doi:10.1017/S1431927616005973
Microsc. Microanal. 22 (Suppl 3), 2016

AINTEGUMENTA-LIKE6 Can Functionally Replace *AINTEGUMENTA* but Alters Arabidopsis Flower Development When Misexpressed at High Levels

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Flowers of the model plant *Arabidopsis thaliana* consists of four types of floral organs that are arranged in four concentric rings called whorls. From the outside whorl to the center of the flower are four sepals, four petals, six stamens and two carpels (Fig. 1A). Four classes of floral organ identity genes (i.e. the class ABCE genes) act in different combinations to specify these four floral organ identities. While much is known about genes that specify floral organ identity, the molecular mechanisms regulating floral organ initiation, growth and patterning remain largely unknown. As flowers give rise to essential components of the human diet (i.e. grains, fruits and seeds), an understanding of flower development can contribute toward increases in crop yields needed to feed the world's growing population.

AINTEGUMENTA (*ANT*) and *AINTEGUMENTA-LIKE6* (*AIL6*) encode related transcription factors with partially overlapping roles in floral organ development in *Arabidopsis*. *ant ail6* double mutant flowers consist of small sepals, filamentous organs, stamen-like organs, undefined organs and unfused carpel valves, which arise in random positions within flower primordia [1]. Thus *ANT* and *AIL6* contribute to the establishment of floral organ initiation, identity, and floral organ growth as well as carpel patterning. *ANT* and *AIL6* do not make equivalent contributions to these processes. Loss of *ANT* function by itself results in smaller flowers (Fig. 1B) [2,3], demonstrating that the role of *ANT* in organ size control cannot be provided by *AIL6*. Loss of *AIL6* function on its own has no phenotypic consequences indicating that all of its roles in flower development can be provided by *ANT* or some other gene [1]. Some of the functional differences between *ANT* and *AIL6* may arise from differences in gene expression, as *ANT* mRNA is present at higher levels and in a broader domain than *AIL6* mRNA in young flowers, and *ANT* mRNA persists much longer in developing floral organs [2,4].

To further probe the function of AIL6 in flower development, we investigated whether the functional differences between *ANT* and *AIL6* are a consequence of differences in gene expression and/or protein activity. We made transgenic plants in which a genomic copy of *AIL6* was expressed under the control of the *ANT* promoter. *ANT:gAIL6* can rescue the floral organ size defects of *ant* mutants when *AIL6* is expressed at similar levels as *ANT* in wild type (Fig. 1A-C). The anthers of *ANT:gAIL6 ant* stamens consist of four locules like wild type, rather than the two locules present in *ant* mutants (Fig. 2). Thus, the functional differences between *ANT* and *AIL6* result primarily from gene expression differences. However, *ANT:gAIL6 ant* lines that express *AIL6* at higher levels display additional phenotypes including reduced numbers of floral organs, mosaic floral organs, and subtending filaments or bracts. The severity of these phenotypes correlates with overall *AIL6* mRNA levels suggesting that different levels of AIL6 activity can evoke different developmental outcomes. Furthermore, such phenotypes (mosaic organs, bracts etc) were not observed in previously characterized transgenic lines in which the coding region of *AIL6* was expressed under the *35S* promoter. In some *35S:cAIL6* lines, larger flowers are produced, similar to transgenic plants that misexpress *ANT* [5,6].

To investigate the basis for these distinct *AIL6* misexpression phenotypes, we expressed *AIL6* in L*er* using a steroid inducible *AIL6* line under the control of the *35S* promoter (i.e. *35S:gAIL6-GR*).

Treatment of *35S:gAIL6-GR* lines with the steroid dexamethasone results in distinct floral phenotypes depending on the developmental stage of the flower at the time of treatment. Induction of high AIL6 activity in older flowers results in larger floral organs while induction of high AIL6 activity in younger flowers resulted in the production of petaloid sepals. Thus the developmental stage at which a flower receives high AIL6 activity determines the phenotypic outcome. The distinct phenotypes observed in different *AIL6* misexpression lines can be explained by differences in both the levels and spatial/temporal accumulation of *AIL6* expression. Our results contribute to our understanding of flower development and identify potential genetic tools to engineer flowers with altered floral organ morphology and size. [7]

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[7] The authors acknowledge funding from NSF.

Figure 1. *ANT:gAIL6 ant* flowers rescue the petal size defects of *ant*. (A) L*er* flower. (B) *ant-4* flower. (C) *ANT:gAIL6 ant-4* line C1-69 flower. Pictures in A-C are taken at the same magnification.

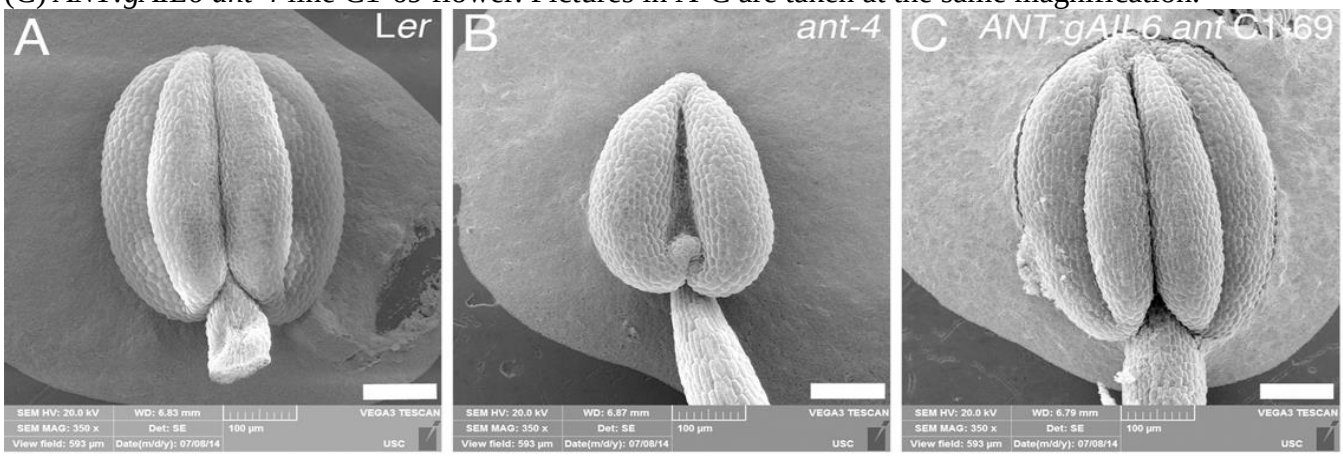

Figure 2. SEM of *ANT:gAIL6 ant* anthers. Stamen anthers from L*er* (A), *ant-4* (B), *ANT:gAIL6 ant-4* line 69 (C). Size bars are 100μm.

https://doi.org/10.1017/S1431927616005973 Published online by Cambridge University Press