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Journal:	<i>WIREs Developmental Biology</i>
Manuscript ID:	Draft
Wiley - Manuscript type:	Advanced Review
Date Submitted by the Author:	n/a
Complete List of Authors:	Sehra, Bhupinder; North Carolina State University, Franks, Robert; North Carolina State University, Plant and Microbial Biology
Keywords:	Auxin transport, Carpel margin meristem, Arabidopsis gynoecium, PINFORMED, PGP/ABCB type auxin transporters
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**Article type:** Advanced Review

## **Auxin and cytokinin act during gynoecial patterning and the development of ovules from the meristematic medial domain**

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Authors declare no conflicts of interest

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### **Abstract**

The gynoecium is the female reproductive structure of flowering plants, and is the site of ovule and seed development. The gynoecium is critical for reproductive competence and for agricultural productivity in many crop plants. In this review we focus on molecular aspects of the development of the *Arabidopsis thaliana* gynoecium. We briefly introduce gynoecium structure and development and then focus on important research advances published within the last year. We highlight what has been learned recently with respect to: 1) the role of auxin in the differential development of the medial and lateral domains of the *Arabidopsis* gynoecium; 2) the interaction between cytokinin and auxin during gynoecial development; 3) the role of auxin in the termination of the floral meristem and in the transition of floral meristem to gynoecium and 4) recent studies that suggest a degree of evolutionary conservation of auxin mechanisms during gynoecial development in other eudicots.

## Introduction

### Structure of the mature *Arabidopsis* gynoecium

The *Arabidopsis thaliana* gynoecium is made up of two fused carpels, generating a tube-like structure<sup>1-6</sup>. Along its apical to basal extent, the mature *Arabidopsis* gynoecium can be divided into four structurally and functionally different regions: the stigma, the style, the ovary and the gynophore (Figure 1A). The most apically located structure, the stigma, allows for pollen to be received and to germinate. The stigma and the style together comprise the upper-most portion of the transmitting tract. The transmitting tract is a specialized tissue that supports pollen tube growth and allows the pollen cell to reach the ovules located in the ovary. The gynophore, the most basally located portion of the gynoecium, attaches the gynoecium to the rest of the plant.

The ovary makes up the largest portion of the gynoecium. Within the ovary, about 50 ovules will form attached to a septum that separates the ovary into two locules (Figure 1B). Each ovule contains a female gametophyte, a multi-cellular haploid structure that includes both the egg cell and the central cell. After a double fertilization event, the egg cell will form into the embryo and the central cell into the endosperm. Thus the fertilized ovules mature into seeds containing embryo and the endosperm components.

Unlike the stigma, style and gynophore regions that are radially symmetric, the ovary is bilaterally symmetric to an imaginary plane that bisects the septum (Figure 1B). The ovary can be divided along this axis of symmetry into medial positions (those close to the axis of symmetry) and lateral positions (further from the axis)(Figure 1C). Within the mature gynoecium the structures located in the lateral positions are termed valves and make up the majority of the wall of the gynoecial tube. The ovules, septum and abaxial replum are located in medial positions. The septum contains the lower portions of the transmitting tract that are required for pollen tube growth and ovule fertilization. Late in development of the maturing fruit, cells of the valve margins, (where the valves meet the abaxial replum) differentiate into a dehiscence zone (Figure 1). Cells of the dehiscence zone undergo a set of specialized differentiation programs so that the valves can separate from the replum (dehisce) and allow seed dispersal<sup>7</sup>.

### Development of the *Arabidopsis* Gynoecium

The *Arabidopsis* gynoecium arises as a single, mound-shaped primordium in the center of the floral meristem, thus marking the beginning of stage 6 of floral development<sup>6,8</sup>. Floral stages are according to Smyth et al.<sup>8</sup>. At this early stage of development a degree of positional identity has already been assigned that distinguishes the medial and lateral regions of the primordium<sup>1</sup>. Different positional identities are evidenced by the expression of several genes including *CRABSCRAW* (*CRC*) and *YABBY1* (*YAB1*)<sup>9-11</sup> in the lateral domains and *SHOOTMERISTEMLESS* (*STM*) and *SHATTERPROOF2* (*SHP2*)<sup>1,12,13</sup> in the medial domain (Figure 1E,F). These different positional domains will give rise to different functional structures. Although a careful clonal analysis of the developmental progression has not been published, based on patterns of cell division and gene expression, the lateral domains are thought to give rise to the valves while the medial domain gives rise to the ovules, the septum and the abaxial replum.

