Non-inductive conditions expose the cryptic bract of flower phytomeres in Arabidopsis thaliana

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The aerial plant architecture is built by phytomeres which are metameric units, each composed of a stem segment (internode) and a leaf with axillary meristem (node). In Arabidopsis thaliana, fully developed flower phytomeres lack the leaf even if they temporarily exhibit a cryptic bract (CB) during early development. Recently, we demonstrated that the CB becomes more prominent under non-inductive short-day conditions. However, a full outgrowth as cauline leaf is prevented by Polycomb-group (Pc-G) proteins which silence the MADS gene FLOWERING LOCUS C (FLC) encoding a repressor of FLOWERING LOCUS T (FT). Also the loss of SHORT VEGETATIVE PHASE (SVP) supresses the ectopic leaf formation and/or FM-to-IM reverision (Figs. 1B-F and 2C-D). The lack of the Pc-G target SVP suppresses the ectopic leaf growth even more strongly than the loss of FLC but not the RB formation itself (Fig. 2B). Here, we use the floral commitment deficient lines iCLF and ev as genetic and morphological tools to investigate fate decisions of both CB and FM during early floral primordia development by re-analyzing SD induced floral reversion nodes.

Keywords: Arabidopsis, cell specification, floral primordia, floral reversion, plant morphology

Abbreviations: CB, cryptic bract; ev, emf2-10 vrn2-1; FLC, FLOWERING LOCUS C; FM, floral meristem; iCLF, clf-28 sun-7 CLF-GR; IM, inflorescence meristem; FT, FLOWERING LOCUS T; LFY, LEAFY; P0, P1, etc., numbering of floral primordia; Pc-G, Polycomb-group; rLM, reverted inflorescence meristem; RB, rudimentary bract; SAM, shoot apical meristem; St1, floral stage 1 etc; Ste2, early floral stage 2; Stl2, late floral stage 2; STM, SHOOT MERISTEMLESS; SVP, SHORT VEGETATIVE PHASE; TSF, TWIN SISTER OF FT.

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Figure 1. For figure legend, see page 3.
Figure 1 (See previous page). Data and model for reversion nodes at the shoot axis of floral commitment deficient Arabidopsis plants. (A) Phytomers are metameric units that are composed of internode and node (leaf plus axillary meristem). (B-G) Different types of nodes at Arabidopsis shoot axis during normal development (C and G) and floral reversion (B-F). r.l., not indicated in (H). (H) Schematic representation of ev mutants which reverted after transfer from LD to non-inductive SD conditions. Every column represent the main shoot axis of one plant, every square a node. The plants are sorted by the position of the 1st reversion node and split in 2 equal fractions: (1) early and (2) late reverted plants. This raw data set of 86 plants was used in (I and J) and Müller-Xing et al.4 (I) Average of cauline leaves (light green), pre-reversion flowers (yellow) and reversion nodes (dark green) of all ev plants in (H), and the early reversion (1) and the late reversion subgroup (2). Note that all node positions (horizontal numbers) and node numbers (vertical) are significant different between (1) and (2) (Student t-test, P < 0.01) with the exception of the last reversion node (asterisk; p = 0.09). (J-K) Identity of the first 7 and the last 7 reversion nodes in ev (J; N = 85) and CLF (K; N = 35). (L) Floral primordia development in wild-type. Floral primordia stage 0 to 5, St0 - St5; St2e, early St2; St2l, late St2. (M) Model of the origin of different classes of reversion nodes in the early primordia development. The gray gradient indicates dropping and gradual recovery of floral commitment overtime. Note that floral stage (St; defined by morphological criteria by Smyth et al.10 and numbering of floral primordia (P1 is the smallest visible primordia) are not direct linked and that in real plants, different primordia (P) can have the same floral stage (St). Further note for (M) that individual floral primordia keep their numbering which they got at the first place in the time course.

