

Shaping up the fruit

Control of fruit size by an Arabidopsis B-sister MADS-box gene

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The final size and shape of fruits is determined by organogenesis. Organogenesis is the coordination of cell growth, cell differentiation and pattern formation. Individual genes have been identified that affect lateral organ growth. A majority of these characterized growth genes in Arabidopsis affect all lateral plant organs and few of these have been placed into a regulatory network controlling organ growth. We have recently characterized GORDITA (GOA), a MADS-box transcription factor, which represses cell expansion specifically in fruits and affects overall fruit size.¹ Here we provide insights into a possible regulatory network in which GOA can function to regulate fruit growth. We further suggest how duplicated B-sister genes; *GOA* and *TRANSPARENT TESTA 16 (TT16)* could have acquired distinct regulatory roles.

Organ morphogenesis occurs through coordinated cell growth, proliferation and cell expansion in multi-cellular organisms. Although several genes have been isolated that affect organ shape by a change in the duration of cell proliferation or a change in cell expansion (Fig. 1), there appears to be some cross-talk between cell proliferation and cell expansion by mutants that exhibit compensation.²⁻⁴ For example, *an3* leaves have far fewer cells however the leaf is nearly as large as wild type because the leaf cells are larger.³⁻⁵ This compensation phenomenon suggests that there is a mechanism whereby the organ can measure its overall size and adjust its growth accordingly.⁴ Fruits, which are modified leaves, provide a specialized example to

study the mechanisms that control lateral organ size.

A Possible Regulatory Framework of a B-Sister MADS Box Protein; GOA Action

Studies on MADS-box transcription factors have demonstrated its role in controlling organ identity not only in Arabidopsis but in many flowering plant species.⁶ Cell fate determining B-function MADS box transcription factors are expressed in second whorl sterile organs and third whorl male reproductive organs and they are necessary to determine the organ identity in these two whorls.^{7,8} Interestingly, a member of the B-sister MADS-box genes, *TT16*, regulates the development and pigmentation of the seed coat.⁹ Duplication and diversification of evolutionarily conserved transcription factors such as MADS-box proteins can bring about morphological variations among related organisms.¹⁰ Recently, we have demonstrated the functional importance of one of the duplicated B-sister MADS-box genes, *GORDITA (GOA)*.¹ We showed that the dynamic spatiotemporal expression pattern of *GOA* during fruit development is required to determine the final Arabidopsis fruit size largely by repressing cell expansion¹ (Fig. 1). Our findings also reveal that *GOA* likely regulates integument differentiation additively with its paralog *TT16*.^{1,9}

The final size and shape of an organ is likely determined by the interaction of pattern formation and growth.¹¹ Much is known about the genes necessary for proper differentiation and pattern

Key words: GORDITA, AGL63, TT16, MADS-box, B-sister, growth, fruit development, fruit size

Submitted: 04/14/10

Accepted: 04/15/10

Previously published online:

www.landesbioscience.com/journals/psb/article/12095

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Addendum to: Prasad K, Zhang X, Tobón E, Ambrose BA. The Arabidopsis B-sister MADS-box protein, GORDITA, represses fruit growth and contributes to integument development. Plant J 2010; 62:203-14; PMID: 20088901; DOI: 10.1111/j.1365-313X.2010.04139.x.

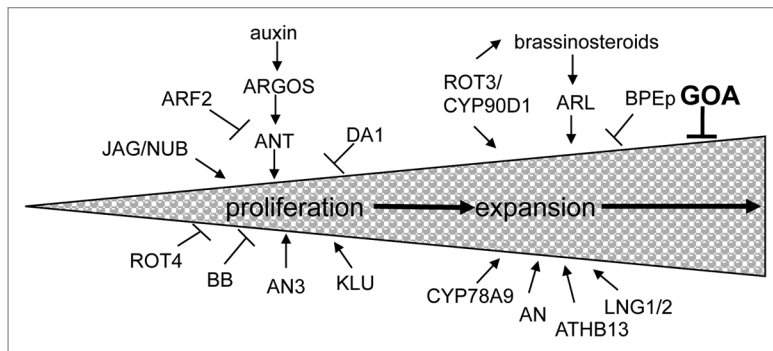


Figure 1. The size of an organ is determined by cell proliferation and cell expansion. Many proteins have been identified in Arabidopsis that play a role in these processes, however, little is known about the regulatory networks that control these processes.

