)*

PAIR1 (OsPAIR1)	Os03g01590	Essential for initiation of meiotic recombination via DNA double-strand break formation used in combination with *OsOSD1* and *OsPAIR1* mutant alleles to induce the formation of clonal gametes in rice	
PATRONUS (PANS1)	At3g14190	Regulator of meiotic sister chromatid cohesion. May interact with the APC/C and OSD1	Cromer et al., 2013; Singh et al., 2015
RECQ4A	At1g10930	DNA helicase with anticrossover activity, participates in the disassembly of D loops and decatenation of double Holiday junctions	Serra et al., 2018b
RECQ4B	At1g60930	Similar to *RECQ4A*	Serra et al., 2018b
REC8 (OsREC8)	Os05g50410	Required for proper separation of sister chromatids during meiosis I, used in combination with *OsOSD1* and *OsPAIR1* mutant alleles to induce the formation of clonal gametes in rice	Mieulet et al., 2016
SAMBA	At1g32310	Plant A-type cyclin stabilizer, negative regulator of the Arabidopsis APC/C, regulator of male gametogenesis	Eloy et al., 2012
SOLO DANCERS (SDS)	At1g14750	Plant-specific cyclin, has a role in meiotic recombination and pollen mother cell wall metabolism	Azumi et al., 2002
SUPPRESSOR WITH MORPHOGENETIC EFFECTS ON GENITALIA7 (SMG7)	At5g19400	Regulator of the first to second meiotic division transition, may operate by downregulating or inducing the degradation of CDKA;1. It is epistatic to OSD1 and TAM	Bulankova et al., 2013
THREE DIVISION MUTANT1/MALE STERILE 5 (TDM1/MS5)	At4g20900	Presumed APC-interacting protein which may regulate termination of meiosis at the end of the second meiotic division and whose activity during the first division may be inhibited by TAM	Cifuentes et al., 2016

shares a C-terminal MR-tail with Mes1, which is a methionine and arginine sequence that in kinase Nek2a is essential for binding and inhibition activities against the APC/C; a similar RL-tail in vertebrate Emi2 is essential for inhibiting APC/C during meiosis (Cromer et al., 2012).

Moreover, results from yeast two-hybrid assay and tandem affinity purification experiments indicate that *Arabidopsis* OSD1 interacts directly with APC/C activators CDC20.1 (At4g33270), CDC20.5 (At5g27570), CCS52A1 (At4g22910), CCS52A2 (At4g11920) and CCS52B (At5g13840) (see Table 2 and Fig. 2), possibly through its D-box and the MR-tail (d'Erfurth et al., 2010; Iwata et al., 2011) but not with APC/C core units APC2, APC7, APC10, CDC27a, and CDC27b (also known as HOBBIT/APC3b) (Iwata et al., 2011; Cifuentes et al., 2016). Crossing of *osd1-3*, *cyca1;2/tam-2* and *tdm-3* indicated that the corresponding wild-type loci cooperate in the control of the meiotic cell cycle, with *OSD1* and *TAM* controlling the first- to second-division transition and *TDM* controlling exit from the second meiotic division (d'Erfurth et al., 2010) (see Fig. 2). Additional layers of genetic regulation involve the following:

a. Interaction with the mRNA decay factor *SUPPRESSOR WITH MORPHOGENETIC EFFECTS ON GENITALIA7* (*SMG7*: At5g19400) (see Table 2 and Fig. 2), a regulator of the first to second meiotic division transition, which is epistatic to *OSD1* and *TAM* and may operate by downregulating or inducing the degradation of CDKA;1, as suggested by experiments with the proteasome inhibitor MG115 (Bulankova et al., 2013).
b. The regulator of meiotic sister chromatid cohesion *PATRONUS* (*PANS1*: At3g14190) (see Table 2 and Fig. 2), whose sequence contains D and KEN boxes, can interact with the APC/C subunits CDC20.1 (At4g33270) and CDC27B (At2g20000) and is synthetically lethal with *OSD1* (Cromer et al., 2013; Singh et al., 2015). Therefore, the *pans1-1/osd1-3* mutants are totally pollen-sterile. The opposite occurs with *pans1-1/tam1-2* mutants, with *pans1-1* infertility rescued (Singh et al., 2015).
c. The CDKA;1-TAM complex may also phosphorylate OSD1 *in vitro* (Cifuentes et al., 2016).

