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FLOWERING NEWSLETTER REVIEW

Auxin regulation of *Arabidopsis* flower development involves members of the AINTEGUMENTA-LIKE/PLETHORA (AIL/PLT) family

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Abstract

Auxin is an important regulator of many aspects of plant growth and development. During reproductive development, auxin specifies the site of flower initiation and subsequently regulates organ growth and patterning as well as later events that determine reproductive success. Underlying auxin action in plant tissues is its uneven distribution, resulting in groups of cells with high auxin levels (auxin maxima) or graded distributions of the hormone (auxin gradients). Dynamic auxin distribution within the periphery of the inflorescence meristems specifies the site of floral meristem initiation, while auxin maxima present at the tips of developing floral organ primordia probably mediate organ growth and patterning. The molecular means by which auxin accumulation patterns are converted into developmental outputs in flowers is not well understood. Members of the AINTEGUMENTA-LIKE/PLETHORA (AIL/PLT) transcription factor family are important developmental regulators in both roots and shoots. In roots, the expression of two AIL/PLT genes is regulated by auxin and these genes feed back to regulate auxin distribution. Here, several aspects of flower development involving both auxin and AIL/PLT activity are described, and evidence linking AIL/PLT function with auxin distribution in reproductive tissues is presented.

Key words: AIL/PLT proteins, auxin gradients, floral meristem initiation, floral organ identity, floral organ growth, gynoecium patterning.

Introduction

Recent advances in our understanding of the roles of auxin during plant development have resulted from a variety of approaches including genetic and molecular studies of mutants disrupted in auxin physiology, cellular imaging of auxin transport proteins, expression of auxin-responsive reporters, and the use of chemical inhibitors of auxin transport. Together these studies demonstrate that auxin gradients can be instructive for tissue patterning in embryos and roots, and suggest that auxin can act as a morphogen (reviewed in Benkova *et al.*, 2009). For example, a graded distribution of auxin within the root tip acts to specify and maintain the root apical meristem and, correspondingly, alteration of this gradient disrupts regional cell fate patterning (Sabatini *et al.*, 1999). In addition, auxin can act as a

trigger for the specification of lateral organ founder cells and subsequent primordium outgrowth (reviewed in Benkova *et al.*, 2009). Auxin accumulation in pericycle cells specifies the site of lateral root initiation (Benkova *et al.*, 2003) while auxin accumulation in groups of cells in the periphery of the shoot apical meristem specifies the site of leaf or floral primordium initiation (Reinhardt *et al.*, 2000).

In addition to floral meristem initiation, auxin regulates other aspects of flower development including floral organ initiation, growth, and patterning, and later events that ensure reproductive success of the mature flower (reviewed in Nemhauser *et al.*, 1998; Cheng and Zhao, 2007; Sundberg and Ostergaard, 2009). Several mutants disrupted in either auxin biosynthesis, transport, or signalling exhibit

flowering defects that are variable but typically involve alterations in organ numbers, organ spacing, and gynoecium morphology. While these studies clearly indicate the importance of auxin accumulation during flower development, it remains to be determined how auxin gradients within floral meristems and developing floral organ primordia regulate pattern formation.

Recent data have linked several auxin-regulated processes during flower development with the functions of *Arabidopsis* AINTEGUMENTA-LIKE/PLETHORA (AIL/PLT) transcription factors that are members of the larger APETALA2/ETHYLENE RESPONSE FACTOR (AP2/ERF) family. The AIL/PLT gene family consists of eight members, five of which are expressed in distinct but overlapping domains within inflorescences: *AINTEGUMENTA* (*ANT*), *AIL1*, *AIL5*, *AIL6/PLT3*, and *AIL7* (Nole-Wilson *et al.*, 2005). Partially overlapping functions for some of these genes are beginning to be revealed. While *ant* single mutants primarily show defects in floral organ number and size (Elliott *et al.*, 1996; Klucher *et al.*, 1996), *ant ail6* flowers have more dramatic defects in floral organ number, size, identity, and position (Krizek, 2009) (Fig. 1A, D). In addition, *ant ail6* plants exhibit decreased apical dominance, reduced stature, and altered vascular patterning, phenotypes similar to those found in plants disrupted in auxin physiology (Krizek, 2009). Altered expression of the auxin-responsive reporter *AGH3-2:GUS* in *ant ail6* inflorescence meristems and flowers suggests that these floral defects may be a consequence of altered patterns of auxin accumulation and/or responsiveness (Krizek, 2009); however, the nature of the relationship between AIL/PLT transcriptional regulators and auxin within flowers is not clear.

