Loss-of-function mutants and overexpression lines of the Arabidopsis cyclin CYCA1;2/TAM exhibit different defects in prophase-I meiocytes but produce the same abnormal meiotic products

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ABSTRACT

Cyclins are known regulators of both meiotic and mitotic cell cycle progression in diverse organisms. In Arabidopsis, loss-of-function mutations in the A-type cyclin *CYCA1;2/TARDY ASYNCHRONOUS MEIOSIS* (*TAM*) gene lead to the production of abnormal meiotic products including triads and dyads. To investigate the effect of overexpression of *TAM* on meiosis, we generated Arabidopsis plants that harbored the *ASK1:TAM* transgene that increased *TAM* expression. 14 of 36 T_1 plants, representing five of six independent transformations, produced dyads or a mixture of dyads, triads and tetrads in male meiosis, indicating that the transgene had a dominant-negative effect. 34 of the 36 T_1 plants with or without the abnormal male meiotic products produced shriveled seeds, suggesting that the seed phenotype was more sensitive to the overexpression of *TAM* than, and independent of, the meiotic phenotype. To understand how overexpression of *TAM* produced the same meiotic products as the loss-of-function mutants, we conducted detailed analysis of spread prophase-I chromosomes in the wild type (WT), *tam-1*, and an *ASK1:TAM* line. We found that both the synapsed and unsynapsed chromosomal regions in zygotene were thicker in *tam-1* and the *ASK1:TAM* line than in the WT, but in pachytene, synapsed chromosomes in *tam-1* and the *ASK1:TAM* line were thinner and thicker than in the wild type, respectively. In diplotene, only the synapsed chromosomal regions in the *ASK1:TAM* line continued to be thicker than in the wild type while the desynapsed chromosomal regions among the three genotypes and the synapsed chromosomal regions between the wild type and *tam-1* were not statistically different. We also found that the pericentromeric heterochromatin regions in *tam-1* and *tam-2* formed a tight cluster at the pachytene and diplotene stages in approximately 40 % of the male meiocytes, much higher than the 6% found in the wild type and the *ASK1:TAM* line. Because the levels of TAM affects chromosomal morphology in specific phases in prophase I, we examined the expression and localization of a prophase-I marker protein ASY1 by immunolocalization in male meiocytes in the wild type, *tam-1*, *tam-2*, and two *ASK1:TAM* lines with different expression levels of *TAM*. The timing of ASY1 expression in the *tam* mutants appeared to match that in the wild type, but ASY1 in the null mutant *tam-2* was not found to be colocalized with the chromosomes as in the wild type and the partial loss-of-function mutant *tam-1*. The level of ASY1 was severely reduced in the highly expressed *ASK1:TAM* line while moderately reduced in the relatively less overexpressed *ASK1:TAM* line. Our results indicate that the reduction and increase in the activity of TAM differentially affect chromosomal morphology and the action of ASY1 in prophase I. Based on these results, we propose that either the different meiotic defects trigger a common cell cycle checkpoint, or the different defects later lead to a common defect such as missing ASY1 on the chromosomal axes, which further causes the production of the same abnormal meiotic products in the *tam* mutants and *ASK1:TAM* lines.

WHAT IS KNOWN ABOUT THE ROLE OF TAM IN MEIOSIS

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• TAM is predominantly expressed at the pachytene stage in male meiosis but it regulates cell cycle progression at the pachytene and post-pachytene stages; *tam* mutants produce abnormal meiotic products such as triads and dyads.

•. TAM acts in the same pathway as OSD1, TDM1, and SMG7; OSD1 is an APC/C inhibitor, TDM1 has no known functional domain, and SMG7 functions in nonsense-mediated RNA decay.

• An epistasis order for the mutants of these genes is, starting from the most epistatic, *osd1*, *tdm1*, *smg7*, and *tam*.

CURRENT FINDINGS

Overexpression of *TAM* has a dominant effect on meiosis and seed development (Fig. 1 and Table 1)

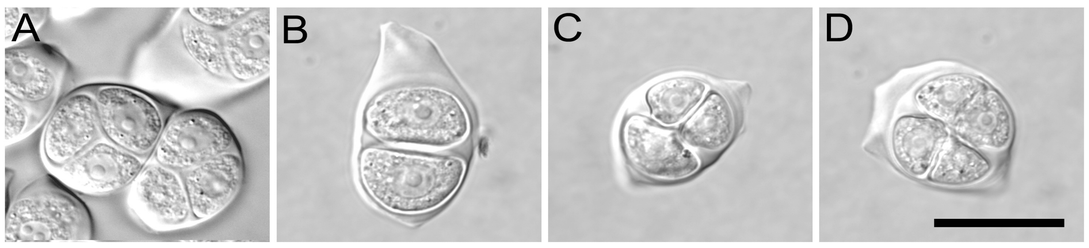

Figure 1. Male meiotic products in the wild type (Col) and T_1 of *ASK1;TAM2*. (**A**) Normal tetrads in Col. (**B-D)** A dyad, a triad, and a tetrad in *ASK1;TAM2*, respectively. Bar = 25 μm.

