FEHLSTART, encoding a land plant-specific transcription factor, is essential for meiotic synchronization in *Arabidopsis*

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Summary

FEHLSTART (FST) is a newly isolated gene in *Arabidopsis* that is preferentially expressed in meiosis and encodes a land plant-specific transcription factor. The mutation of *FST* causes a defect in fertility (Fig. 1) which is due to a novel phenotype in male meiocytes (the cells that undergo meiosis): Meiosis can be completed but the synchronization failes in ~15% of the meiocyte clusters. Together with other meiotic defects, this leads to ~23% abnormal tetrads.

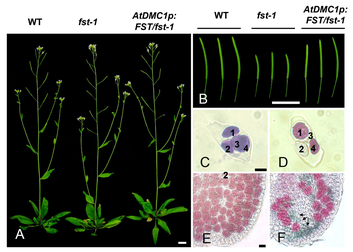


Figure 1. *Arabidopsis* wild-type and *fst-1* mutant plants

A: Wild-type, *fst-1* and rescued *fst-1* plants. B: Siliques of wild type, *fst-1* mutant and rescued *fst-1* mutant plants. C, D: Tetrads stained with toluidin blue (C: WT, D: *fst-1*). Note the unstained sectors in the mutant. E, F: Pollen stained with improved Alexander solution (E: WT, F: *fst-1*). Vital pollen are red; in the mutant, some are degraded (blue-green). Scale bar = 5 mm (A, B), 10 µm (C-F).

Introduction

Although meiosis, the cell division that produces gametes, can be found in animals as well as in plants, land plants have a unique feature: Male meiosis is highly synchronized in each spore sac and the meiotic progression is regulated by a number of cell cycle regulators, e.g. MEL2, SWI1/DYAD/AM1, CDC45, TAM, SDS, SMG7, MS5/TDM1.

What is so special about the *FEHLSTART (FST)* gene and the corresponding protein?

• *FST* encodes a putative **bHLH transcription factor**. So far, only ~ 70 meiosis genes have been characterized in *Arabidopsis* and only one of them (MMD1) is a transcription factor.

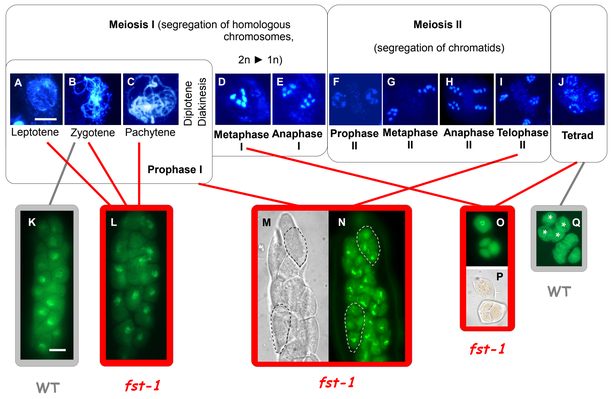
• *FST* is **land plant-specific** - homologs are found in almost all taxa (with the exception of gymnosperms), but not in animals, fungi or algae.

• *fst* mutants show a clear disruption of the normally precisely regulated timing of meiosis, resulting in **asynchrony** of male meiocytes.

Results

Figure 2. Meiosis progression and asynchrony in *fst-1* mutants

A-J: Meiotic phases, chromosomes stained with DAPI. Scale bar = 10 µm. K-Q: Whole collected meiocyte clusters ("worms", in K-N) and tetrads (O-Q) of wild-type (grey frame) and *fst-1* mutant plants (red frame). Scale bar = 10 µm. Shown are pictures taken in brightfield or stained with SYTOX Green which emphasizes DNA but also stains the cytoplasm. While the cells of a wild-type meiocyte cluster are always in the same phase, *fst-1* mutant clusters can show different stages at the same time. In addition, callose wall formation and cell division are tightly associated with certain meiotic phases in wild-type plants, while the connection can be lost in *fst-1* mutants.



- asynchronous meiosis (Figure 2, 3)
- chromosome fragments and asymmetric cell divisions (Figure 3 B, C)
- decreased expression level for *RCK/MER3, MUS81* and *PTD* (Figure 4)
- no significantly altered sensitivity in genotoxicity tests with MMS and MMC (data not shown)

Phenotype of the *fst-1* mutant

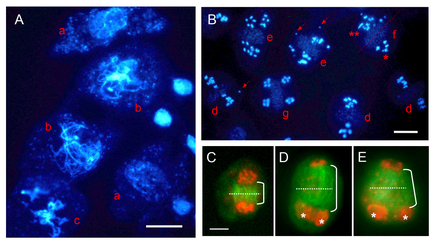


Figure 3. Asynchrony and asymmetry in *fst-1* mutants

A, B: Chromosome spread of *fst-1* meiocytes from a single anther per image, showing different stages (a – leptotene, b – diplotene, c – late diplotene, d – metaphase II, e – anaphase, f – mixed meta- & anaphase II, g – telophase). Arrows point to chromosome fragments. C-F: Immunolocalization of tubulin (green), showing abnormal patterns in the *fst-1* mutant (E, F, three chromosome sets, red), while there is only one chromosome set on each side of the resolving spindle in the wild type (C). Scale bars = 10 µm

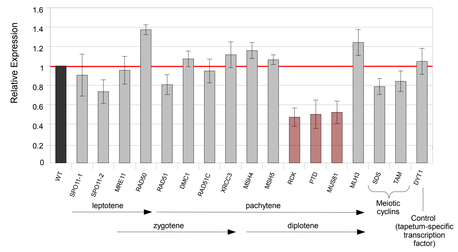


Figure 4. Gene expression (Real-time RT-PCR) in collected meiocytes of *fst-1* mutants

The comparison of expression levels of genes involved in meiotic processes shows that *RCK, MUS81* and *PTD* are significantly reduced in *fst-1* mutants in relation to the wild type. These three genes have not been implicated in progression of meiosis but instead are thought to be mechanistically important, especially in crossover formation and resolution.

Conclusions

• FST is involved in the **regulation of meiotic synchronization** in anthers:

  • among meiocytes in the same anther

  • among processes in a single meiocyte (callose wall formation and chromosome behavior; cell division)

• Loss of FST function leads to defects in fertility and asynchronous meiosis. In addition, asymmetric cell divisions and chromosome fragments occur – either an indirect consequence of the overall asynchrony or more directly caused e.g. by the decreased expression of *MER3, MUS81* and *PTD*.

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