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3 Early during floral stage 7, the mound shaped gynoecial primordium begins to morph into a tube-  
4 shaped structure that is still open at the apex<sup>8</sup>. Inside the tube, two meristematic ridges of tissue  
5 that span the apical-basal extent of the gynoecium form within the medial domain (Figure 1 C, D)<sup>1</sup>.  
6 These have been termed the medial ridges or alternatively the carpel margin meristems (CMMs).  
7 The meristematic nature of the medial ridge is indicated by the expression of *STM*<sup>12</sup> (Figure 1F), a  
8 marker of meristematic regions, as well as by the ability of the cells of the medial ridge to give rise to  
9 organ primordia (e.g. ovules). Ovule primordia arise from the medial ridge during late stage 8, as  
10 finger-like projections along the apical-basal extent of the ridge. These ovules continue to mature  
11 until stage 12 when the flower opens and the ovules are competent to be fertilized. Style and  
12 stigmatic tissues begin to differentiate in apical positions starting at late stage 9<sup>1,6</sup>.  
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### 16 **Evolutionary origin of the meristematic medial domain**

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18 The *Arabidopsis* gynoecium is typically represented as a composite structure derived from the  
19 congenital fusion of two component carpels (Figure 1B)<sup>14</sup>. In an evolutionary sense the two carpels  
20 are thought to be modified leaves<sup>15,16</sup>. In this representation the margins of the two component  
21 carpels are seamlessly fused along the medial domain, and the medial domain is thought to  
22 represent the marginal regions of the component carpels. In this case the medial domain of the  
23 gynoecium would be evolutionarily derived from the leaf margin and the generation of meristematic  
24 fates in this position would likely reflect a developmental redeployment of the meristematic  
25 program along the organ margin.  
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29 An alternative possibility is that the ancestral Angiosperm carpel was ascidiate (cup-shaped)<sup>17</sup> and in  
30 *Arabidopsis* the meristematic medial ridge that sits between the two carpels would reflect tissue  
31 that is derived from a shoot or an axis. In this representation, the meristematic medial domain  
32 would arise directly from the terminating floral meristem or could arise in an axillary position  
33 relative to the two component carpels. In either of these cases, the meristematic identity of the  
34 medial domain would not reflect a re-deployment at the leaf margin, but would be more similar to  
35 an inflorescence branch meristems forming in the axil of a rosette leaf or a floral meristem forming  
36 in the axil of a subtending bract. Although the “modified leaf margin” representation is much  
37 favored in the current literature, the matter is not fully resolved. Future investigations of the  
38 molecular developmental mechanisms of female reproductive meristem development in basal  
39 angiosperms and gymnosperms may help to resolve this question.  
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### 43 **Distinctive patterns of auxin transport and response differentiate lateral gynoecial domains from** 44 **the meristematic medial domain**

#### 45 **The auxin gradient model**

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47 A variety of experiments over the past several decades indicate that proper synthesis, transport and  
48 response to the plant hormone auxin are required within the developing gynoecium for proper  
49 development and female reproductive competence (See sidebar). See Larsson *et al.* and Dresselhaus  
50 and Schneitz for a recent reviews<sup>18,19</sup>. One set of key experiments were those published by  
51 Nemhauser *et al.*<sup>20</sup> in which developing gynoecia were transiently treated with the auxin transport  
52 inhibitor 1-N-naphthylphthalamic acid (NPA)(See sidebar). An analysis of the resulting morphological  
53 defects demonstrated an important role for auxin transport in the patterning of the gynoecium and  
54 lead to a model that proposed that patterning along the apical/basal axis of the gynoecium required  
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3 the formation and action of an auxin gradient along this axis of the developing organ. In this model,  
4 high levels of auxin synthesis would be found at the apex of the gynoecium. Then through the action  
5 of basipetal (toward the base) transport of auxin, a gradient would form with high auxin  
6 concentrations at the apex and low concentrations at the base. The response of the cells to different  
7 threshold levels of auxin along this gradient would lead to the formation of gynophore, ovary, style  
8 and stigmatic tissues along the basal to apical extent of the gynoecium. Later efforts to visualize this  
9 auxin response gradient with the DR5-based auxin response reporters (see side bar) did reveal an  
10 auxin response maximum at the gynoecial apex, but no gradient of auxin response could be detected  
11 in more basal regions, thus calling into question tenets of this model<sup>18, 21-24</sup>

### 14 **Early patterns of auxin response differ in medial and lateral gynoecial domains**

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17 In 2014, two papers were published that carefully examined the patterns of auxin transport, and  
18 auxin response in the developing gynoecia<sup>21, 22</sup>. Larsson et al. (2014) report the formation of two  
19 DR5 auxin response foci at the apex of the stage 5/6 gynoecium, one focus located within each  
20 lateral domain<sup>21</sup>. These DR5 foci likely represent the positions of the individual carpel primordia as  
21 they are forming. Two additional apical foci located in the medial domain can be detected, however  
22 these form later in development. These medial DR5 foci are weakly and inconsistently detected in  
23 stage 6<sup>21</sup> and become established more strongly and consistently during stage 7<sup>21, 22</sup>. Thus the  
24 formation of the medial DR5 foci is delayed by 24-48 hours relative to the lateral foci. During stage 8,  
25 the four apical foci of DR5 expression are transformed into a ring of apical expression that  
26 encompasses the entire apical tip of the gynoecium<sup>18, 21-24</sup>.