Table 1. Male meiotic products in *ASK1:TAM* at the T_1 generation

Experiment	Number of T_1s	Meiotic products
1	9	Tetrads
	5	Dyads
2	6	Tetrads
	3	Dyads
	1	Mixture of dyads, triads, and tetrads
3	4	Tetrads
4	2	Tetrads
	2	Dyads
5	1	Tetrads
	1	Dyads
6	1	Dyads
	1	Mixture of dyads, triads, and tetrads

34 of the above 36 plants produced variable amounts of shriveled seeds (Fig. 2)

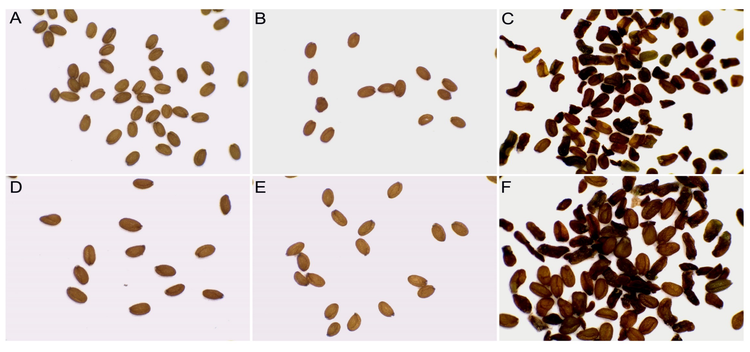

Figure 2. Seeds from Col and T_2s of *ASK1:TAM* lines. (**A**) Diploid Col. (**B**) Likely normal and diploid seeds from an *ASK1:TAM2* plant. (**C**) Mostly shriveled and likely diploid seeds from another *ASK1:TAM2* plant. (**D**) Seeds from tetraploid Col. (**E**) Likely normal and tetraploid seeds from an *ASK1:TAM1* plant. (**E**) A mixture of shriveled and normal-looking seeds from another *ASK1:TAM1* plant. These seeds appeared larger than the tetraploid seeds on the left.

Differential effects of *tam-1 and ASK1:TAM2* on chromosomal thickness in zygotene-to-diplotene male meiocytes (Figs. 3 and 4)

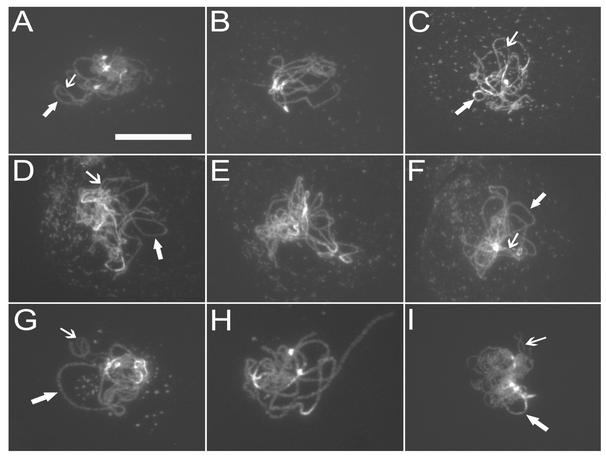

Figure 3. Subtle differences in chromosome thickness among Col, *tam-1* and *ASK1:TAM2*. (**A-C**) Zygotene, pachytene, and early diplotene chromosomes in Col, respectively. (**D-F**) Zygotene, pachytene, and early diplotene chromosomes in *tam-1*, respectively. (**G-I**) Zygotene, pachytene, and early diplotene chromosomes in *ASK1:TAM2*, respectively. The quantitative differences in chromosome thickness was summarized in Fig. 4. Thin arrows, unsynapsed zygotene or desynapsed diplotene chromosome regions. Thick arrows, synapsed chromosome regions. Bar = 25 μm.

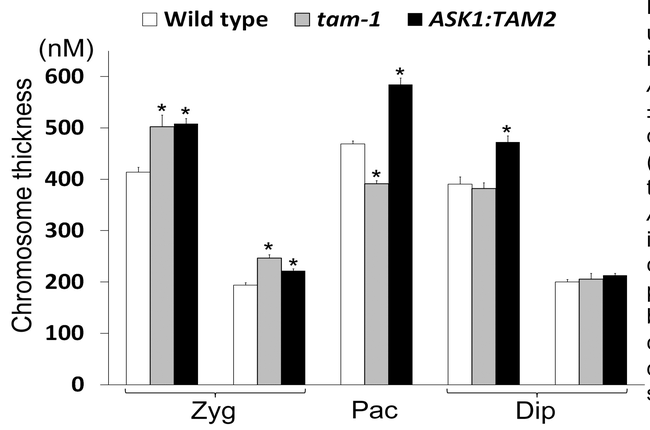

Figure 4. Thicknesses of synapsed, unsynapsed, and desynapsed chromosomes in male meiocytes in Col, *tam-1*, and *ASK1:TAM2*. Shown are average thicknesses ± standard errors. *The values are statistically different from the corresponding values of Col (t-test, $p < 0.01$, $11 \le n \le 78$). Also note that the synapsed chromosomes in Col and *ASK1:TAM2* underwent a thickening process in pachytene whereas synapsed chromosomes in *tam-1* underwent a thinning process. Zyg, zygotene, with the left and right being synapsed and unsynapsed chromosomes. Pac, pachytene. Dip, diplotene, with the left and right being synapsed and desynapsed chromosomes.