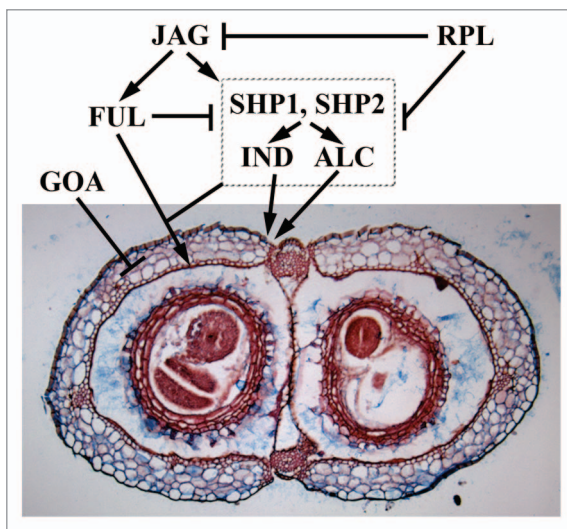


Figure 2. Patterning and growth during Arabidopsis fruit development. SHP1, SHP2, IND and ALC are necessary for patterning cells within the valve margin (vm). FUL represses the expression of these vm genes in the valves and RPL represses these vm genes in the replum. This sets up boundaries for patterning the various tissues of the fruit. JAG and other genes are necessary for proper FUL and vm gene expression. GOA represses fruit growth in the valves and FUL could repress GOA expression in the valves. Figure adapted from Dinneny et al. 2005.

formation within the Arabidopsis fruit.¹² FRUITFULL (FUL) is important for the proper differentiation of the valve cells and repressing the valve margin identity genes *SHATTERPROOF 1* and *2* (*SHP1/2*)¹³⁻¹⁵ (Fig. 2). *ful* fruits are indehiscent and the valve cells are small and lignified due to the ectopic expression of *SHP1/2*. *SHP1/2* are valve margin identity genes and *shp1 shp2* double mutants are indehiscent due to a lack of lignification of the valve margin cells.¹⁵ *INDEHISCENT* (*IND*) is also important for valve margin identity and *ind* fruits are indehiscent.¹⁶ *REPLUMLESS* (*RPL*) is required for replum identity and repressing *SHP1/2* in the replum.¹⁷ *rpl*

fruits have valve margin cells that develop in place of the replum due to the ectopic expression of *SHP1/2*. JAG and additional regulatory proteins activate both *FUL* and *SHP1/2*.¹² Further genetic interaction of these regulators with *GOA* should elucidate the regulatory frame work of fruit size control via coordinated cell proliferation and cell expansion (Fig. 2). Such studies should further reveal how organ size controlling mechanisms is linked to patterning and organogenesis.

Plant hormones likely act as morphogens that play a crucial role in the final size and shape of an organ.¹⁸ Several genes have been isolated that regulate cell

