The CYCLIN-A CYCA1;2/TAM Is Required for the Meiosis I to Meiosis II Transition and Cooperates with OSD1 for the Prophase to First Meiotic Division Transition

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Abstract

Meiosis halves the chromosome number because its two divisions follow a single round of DNA replication. This process involves two cell transitions, the transition from prophase to the first meiotic division (meiosis I) and the unique meiosis I to meiosis II transition. We show here that the A-type cyclin CYCA1;2/TAM plays a major role in both transitions in Arabidopsis. A series of tam mutants failed to enter meiosis II and thus produced diploid spores and functional diploid gametes. These diploid gametes had a recombined genotype produced through the single meiosis I division. In addition, by combining the tam-2 mutation with AtSpo11-1 and Atrec8, we obtained plants producing diploid gametes through a mitotic-like division that were genetically identical to their parents. Thus tam alleles displayed phenotypes very similar to that of the previously described osd1 mutant. Combining tam and osd1 mutations leads to a failure in the prophase to meiosis I transition during male meiosis and to the production of tetraploid spores and gametes. This suggests that TAM and OSD1 are involved in the control of both meiotic transitions.

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Introduction

Meiosis is a central feature in the reproductive program of all sexually reproducing eukaryotes. The process of meiosis involves two rounds of chromosome segregation that follow a single round of chromosome duplication leading to the production of haploid gametes. Meiosis differs from mitosis, somatic cell division, in a number of ways. In meiosis:

(i) Homologous chromosomes pair and closely associate along a proteinaceous structure called the synaptonemal complex (SC). This process culminates at a substage of prophase called pachynema.

(ii) Crossovers occur between homologs during prophase.

(iii) Homologous chromosomes separate at anaphase of the first division. To ensure accurate chromosome segregation at anaphase I, each homolog must remain connected to the other through metaphase I. Since the SC disappears before the end of prophase, it cannot ensure linkage of homologs at metaphase I. This connection is maintained until anaphase I by chiasmata, the cytological manifestation of crossovers.

(iv) Meiosis has a second division with no intervening DNA synthesis. Sister chromatids, resulting from the meiotic S phase, remain associated until metaphase II and are separated from each other at anaphase II, leading to the production of four haploid spores [1,2].

To generate haploid spores the meiocyte must enter meiosis I, pass through the meiosis I to meiosis II transition and exit meiosis II. Errors in these transitions, are not uncommon and may lead to parthenogenesis or teratoma formation, or to the production of gametes with the somatic number of chromosomes (2n gametes) [3,4]. The formation of 2n gametes is thought to be an important mechanism for generating polyploids. Polyploidy has played a key role in the evolution of many fungal, plant, invertebrate and vertebrate lineages, and is particularly frequent in plants [5,6]. 2n gametes are also an important tool for plant breeding [7].

Cyclins and cyclin-dependent kinases (Cdk) form complexes that are essential for progression through both the mitotic and meiotic cell cycles. The transition from meiosis I to meiosis II requires a fine balance in Cyclin–Cdk activity: it must be sufficiently low to exit meiosis I but must nonetheless be maintained at a level sufficiently high to suppress DNA replication.
and promote entry into meiosis II [8,9]. Precisely how the mitotic machinery is modified for the purpose of meiosis is not fully understood. Most of the knowledge currently available originates from studies carried out in unicellular fungi, *Xenopus laevis* or mouse oocyte systems.

In oocytes, entry into both meiosis I and meiosis II is driven by Cdc2/Cyclin B complexes (the molecular components of the maturation promoting factor, MPF) [10]. The rate of Cyclin B synthesis and degradation by the APC (anaphase promoting complex) determines the timing of the transitions occurring during meiosis [10,11]. At the end of meiosis I, Cyclin B is only partially degraded [12] and the residual, low level of Cdc2/CyclinB activity is essential for entry into meiosis II [13]. This partial Cyclin B degradation is fine-tuned by the Erp1/Emi2 APC inhibitor [14–16]. In *Schizosaccharomyces pombe*, Mes1 is a key player in the meiosis I to meiosis II transition. Like Erp1/Emi2, the Mes1 protein partially inhibits cyclin degradation by the anaphase promoting complex (APC), thereby allowing entry into meiosis II [17,18]. In *Sacharomyces cerevisiae*, the simultaneous deletion of two of the six B-type cyclins results in a single reductional division involving a non redundant Cyclin-cdk activity. Furthermore, combining *tam* and *osd1* mutations leads to a failure of the transition from prophase to the first meiotic division (meiosis I) during male meiosis and to the production of single-spore meiotic products and tetraploid pollen grains. This shows that *CYCA1;2* and *OSD1* are also involved in the prophase to meiosis I transition. The implications of these results for the control of meiotic transitions are discussed.

In addition, taking advantage of a genetic strategy we used previously to investigate *OSD1* function [23], we combined *tam* mutants with mutations that affect homologous recombination (*Atspo11-1*) and chromosome segregation (*Atrec8*), essentially converting meiosis into a mitotic-like division. The *tam/Atspo11-1/Atrec8* line, called *MiMe*-2, produces diploid gametes that are genetically identical to their parents, mimicking apomixis, a key element of apomixis or asexual reproduction through seeds. However, *MiMe*-2 also resulted in the production of diploid and aneuploid gametes, probably due to the lower penetrance of *tam* mutations compared to *osd1* mutations.