### 29 **PINFORMED (PIN) family of auxin transporters**

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32 Based on the expression patterns and timing of expression of genes required for auxin synthesis (i.e.  
33 *TRYPTOPHAN AMINOTRANSFERASE OF ARABIDOPSIS1* [TAA1]/ (*TRYPTOPHAN AMINOTRANSFERASE*  
34 *RELATED 2*)[TAR2] and *YUCCA*-family genes –See side box) relative to the timing of the appearance  
35 of the DR5 foci, it is unlikely that the apical DR5 response foci are formed entirely by local auxin  
36 synthesis<sup>21, 22</sup>. A variety of evidences suggest that the DR5 expression foci require directional  
37 transport of auxin. Both Larsson *et al.* and Moubayidin and Ostergaard report the expression  
38 patterns of PIN1, PIN3 and PIN7 auxin transporters during gynoecial development and highlight the  
39 functional importance of PIN1. At the earliest stage of gynoecium development, as the central  
40 portion of the floral meristem is transitioning into the gynoecial primordium (Stage 5 to 6), PIN1  
41 protein is expressed in the epidermal cells and appears to transport auxin toward the apex and  
42 center of the developing gynoecial mound and toward the developing lateral DR5 foci<sup>21</sup>. The  
43 reduced expression of the DR5 lateral foci in the hypomorphic *pin1-5* allele<sup>22</sup> and the loss of valves  
44 observed in *pin1* mutant gynoecia<sup>25</sup> indicate that the PIN1 transporter is indeed important for the  
45 generation of the lateral DR5 foci, and subsequent valve growth.

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50 As the gynoecial tube develops further, PIN1 transporters are expressed in the lateral domains in the  
51 L1 epidermal cell layer. Here the PIN1 transporter protein is polarly localized on the apical surface of  
52 the epidermal cells, orientated so as to transport auxin toward the apex of the gynoecium<sup>21, 22, 26</sup>.  
53 Within the medial domains, PIN 1 is not a strongly localized to just the apical surface of the cell, but  
54 rather is distributed evenly on all plasma membrane surfaces, suggesting a less polarized transport  
55 of auxin within the medial domain<sup>21</sup>. In addition, in the medial domain PIN1 is expressed  
56 throughout the internal cell layers (i.e. in the L2 and L3 mesophyll cell layers). This is in contrast to in  
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3 the lateral domains where PIN1 is expressed chiefly in the epidermis and in the developing  
4 vasculature. Thus PIN1 localization and DR5 reporter expression data suggest that the auxin  
5 transport and response in medial and lateral domains is different from an early developmental  
6 stage.  
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9 Taken together these data suggest that the flow of auxin in the epidermis of the lateral domains is  
10 acropetal (toward the tip of the gynoecium)<sup>21,22</sup>, then back down through the forming vasculature<sup>21</sup>  
11 similar to the reverse fountain model as proposed in the developing leaf<sup>27</sup> (Figure 2). In contrast, in  
12 the medial domains it appears that the net flow of auxin may be less strongly directed when  
13 compared to the flows in the lateral domains. Based on patterns of expression of auxin synthesis  
14 genes (e.g. *TAA1/TAR2/YUCCA*-family members), both Larsson *et al.* (2013) and Hawkins and Liu  
15 (2014) suggest that auxin synthesis in basal portions of the gynoecium or in the receptacle of the  
16 flower may be important for the proper formation of the gynoecium<sup>18,28</sup>. Furthermore, Larsson *et al.*  
17 (2014) report a difference in the timing of the development of the vasculature in the medial and  
18 lateral domains using the *IAA2:GFP* reporter as a marker of early vasculature development; the  
19 lateral domain vasculature starts to develop by stage 5 while in the medial domain *IAA2:GFP*  
20 expression is not detected until stage 7-8<sup>21</sup>. Thus, the patterns of auxin transport and the timing of  
21 vascular development differs between the medial and lateral gynoecial domains during early  
22 development.  
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### 27 **Regulation of PIN1 subcellular polarity by PINOID kinase is required for proper medial domain** 28 **development**

