The role of *BELL1* (*BEL1*) and *AINTEGUMENTA* (*ANT*) in megasporogenesis.

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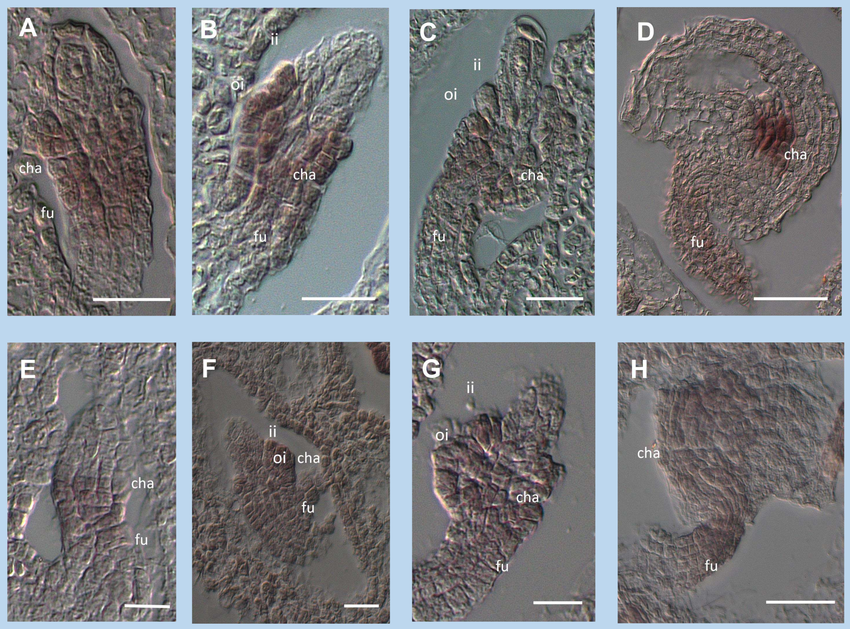
ABSTRACT

In the ovules female germline development initiates with the differentiation of the Megaspore Mother Cell (MMC) in the nucellus. MMC upon meiosis formed four spores, three of which degenerate and the one surviving differentiates into the Functional Megaspore (FM). At the same time from the chalaza the two integuments are formed. The transcription factors *BELL1* (*BEL1*) and *AINTEGUMENTA* (*ANT*) have been shown to be involved in the control of integuments development (1-5). We have investigated in the role of *BEL1* and *ANT* during the megasporogenesis since in both mutants this process is impaired. Indeed, the degeneration of spores and the FM specification are altered. Our results suggest that alteration in Auxin distribution might cause defects in sporophytic tissues leading to Functional Megaspore specification defect.

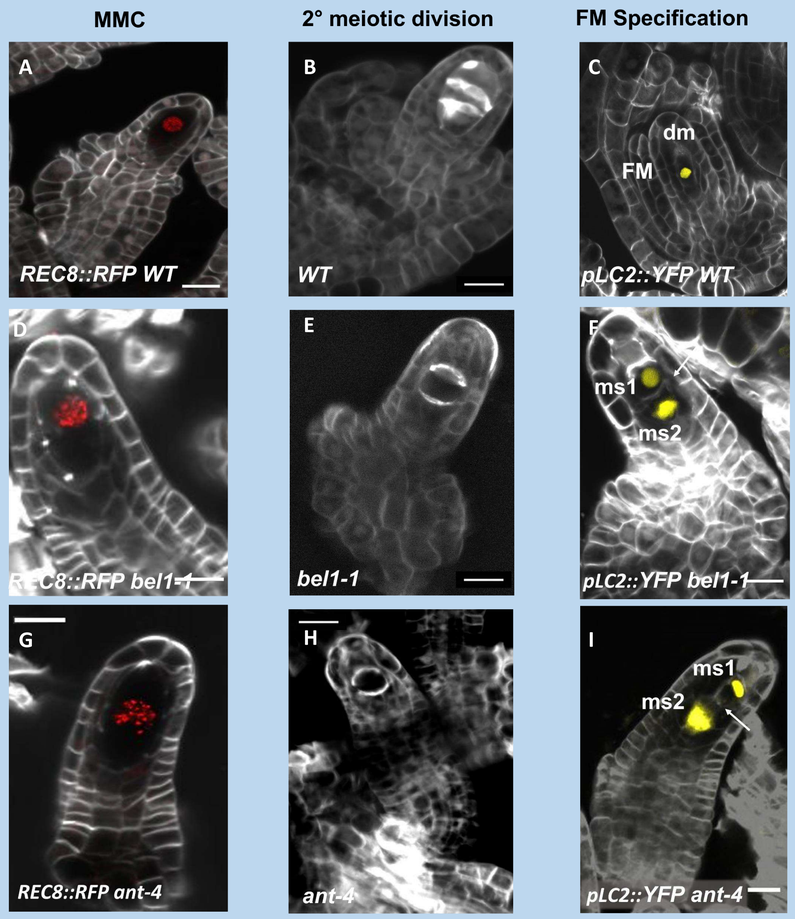
BEL1 and *ANT* are expressed in the chalaza



In the ovules, the two transcription factors *BEL1* (*Fig*. 1A-D) and *ANT* (*Fig*. 1E-H) are expressed in the region where the inner (ii) and the outer (oi) integuments arise and then are formed, and throughout the ovule development they localized in correspondence of the chalaza region (cha) and in the funiculus (fu).