**Results**

**The *tam* Mutants Produce Diploid Gametes**

The *Arabidopsis* TAM (Tardy Asynchronous Meiosis) gene has been implicated in the control of meiotic progression [26,27]. The *tam-1* mutant displays slower cell cycle progression during male meiosis, although it does eventually, like wild type, produce tetrad. However, previous to this study, only one mutant allele with a point mutation leading to a single amino-acid substitution in the protein had been studied. As this point mutation is temperature-sensitive and probably corresponds to a hypomorphic allele, we revisited the role of the TAM gene by isolating and characterizing three independent insertion mutants from public mutant collections and three additional point mutations (Figure 1A and Figure S1). The *tam-2* (sail_505_C06 [28,29]) T-DNA insertion is in the fourth exon (ATG+1130 pb) and is accompanied by a large deletion (corresponding to half of the TAM coding sequence, the entire TAM promoter region and the first 52 bp of the next gene, At1g77400), the *tam-3* (SALK_080686 [30]) T-DNA insertion is in the seventh intron (ATG+1690 bp) and the *tam-4* (CSHL_ET12273 [31]) Ds insertion is in the first exon (ATG+62 bp). The *tam-2* and *tam-3* mutations are in the *Columbia* (Col-0) background, whereas the *tam-4* mutation is in the *Landberg erecta* (Ler) background (Figure 1A). Additionally, three point mutations were isolated in an ethyl methanesulfonate (EMS) chemical mutagenesis screen. The *tam-5* mutation is a G to A transition at nucleotide 378 in the coding sequence that creates a stop codon (TGG → TAG; Trp77 → stop). The *tam-6* mutation is also a G to A transition at the -1 position of the 3′ splice site between intron 2 and exon 3. The *tam-3* and *tam-6* alleles are both recessive and do not complement one another. The *tam-7* mutation is a G to A transition at nucleotide 378 in the coding sequence that creates a stop codon (TGG → TAG; Trp77 → stop). The *tam-6* mutation is also a G to A transition at the -1 position of the 3′ splice site between intron 2 and exon 3. The *tam-3* and *tam-6* alleles are both recessive and do not complement one another. The *tam-7* mutation is a G to A transition at position -1 of the 3′ splice site between intron 2 and exon 2. Similar splice site proximal mutations have been observed in other *Arabidopsis* mutants including *del1-1* and *del1-3* [32]. The *tam-7* allele is dominant and thus could not be used for genetic complementation of the other *tam* alleles. All three EMS *tam* alleles are in the *Columbia* (Col-0) background. The T-DNA alleles were used preferentially for detailed analysis.
The *tam* mutants displayed no defects during somatic development. In *Arabidopsis*, male meiosis produces a group of four spores, organized in a tetrahedron, called a tetrad (Figure 1B). Each spore gives rise to a pollen grain. In these six independent *tam* mutants, the products of male meiosis were mostly dyads of spores instead of tetrads (Figure 1B and Table 1). In addition to balanced dyads, the stronger mutants also produced triads and unbalanced products together with a very small number of tetrads. Complementation tests with *tam-2*, *tam-3* and *tam-4* mutants confirmed that these mutations were allelic. Unlike the previously described temperature sensitive *tam-1* mutant, these six mutants expressed the dyad phenotype at normal growing temperatures (20°C). Furthermore, the dyad stage in *tam-1* does not appear to be terminal, as meiosis always progresses to tetrad production [27], whereas the *tam-2*, *tam-3*, *tam-4*, *tam-5*, *tam-6* and *tam-7* systematically produced mostly dyads. This suggests that the *tam-1* mutant presents only a delay in the progression of meiosis, whereas the other mutants do not progress beyond the dyad stage (as confirmed by pollen analysis, see below). This phenotype is reminiscent of the phenotype of the *osd1* mutant, which produces spores directly after meiosis I. Its gametes are thus diploid and its offspring polyploid. Thus, we determined ploidy levels among the offspring of diploid *tam-2* and *tam-4* mutants. In the progeny of selfed homozygous mutants, we observed tetraploids, triploids, and occasionally diploid plants (Table 2). If pollen from *tam-2* or *tam-4* mutant plants was used to fertilize a wild-type plant, almost all the resulting progeny were triploid, with only a few diploid plants identified (Table 2). If *tam-2* or *tam-4* mutant ovules were fertilized with wild-type pollen grains we isolated diploid and triploid plants (Table 2). Thus, the frequency of diploid spores, resulting in functional gametes, was high in the *tam-2* and *tam-4* mutants, for both the male (~90%) and female (~30%) lineages. *Tam* mutants produced only slightly fewer seeds than the corresponding wild-type lines (36±4 seeds/silique in *tam-2; 42±4 in wild type Col-0; 43±4 in *tam-4; 52±3 in wild type Ler), but many (>50%) of the seeds produced by *tam-2* mutants and a few (<10%) of those produced by *tam-4* mutants were shriveled. This finding was not unexpected, because *tam* mutants produce triploid seeds with an excess of the paternal genome that is associated with the production of shriveled seed, particularly in the Col-0 background [33]. The germination rate of seeds produced by *tam-2* was 63% (n = 264), suggesting that the proportion of triploid seeds may be underestimated in *tam-2* progeny.