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30 *PID* (*PINOID*) encodes an AGC-3 type protein kinase that is required for apical (polar) localization of  
31 the PIN transporter proteins within the apical/basal context of individual cells<sup>29-31</sup>. *PID* acts to  
32 phosphorylate serine residues in the PIN transporters, thus resulting in the apical (polar) localization  
33 of the PIN1 protein<sup>32</sup>. Mutation of two of the PIN1 serine residues (substitution of serine with  
34 alanine and thus preventing phosphorylation) results in a constitutive apolar localization of the PIN1  
35 transporter<sup>32</sup> and in apical/basal patterning defects in the gynoecium that are similar to those  
36 observed in the hypomorphic *pin1-5* mutant or after NPA treatment<sup>22</sup>. Furthermore the serine-to-  
37 alanine substitutions in PIN1 condition the reduced expression of the DR5 reporter in the lateral  
38 apical foci.  
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42 Interestingly the DR5 medial foci and the DR5 apical ring are largely maintained in the *pin1-5* and  
43 *pid-8* mutants indicating that they are independent of polar PIN1 localization<sup>22</sup>. Moubayidin and  
44 Ostergaard propose that the medial foci are the result of the action of the transcription factors  
45 *SPATULA* (*SPT*) and *INDIHISCENT* (*IND*) that function to repress *PID* transcription in the medial  
46 domains<sup>23,26</sup> thus resulting in the observed less polarized localization of PIN transporters in the  
47 medial domain.  
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50 Furthermore, the phosphorylated state of a serine residue can be mimicked by changing the serine  
51 residue to a glutamate residue, thus generating a constitutively pseudo-phosphorylated phospho-  
52 mimic<sup>32</sup>. When Moubayidin and Ostergaard expressed such a mutant PIN1 protein (*PIN1:GFP*  
53 *S1,2,3E*) from the PIN1 promoter, the phospho-mimic PIN1 protein was constitutively apically  
54 polarized, even in the medial domain, resulting in a split style phenotype similar to that observed in  
55 *spt-12*<sup>22</sup>. In the *PIN1:GFP S1,2,3E* expressing plants, the medial DR5 foci were not detected, while  
56 the lateral foci were detected as in wild type. Thus the medial DR5 foci do not require strongly apical  
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3 (polar) localization of PIN1, and in fact may require a greater degree of apolar PIN1 distribution. This  
4 greater degree of apolar PIN1 in the medial domains is important for DR5 foci in the medial domains  
5 and for the subsequent formation of the DR5 apical expression ring at stage 8 and the proper  
6 formation of style and stigmatic tissues. The Moubayidin and Ostergaard model further suggests  
7 that the medial DR5 foci promote the bilateral to radial transition at the apex of the gynoecium and  
8 the subsequent formation of the style, while the apical/basal gynoecial patterning defects are a  
9 result of a failure to form the lateral foci and the associated reduced growth of the lateral/valve  
10 domains.  
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14 Larsson et al. suggest that the drainage of auxin through the forming pre-vasculature may be  
15 required for carpel organ outgrowth in the lateral domains<sup>21</sup>. This is similar to what has been  
16 proposed to occur in developing leaves<sup>33</sup>. A disruption of auxin removal through the pre-vasculature  
17 (due to NPA treatment) would be expected to affect the lateral domains more severely than the  
18 medial domains, as the “reverse fountain” flow of auxin appears more predominant in the lateral  
19 domains. This may also contribute to the preferential growth of the medial domains over the lateral  
20 domain upon NPA treatment. This would provide a mechanistic explanation for the observation that  
21 the medial and lateral domains experience differential responses to the application of NPA<sup>21</sup>.  
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#### 24 **Transient treatment with NPA results in ectopic expression of DR5 and expansion of medial** 25 **domain markers** 26

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28 Larsson et al. tested the effects of a transient NPA treatment on development of the gynoecia and  
29 expression from the DR5 reporter<sup>21</sup>. In general, treatment of the floral buds with NPA resulted in a  
30 spreading or delocalization of the DR5 signal. More defined patterns of expression seen in the wild  
31 type such as lateral foci and pre-vascular strand specific expression patterns were broadened into  
32 more diffuse patterns that encompassed a greater proportion of the developing primordium. The  
33 ectopic DR5 response was often quite pervasive in the valve tissues suggesting that NPA treatment  
34 increased the level of auxin signaling generally throughout the valve. These gene expression changes  
35 were observed between 7 and 24 hours after treatment and preceded morphological changes.  
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39 NPA treatment also resulted in alterations of patterning along the medio-lateral extent of the  
40 gynoecium<sup>21</sup>. After NPA treatment, expression from medial-domain expressed reporters (i.e.  
41 *SHATTERPROOF2* [*SHP2*], *KNOTTED-LIKE FROM ARABIDOPSIS THALIANA1/BREVIPEDICELLUS*  
42 [*KNAT1/BP*], and *TAA1*) was expanded into lateral positions. These experiments suggest that  
43 blocking of polar auxin transport results in a partial reassignment of lateral fates into medial fates or  
44 in an expansion of the extent of the medial domain at the expense of the lateral domain.  
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47 *SHOOT MERISTEMLESS* (*STM*), like *KNAT1/BP*, encodes a Class I KNOX transcription factor and is  
48 expressed early within the developing medial domain of the gynoecium as well as within the shoot  
49 apical meristem<sup>12</sup>. *STM* has a key function in the formation, organization and maintenance of  
50 meristematic potential in the shoot apical meristem<sup>12, 34-36</sup>. *STM* maintains the pool of meristematic  
51 cells within the shoot apical meristem and more transiently at the center of the floral meristem,  
52 allowing carpel and placental development<sup>37</sup>. It would be interesting to look at the expression  
53 pattern of *STM* in response to NPA treatment. Based on the observed expansion of the medial  
54 domain markers (i.e. *SHP2*, *KNAT1/BP*, *TAA1*) upon NPA treatment, one might expect an expansion  
55 of *STM* expression upon NPA treatment, however to our knowledge this has not yet been  
56 investigated. Additionally, the recent report of a role for *STM* and *KNAT1/BP* during cell expansion  
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3 and differentiation of xylem in the cambium of *Arabidopsis* hypocotyls<sup>38</sup> suggests that, in addition to  
4 their role in maintenance of meristematic potential in the medial domain, *STM* or *KNAT1/BP* might  
5 function in the later differentiation of medial domain structures or vasculature.  
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### 7 **PGP/ABCB-type auxin transporters are also important for proper auxin responses in the** 8 **gynoecium**