It is useful to consider the functions of related AIL/PLT genes in the root which appear to act downstream of auxin

accumulation. Four AIL/PLT genes are required for root development: *PLT1*, *PLT2*, *AIL6/PLT3*, and *BBM* (Aida *et al.*, 2004; Galinha *et al.*, 2007). *PLT1* and *PLT2* are regulated at the transcriptional level by auxin, through the direct or indirect action of AUXIN RESPONSE FACTORS (ARFs) (Aida *et al.*, 2004), which mediate auxin-regulated gene expression (reviewed in Chapman and Estelle, 2009). Two ARFs, MONOPTEROS (MP)/ARF5 and NPH4/ARF7, have been implicated in *PLT1* and *PLT2* gene regulation (Aida *et al.*, 2004). In addition to being regulated by auxin, *PLT1* and *PLT2* activity feeds back to regulate auxin distribution, helping to stabilize an auxin maximum in the root tip (Blilou *et al.*, 2005). AIL/PLT activity is detected in a gradient along the longitudinal axis of the root where it appears to be instructive for distinct cell behaviours (Galinha *et al.*, 2007). One exciting idea is that auxin gradients within the root are converted to a gradient in AIL/PLT activity which mediates root patterning and growth (Galinha *et al.*, 2007).

Auxin maxima specify the site of floral meristem initiation

During reproductive development, floral meristems are initiated in a precise and reiterative manner around the periphery of the inflorescence meristem. These initiation sites correspond to transient auxin maxima that probably result from both local biosynthesis and directional transport of the hormone within the shoot apex (Reinhardt *et al.*, 2003; Heisler *et al.*, 2005; Cheng *et al.*, 2006). Biosynthesis of the major auxin in plants, indole acetic acid (IAA), is thought to occur through multiple tryptophan-dependent pathways and a tryptophan-independent pathway, none of which has been fully characterized (reviewed in Woodward and Bartel, 2005). Genes encoding several auxin biosynthetic enzymes are expressed in the inflorescence meristem and flowers, and are likely to contribute to the production of auxin maxima/gradients within these tissues (Cheng *et al.*, 2006; Stepanova *et al.*, 2008). Auxin distribution within the inflorescence apex also involves polarly localized PINFORMED (PIN) proteins, auxin effluxers through which anionic IAA exits the cell (Galweiler *et al.*, 1998; Reinhardt *et al.*, 2003), and auxin influx carriers (AUX1, LAX1, LAX2, and LAX3) which mediate active uptake of IAA (Bainbridge *et al.*, 2008). Reversals in the polarity of PIN localization underlie the dynamic cycles of auxin accumulation and depletion within the inflorescence meristem periphery (Heisler *et al.*, 2005). PIN1 polarity is regulated by the protein kinase PINOID (PID) and the protein phosphatase PP2A (Christensen *et al.*, 2000; Friml *et al.*, 2004; Michniewicz *et al.*, 2007).

Auxin is both required and sufficient for floral meristem initiation, as demonstrated by the absence of flower initiation in *pin1* mutants and the rescue of this phenotype by application of auxin paste to *pin1* shoot apices (Okada *et al.*, 1991; Reinhardt *et al.*, 2000). The site of primordium initiation within the periphery of the shoot apical meristem