**The *tam* Mutants Skip the Second Division of Meiosis**

We characterized the mechanisms underlying dyad production in *tam* mutants, by investigating chromosome behavior during male meiosis using a meiocyte spreading technique (Figure 2 and Figure 3). In wild type, the ten chromosomes appeared as threads at leptotene, underwent synopsis at zygotene and were fully synapsed, along the SC at pachytene (Figure 2A). After the disappearance of the SC at diplotene the resulting five bivalents condensed, revealing the presence of chiasmata (Figure 2B). The bivalents organized on the metaphase I plate (Figure 2C) and homologs segregated to opposite poles at anaphase I (Figure 2D and 2E). The two sets of five homologs aligned on the two metaphase II plates (Figure 2F). The second round of segregation at anaphase II (Figure 2G) led to the formation of four sets of five chromosomes, that decondensed to form the spore nuclei (Figure 2H). Meiosis I in *tam* mutants was indistinguishable from that in the wild type. All stages of prophase were observed.

**Table 1. Male meiotic products in *tam* mutants.**

| Mutant | Dyads (%) | Tetrads | Triads (%) | Unbalanced (%) | Aneuploid (%) | Aneuploid frequency |
|--------|-----------|---------|------------|----------------|--------------|-------------------|
| *tam-2* | 89%       | 0.3%    | 11%        | 26%            | 39%          | 62%               |
| *tam-3* | 87%       | 0.4%    | 13%        | 28%            | 25%          | 11%               |
| *tam-4* | 84%       | 5%      | 11%        | 3%             | 3%           | 15%               |
| *tam-5* | 62%       | 1.1%    | 26%        | -              | -            | -                 |
| *tam-6* | 70.1%     | 1.9%    | 28%        | 3%             | -            | -                 |
| *tam-7* | 82%       | 15%     | -          | -              | -            | -                 |

**Table 2. Ploidy of *tam*, *tam/osd1*, and MiMe-2 progenies.**

| Lineage | selfed progenies | *tam-2* | *tam-4* | *tam-2/ osd1* | *MiMe-2* |
|---------|------------------|---------|---------|---------------|----------|
|         | 2N               | 7%      | 3%      | 8%            | 7%       |
|         | 3N               | 67%     | 58%     | 7%            | 7%       |
|         | 4N               | 60%     | 40%     | 8%            | 8%       |
|         | 6N frequency     | 32%     | 32%     | 32%           | 32%      |
|         | aneuploid frequency | 15%    | 15%     | 15%           | 15%      |
|         | number of plants | 27      | 27      | 27            | 27       |
|         | wt♀ x mutant ♂   | 12%     | 12%     | 12%           | 12%      |
|         | 3N frequency     | 88%     | 88%     | 88%           | 88%      |
|         | aneuploid frequency | 7%     | 7%      | 7%            | 7%       |
|         | number of plants | 45      | 45      | 45            | 45       |
|         | mutant♀ x wt♂    | 1%      | 1%      | 1%            | 1%       |
|         | 3N frequency     | 99%     | 99%     | 99%           | 99%      |
|         | aneuploid frequency | 9%     | 9%      | 9%            | 9%       |
|         | number of plants | 45      | 45      | 45            | 45       |
including full synapsis at pachytene (Figure 3A) and chiasmata at diakinesis (Figure 3B). The bivalents observed at diakinesis, condensed and aligned on the metaphase plate (Figure 3C). We quantified the chiasma frequency by studying the shape of metaphase I bivalents, as described by [34,35], and found no difference between tam-2 (9±0.8 chiasmata per cell, n = 70) and the wild type (9.1±1 chiasmata per cell, n = 53). The bivalents segregated at anaphase I and decondensed at telophase I (Figure 3D–3F). However, we found no meiosis II figures (among >1000 male meiocytes for each tam-2, tam-3 and tam-4, from prophase to spore formation), consistent with the observed production of ~70% haploid female gametes in tam mutants and the notion that female diploid megaspores are also produced by skipping the second meiotic division.

Figure 3. Male meiosis in the tam-2 mutant. Micrographs of DAPI stained Arabidopsis chromosomes show that tam-2 Meiosis I (Figure 3A–3C) including Pachytene; (B) Diakinesis; (C) Metaphase I; (D, E) Anaphase I; (F) Telophase I; (G) Metaphase II; (H) Anaphase II; and (I) Telophase II. Scale bar = 10 μm.
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If diploid gametes are indeed generated by skipping the second meiotic division, any parental heterozygosity at centromeres should be lost in the tam diploid gametes, because sister centromeres cosegregate at division I. Conversely, we would also expect heterozygosity to increase towards telomeres, due to recombination between the locus concerned and the centromere. We tested this hypothesis in two ways, using the fluorescent tagged line (FTL) Arabidopsis tetrads system providing direct information about the genetic content of pollen grains (Figure 6, Figure 7, Figure 8) and by genotyping male and female tam-2 triploid offspring for polymorphic molecular markers (Figure 8) [23,36,37].