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10 In addition to the PIN family transporters, the ATP-binding cassette subfamily B (ABCB)/MULTI-DRUG  
11 RESISTANT/ P-GLYCOPROTEIN (PGP) family proteins also function as auxin efflux transporters<sup>39,40</sup>.  
12 These transporters are typically localized in an apolar fashion and are thought to alter polar auxin  
13 flux by regulating the intracellular auxin concentration that is available for polar transport by PIN  
14 proteins<sup>41,42</sup>. *PGP1* and *PGP19* are expressed in the developing gynoecium and are differentially  
15 required in the medial and lateral domains. Larsson et al. reported that *pgp1/pgp19* double  
16 mutants displayed a reduced ability to generate the lateral DR5 apical foci while the medial DR5 foci  
17 were largely unaffected in this double mutant<sup>21</sup>. These results suggest a role for *PGP1* and *PGP19* in  
18 auxin transport in lateral domains. Larsson et al. suggest that when *PGP*-dependent functions are  
19 blocked by NPA treatment or in the *pgp1/19* double mutant, auxin becomes trapped in internal cell  
20 layers of the carpel and thus is not available for transport by PIN transporters in the epidermis. The  
21 broader expression domain of the PIN transporters in the medial domain (expression in epidermis as  
22 well as internal mesophyll layers) may in part explain why the medial domain is less sensitive to the  
23 application of NPA or the loss of *PGP1* and *PGP19* activity.  
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### 31 **Interactions between cytokinin and auxin during gynoecial patterning, medial domain** 32 **development and ovule initiation**

