The role for CYCLIN A1;2/TARDY ASYNCHRONOUS MEIOSIS in differentiated cells in Arabidopsis

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ABSTRACT

The Arabidopsis A1-type cyclin, CYCA1;2, also named TARDY ASYNCHRONOUS MEIOSIS (TAM), is known for its positive role in meiotic cell cycle progression, but its function in other cells has not been characterized. Here we report the role of TAM in cell differentiation in vegetative organs. The expression pattern of TAM was investigated by promoter and protein fusions using the β-glucuronidase (GUS) and the green fluorescent protein (GFP), respectively. Based on the expression pattern, morphologies of cells expressing TAM were studied in various plants including loss-of-function *tam-1* and *tam-2* mutants, a *TAM*-overexpression line, *tam-1* complemented by the wild type *TAM*, and the wild-type. In addition, ploidy profiles in mature leaves of these plants were determined by flow cytometric analysis. *TAM* expression was found primarily in non-proliferating cells such as guard cells, trichomes, and mesophyll cells, and in vascular tissue, but its protein is kept at low levels. The *tam* mutants exhibited a reduction in trichome branch number but had a normal ploidy profile in the leaves. Compared to the wild type, these mutants also exhibited more pavement cell swelling, and the null allele, *tam-2*, had a larger average guard cell size but a normal ploidy level in the guard cells. Overexpression of *TAM* produced phenotypic changes opposite to those in the loss-of-function mutants, in addition to an increase in the relative portion of diploid cells. These results suggest that the primary role of TAM in vegetative organs is to regulate cell differentiation or cell turgidity in a cell-cycle-independent manner.

INTRODUCTION

- Cyclins and cyclin-dependent kinases (CDKs) are conserved regulators of the cell cycle and cell-cycle-independent processes such as transcriptional RNA elongation, RNA splicing, and vesicle transport
- Much less is known about cell-cycle-independent functions of cyclins and CDKs than their functions in cell cycle regulation in plants
- The A1-type cyclin CYCA1;2/TARDY ASYNCHRONOUS MEIOSIS (TAM) protein regulates meiotic cell cycle progression but its function outside of meiosis is unknown

RESULTS

TAM is expressed in trichomes, pavement cells, guard cells, mesophyll, and vascular tissue

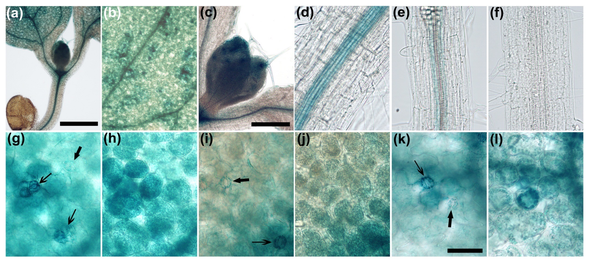

Fig. 1 GUS staining pattern in *TAM:GUS*. **a** 6-day old seedling. **b** Abaxial epidermis of a cotyledon, showing high-level GUS signals in a spotty appearance. **c** Larger magnification of **a** showing GUS staining in the leaf tips and trichomes. **D-f** Root segments near the hypocotyl, the mid-maturation zone, and near the elongation zone. **g** Abaxial epidermis of a cotyledon, showing two clusters of intensely GUS-stained cells (thin arrows: stoma; thick arrow: a stoma outside the cluster). **h** The same area as in **g** but focused into the mesophyll. **i** Abaxial epidermis of a cotyledon, showing transition from a zone of a high GUS-staining level to a zone of a low GUS-staining level (thin arrow: a stoma in the intensely GUS-stained cluster; thick arrow: a stoma in the low GUS-staining zone). **j** The same area as in **i** but focused into the mesophyll. The transition from a high GUS-staining zone to a low GUS-staining zone also occurred in the mesophyll. **k** Intensely GUS-stained single stoma (thin arrow), with a relatively lightly stained stoma (thick arrow) nearby. **l** The same area as in **k** but focused into the mesophyll. Bar in **a** = 500 μm, bar in **c** for **b**, **c** = 200 μm, and bar in **k** for **d-l** = 50 μm.