The FTL system is a visual assay based on the use of reporter constructs encoding fluorescent proteins produced in the pollen of the quartet mutant (qrt1-2) [38]. In this mutant, the pollen grains from each meiosis remain physically attached. We carried out crosses to generate plants with both the qrt1-2 and tam-2 mutations, heterozygous for one or two reporter transgenes.
conferring pollen fluorescence. As controls, we used both wild type and osd1 mutant plants. We first used an FTL transgene located close to the centromere on chromosome 5 (FTL3253 encoding AmCyan) [39]. In wild-type (qrt1-2 background) control plants, all tetrads consisted of two fluorescent and two non-fluorescent pollen grains (Figure 6A), reflecting the segregation of the four chromatids, two of which carried the transgene (n = 120). In the osd1-1 and tam-2 mutants, we observed mostly dyads of pollen (Figure 6B–6E). Furthermore, in both mutants, the vast majority of dyads (91%, n = 526 and n = 738, in osd1-1 and tam-2 respectively) consisted of one fluorescent pollen grain and one non-fluorescent pollen grain (Figure 6B and 6C). Thus, in these dyads, one of the pollen grains contained the two sister chromatids carrying the transgene, whereas the other pollen grain inherited the other two sister chromatids. This segregation pattern is fully consistent with the absence of a second meiotic division. In a small proportion of dyads (9% in both osd1-1 and tam-2 mutants) both pollen grains were fluorescent, indicating recombination between the transgene and the centromere (Figure 6D and 6E).

For confirmation of this genetic analysis, we genotyped triploid offspring generated from male and female gametes from the tam-2 mutant. We first introduced genetic polymorphisms into tam-2 (Col-0 background) by crossing this mutant to the No-0 accession. In the F2 generation, we used PCR to select plants homozygous for the tam-2 mutation but heterozygous for a series of microsatellite markers. These plants were crossed, as male or female parents, with plants from a third genetic background (Landsberg erecta, Ler). Genotyping of the resultant triploid progeny for the trimorphic molecular markers revealed the genetic make-up of the 2n gametes produced by the mutant. The Ler allele was present in all the plants (brought by the wild type Ler haploid gamete), while the presence/absence of the Col-0/No-0 allele in the triploids corresponds to the genotype of the 2N mutant gametes. All the diploid gametes tested had the predicted genetic characteristics, similar to those of the diploid gametes produced by the osd1 mutant (Figure 8). The markers were homozygous at the centromere, but displayed segregation at other loci, due to recombination. These results confirmed that the absence of a second meiotic division was indeed responsible for the production of both male and female 2n gametes in tam-2.
Table 2). Backcrossing MiMe-2 of sister chromatids at anaphase (Figure 9). The selfed progeny of 10 univalents aligned on the metaphase plate, with the separation of either the wild-type or the tam-2 mutant (wild type: 42±4 seeds/fruit; tam-2: 37±4, MiMe2: 20±3). This finding was not unexpected, as the tam mutation does not display full penetrance. In meiocytes lacking Atrec8 and Atspo11 that undergo a second division, this division is unbalanced, probably leading to the production of aneuploid gametes in MiMe-2 plants [42]. We analyzed the genetic content of the MiMe-2 diploid gametes, using both the FTL lines and molecular markers (Figure 7K and 7L and Figure 8). We introduced the same FTL transgenes as described above (FTL1273 DoRed2, FTL993 ECFP) into MiMe and MiMe-2 plants. Almost all the pollen grains of both genotypes displayed both types of fluorescence (Figure 7K and 7L; 98% and 95%, respectively), indicating that they had inherited both transgenes, and confirming the occurrence of a mitosis-like division, rather than meiosis, in both lines. The few cases in which the two pollen grains were not expressing both fluorescent proteins may be explained by chromosome missegregation or occasional extinction of the transgenes. In addition, all the diploid MiMe-2 gametes (male and female) systematically retained heterozygosity for each genetic marker tested (Figure 8). They were thus genetically identical to the mother plant. These results confirm that MiMe-2 plants undergo a mitosis-like division instead of a normal meiotic division, generating gametes genetically identical to the parent plant, but at a lower regularity than MiMe plants [23].

tam/osd1 Double Mutant Skip Division II at Female Meiosis and Skip Both Divisions at Male Meiosis

Our tam alleles displayed phenotypes very similar to that of the osd1 mutant, with no second meiotic division, leading to the production of viable diploid male and female gametes. We then combined tam-2 and osd1-1 mutations. The double mutant was almost sterile, producing very few seeds by selfing. Reciprocal crosses with wild type revealed that tam-2/osd1-1 double mutant was female fertile but male sterile. If tam-2/osd1-1 mutant ovules were fertilized with wild-type pollen grains we isolated almost exclusively triploid plants (Table 2). Observation of female meiosis in the double mutant revealed normal meiosis I but an absence of meiosis II (Figure 10). The genetic analysis of the female gametes, performed as above, showed that all the diploid ovules tested had the predicted genetic characteristics for an absence of second meiotic division (Figure 8). These results show that tam-2/osd1-1 double mutants display the same female meiosis phenotype as single mutants, with female meiocytes failing to enter meiosis II, leading to the production of 2n ovules.