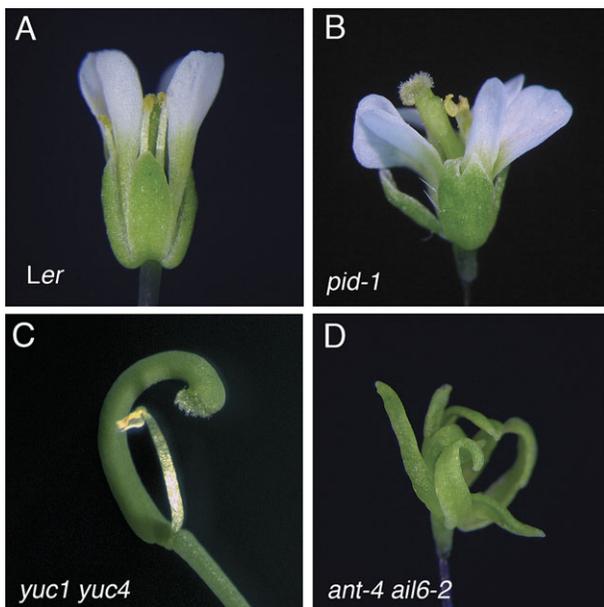


Fig. 1. Flowers of *Arabidopsis* wild-type plants and mutants disrupted in auxin physiology or AIL/PLT gene function. (A) *Ler* (wild type), (B) *pid-1*, (C) *yuc1 yuc4*, and (D) *ant-4 ail6-2*.

occurs at the location of auxin application, indicating that auxin specifies primordium positioning (Reinhardt *et al.*, 2000). In *pin1* mutants, the shoot continues to grow in the absence of floral meristem initiation, leading to the development of a naked pin-like inflorescence (Okada *et al.*, 1991). Similar pin-like inflorescences are produced in other backgrounds in which auxin transport, signalling, or biosynthesis is disrupted: *pid* mutants, wild-type plants treated with the auxin transport inhibitor *N*-1-naphthylphthalamic acid (NPA), *mp* mutants, and in plants with mutations in four *YUCCA* (*YUC*) auxin biosynthetic genes. Pin-like inflorescences are also produced in *npyl npy3 npy5* triple mutant plants, although the exact role of *NPY* genes in auxin physiology is not known (Cheng *et al.*, 2008).

AIL/PLT proteins and floral meristem initiation

The expression pattern of several *AIL/PLT* genes is correlated (either positively or negatively) with auxin distribution in the inflorescence meristem. *ANT* expression is associated with incipient floral primordia (Elliott *et al.*, 1996) that correspond to auxin maxima (Benkova *et al.*, 2003; Reinhardt *et al.*, 2003; Heisler *et al.*, 2005). While *AIL5* and *AIL6* mRNA are distributed more broadly in the inflorescence meristem, both genes are up-regulated in floral anlagen (Nole-Wilson *et al.*, 2005). *AIL7* mRNA is restricted to the central region of the inflorescence meristem in a pattern inversely correlated with auxin accumulation (Nole-Wilson *et al.*, 2005). Whether these hormone and transcript distribution patterns reflect a causal relationship between auxin and *AIL/PLT* regulation awaits further studies. Further supporting a role for auxin in *ANT* regulation are the observations that *ANT* expression within the inflorescence meristem is altered in *pin1* mutants (Vernoux *et al.*, 2000) and in plants treated with NPA (Krizek, 2009).

While the expression patterns of *ANT*, *AIL5*, and *AIL6* suggest roles for these genes in floral meristem initiation downstream of auxin, genetic evidence has so far been lacking. *ant*, *ail5*, and *ail6* single mutants show no defects in floral meristem initiation (Elliott *et al.*, 1996; Klucher *et al.*, 1996; Nole-Wilson *et al.*, 2005; Krizek, 2009). While *ant ail6* double mutants do exhibit some inflorescence meristem defects, these plants initiate a large number of flowers prior to growth arrest of the entire shoot apex (Krizek, 2009). This is quite distinct from *pin1* mutants in which the shoot apex continues to grow in the absence of primordium initiation (Okada *et al.*, 1991). However, recent evidence supports functions for both *ANT* and *AIL6* in floral meristem initiation. While *pid-1* and *pid-2* make a few flowers prior to termination of floral initiation (Bennett *et al.*, 1995), no flowers are initiated in *ant ail6 pid-1* or *ant ail6 pid-2* triple mutants (BAK, unpublished results). Additionally, floral meristem initiation is terminated in *ail6* mutants treated with 10 μ M NPA, a concentration that has

no effect on flower initiation in wild-type *Arabidopsis* inflorescences (BAK, unpublished results).