#### 33 **Auxin and cytokinin regulate gynoecial patterning**

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35 The plant hormone cytokinin (see side bar) regulates the development of the shoot and root apical  
36 meristems<sup>43,44</sup> and is required for proper development of key medial domain derived structures,  
37 including the ovules, as well as the valve margin (dehiscence zone)<sup>45</sup>. Reduced ovule formation is  
38 observed when cytokinin biosynthesis or perception is impaired<sup>46-51</sup>. Conversely, increasing  
39 cytokinin levels delay cell differentiation and enhance cell proliferation in the CMM-derived  
40 placental tissues and increase the number of ovules formed per gynoecium<sup>52,53</sup>. The exogenous  
41 application of the cytokinin benzylaminopurine (BAP) as well as the analysis of transgenic cytokinin  
42 overexpression lines suggest that cytokinin plays at least three roles during gynoecial development:  
43 1) an early developmental role in stimulating cell proliferation in the medial domain 2) a later role in  
44 the development of the valve margin<sup>45</sup>, and a role in patterning along the apical-basal axis of the  
45 gynoecium<sup>54</sup>.  
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51 Zuniga-Mayo et al. recently reported that the treatment of developing flowers with exogenous  
52 cytokinin, in addition to causing proliferation of medial domain structures, also alters apical-basal  
53 patterning within the gynoecium<sup>54</sup>. The observed apical-basal patterning defects were characterized  
54 by a reduction or loss of the valves and were similar to those caused by the blocking of auxin  
55 transport via NPA treatment. These results suggest interplay between auxin and cytokinin signaling  
56 pathways in the gynoecium. The similarity of the cytokinin and NPA treatment phenotypes suggests  
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3 that cytokinin reduces polar auxin transport in the gynoecium. Furthermore, the enhanced  
4 sensitivity of *auxin response factor19* (*arf19*) and *arf7* mutant gynoecia to the application of BAP  
5 suggests that these two *ARFS*, in addition to *ARF3/ETTIN*<sup>3,55</sup>, function in apical-basal patterning of  
6 the gynoecium<sup>54</sup>.  
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10 A large degree of cross-talk between the cytokinin and auxin signaling pathways has been shown to  
11 influence the development of the shoot and root apical meristem<sup>43,44</sup>. In peripheral portions of the  
12 SAM, auxin down regulates *STM* expression<sup>56,57</sup> and reduces cytokinin biosynthesis<sup>58</sup>. Additionally  
13 auxin, acting through *MP/ARF5*, suppresses the expression of *ARABIDOPSIS RESPONSE REGULATOR7*  
14 (*ARR7*) and *ARR15*, two components of the cytokinin response pathway<sup>59</sup>. Given the meristematic  
15 nature of the gynoecial medial domain it seems likely that similar mechanisms of cross talk may  
16 shape medial domain development. Visualization of cytokinin and auxin responses in the gynoecium,  
17 using synthetic reporters (TCS-based and DR5-based, respectively), showed that auxin and cytokinin  
18 responses in the gynoecium tend to have complementary and mutually-exclusive expression  
19 patterns suggesting an antagonistic regulatory relationship between auxin and cytokinin<sup>15,45,60</sup>.  
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23 The *NO TRANSMITTING TRACT* (*NTT*) zinc finger-containing transcription factor previously identified  
24 as a regulator of transmitting tract development<sup>61</sup> also plays a role in the development of the valve  
25 margin and the replum<sup>62,63</sup>. *NTT* activates the expression of *KNAT1/BP1* in the medial domain of the  
26 gynoecium, likely through the direct binding to the *KNAT1/BP* promoter sequences<sup>63</sup>. The *KNAT1/BP*  
27 and *REPLUMLESS* (*RPL*) transcriptional regulators form a protein complex that is important for  
28 replum development<sup>64</sup>. *NTT* physically interacts with itself as well as *RPL*, *KNAT1/BP* and *STM* and  
29 several other regulators of gynoecial development suggesting that it is a member of a multi  
30 component complex that regulates development within the medial domain<sup>63</sup>. *KNAT1/BP* and *STM*  
31 stimulate cytokinin synthesis in the SAM via the transcriptional upregulation of *AtIPT7* in the  
32 cytokinin biosynthesis pathway<sup>65,66</sup>. Thus the stimulation of *KNAT1/BP* expression that is brought  
33 about by *NTT* action may support higher levels of cytokinin in the medial domain. This is consistent  
34 with the increase in the size of the replum that is observed upon exogenous cytokinin application as  
35 well as in response to *NTT* overexpression.  
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41 The splicing factor *CLUMSY VEIN* (*CUV*), the Arabidopsis ortholog of the eukaryotic DEAH-box RNA-  
42 dependent ATPase Pre-mRNA-processing factor 16 (*Prp16*), affects the splicing and differential  
43 expression of key genes involved in auxin-mediated development<sup>67</sup>. *CUV* is required for efficient  
44 pre-mRNA splicing of several auxin biosynthesis genes including *TAA1*, *TAR2* and several *YUCCA*  
45 family members. The levels of properly spliced transcripts of a number of *PIN* genes, auxin receptor  
46 genes and auxin signaling genes were also reduced in *cuv* mutants. The expression and subcellular  
47 localization of the *PIN1* protein was also altered in the *cuv* mutant roots. Consequently, *cuv* mutants  
48 exhibit a number of phenotypes that arise from altered auxin spatial distribution, as is corroborated  
49 by aberrant DR5-reporter expression. The *cuv* mutants exhibit embryonic defects, leaf vasculature  
50 defects, and ectopic vein formation in cotyledons, flowers and gynoecia. The apical-basal patterning  
51 of the gynoecium is also disrupted in *cuv* plants, with *cuv* mutants displaying shorter valves and  
52 longer gynophore and style domains.  
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57 **Auxin and cytokinin regulate CMM development and ovule initiation from the meristematic**  
58 **medial domain**  
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3 *CUP-SHAPED COTYLEDON 1 (CUC1)* and *CUC2* encode a pair of paralogous NAC-domain containing  
4 transcription factors that are expressed within adaxial portions of the gynoecial medial domain<sup>68-71</sup>.  
5 In order to study the functions of *CUC1* and *CUC2* during the development of the meristematic  
6 medial domain, Kamiuchi *et al.* examined gynoecium development in *cuc1 cuc2* double mutant  
7 plantlets that were regenerated from *cuc1 cuc2* calli<sup>69</sup>. The gynoecial phenotypes (i.e. reduced and  
8 misplaced CMMs) indicate a redundant role for *CUC1* and *CUC2* in the initiation and placement of  
9 the CMM within the medial domain. *STM* expression was greatly reduced in the *cuc1 cuc2* double  
10 mutant gynoecia, particularly in adaxial portions of the medial domain, indicating the *CUC1* and  
11 *CUC2* are required for the initiation of *STM* expression in the CMM. This is reminiscent of their role  
12 in the initiation of *STM* expression during the development of the embryonic shoot apical meristem  
13<sup>71,72</sup>. In addition to its role in shoot apical meristem development (see above), *STM* plays a role in the  
14 maintenance of undifferentiated cells in the floral meristem<sup>34</sup> as well as in the initiation of carpel  
15 development and in the formation or maintenance of the placental tissue of the medial domain<sup>37</sup>.  
16 Thus, much of the *cuc1 cuc2* double mutant gynoecial phenotype may be due to the failure to  
17 activate *STM* in the developing CMM<sup>69</sup>. The expansion of *FILAMENTOUS FLOWER (FIL)* expression  
18 into portions of the medial domain in the *cuc1 cuc2* double mutant suggests that *CUC1* and *CUC2*  
19 activities normally prevent valve differentiation in the medial domain, presumably through the  
20 activation of *STM* expression<sup>69</sup>.  
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26 Kamiuchi *et al.* also generated microRNA-resistant versions of *CUC1* and *CUC2* that were expressed  
27 at higher levels and in expanded domains within the gynoecium relative to the expression of the wild  
28 type genes. Plants expressing these microRNA-resistant *CUC* constructs displayed supernumerary  
29 CMMs in adaxial portions of the medial domain and generated filamentous structures from ectopic  
30 CMM-like structures in abaxial portions of the medial domain<sup>69</sup>. Correspondingly, *STM* expression is  
31 expanded. The formation of filamentous structures from abaxial replum is similar to, albeit less  
32 severe than, the phenotype of BAP-treated gynoecia<sup>45</sup>, again suggesting a role of cytokinin  
33 downstream of *STM* that is important for proliferation of cells of the CMM<sup>45,52</sup>.  
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37 In order to look at the function of *CUC1*, *CUC2* and *ANT* during ovule initiation Galbiati *et al.*  
38 generated an RNAi construct to reduce the activity of *CUC1* specifically in the developing ovules<sup>73</sup>.  
39 As *cuc1 cuc2* double mutants don't form a shoot apical meristem, this *CUC1\_RNAi* construct allowed  
40 the ovule specific reduction of *CUC1* activity and thus enabled the analysis of *cuc1 cuc2* double and  
41 *cuc1 cuc2 ant* triple mutant ovules. The analysis of the *pPIN1:PIN1::GFP* reporter in a *cuc1\_RNAi cuc2*  
42 double mutant revealed that *CUC1* and *CUC2* are redundantly required for the expression and  
43 proper localization of PIN1 in the developing ovules. In wild type plants *PIN1* is detected in  
44 epidermal cells and displays a polarized subcellular localization on the plasma membrane surface  
45 orientated toward the apex of the growing ovule primordium<sup>27</sup>. In the *cuc1\_RNAi cuc2* double  
46 mutants, PIN1 protein was detected weakly and throughout the ovule primordium and failed to  
47 efficiently localize to the plasma membrane in an polar manner, instead being detected in vacuoles  
48 and diffusely on plasma membranes in an apolar fashion<sup>73</sup>. Previous work had demonstrated that  
49 exogenous application of cytokinin could stimulate the expression of PIN1<sup>74</sup>, and Galbiati *et al.*  
50 demonstrated that ovule loss and the reduction of PIN1 expression that was observed in the  
51 *cuc1\_RNAi cuc2* double mutant could be partially rescued by the exogenous application of cytokinin.  
52 Interestingly the loss of ovule primordia in the *ant* mutant was not rescued by the application of BAP  
53 suggesting that *ANT* functions in a *CUC1/CUC2*-independent pathway.  
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3 The expression of *CUC1*, *CUC2* and *ANT* are stimulated by the MP/ARF5 auxin response factor  
4 through the direct interaction of MP/ARF5 with the cis-regulatory regions of these genes<sup>73,75</sup>.  
5 Galbiati *et al.* recently presented a model that proposes that auxin via the MP/ARF5 protein  
6 stimulates *ANT* expression, and thus promotes cell proliferation in the placenta and ovules during  
7 early gynoecial development<sup>73</sup>(Figure 3). In this model *MP/ARF5* also directly stimulates the  
8 expression of *CUC1* and *CUC2* that redundantly regulate *PIN1* expression through a non-cell  
9 autonomous action, allowing the formation of auxin maxima at the growing tip of the new ovule  
10 primordium. Cytokinin response, downstream of the *CUC* genes, is likely in part responsible for the  
11 *CUC1/CUC2* promotion of *PIN1* expression. At later stages of ovule development the auxin maxima  
12 at the apex of the developing ovule may repress *CUC* gene expression, as has been demonstrated in  
13 leaves<sup>76</sup>.  
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### 20 Transition from floral meristem to gynoecial primordium