In rice, combining the *Ososd1-1* mutant allele (Os02g37850) with mutant alleles for *REC8* (required for orientation of kinetochores; Os05g50410) (see Table 2), and *PAIR1* (required for formation of DNA double-strand breaks, Os03g01590) (see Table 2) led to the formation of male and female clonal diploid gametes, a finding that may contribute greatly to plant breeding (Mieulet et al., 2016).

MMD1/DUET IS A KEY REGULATOR OF MEIOTIC PROGRESSION

Arabidopsis MALE MEIOCYTE DEATH1/DUET (*MMD1/DUET*: At1g66170) (see Table 2) is involved in the development and viability of meiocytes, as seen in *mmd1* male meiocytes by cytoplasmic shrinkage and DNA fragmentation at diakinesis (Reddy et al., 2003; Yang et al., 2003), and its expression peaks at late diplotene during male meiosis (Andreuzza et al., 2015). Biochemical characterization of this protein indicated that MMD1/DUET is able to bind *in vitro* and *in vivo* to H3K4me2, H3K4me3 (and possibly H3K9me2 and H3S10ph) via its Plant Homeo Domain (PHD) finger (amino acids 606 to 656) and that it activates transcription of APC/C-interacting protein TDM1 (At4g20900) (see Fig. 2) (Andreuzza et al., 2015; Wang et al., 2016). Chromatin immunoprecipitation (ChIP) experiments showed that MMD1/DUET binds to the *TDM1* promoter, whereas transcriptional analyses showed that the onset of *TDM1* expression coincides with the timing of *DUET* expression, which suggests that *TDM1* is indeed a direct target of MMD1 during male meiosis (Andreuzza et al., 2015). Analysis of gene expression profiles in *mmd1/duet* meiocytes by quantitative PCR (qPCR) indicated significantly reduced transcription of *TDM1* along with other 34 meiotic genes, a mild decrease in *OSD1,* and no change in expression of *TAM* (Andreuzza et al., 2015). RNA sequencing and qPCR results revealed that up to 756 genes may show transcriptional regulation in *mmd1* meiocytes, including several condensin genes (*CAP-D2:* At3g57060*, CAP-D3:* At4g15890*, CAP-H:* At2g32590, and *CAP-H2:* At3g16730) (Wang et al., 2016). Therefore, MMD1 may affect meiotic chromosome condensation by regulating the expression of condensin I and II complexes (Wang et al., 2016). Notably MMD1/DUET is a versatile protein also involved in microtubule organization, homologous recombination and chromosome condensation at prophase and metaphase I (Wang et al., 2016).

APC/C ACTIVATOR CDC20.1 HAS A ROLE IN MEIOSIS

Tandem affinity purification (TAP) results indicated that *Arabidopsis* APC/C contains at least 11 core subunits, all of them are required for the ubiquitin transfer reaction (Heyman and De Veylder, 2012). The processing and timely activation of the entire complex depends on docking by the CDC20 and CCS52 APC/C activator subunits as well as the APC10 co-activating subunit (see Fig. 2) (Heyman and De Veylder, 2012). Both CDC20 and CCS52 contain seven WD40 repeats that facilitate protein–protein interactions; each targets proteins that contain a D-box sequence (RxxLxxxN/Q) (Kevei et al., 2011). CDC20 is believed to be actively sequestered by spindle assembly checkpoint (SAC) proteins such as protein kinase Aurora B to allow for correction of errors in the attachment of kinetochores to the microtubules (Niu et al., 2015). Cytological observation of anthers from T-DNA insertional alleles *cdc20.1-3* and *cdc20.1-4* corresponding to *Arabidopsis CDC20.1* (At4g33270) (see Table 2) showed that anthers contained mostly unviable microspores and meiocytes mostly developed into polyads (see Fig. 1). Moreover, mutant meiocytes showed defects in the alignment of bivalents and segregation of chromosomes at metaphase I and anaphase I and II (see Fig. 1), possibly caused by poor alignment of chromosomes, as suggested by unequal distribution of the kinetochore marker HISTONE H3-LIKE CENTROMERIC PROTEIN (HTR12, At1g01370) (Niu et al., 2015). Analysis of the Aurora marker H3S10ph at centromeres indicates that in the mutant, its distribution is diffuse during diakinesis, which suggests that CDC20.1 is required for proper localization of Aurora 1. A similar phenotype is observed in meiocytes from transgenic *ProDMC1:Aurora1RNAi* plants (Niu et al., 2015), further supporting the idea that CDC20.1 is required for meiotic chromosome segregation likely through influencing Aurora localization.