We investigated the origin of the male sterility phenotype observed in tam-2/osd1-1 double mutant plants, by assessing pollen viability [43]. Figure 11A shows a wild-type anther treated with Alexander stain which produces a red pigment in viable pollen. Anthers of tam-2 and osd1-1 single mutants contained viable pollen grains, although less numerous and slightly bigger than wild type (Figure 11B and 11C). In contrast, tam-2/osd1-1 anther contained very few pollen grains (9±2) with variable size (Figure 11D–11F). In wild type, meiosis produces four spores (Figure 12A), whereas both tam and osd1 mutant produces dyads of spores (Figure 12B and 12C). In contrast, observation of male meiotic products in tam-2/osd1-1 revealed only “monads”, with a single-spore product (Figure 12D, n = 498). We then investigated the behavior of male meiotic chromosomes in tam-2/osd1-1 mutants (Figure 13). Prophase was indistinguishable from
wild type: the ten chromosomes appeared as threads at leptotene, underwent synapsis at zygotene and were fully synapsed at pachytene. After the disappearance of the SC at diplotene, the resulting five bivalents condensed, revealing the presence of chiasmata. However, we observed spores with a single nucleus (Figure 13E and 13F), consistent with the observation of monads. Furthermore, only two figures typical of metaphase/anaphase I and no figures of telophase I or meiosis II were found among 1600 meiocytes. This suggests that most male meiocytes skip both meiosis I and II and produce spores directly after replication and prophase, without chromosome segregation. An expected consequence of such a defect is the production of 4n pollen grains. We did not succeed in crossing tam-2/osd1-1 mutants as male but we determined ploidy levels among the seeds produced by selfing and found a large proportion of 6n plants in the progeny of 2n plants (Table 2). As crosses with wild type showed that tam-2/osd1-1 produce 2n ovules, the occurrence of 6n plants strongly suggests that 4n pollen grains are produced, in accordance with the skipping of both rounds of segregation at male meiosis in tam-2/osd1-1. A large proportion of 4n plants is also found in the selfed progeny showing that tam-2/osd1-1 also produces 2n pollen grains. These 2n pollen grains likely originate from the few meiocytes that enter meiosis I and produces 2n spores, that later outcompete 4n pollen.

**Discussion**

A Cyclin A Mediates the Meiosis I to Meiosis II Transition

We show here that one of the ten known type A cyclins in Arabidopsis, CYCA1;2/TAM, is required for the transition between meiosis I and meiosis II. No phenotype has been reported for mutants lacking any of the other CVCA, probably due to the high level of redundancy [24]. By contrast, none of the other cyclins were able to compensate for CYCA1;2 in the meiosis I to meiosis II transition. CYCA1;2 may have a specialist function or pattern of expression, required for this transition, that cannot be supplied by any of the other cyclins from Arabidopsis. Alternatively, the lack of CYCA1;2 may decrease generic cyclin/CDK complex activity, causing the meiosis I-meiosis II transition to fail.

CYCA1;2 and OSD1 Are Involved in the Prophase to Meiosis I Transition at Male Meiosis

Both cyca1;2/tam and the previously described osd1 mutants fail to enter meiosis II and produce spores after meiosis I. Remarkably,
male meiocytes lacking both OSD1 and CYCA1;2/TAM genes fail to enter meiotic division I, producing spores directly after prophase. This shows that in addition to their crucial function in driving meiosis I to meiosis II transition, these two genes are involved in the prophase to meiosis I transition. This suggests that they both contribute to an activity promoting entry into meiosis I and entry into meiosis II, most likely a CDK activity. The molecular function of OSD1 is currently unknown, however, it has been proposed by analogy to Erp1/Emi2 and Mes1 that it may inhibit APC activity, thus promoting CDK activity [23]. TAM/CYCA1;2 being a cyclin, may directly promote CDK activity. We believe that the meiosis I to meiosis II transition is easily disturbed, because fine regulation of the levels of cyclin/CDK activity is required to ensure both exit from meiosis I and entry into meiosis II [8]. Thus a moderate decrease of CDK activity in osd1 and cyca1;2/tam single mutants may cause failure to enter meiosis II without impairing the prophase to meiosis I transition. In contrast the coincident depletion of OSD1 and CYCA1;2/TAM, may further decrease CDK activity, impairing entry into meiosis I. Unfortunately the direct measurement of CDK activity during meiosis in Arabidopsis is currently not possible. The combination of osd1 and/or cyca1;2/tam mutants with other mutants affecting CDK activity may help to test this model.