Auxin regulates floral organ number and positioning

A wild-type *Arabidopsis* flower consists of four types of floral organs that arise in concentric whorls: four sepals in the outermost whorl one, four petals in whorl two, six stamens in whorl three, and two carpels are fused in the fourth whorl to form the female gynoecium (Fig. 1A). Floral organ primordia are initiated in precise positions within these whorls. For example, four sepal primordia arise in a cross pattern equidistant from each other in whorl one, while in the second whorl four petal primordia arise just inside and between adjacent sepal primordia. While strong disruptions in auxin physiology can preclude flower formation, many mutants make a few abnormal flowers prior to this termination. Although the phenotypes of these flowers vary considerably (Fig. 1B, C), they all exhibit alterations in floral organ number and positioning together with gynoecium defects. Defects in gynoecium patterning will be discussed later. As auxin is required for lateral organ positioning and primordium outgrowth from the shoot apical meristem, it probably plays a role in the somewhat analogous process of floral organ positioning and primordium outgrowth from floral meristems. However, there are two important differences between shoot and floral meristems in *Arabidopsis*. Lateral organs arise with spiral phyllotaxis within the shoot apical meristem while floral organs arise with whorled phyllotaxis within the floral meristem. Since multiple organ primordia are initiated simultaneously in the periphery of the floral meristem, several auxin maxima would have to be generated concurrently. Observed PIN1 localization in regions corresponding to incipient sepal and stamen primordia in *Arabidopsis* floral meristems appears to be consistent with this model (Reinhardt *et al.*, 2003). In addition, floral meristems are determinate structures in which all meristematic cells are consumed in the initiation of floral organ primordia while shoot apical meristems are indeterminate. So rather than auxin maxima being created in a regular and self-sustaining manner in the periphery of the shoot apical meristem, the specification of carpel primordia in the centre of the floral meristem would involve the generation of a final auxin maximum in the remaining meristematic cells.

The effects on floral organ number vary for different classes of mutants disrupted in auxin physiology. Mutations in gene-encoding components of the auxin transport system such as *PIN* and *PID* produce a few flowers that typically have fewer sepals, stamens, and carpel valves than the wild type, but more petals (Bennett *et al.*, 1995) (Fig. 1B). In contrast, mutations in genes encoding auxin biosynthetic enzymes, such as the *Arabidopsis* *YUC* genes and petunia *YUC* homology *FLOOZY* (*FZY*) result in flowers with very few floral organs (Tobena-Santamaria *et al.*, 2002; Cheng *et al.*, 2006). *yuc1 yuc4* flowers typically consist of one to a

few outer whorl organs of variable identity and an abnormal gynoecium (Cheng *et al.*, 2006) (Fig. 1C). Mutations in *FZY* result in flowers that consist solely of a sepal-like organ and two carpels (Tobena-Santamaria *et al.*, 2002). Dramatic reductions in floral organ number are also observed in the few flowers formed in *mp* mutants, which typically consist of one or two carpels (Przemeck *et al.*, 1996). However, defects in another *AUXIN RESPONSE FACTOR*, *ETTIN (ETT)/ARF3*, result in flowers with increased numbers of sepals and petals but reduced numbers of stamens and abnormal gynoecia (Sessions, 1997; Sessions *et al.*, 1997). In mutants such as *pid* and *ett* that make significant numbers of floral organs, the relative spacing and position of organ primordia is also altered (Bennett *et al.*, 1995; Sessions, 1997; Sessions *et al.*, 1997).