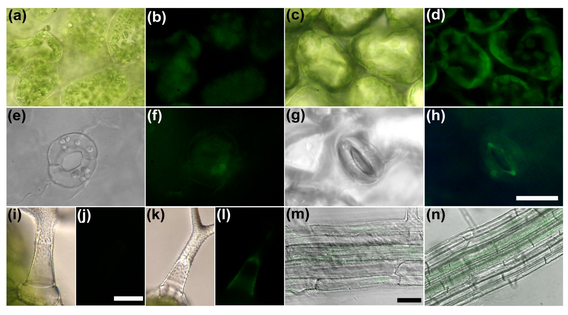

Fig. 2 TAM-GFP in *TAM:TAM-GFP* seedlings. **a,b** Mesophyll in Col (negative control). **c,d** Mesophyll in *TAM:TAM-GFP*. **e,f** Stoma in Col. **g,h** Stoma in *TAM:TAM-GFP*. **i,j** Trichome in Col. **k,l** Trichome in *TAM:TAM-GFP*. **m** Superimposed confocal laser scanning fluorescence and bright-field images of vascular tissue in Col root. **n** Superimposed *TAM:TAM-GFP* images corresponding to **m**. Bar in **h** for **a-h** = 20 μm, bar in **j** for **i-l** = 20 μm, and bar in **m** for **m**, **n** = 40 μm. **O** Relative GFP intensities in guard cells (mean ± standard error, n ≥ 24).

The TAM protein is kept at low levels in differentiated cells

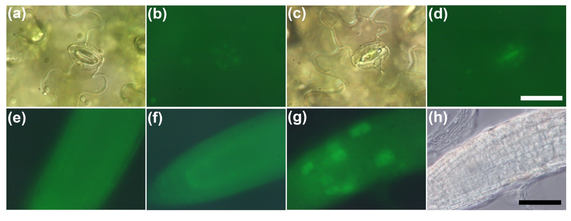

Fig. 3 GFP in *35S:TAM-GFP*, *TAM:TAM-GFP*, and Col. **a** DIC of abaxial epidermis of a cotyledon of Col. **b** GFP of **a**. **c** DIC of abaxial epidermis of a cotyledon of *35S:TAM-GFP*. **d** GFP of **c**. **e** GFP of a root tip in Col. **f** GFP of a root tip in *TAM:TAM-GFP*. **g** GFP of a root tip in *35S:TAM-GFP*. **h** DIC of **g**. Bar in **d** for **a-d** = 20 μm, and bar in **h** for **e-h** = 50 μm.

TAM is a positive and limiting factor for trichome branching

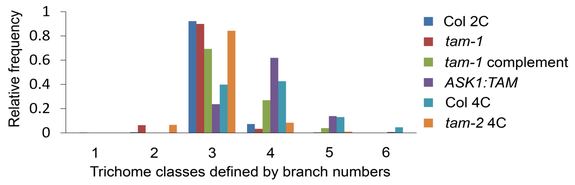

Fig. 4 Relative frequency of trichomes with different branch numbers in six plant lines. For each genotype, 108 or more trichomes were examined.

TAM is not required for endoreduplication and suppresses pavement cell swelling in rosette leaves

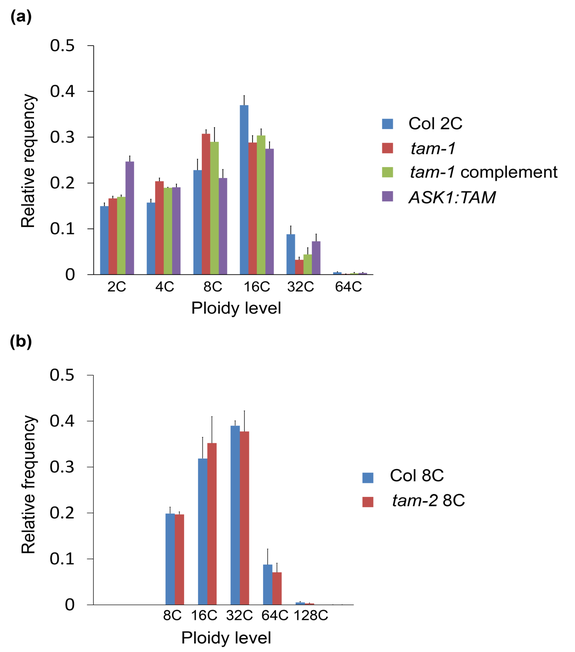

Fig. 5 Ploidy profiles of leaf cells in six plant lines. **a** Comparison of the ploidy profiles in four diploid plant lines. **b** Comparison of the ploidy profiles in two octaploid plant lines. For each genotype, three or more leaves were analyzed, and 6600 or more nuclei were counted for each leaf sample