In S. cerevisiae, the simultaneous deletion of two (out of six possible) B-type cyclins (Clb1 and Clb3 or Clb1 and Clb4) leads to a failure to enter meiosis II [20,21]. However, although Clb3 activity is specific to meiosis II, this is not the case for Clb1 and Clb4 [19], and the Clb1 Clb3 Clb4 triple mutant barely sporulates, suggesting that all three proteins have functions in meiosis progression other than the meiosis I-meiosis II transition, similar to OSD1 and CYCA1;2/TAM.

Different Control of Male and Female Meiosis

In the osd1/tam double mutant, male meiocytes fail to enter meiosis I after prophase, whereas female meiocytes proceed to meiosis I and fail to enter meiosis II revealing a striking difference in the control of male and female meiosis progression. We suggest that other cyclins may partly compensate for the absence of CYCA1;2, during female meiosis, and that the meiocytes I to meiosis II transition may be driven by different mixtures of cyclins in male and female meiosis. This possibility is supported by the cyca1;2/tam single mutants being weakly affected in the female lineage, compared to the male lineage and to osd1 male and female lineage. An analysis of the effects on meiosis of the depletion of other cyclins, either alone or together with TAM/CYCA1;2, is required to test this hypothesis.

Cyclin A proteins specific to male meiosis have already been described in mammals. The mouse and human genomes each contain two different A-type cyclins. One of these cyclins, Cyclin A1, is restricted to the germ line whereas Cyclin A2 is ubiquitously expressed [44–47]. The loss of Cyclin A1 function has no effect on viability and results in male meiosis progressing to the late prophase and then leading to apoptosis; it has no effect on female fertility [48]. Control of male and female meiosis by a different set of cyclins may thus be a general phenomenon.

Plant Cyclins and Meiosis

The Arabidopsis genome encodes 50 cyclins or putative cyclins [25]. Two of these cyclins, SDS and CYCA1;2/TAM, have been directly implicated in meiosis. CYCA1;2/TAM is a type A cyclin involved in cell cycle progression [27] [25 and this study], whereas SDS forms an outgroup, and plays a specific role in recombination with no evidence for any role in cell cycle progression [49,50]. SDS is required to bias crossovers between homologous chromosome at meiosis rather than between sister chromatids, as in mitosis [50]. A viable hypomorphic mutant of CDKA displays meiotic defects potentially corresponding to a combination of the sdi and tam defects [51] (e.g. recombination and progression defects), consistent with both SDS and TAM/CYCA1;2 being involved in CDKA activation. In wheat, Cdc2/CDKA genes are essential
components of the \( \Phi 1 \) locus, which is responsible for preventing recombination between homologous chromosomes [52]. This suggests that cyclin/CDK activity may finely regulate various meiotic events.

Relevance for Apomixis

Apomixis, or asexual clonal reproduction through seeds, has great potential for agricultural applications [40,41]. It can be separated into three developmental components: an absence or alteration of meiosis (apomeiosis), the fertilization-independent development of the embryo from the egg cell (parthenogenesis), and the initiation of endosperm development with or without fertilization [40,41,53]. We recently showed that fully penetrant apomeiosis can be induced in \( \text{Arabidopsis} \) when meiosis is replaced by a mitosis-like division in the MiMe genotype, which combines mutations in three genes, \( \text{AtSPO11-1} \), to eliminate recombination, \( \text{AtREC8} \), to ensure the separation of sister chromatids rather than homologues and \( \text{OSD1} \) to abolish the second division [23]. We have now identified a second gene, \( \text{CYCA1;2/TAM} \), the mutation of which abolishes the second division of meiosis. The \( \text{MiMe-2} \) genotype, which combines the \( \text{Atspo11-1} \), \( \text{AtRec8} \) and a newly described \( \text{tam} \) mutation, gives rise to the same phenotype as the \( \text{MiMe} \) genotype, with the conversion of meiosis into a mitosis-like division. Thus, apomeiosis can be engineered by combining various mutations. However, as \( \text{tam} \) mutations have a lower penetrance than \( \text{osd1} \) mutations, \( \text{MiMe-2} \) plants produce apomeiotic gametes less frequently than \( \text{MiMe} \) plants. Thus, \( \text{osd1} \) mutations may be more suitable than \( \text{tam} \) mutations for agricultural applications, if the results obtained in \( \text{Arabidopsis} \) can be extrapolated to crop plants. In addition to apomeiosis in \( \text{MiMe} \) or \( \text{MiMe-2} \) plants, apomixis will require the introduction of parthenogenesis and autonomous endosperm formation.