expansion (Fig. 1). *ARGOS*-like (*ARL*) promotes cell expansion and is induced by plant hormone brassinosteroids.¹⁹ BIG PETALp (*BPEp*), a bHLH transcription factor, inhibits cell expansion specifically in petals.²⁰ Although it has been suggested that the duration of cell proliferation is the main determinant of organ size and shape, several genes have been isolated that promote cell expansion and when mutated change the size and shape of lateral organs. Several molecular genetic studies in leaf growth have shown that cell expansion occurs in a polar direction. *ANGUSTIFOLIA* (*AN*) promotes polar cell expansion in the leaf width direction while *ROTUNDIFOLIA3* (*ROT3*) and the closely related *CYP90D1* promotes polar cell expansion in the leaf length direction independently of *AN*.²¹⁻²⁵ *ROT3* encodes a cytochrome P450 involved in brassinosteroid synthesis. *LONGIFOLIA 1* and *2* (*LNG1* and *LNG2*) belong to a small family of novel plant proteins.²⁶ *LNG1* and *LNG2* are important regulators of leaf growth by polar cell expansion. Interestingly, *LNG1* and *LNG2* promote longitudinal cell expansion independently of *ROT3* however *LNG1/2* and *ROT3* promote longitudinal cell expansion at the expense of transverse cell expansion (Fig. 1). *CYP78A9*, a member of the cytochrome P450 *CYP78A* family promotes fruit cell expansion in a longitudinal direction.²⁷ *ATHB13* is a homeobox leucine zipper protein that regulates leaf expansion by promoting transverse cell expansion.²⁸ Although individual growth genes have been identified, little is known about the interaction of these growth genes and the pathways that control overall organ size and shape. Our findings of a role for *GOA*, a transcription factor, controlling fruit size largely by regulating cell expansion presents a starting point to investigate its function in conjunction with known cell expansion regulators.¹ Intriguingly, overexpression of *GOA* deregulates cell expansion controlling genes such as *LNG1*, *LNG2* and *ROT3*.¹ Though perturbation in the level of these genes might reflect indirect regulatory action of *GOA*, the implication of *ROT3* in brassinosteroid signaling makes it tempting to speculate that *GOA* controls fruit size via brassinosteroid signaling, at least in part.



Figure 3. Schematic diagram displaying distribution patterns of putative cis-regulatory elements in the promoter region of *TT16* (predicted promoter 3.9 kb) and *GOA* (1.7 kb). These analyses were carried out using PLACE (www.dna.affrc.go.jp/PLACE; Plant Cis-acting Regulatory DNA Elements). Asterisks at vertical bars represents predicted putative cis-regulatory element present only in *pGOA* but not in *pTT16*.

It will be interesting to uncover the regulatory mechanisms by which *GOA* controls final fruit size. Identifying upstream regulators that control the dynamic spatial and temporal distribution pattern of *GOA* transcripts and downstream targets of *GOA* protein should reveal the regulatory framework of *GOA* action.

B-Sister Gene Duplication and Acquisition of New Regulatory Role

Duplicated genes can acquire new divergent functions by their ability to regulate a distinct set of downstream targets or by acquiring new domains of expression.²⁹ *TT16* and *GOA* are paralogs that have unique regulatory functions as reflected in their individual loss-of-function mutants, which suggest their unique targets.^{1,9} It is important to note that overexpression of either *TT16* or *GOA* results in reduced plant organ growth suggesting both transcription factors should have significant overlapping regulatory targets.^{1,9} Isolation and further characterization of overlapping yet distinct downstream targets of *TT16* and *GOA* should shed light on the evolutionary significance of B-sister gene duplication. Though the spatiotemporal expression pattern of *TT16* is yet not known, its functional analysis suggests

that some of *TT16* expression domains should be distinct from that of *GOA*.⁹ In accordance we find some distinct putative cis-regulatory elements present in the promoter of *GOA* but not in *TT16* and vice versa (Fig. 3). These predictive analyses exemplify how duplicated genes could acquire new domains of expression. Although the functional validation and importance of putative cis-elements unique to the *GOA* promoter still need to be characterized, the presence of potential binding sites (HD motif PCPR2) for Arabidopsis homeodomain protein PRHA and the MYB2AT motif of Persimmon DkMyb4 provide a starting point for unraveling the network involved in the *GOA* mediated control of fruit size.^{30,31} It would not be surprising if a combination of both, altered protein sequence and unique cis-regulatory sequences, are utilized by Arabidopsis B-sister duplicated paralogs to deliver new regulatory roles in addition to their conserved function.

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