These mutant phenotypes indicate that local auxin biosynthesis, auxin distribution, and auxin responsiveness within floral meristems are all critical for floral organ initiation and positioning. The dramatic reductions in floral organ number observed in auxin biosynthetic mutants probably indicate that most floral meristem cells possess auxin levels below a threshold concentration required for founder cell specification and primordium outgrowth. Occasionally auxin may accumulate to a level above that required for organ initiation, but the exact position of this auxin gradient within the floral meristem is random, leading to variability in the identity of the floral organ(s) that are formed. In other mutants such as *pid* and *ett* that exhibit increases in the numbers of some floral organs, alterations in auxin distribution presumably lead to the formation of more but incorrectly spaced auxin maxima in some whorls and fewer auxin maxima in other whorls. It has been difficult to examine auxin distribution in early stages of flower development using currently available technologies. First of all it is technically challenging to image floral meristems that are hidden as sepal primordia grow to enclose the developing flower. Secondly, the long half-life of reporters such as green fluorescent protein (GFP) obscures dynamic changes in auxin accumulation. In addition, the synthetic *DR5* promoter element often used to characterize the auxin response is also induced by brassinosteroids (Nakamura *et al.*, 2003; Nemhauser *et al.*, 2004). A challenge for future work will be the development of improved auxin-responsive reporters and biosensors for visualizing auxin accumulation directly in living plant tissues during development (Jaillais and Chory, 2010).

ANT and AIL6 regulate floral organ number, position, and identity

Mutations in *ANT* and *AIL6* resemble the mutants described above in several ways. *ant ail6* flowers consist of fewer and smaller floral organs, typically four sepals, one to two filamentous or flat organs of undefined identity, and two unfused carpel valves (Krizek, 2009) (Fig. 1D). The spacing of sepal primordia in the periphery of the floral meristem is random, and the remaining organ primordia do

not arise with any regular positioning. Once initiated, lateral organ primordia exhibit an auxin maximum at their tip that presumably guides subsequent growth and patterning (Benkova *et al.*, 2003). In *ant ail6* flowers, expression of the auxin-responsive reporter *AGH3-2:GUS* is no longer confined to the tips of developing floral organ primordia but is often detected throughout these primordia (Krizek, 2009). The presence of filamentous or flat organs that do not resemble any normal floral organ in *ant ail6* flowers suggests defects in floral organ identity specification that are not observed in mutants disrupted in auxin physiology.

According to the ABCE model, floral organ identity is specified by the combined action of four classes of floral organ identity genes (A, B, C, and E) that act in distinct regions within a floral meristem (reviewed in Krizek and Fletcher, 2005). The following combinations of class A, B, C, and E activities: AE, ABE, BCE, and CE, specify sepal, petal, stamen, and carpel identity, respectively, in whorls one to four. The A and C functions are mutually antagonistic, acting to repress each other in their respective domains. Loss of either the class A, B, or C genes results in homeotic transformations in floral organ identity, while loss of either the class E activity alone or the ABC activities together produce flowers composed solely of leaf-like organs (Bowman *et al.*, 1991a; Ditta *et al.*, 2004). Thus these gene activities act upon a leaf-like ground state to confer distinct floral organ identities. The floral organ identity genes are expressed throughout floral organ development where they are continuously required for proper organ development (Bowman *et al.*, 1989, 1991b). These genes encode transcriptional regulators that appear to regulate distinct sets of target genes at different times during organ development (reviewed in Ito, 2011).

ant ail6 flowers do not exhibit homeotic transformations but rather produce some organs that lack any distinct floral organ identity (Krizek, 2009). Some are filamentous and can be swollen at their apex and thus stamen-like, while others are flat green laminar structures. Many arise between the sepals and carpel valves. These organs might be the consequence of insufficient floral organ identity gene activity and/or the loss of this activity during development. Expression of class B and class C genes is reduced and spatially altered in early floral meristems and often absent in many developing organ primordia (Krizek, 2009). A low level of floral organ identity gene activities may preclude leaf development but be insufficient to confer petal or stamen identity. *ANT* and *AIL6* thus contribute to both the initiation and maintenance of floral organ identity gene expression.

Floral organ identity genes regulate auxin homeostasis

While mutants disrupted in auxin physiology do not exhibit alterations in floral organ identity, several pieces of evidence suggest tight coordination between specification of floral organ identity and auxin accumulation and signalling

during floral organogenesis. Genome-wide analysis of *in vivo* binding sites of the class E protein SEPALLATA3 (SEP3) identified genes encoding the auxin conjugation enzyme GH3.3, auxin transport proteins (PID and PIN4), auxin signalling proteins (including ETT, ARF6, ARF8, and IAA4), and transcription factors that regulate auxin biosynthesis during carpel development [STYLISH1 (STY1) and NGA1] (Kaufmann *et al.*, 2009). Expression of a dominant repressor form of SEP3 in transgenic *Arabidopsis* plants produced phenotypes similar to *ett* mutants, further supporting an *in vivo* role for SEP3 in mediating auxin responses (Kaufmann *et al.*, 2009). These studies also identified auxin response elements (AuxREs) as being overrepresented in genomic regions bound by SEP3, suggesting that ARFs (which bind to AuxREs) act in combination with SEP3 to regulate gene expression during flower development. These results indicate close association between master regulators of organ identity and auxin signalling pathways that mediate organ growth and patterning.