26S PROTEASOME AND E3 UBIQUITIN LIGASES AFFECT MEIOTIC RECOMBINATION

The 26S proteasome plays an essential role in meiosis. Both pairing and chromosome segregation require proper proteasome function during budding yeast meiosis and mouse spermatogenesis (Ahuja et al., 2017; Rao et al., 2017). In *Saccharomyces cerevisiae*, the proteasome regulates axis morphogenesis and synapsis, disassembling non-homologous SCs from clustered centromeres at early prophase I (Ahuja et al., 2017). Additionally, the proteasome is needed for removing SCs at the end of prophase I. In mice, the ubiquitin–proteasome system controls meiotic recombination indirectly by influencing the turnover of several recombination factors (Rao et al., 2017).

Humans have two important E3 ligases that control meiotic recombination: RING FINGER PROTEIN 212 (RNF212) and HUMAN ENHANCER OF INVASION CLONE 10 (HEI10) (Kong et al., 2008). RNF212 is the homologue of yeast Zip3 and worm ZHP-3, a SMALL UBIQUITIN-LIKE MODIFIER (SUMO) RING-finger ligase (Kong et al., 2008; Zhang et al., 2017). HEI10 is a ubiquitin E3 ligase important for CO designation and cell cycle (Toby et al., 2003). RNF212 and HEI10 show haploinsufficiency and play antagonistic roles in CO formation. RNF212 stabilizes recombination factors in the meiotic axes and HEI10 limits the colocalization of RNF212 and pro-CO factors, thereby allowing for resolution of recombination intermediates (Reynolds et al., 2013).

What happens in *Arabidopsis*? Most proteins involved in the ubiquitination pathway correspond to RING E3 ligases that specifically recognize target proteins (Mazzucotelli et al., 2006). Although no homolog of RNF212 has been found, *Arabidopsis* HEI10 (At1g53490) (see Table 2) has an essential role during meiosis, because it is required for most COs between homologs (~85%) (Chelysheva et al., 2012). The HEI10 E3 ligase is a ZMM protein (from the proteins Zip1,2,3,4; Msh4,5; Mer3, implicated in interference-sensitive COs) that appears as numerous foci on the chromosome axes during early prophase I and then is maintained at only ~10 sites, co-localizing with the CO marker MUTL HOMOLOG 1, COLON CANCER, NONPOLYPOSIS TYPE 2 (MLH1) at pachytene (Chelysheva et al., 2012). The corresponding mutant displayed fertility defects that are due to the presence of univalents at metaphase I (Chelysheva et al., 2012). HEI10 is believed to interact directly with MHS5, to stabilize MSH4 and MSH5, and to regulate the recruitment of MLH1 and MLH3 (see Fig. 2) (Ziolkowski et al., 2017). The *HEI10* homolog (Os02g13810) was also identified in rice, a monocot (Wang et al., 2012), the *oshei10* mutant shows a similar meiotic defect to the *Arabidopsis hei10* mutant, indicating that the HEI10 function in meiotic crossover formation is highly conserved (Wang et al., 2012). Recently, natural polymorphisms in the coding sequence of *HEI10* were found associated with variations in chiasma frequencies between different *Arabidopsis* accessions (Ziolkowski et al., 2017). Furthermore, *HEI10* was characterized as haploinsufficient in heterozygotes, and having extra *HEI10* copies led to double the number of MLH1 recombination foci, more compact bivalents, and increased subtelomeric recombination (Ziolkowski et al., 2017). This suggests its use as a tool for increasing recombination in key crop genomes, especially by combining it with mutation of the anticrossover helicase *recq4a recq4b* (*RECQ4A*: At1g10930, *RECQ4B*: At1g60930) (see Table 2) (Serra et al., 2018b). In the latter mutant background, unrepaired joint DNA molecules (D loops and double Holiday Junctions) are likely to persist and be repaired as non-interfering class II crossovers (Serra et al., 2018b). Moreover, *Arabidopsis* anticrossover pathways are not completely redundant (Serra et al., 2018b; Ziolkowski et al., 2017); hence, additional mutations in *FANCM* (At1g35530), *FIGL1* (At3g27120), *FANCD2* (At1g48360) and *MSH2* (At3g18524) (see Table 2), may further enhance crossover formation (Serra et al., 2018b; Kurzbauer et al., 2018; Serra et al., 2018a; Girard et al., 2015; Crismani et al., 2012; Fernandes et al., 2018). The efficiency of such mutations in causing an increase in recombination appears to be universal, as shown by the characterization of *FANCM*, *RECQ4* and *FIGL1* mutants in several crop species such as rice (*Oryza sativa*), pea (*Pisum sativum*) and tomato (*Solanum lycopersicum*) (Mieulet et al., 2018).