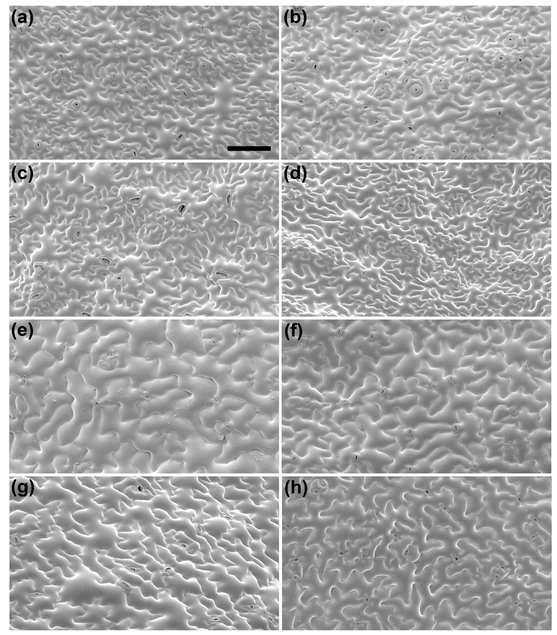

Fig. 6 Scanning electron microscopy images of epidermis of mature rosette leaves**. a** Abaxial side of Col 2C. **b** Abaxial side of *tam-1***. c** Abaxial side of *tam-2* 8C. **d** Abaxial side of *ASK1:TAM*. **e** Adaxial side of Col 2C. **f** Adaxial side of *tam-1*. **g** Adaxial side of *tam-2* 8C. **h** Adaxial side of *ASK1:TAM*. Bar in **a** for **a-h** = 100 μm.

TAM suppresses guard cell expansion but it does not affect the ploidy level of guard cells

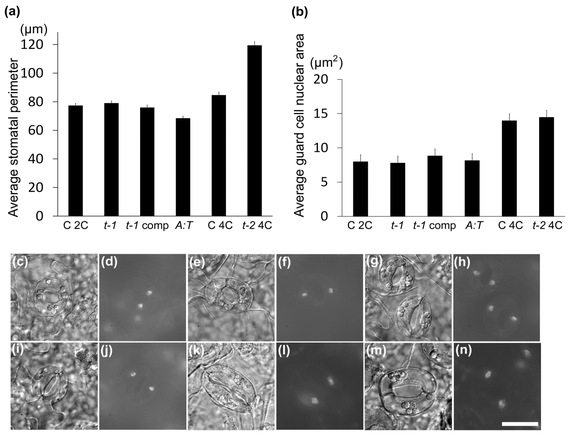

Fig. 7 Stomatal perimeters and guard cell nuclear areas in six plant lines. **a** Average stomatal perimeters. 45 or more stomata were measured for each genotype. **b** Average guard cell nuclear areas. 50 or more nuclei were measured for each genotype. C 2C: Col 2C; *t-1*: *tam-1*; *t-1* comp: *tam-1* complement; *A:T*: *ASK1:TAM*; C 4C: Col 4C; and *t-2* 4C: *tam-2*, 4C. **c**, **e**, **g**, **i**, **k**, **m** DIC images of stomata in the abaxial epidermis of cotyledons in Col, *tam-1*, *tam-1* complement, *ASK1:TAM*, Col 4C, and *tam-2* 4C seedlings, respectively. **d**, **f**, **h**, **j**, **l**, **n** Fluorescence images from DAPI staining that correspond to the DIC images on their left, respectively. Bar in **n** = 20 μm for **c-n.**

CONCLUSIONS

- *TAM* is transcribed in mature differentiated cells and the production of TAM is post-transcriptionally controlled
- TAM promotes trichome branching and suppresses expansion of other epidermal cells
- TAM may exerts its effect on cell morphology and cell size by modulating cell turgidity
- TAM may regulate trichome branching via an endoreduplication-independent manner

ACKNOWLEDGEMENTS

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