Auxin and ANT regulate gynoecium patterning

Gynoecium patterning and the role of auxin in this process have been reviewed previously (Balanza *et al.*, 2006; Ostergaard, 2009; Staldal and Sundberg, 2009). Here, several aspects of gynoecium development that involve both auxin and ANT are described. The female gynoecium of *Arabidopsis* is composed of two congenitally fused carpels that arise as a hollow tube from the centre of the floral meristem. Elaboration of distinct tissues within the gynoecium occurs along three axes: apical–basal, adaxial–abaxial, and medial–lateral (Fig. 2A, B). The apical–basal axis consists of a short stalk-like structure called the gynophore on which a two-chambered ovary topped with a style and stigma is positioned (Fig. 2A). A cross-section of the gynoecium through the ovary reveals lateral (valve and valve margin) and medial tissues, which can be further characterized as abaxial (replum) or adaxial (septum, placenta, ovules, and transmitting tract) (Fig. 2B). Meristematic tissue derived from the medial domain gives rise to the adaxial-positioned medial tissues and also contributes to apical tissues (stigma and style) (Bowman *et al.*, 1999).

Auxin plays a critical role in patterning along the apical–basal axis of the gynoecium. In both *ett* mutants and wild-type flowers treated with NPA, central ovary tissue is lost and replaced by more apical (stigma and style) and basal (gynophore) tissues (Sessions *et al.*, 1997; Nemhauser *et al.*, 2000). A similar loss of ovary identity and expanded regions of apical and basal tissues are observed in auxin transport mutants (*pin* and *pid*) and in auxin biosynthetic mutants (*yuc* and *taal*) (Okada *et al.*, 1991; Bennett *et al.*, 1995; Cheng *et al.*, 2006; Stepanova *et al.*, 2008). Nemhauser and colleagues proposed the existence of an auxin gradient along the apical–basal axis of the gynoecium in which auxin synthesized at the apical end of the primordium is transported downward (Nemhauser *et al.*, 2000). In this

model, high auxin levels specify stigma and style identity, intermediate auxin levels specify ovary identity, and lower auxin levels specify gynophore identity. Disruptions in auxin transport result in pooling of auxin at the apex of the primordium and consequently decreased auxin concentrations in lower regions, leading to the observed phenotypes.

This model is supported by the detection of auxin maxima at the tips of developing gynoecium primordia (Benkova *et al.*, 2003). Furthermore, several members of the *SHORT-INTERNODES/STYLISH* (*SHI/STY*) gene family that are expressed in apical regions of the developing gynoecium promote auxin biosynthesis through regulation of *YUC4* expression. (Kuusk *et al.*, 2006; Sohlberg *et al.*, 2006). STY1 acts as a transcriptional activator that binds directly to the *YUC4* promoter (Eklund *et al.*, 2010). *sty1 shi* and other mutant combinations involving additional members of the *SHI/STY* family show reductions in the amount of stigma and style tissues (Kuusk *et al.*, 2006). The style fusion defects in *sty1 sty2* can be rescued by treatment with exogenous auxin, further suggesting that reduced auxin level is the cause of the phenotypes observed in these mutants (Staldal *et al.*, 2008). Other factors that may act in parallel with SHI/STY to promote *YUC* expression in the gynoecium apical region are the NGATHA B3 domain transcription factors. Quadruple *nga* mutants completely lack style and stigma tissue, and different *nga* mutant combinations show reductions in several classical auxin responses (root gravitropism, lateral root formation, and apical dominance) (Alvarez *et al.*, 2009; Trigueros *et al.*, 2009).