Figure 2. Morphological changes in wild-type and Pc-G deficient plants triggered by non-inductive conditions (after LD-to-SD shifts). (A-B) Exposure of the CB as RB in wild-type (A, La-0) and ev svp-32 triple mutants (B). Arrow, leaf-like structure; arrowhead, RB, some with stipules. (C) A reverted (left) and a non-flowering induced iCLF plant (right, arrow). Asterisk, pre-reversion flower; arrowheads, reversion nodes. (D) Reverted ev mutant plant. Note the arrangement of the pre-reversion flowers (asterisks) and the "empty" reversion leaf-nodes (arrowheads) in whorls. (E-G) in situ RNA hybridisations of longitudinal sections wild-type (continuous LD) and ev inflorescence apices (6 days after LD-to-SD shift). (E) SVP and STM expression in wild-type St2 primordia (left) and morphologic transformed primordia of ev (right). Arrowhead, CB without expression. (F-G) In the flattened ev St2 equal primordia (2), LFY expression is almost distinct, although LFY is strongly expressed in ev St0 (0) and St5 (5) primordia (G) as well as St2 primordia of the wild-type control (F). rIM, reverted IM; arrowhead, CB without LFP expression. (H-I) SEM pictures ev inflorescences 6 days after LD-to-SD shift, top view. rIM, reverted IM; arrow, CB/RB without axillary meristem; arrowhead, CB with axillary meristem (hashtag); L, reversion leaf; 3, ≥6, ≤13, pre-reversion floral primordia/flower (St3, ≥St6, ≤St13). (J) Quantitative RT-PCR analyses of TSF mRNA expression in ev inflorescence apices (harvested 8 h after lights on) normalized to elf4, relative to expression in La-0 (LD). N ≥ 3; ± s.e.m. Asterisks indicate significant decrease of expression (Student’s t test; P ≤ 0.05) compared with the equally treated wild-type control (La-0). evs, ev svp-32; evf, ev flc-5; evfs, ev flc-5 svp-32. DAS, days after LD-to-SD shift. Bars = 10 mm (C-D), 1 mm (A-B) and 50 μm in (E-I).
independent of the day-length. Conversely, LFY, similar to SHOOT MERISTEMLESS (STM), is nearly undetectable in flattened Ste2-equal ev primordia (Fig. 2G), but it remains unclear whether this is the cause or the result of the meristem loss. Similar to its homolog FT, the expression of TSF drops in ev mutants (Fig. 2J). However, in contrast to FT, TSF does not decrease in wild-type after shift to SD (Fig. 2J) which could be one reason why wild-type does not revert. ev fi triple mutants as well as the SD phenotype of ev copy the multi cauline leaf phenotype of ft lfy and fi stm double mutants. Interestingly, LFY and STM are both expressed in the rising FM but not in the CB in St2 primordia (Fig. 2E-F). Therefore, floral reversion in ev could be the result of combined downregulation of the mobile FT/TSF signal and of LFY and/or STM in individual floral primordia under non-inductive conditions.

“Flowers can be placed in order of age and developmental stage by their position on an inflorescence.”

The same is true for every organ produced by the SAM at the shoot axis, which therefore represents a time axis (Fig. 1H and L). Concerning that the type of nodes produced by the SAM reflects its identity, the entire past of the SAM identity and identity changes, respectively, can be read out by examining both, node identity and position at the shoot axis of a mature plant. The clustering of the nodes of reverted ev main shoots (Fig. 1H) results in the following progression: cauline leaves, pre-reversion flowers, reversion nodes and post reversion flowers (Fig. 1I). Notably, SD-triggered flower reversions in ev and iCLF are limited in time, afterwards only flowers are produced (Fig. 1H-I; data not shown). Furthermore, we compared early (1) with late reverted plants (2) to reveal that the number of pre-reversion nodes does not influence the position of the last reversion node (Fig. 1I). One explanation could be the declining importance of FT for maintaining flower formation; even ft mutants start flower production in later development. Suppressed internode elongation of reversion phytomeres causes occasionally leaf whorls reminiscent of normal leaf rosettes produced by vegetative SAMs (Fig. 2C and D). The formation of whorls can be caused by perturbation of the meristem function but here post-meristic mechanisms are more likely, because pre-reversion flower nodes, which are mainly established before the drop in the floral commitment, also form whorls (Fig. 1D).

The four main reversion node classes in ev and iCLF (Fig. 1B-E) are the result of either FM-to-IM reversion, which is visible by flower-to-paracledale transformation, and/or outgrowth of the primary derivate of the IM, the CB as cauline leaf. The analysis of these classes, with respect to the relative position at the main axis, provides direct conclusions on the cell specifications of CB and FM (see model Fig. 1M), 2 tissues that first clearly distinguishable by expression pattern in St2e primordia (Fig. 1L). Paraclade node and leaf/flower node are in some ways contrary to each other (Fig. 1B and E). Paraclade nodes, which miss a fully developed cauline leaf are over represented at the beginning of floral reversions (Fig. 1H and J-K). On the other hand, leaf/flower nodes are found only late during floral reversions (Fig. 1H and J-K). That spatiotemporal pattern, FM-to-IM reversion without outgrowth of the CB in the beginning of decreased floral commitment and cauline leaf formation without FM-to-IM reversion at the end, suggests that the decision for suppression of cauline leaf development is specified earlier than the final identity of the axillary meristem.

LFY is well-known as FM identity gene. Recently, Chahtane et al. demonstrated that LFY also controls axillary meristem formation by direct induction of REGULATOR OF AXILLARY MERISTEMS (RAXI). Interestingly, genetic ablations of LFY expressing cells promotes ectopic bract development revealing a link between FM and bract suppression in Arabidopsis. Therefore, LFY function controls at different levels meristem formation, FM identity and indirect, bract suppression during normal flower development. In the floral commitment deficient lines ev and iCLF the 2 most frequent classes of reversion nodes, leaf/paracledale and leaf node (Fig. 1C-D, H and J-K), could be explained by different decreasing levels of LFY and consequently, weakening of FM identity (FM-to-IM reversion triggering ectopic bract formation) and a complete failure of meristem formation (triggering ectopic bract formation as well), respectively. Nevertheless, further studies will be necessary to get a deeper mechanistic understanding of why LFY is downregulated in the Pc-G and floral commitment deficient lines ev and iCLF and how LFY, in parallel with FT, maintain commitment to flowering in general.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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