Materials and Methods

Growth Conditions and Genotyping

\( \text{Arabidopsis} \) plants were cultivated as previously described [54]. For cytometry experiments, \( \text{Arabidopsis} \) plants were cultivated on...
were described in a previous study [23]. The qrt1-2 (CAPS) used to genotyperespectively. The cleaved amplified polymorphic sequences products. For DNA right border junction was analyzed by sequencing PCR [39]. The primers used to genotype

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The seeds for FTL analysis were obtained from G.P. Copenhaver [36,57]. We obtained tam-2/qrt1-2 plants by selfing double heterozygous tam-2/qrt1-2, FTL3253/+ plants. We obtained osd1-1/qrt1-2 and FTL3253 +/+ plants by selling a double heterozygote osd1-1/qrt1-2 FTL3253 +/+ plants. We obtained tam-2/spo11-1-3/rec8-3/qrt1-2 and FTL993 +/+ FTL1273 +/+ plants by crossing a qrt1-2 mutant, triple heterozygous tam-2/spo11-1-3/ rec8-3 and FTL993 +/+ FTL1273 +/+ plants. We obtained osd1-1/spo11-1-3/rec8-3/qrt1-2 and FTL993 +/+ FTL1273 +/+ plants by crossing a qrt1-2 mutant, triple heterozygote osd1-1/spo11-1-3/rec8-3 FTL993 +/+ FTL1273 +/+ plant with a qrt1-2 mutant, triple heterozygous osd1-1/spo11-1-3/rec8-3 FTL993 +/+ FTL1273 +/+ plant.

Plants of interest were selected by PCR genotyping. For each line, the first pair of primers is specific to the wild-type allele and the second pair is specific to the T-DNA insertion:

FTL993 (AmCyan, nucleotide position 9304032 on chromosome 3); FTL1273 (AmCyan, nucleotide position 9304032 on chromosome 3); FTL3253 (AmCyan, nucleotide position 9304032 on chromosome 3); FTL993 (AmCyan, nucleotide position 9304032 on chromosome 3); FTL1273 (AmCyan, nucleotide position 9304032 on chromosome 3); FTL3253 (AmCyan, nucleotide position 9304032 on chromosome 3); FTL993 (AmCyan, nucleotide position 9304032 on chromosome 3); FTL1273 (AmCyan, nucleotide position 9304032 on chromosome 3); FTL3253 (AmCyan, nucleotide position 9304032 on chromosome 3); FTL993 (AmCyan, nucleotide position 9304032 on chromosome 3); FTL1273 (AmCyan, nucleotide position 9304032 on chromosome 3); FTL3253 (AmCyan, nucleotide position 9304032 on chromosome 3);

Fluorescent Tagged Lines

Figure 13. Male meiosis in tam-2/qrt1-2. Prophase was indistinguishable from wild type. (A) Leptotene. (B) Pachytene. (C) Diplotene. (D) Diakinesis. (E) Spore formation. (F) Spore. Scale bar = 10 μm.
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Figure 12. Male meiotic products stained with toluidine blue. (A) Wild type. (B) tam-2. (C) osd1-2. (D) tam-2/qrt1-2. Scale bar = 10 μm.
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Arabidopsis medium [55], at 21°C, under a 16-h to 18-h photoperiod and 70% relative humidity. The tam-2 (Sail_505-C06 Columbia accession), tam-3 (Salk_080686 Columbia accession), tam-4 (CSHL_E12273 Landsberg erecta accession), tam-5, tam-6, and tam-7 mutants were genotyped by PCR. For tam-2, tam-3 and tam-4 two primer pairs were used. The first pair is specific to the wild-type allele and the second pair is specific to the left border of the inserted sequence. tam-2: N874380U (5’ GACTTGATG-GATCCACAGG 3’) & N874380L (5’ CAGAATGCCTC-CAGTTGCAG 3’); N874380L & LB3Sail (5’ TAGCATGCTG- ATTTCCAACCCAGCTTAGAC 3’). tam-3: N580686U (5’ GAAGATATAGGCCTTGCAGG 3’) & N580686L (5’ TGCAACCACATCAGATGAGA 3’); N580686L & LB3Sail2 (5’ GCTTTTCCTCCTCTTCTGAG 3’). tam-4: ET12273R (5’ TAATGGGACCCCTGAGATC 3’) & ET12273L (5’ ACCTCATAGACAGGCAAATTG 3’); ET12273L & Ds3-4 (5’ GAAGATATAGGCCTTGCAGG 3’); N874380U (5’ GAAGATATAGGCCTTGCAGG 3’); N874380L & LB3Sail (5’ TAGCATGCTG- ATTTCCAACCCAGCTTAGAC 3’). tam-5: F2P24.10_4 (5’ GAACGACCACCTGAGACCG 3’) & F2P24.10_y (ATGTAATGACTAGCCGTTCTTTGTCCAA). The PCR products for tam-5, tam-6 and tam-7 were designed as derived amplified polymorphic sequences (dCAPS) [56] using the Sainsbury atPRIMER webtool (http://www.atprimer.tsl.ac.uk/cgi-bin/ form1.cgi). The dCAPS for tam-5, tam-6 and tam-7 are cleaved with Mfe I (125 bp versus 103 bp+22 bp), Xba I (316 bp versus 136 bp+180 bp) and Mfe I (396 bp versus 365 bp+31 bp) respectively. The cleaved amplified polymorphic sequences (CAPS) used to genotype qrt1-2 were described by Francis et al [39]. The primers used to genotype osd1-1, Atspo11-1-3 and Atrec8-3 were described in a previous study [23]. The tam-2 and tam-4 T-DNA right border junction was analyzed by sequencing PCR products. For tam-2, the specific primers used were 77400F (5’ TTGGGAAATCGTGCGGAGA 3’) and Rb3Sail (5’ TAAACATTTCACAGGAAACGCTTAGAC 3’). For tam-4, the specific primers used were ET12273R and Ds3-4 (5’ CCGTCCCCAGAAGTTAAATG 3’).
FTL1273U & LB-TDNA_FTL (5’ GGACATGCAAGCCTGA-TAATTC3’)