ant gynoecia display several defects suggestive of alterations in auxin homeostasis. They occasionally show apical fusion defects (Elliott *et al.*, 1996) that can be rescued upon NPA application (Staldal *et al.*, 2008). Furthermore, they

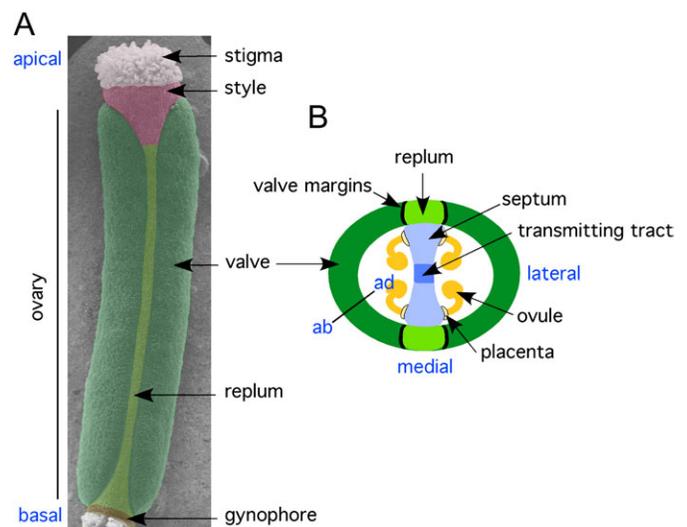


Fig. 2. Tissues types in an *Arabidopsis* gynoecium. (A) Scanning electron micrograph of an *Arabidopsis* gynoecium showing elements along the apical–basal axis: stigma, style, ovary, gynophore. (B) Transverse section through the ovary showing the adaxial (ad)–abaxial (ab) and medial–lateral axes. The adaxial medial domain gives rise to the septum, placenta, ovules, and transmitting tract.

produce reduced numbers of ovules (Elliott *et al.*, 1996; Klucher *et al.*, 1996) and exhibit alterations in gynoecium vascular patterning (Nole-Wilson *et al.*, 2010), phenotypes that mimic effects seen upon NPA treatment of wild-type gynoecia (Nemhauser *et al.*, 2000). In wild-type *Arabidopsis* gynoecia, four internal vascular bundles run the length of the ovary. The two lateral veins terminate at the boundary between the ovary and style, while the two medial veins bifurcate at this boundary into a fan-like arrangement. Basalized medial vein bifurcation is observed in NPA-treated wild-type gynoecium and in *ant*, *styl1*, and *nga3 nga4* mutants (Nemhauser *et al.*, 2000; Kuusk *et al.*, 2002; Trigueros *et al.*, 2009; Nole-Wilson *et al.*, 2010). Finally, the expression of two auxin-induced *AUX/IAA* genes is reduced in *ant* mutants, and *ant* gynoecium displays an enhanced sensitivity to NPA with regard to loss of ovary tissue (Nole-Wilson *et al.*, 2010).

Severe defects in gynoecium patterning along the medial–lateral axis are observed in several *ant* double mutant combinations including *ant ail6*, *ant leunig (lug)*, *ant seuss (seu)*, *ant filamentous flower (fil)*, and *ant shatterproof1 (shp1) shatterproof2 (shp2) crabs claw (crc)* (Liu *et al.*, 2000; Nole-Wilson and Krizek, 2006; Azhakanandam *et al.*, 2008; Krizek, 2009; Colombo *et al.*, 2010). These mutants typically produce two carpel valves that are unfused along most or some of their entire length. There is a complete or nearly complete loss of all medially derived adaxial tissues including septum, placenta, ovules, and transmitting tract, and severe reductions in the amount of stylar and stigmatic tissue. Previous work supports roles for both *LUG* and *SEU* in auxin-mediated growth and patterning. *seu* mutants display classical defects in auxin physiology such as loss of apical dominance, reduced lateral root initiation, and decreased expression of the auxin-responsive reporter *DR5:GUS* (Pfluger and Zambryski, 2004). Furthermore, *SEU* is a transcriptional co-regulator that can physically interact with ETT (Pfluger and Zambryski, 2004) and with *LUG* (Sridhar *et al.*, 2004). Mutations in *STYLOSA (STY)*, the *Antirrhinum LUG* homologue, result in vascular patterning defects and enhanced sensitivity to NPA treatment (Navarro *et al.*, 2004). While treatment of wild-type gynoecium with NPA typically results in loss of ovules, in rare cases a more severe loss of all medial domain-derived adaxial tissue was observed (Nole-Wilson *et al.*, 2010). Taken together these data suggest that auxin plays a key role in patterning along the medial–lateral axis of the gynoecium (Nole-Wilson *et al.*, 2010). Consistent with this model, expression of the auxin biosynthetic enzyme *TAA1* is localized within the medial domain of the gynoecium during early gynoecial development (Stepanova *et al.*, 2008) and *TAA1* expression is significantly reduced in *ant* mutants (Nole-Wilson *et al.*, 2010). Much later during the maturation of the ovary into a fruit, auxin has been shown to specify tissue patterning within the medial–lateral axis. The formation of an auxin minimum in the valve margins is required for formation of a separation layer and silique opening (dehiscence) in *Arabidopsis* (Sorefan *et al.*, 2009).