Measuring recombination through the use of Col/Ler chromosome substitution lines (CSLs) expressing different colors of fluorescent proteins in pollen (Berchowitz and Copenhaver, 2008; Yelina et al., 2013), led to the determination that *HEI10* is part of an *Arabidopsis* quantitative trait loci (QTL) named *rQTL1* that positively affects the formation of MLH1 recombination foci along the meiotic synaptonemal axis (Ziolkowski et al., 2017). Proteins from the HEI10 family possess N-terminal RING domains, central coiled-coil domains and C-terminal regions of unknown function, but are suspected to play a role in substrate/target recognition (Ziolkowski et al., 2017). An R264G polymorphism in the C-terminus of HEI10 is believed to promote recombination, perhaps by promoting protein function or expression timing (Ziolkowski et al., 2017). Crossover modifier loci such as *HEI10* may affect genetic adaptation to diverse environments and conditions (Ziolkowski et al., 2017).

Polymorphisms have a suppressive effect in recombination in *fancm, figl,* and *recq4a recq4b* backgrounds and even in plants transformed with additional copies of *HEI10*. In such plants, recombination occurs preferably within gene transcribed regions and is reduced in highly polymorphic intergenic regions (Serra et al., 2018a). Likely mechanisms are: 1) MSH2-mediated mismatch recognition, which may lead to dissolution of strand-invasion events at the megabase level (see Table 2) (Serra et al., 2018b; Emmanuel et al., 2006), and 2) the presence of the chromatin marker H3K4me3, which in budding yeast is known to interact with meiotic Mer2 (Serra et al., 2018a).

INTERACTION BETWEEN RING LIGASES AND AXR1

AUXIN RESISTANT1 (AXR1: At1g05180) (see Table 2) is a RELATED TO UBIQUITIN1 (RUB1: At1g31340)-activating enzyme. Rubylation is a modification of Cullin-RING E3 ligases that may lead to protein activation (Jahns et al., 2014). The *axr1* mutant displays defects in auxin responses due to impaired degradation of an AUX/IAA repressor (Del Pozo et al., 1998). Notably during meiosis, the absence of AXR1 produces defects in SC formation and reduced bivalent formation. This reduction may be due to a variation in chiasma localization without affecting class I CO frequency (Jahns et al., 2014). AXR1 may operate as an E1 enzyme that modulates the activity of Cullin RING Ligase 4 (CRL4: At1g60986), a protein associated with DNA repair in plants and humans (Jahns et al., 2014).

CONCLUSIONS

Meiosis involves numerous and fine-tuned chromosomal processes that must be regulated in a coordinated fashion. Ubiquitinated targets may include proteins involved in the maintenance of chromosome structure, meiotic recombination, axis assembly and SC formation. Future characterization of key proteins such as HEI10 should provide a better picture of the interplay between ubiquitination, ubiquitin modifiers, meiotic recombination, adaptation and evolution in higher plants. These studies may lead to the development of methods to increase homologous recombination, or to produce gametes with specific genetic make-up (i.e. diploid and clonal) that might accelerate breeding in important crops such as rice. However, this approach remains mostly unexplored in genomes that are much larger than in *Arabidopsis* (Lambing et al., 2017).

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