#### 21 Auxin regulates the termination of the floral meristem and progression into the gynoecium

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23 The earliest steps in the specification of the medial and lateral gynoecial domains, and the degree to  
24 which the termination of the floral meristem and the formation of the gynoecium is mechanistically  
25 coordinated, remain unresolved. A recent paper by Liu *et al.* revealed a role for auxin and the auxin  
26 response factor ARF3 in floral meristem termination<sup>77</sup>. They show that ARF3 functions as a  
27 repressor of *WUS* transcription and this action is in part dependent on the transcription factor  
28 *AGAMOUS (AG)*. Chromatin IP data indicate that ARF3 associates with the *WUS* cis-regulatory  
29 regions and this interaction is promoted by *AG* function. These experiments also argued for a rather  
30 complex role for *ARF3* in floral termination with *ARF3* likely playing a direct role in *WUS* repression in  
31 the cells of the organizing center, while *ARF3* may additionally function in a non-cell autonomous  
32 fashion outside the organizing center, exerting an indirect repression on *WUS*. The experiments of  
33 Liu *et al.* when taken together with those of Larsson *et al.*, suggest that auxin plays a role in both the  
34 termination of the floral meristem<sup>77</sup> as well as the early patterning of the medial and lateral  
35 domains of the gynoecium<sup>21</sup> thus potentially linking these two processes mechanistically. However,  
36 the observation that NPA treatments have not been shown to regulate floral meristem determinacy  
37 is puzzling in this regard.  
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43 Another link between floral meristem termination and medial domain development is suggested by  
44 the work of Wynn *et al.* who recently demonstrated a role for the transcriptional regulator  
45 *PERIANTHIA (PAN)* in medial domain development and the subsequent formation of ovules<sup>78</sup>. *PAN*  
46 activates *AG* at the transcriptional level in the center of the floral meristem and thus promotes the  
47 proper termination of the floral meristem<sup>79,80</sup>. *AG* functions as a repressor of *WUS* both directly and  
48 indirectly, as well as in concert with *ARF3* as described above<sup>77,81-83</sup>. Wynn *et al.* demonstrate that in  
49 *seu pan* double mutants the floral meristem termination defects are enhanced and *WUS* expression  
50 is temporally extended<sup>78</sup>. Thus they propose that *PAN* and *SEU* act as activators of *AG* in central  
51 portions of the flower. The enhanced indeterminacy in the *pan seu* double mutants is correlated  
52 with an enhanced loss of ovules suggesting a possible mechanistic link between these two  
53 developmental events. This apparent connection between floral meristem termination and  
54 ovule/medial domain formation was first noted by Zuniga-Mayo *et al.* in their study of *jaiba crc*  
55 double mutants<sup>84,85</sup>. *JAIBA* and *CRC* are involved in the proliferation of the medial tissues and *jaiba*  
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3 *crc* double mutants display both abnormal floral meristem determination phenotypes, as well as  
4 reduced formation of ovule primordia. A large number of other genes have been shown to function  
5 during floral meristem termination in Arabidopsis [see Ito and Bo (2015) for a recent review <sup>86</sup>]  
6 perhaps providing additional opportunities to explore the proposed mechanistic link between floral  
7 meristem termination and CMM/ovule formation.  
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### 10 11 12 Conservation and divergence of gynoecial developmental mechanisms within eudicots