ANT promotes floral organ growth downstream of auxin

ANT is both necessary and sufficient for lateral organ growth and appears to function downstream of auxin in this role. While mutations in *ANT* result in flowers with smaller floral organs, constitutive expression of *ANT* (i.e. *35S:ANT*) produces flowers with larger floral organs (Elliott *et al.*, 1996; Klucher *et al.*, 1996; Krizek, 1999; Mizukami and Fischer, 2000). It has been proposed that *ANT* acts to maintain meristematic competence during organogenesis (Mizukami and Fischer, 2000). Genetic studies indicate that *ANT* acts downstream of the auxin-inducible gene *ARGOS* (auxin-regulated gene involved in organ size), which acts downstream of *AUXIN-RESISTANT1 (AXR1)* (Hu *et al.*, 2003). Similar to *ANT*, loss- and gain-of-function *ARGOS* plants display opposite effects on lateral organ growth. *ANT* activity is required for the increased size of *35S:ARGOS* lateral organs and *35S:ARGOS* can partially compensate for the organ growth defects observed in *axr1* mutants (Hu *et al.*, 2003). Further linking *ANT* function in organ control growth with auxin, *ANT* expression in maturing organs is repressed by *ARF2*, a negative regulator of organ growth (Schruff *et al.*, 2005). *AIL6* also promotes floral organ growth as *ant ail6* double mutants exhibit more severe defects in organ size than *ant* single mutants (Krizek, 2009). *ant ail6* plants produce smaller flowers than loss-of-function *ARGOS* plants, suggesting that *AIL6* acts in a parallel pathway rather than downstream of *ARGOS*.

Conclusion

Auxin and AIL/PLT transcription factors play critical roles in several aspects of flower development, including floral meristem initiation and floral organ initiation, growth, and patterning. In floral meristem initiation, *ANT* and *AIL6* probably act downstream of auxin to promote floral primordium outgrowth in founder cells specified by an auxin maximum. Evidence also suggests that *ANT* acts downstream of auxin in regulation of floral organ size. However, complementation of the gynoecium apical fusion defect of *ant* mutants by NPA suggests that in this process auxin acts downstream of *ANT* or in a parallel pathway (Staldal *et al.*, 2008). It is possible that *ANT* and *AIL6* are targets of auxin signalling pathways but also act back to regulate auxin levels and/or distribution like AIL/PLT proteins in the root (Aida *et al.*, 2004; Blilou *et al.*, 2005). *PIN4*, *PIN3*, and *PIN7* expression is reduced in *plt1 plt2* roots, suggesting that AIL/PLT proteins control auxin distribution in roots through regulation of *PIN* expression (Blilou *et al.*, 2005). Whether AIL/PLT transcription factors regulate *PIN* expression in shoots is not known. A role for AIL/PLT proteins in auxin distribution in shoots is supported by the altered expression of an auxin-responsive reporter in *ant ail6* floral organs (Krizek, 2009), although this could be an indirect effect of the altered growth of these primordia. AIL/PLT proteins probably contribute to the maintenance of

auxin gradients during floral organogenesis while also regulating growth and pattern in response to auxin maxima.

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