Fig.1. Cha, chalaza; fu, funiculus; ii, inner integument; oi, outer integument.Scale bar 20 µm

In *bel1-1* and *ant-4* the Degeneration of Megaspores and the FM specification are impaired



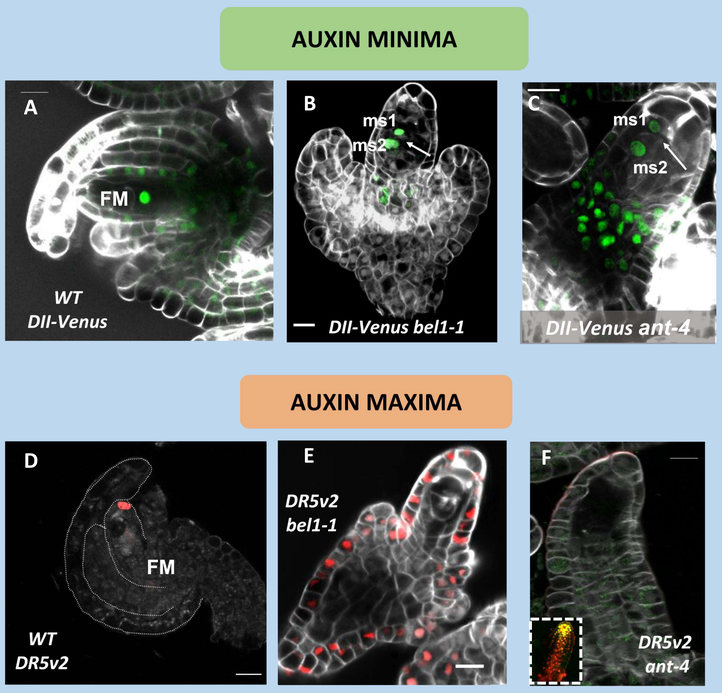
In wild type ovules once the MMC is specified it starts the meiotic program (*REC8::RFP; Fig.2A*) and gives rise to four spores (*Fig*.2B) and after the degeneration of three out of four megaspores (dm) the most chalazal spore acquires FM identity (*pLC2::YFP; Fig 2C*).

In *bel1-1* and *ant-4* ovules the meiotic program is correctly set up (*Fig*. 2D,2G) and the meiotic divisions occur (*Fig*. 2E, 2H).

However, in *bel1-1* and *ant-4* mutant ovules, the megaspores don't degenerate, and the FM identity is acquired randomly by one or more than one megaspores (ms1,ms2; *Fig*. 2F,2I).

Fig.2. ms, megaspore. Arrows indicate cell wall plate formed after meiotic division. Scale Bars 10 µm

Auxin distribution is altered in *bel1-1* and *ant-4* ovules



In the ovules, auxin is accumulated at the tip of the nucella (auxin maxima, *Fig*.3D) whereas in the chalaza and in correspondence of the FM a minimum of auxin is observed (*Fig*. 3A).

In both *bel1-1* and *ant-4* mutants the auxin minima (*DII-Venus*) is expressed in correspondence of one or more than one spores mirroring the different situation observed with the *pLC2::YFP (Fig. 3B,3C)*.

We couldn't observe any expression of the auxin maxima reporter marker (*DR5v2*) in any *ant-4* ovules (*Fig*. 3F) whereas in *bel1-1* ovules, the auxin maximum is accumulated randomly in the ovules (*Fig*.3E).

Fig3. ms, megaspore. Arrows indicate cell wall plate formed after meiotic division. Within the dashed rectangular box, *ant-4* roots used as positive control for *DR5v2* in *ant-4*. Scale Bars 10 µm

CONCLUSION

Our results suggest that the mis-distribution of auxin within *bel1-1* and *ant-4* mutant ovules leads to alteration in degeneration of megaspores and FM specification.

This alteration in auxin distribution might be due to mis-expression of auxin polar transport PIN1, which has been already shown to be important for the correct female germline progression (6)

Fig4. Model for BEL1 and ANT in the control of megasporogenesis and FM specification

References

1. Robinson-Beers, K.; Pruitt, R.E.; Gasser, C.S. Ovule Development in Wild-Type Arabidopsis and Two Female-Sterile Mutants. *Plant Cell,* **1992**, *4,* 1237-1249, doi: 10.1105/tpc.4.10.1237.
2. Reiser, L.; Modrusan, Z.; Margossian, L.; Samach, A.; Ohad, N.; Haughn, G.W.; Fischer, R.L. The BELL1 gene encodes a homeodomain protein involved in pattern formation in the Arabidopsis ovule primordium. *Cell* **1995**, *83*, 735-42. doi: 10.1016/0092-8674(95)90186-8.
3. Elliott, R.C.; Betzner, A.S.; Huttner, E.; Oakes, M.P.; Tucker, W.Q.J.; Gerentes, D.; Perez, P.; Smytha, D.R. AINTEGUMENTA, an APETALA2-like gene of Arabidopsis with pleiotropic roles in ovule development and floral organ growth. *Plant Cell.* **1996**, *8,*155-68. doi: 10.1105/tpc.8.2.155. 10.
4. Baker, S.C.; Robinson-Beers, K.; Villanueva, J.M.; Gaiser, J.C.; Gasser, C.S. Interactions among genes regulating ovule development in Arabidopsis thaliana. *Genetics* **1997**, *145*, 1109-24. PMID: 9093862.
5. Klucher, K.M.; Chow, H.; Reiser, L.; Fischer, L. AINTEGUMENTA gene of Arabidopsis required for ovule and female gametophyte development is related to the floral homeotic gene APETALA2. *Plant Cell* **1996**, *8*, 137-53 doi:10.1105/tpc.8.2.137.
6. Bencivenga, S.; Simonini, S.; Benková, E.; Colombo, L. The transcription factors BEL1 and SPL are required for cytokinin and auxin signaling during ovule development in Arabidopsis. *Plant Cell.* **2012**, *24*, 2886–97, doi: 10.1105/tpc.112.100164.