Pericentromeric heterochromatin regions tend to cluster in *tam-1* and *tam-2* (Fig. 5 and Table 2)

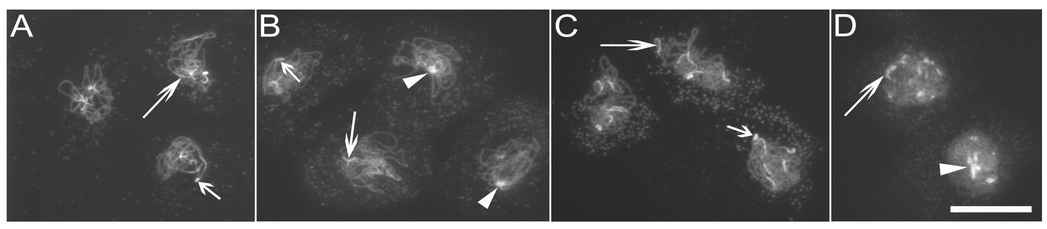

Figure 5. Spread chromosomes at the pachytene or diplotene stage showing scattered and clustered pericentromeric heterochromatin regions. (**A**) Col. (**B**) *tam-1*. (**C**) *ASK1:TAM2*. (**D**) Octaploid *tam-2*. Large arrows, scattered and elongated pericentromeric heterochromatin regions. Small arrows, conjoined telomeric regions containing rDNA on chromosomes 2 and 4. Arrowheads, clustered pericentromeric heterochromatin regions. Bar = 25 μm.

Table 2. Percentage of meiocytes with a single cluster of pericentromeric heterochromatin regions from pachytene to early diplotene

	22°C	28°C
WT	6 (n = 53)	6 (n = 122)
tam-1	23 (n = 96)	40 (n = 88)
tam-2	41 (n = 27)	Not determined
ASK1::TAM	6 (n = 85)	Not determined

Chromosomal loading and abundance of ASY1 are differentially affected in *tam* mutants and *ASK1:TAM* lines (Fig. 6)

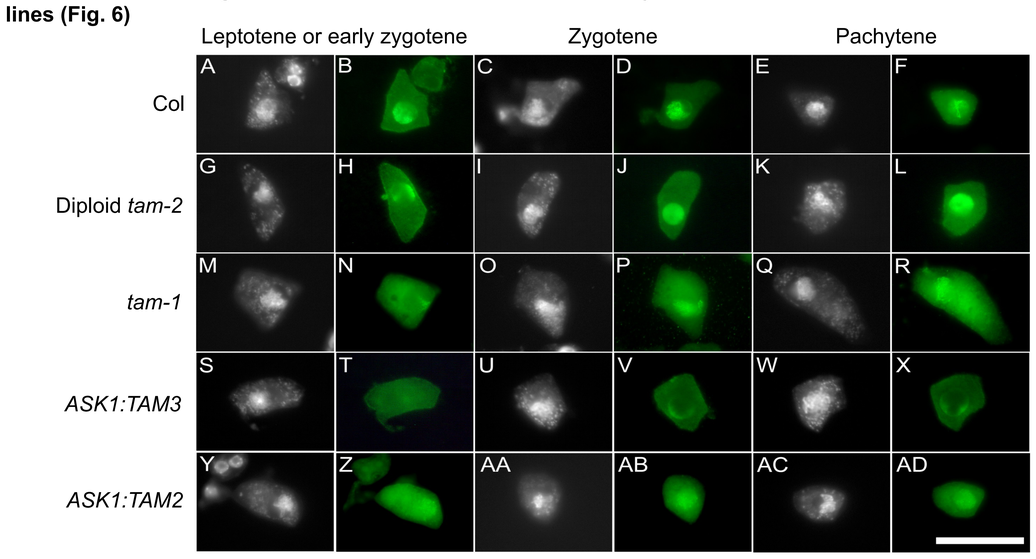

Figure 6. Immunolocalization of ASY1 in male meiocytes. Shown are DAPI and corresponding anti-ASY1 (green fluorescence) images. Note that *ASK1:TAM3* has a much higher expression of *TAM* than *ASK1:TAM2*. Bar = 25 μm. The results show that ASY1 was not loaded onto the chromosomes in *tam-2* and overexpression of *TAM* inhibited the expression of ASY1 in the male meiocytes.

CONCLUSIONS

- The reduction and increase in the activity of TAM cause different defects in prophase I.
- The different defects either trigger a common cell cycle checkpoint or later lead to a common defect such as missing ASY2 on the chromosomal axes, which further causes the production of the same abnormal meiotic products in the *tam* mutants and *ASK1:TAM* lines.

Acknowledgements

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