FTL993 (CFP, nucleotide position 2573131 on chromosome 5); FTL993U (5’ AGTGAGAAGATCTAGTGG 3’ & LB-TDNA_FTL) & LB-TDNA_FTL

FTL1273 (DsRed2, nucleotide position 18164269 on chromosome 3); FTL1273U (5’ TAATTC3’ & LB-TDNA_FTL)

The I3 line (qrt-2 with two insertions on chromosome 3, FTL1500 CFP at nucleotide position 498916 & LB-TDNA_FTL)

Genetic Analysis

Genetic complementation is typically tested by crossing homozygous mutants, but with the tam mutants this would introduce a complicating variable since the progeny would be tetraploid. Instead we assessed complementation by crossing heterozygous tam plants, selecting double heterozygous progeny with PCR and scoring their phenotype.

A tam-2 mutant with Col/No-0 polymorphisms was obtained by crossing a heterozygous tam-2 mutant with a No-0 plant and selling the F1 generation. We then selected tam mutants heterozygous for several Col/No-0 polymorphisms in the F2 generation and crossed them to wild-type Ler plants. The triploid plants obtained were genotyped to infer the genotype of the tam 2n gametes.

We obtained tam-2/ spt11-1-3/ rec8-3 mutants with Col/0/Ler polymorphisms by crossing a triple heterozygous tam-2/ spt11-1-3/ rec8-3 mutant (Col-0) with a wild-type Ler and selling the F1 progeny. Similarly, plants of interest in the F2 generation were crossed with wild-type No-0 and the triploid progeny were genotyped to infer the genotype of the tam mutants this would affect meiosis II.

A tam-2 mutant with a No-0 plant and Col/No-0 polymorphisms was obtained by crossing a heterozygous tam-2 mutant with a No-0 plant and selling the F1 generation. We then selected tam mutants heterozygous for several Col/No-0 polymorphisms in the F2 generation and crossed them to wild-type Ler plants. The triploid plants obtained were genotyped to infer the genotype of the tam 2n gametes.

Cytology and Flow Cytometry

Final meiotic products were observed, chromosome spreads generated, and genome sizes were determined as described previously [23]. Polten fluorescence was analysed as previously described [36]. Images were acquired with a LEICA DM RXA2 epifluorescence microscope using eCFP and DsRed2 filters (Chroma Technologies) and processed with Photoshop 8 (Adobe Systems Inc.).

Mutagenesis

EMS alleles of the TAM locus were generated following the protocol of Weigel and Glazebrook [58]. 0.5 grams of seed were imbibed in 30 ml of sterile water for 4 hrs. and then mutagenized with 0.2% ethyl methane sulfonate for 16 hrs. at room temperature with gentle agitation. Mutagenized seeds were rinsed with 30 ml of sterile water eight times and then dried before planting. Seeds were harvested from individually collected M1 plants. M2 plants were screened for a pollen dyad phenotype (background was qrt-1-2 which produces pollen tetrads).

Supporting Information

Figure S1 The tam-2, tam-3 and tam-4 insertions.

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Author Contributions

Conceived and designed the experiments: IdE GPC RM. Performed the experiments: IdE LC SJ CG GH YS JPCT LEB RM. Analyzed the data: IdE LC CG GMC GM. Contributed reagents/materials/analysis tools: GMC. Wrote the paper: LC GPC RM.

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A tam-2 mutant with Col/No-0 polymorphisms was obtained by crossing a heterozygous tam-2 mutant with a No-0 plant and selling the F1 generation. We then selected tam mutants heterozygous for several Col/No-0 polymorphisms in the F2 generation and crossed them to wild-type Ler plants. The triploid plants obtained were genotyped to infer the genotype of the tam 2n gametes.

We obtained tam-2/ spt11-1-3/ rec8-3 mutants with Col/0/Ler polymorphisms by crossing a triple heterozygous tam-2/ spt11-1-3/ rec8-3 mutant (Col-0) with a wild-type Ler and selling the F1 progeny. Similarly, plants of interest in the F2 generation were crossed with wild-type No-0 and the triploid progeny were genotyped. The trimorphic (Col-0/Ler/No-0) microsatellite markers used to genotype the tam-2 (Col-0/No-0) x Ler population and the tam-2/ spt11-1-3/ rec8-3 (Col-0/Ler) triple mutant x No-0 F1 plant population have been described previously [23]. Microsatellite

The NGA76 microsatellite was amplified (Tm: 57°C with the 5’GGAAAAATGTAGCTCCACCC 3’ and 5’AGGCATGG-
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