#### 13 14 **NGATHA (NGA) gene function is conserved in diverse eudicots indicating aspects of auxin** 15 **regulation during gynoecial development are evolutionarily conserved** 16

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18 The four *Arabidopsis thaliana* *NGATHA* (*NGA*) genes are a part of the RAV clade within the B3 family  
19 of transcription factors <sup>87,88</sup>. The Arabidopsis *NGA* genes act in a redundant manner to specify  
20 stigma and style development. The *nga1,2,3,4* quadruple mutants (hereafter *nga* quad. mutants)  
21 display a severe loss of style and stigmatic tissue as well as apical splitting of gynoecia. The strong  
22 apical ring of DR5-based auxin response that is normally detected at the gynoecial apex, and that  
23 precedes the morphological development of the stigmatic and stylar tissues, is also not detected in  
24 the *nga* quad. mutants <sup>89</sup>. The comparison of transcriptomic profiles of gynoecia from wild type, *nga*  
25 quad. mutant and *NGA3*-overexpression lines has identified nearly 2,500 putative targets of *NGA*  
26 regulation within the gynoecium many of which function in auxin synthesis, transport or signaling <sup>89</sup>.  
27 The expression of the auxin biosynthesis genes *YUCCA2*, *4*, *8*, and *TAA1* as well as *AMIDASE1* (*AMI1*)  
28 are reduced or absent in the apical gynoecial tissues in *nga1,2,3,4* quad. mutants <sup>88-90</sup>. Polar auxin  
29 transport may also be regulated by *NGA* genes through the repression of *PID* expression and the  
30 activation of the expression of *WAG2* <sup>89</sup>.  
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35 Studies in the basal eudicot *Eschscholzia californica* and core eudicot *Nicotiana benthamiana*  
36 revealed that *NGA* function is highly conserved across eudicots <sup>90</sup>. Using degenerate primers, one  
37 *NGA* gene was identified in *E. californica* (*EcNGA*) while BLAST searches of available draft genomic  
38 sequences identified two *NGA* genes in *Nicotiana benthamiana*, termed *NtNGAa* and *NtNGAb*.  
39 Expression patterns of *EcNGA*, *NtNGAa* and *NtNGAb* are similar in floral tissues in both species and  
40 resembles the spatial-temporal expression of *AtNGA* genes, including expression in placenta, ovules  
41 and apical portions of the gynoecium. Knockdown of *NGA* expression using viral induced gene  
42 silencing (VIGS) (see side bar) in *E. californica* and *N. benthamiana* produced reduction of style and  
43 stigmatic tissue and incomplete closure of the gynoecial tube. The similarity of these phenotypes  
44 with those observed in the *A. thaliana nga* quad. mutants <sup>87,88</sup> indicates a conserved function for  
45 *NGA* genes across these eudicot species. This functional conservation also correlates with high level  
46 of putative structural and sequence similarity across *NGA* orthologs investigated <sup>90</sup>. BLAST searches  
47 identified a putative *N. benthamiana YUCCA* gene with high sequence similarity to *A. thaliana YUC2*  
48 and *YUC6* sequences. The expression of this putative *N. benthamiana YUCCA* gene was significantly  
49 reduced in styles and stigmas after VIGS-induced reduction of the expression of *NtNGA*. These data  
50 suggest that the regulatory relationship between *NGA* and *YUC* genes characterized in *A. thaliana*  
51 gynoecia may well be conserved in *N. benthamiana*.  
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### The *miR156-SBP* regulatory module may function differently during gynoecial development in *Arabidopsis* and tomato

In *A. thaliana* the *microRNA156* (*miR156*) regulates expression of several members of the *SQUAMOSA PROMOTER-BINDING PROTEIN-LIKE* (*SPL* or *SBP* box) transcription factor family<sup>91</sup>. This *miR156-SBP* regulatory module functions during gynoecial patterning and female fertility in *Arabidopsis*<sup>92</sup>. Overexpression of the *miRNA156* in an *spb8* mutant results in reduced ovule number, deformed septa and an absence of transmitting tract tissue, as well as a shortened style. These data indicate that the targets of *miR156* act redundantly with *SPB8* (that is not targeted by *miR156*) to affect gynoecial development. These *spb8; miR156* overexpression “double mutants” also displayed a hypersensitivity to NPA and a reduced expression of the *YUCCA4::GUS* reporter. In addition, *SPB8* and targets of *miR156* from the *SBP* family were shown to interact genetically with *ettin/arf3*, *spt* and *crc*, suggesting that the *miR156-SBP* regulatory module functions during gynoecial patterning by regulating auxin homeostasis.

Silva et al. (2014) recently demonstrated that the *miR156-SBP* regulatory module also functions during gynoecial development in tomato<sup>93</sup>. However their results suggest that *miR156-SBP* module regulates tomato gynoecial development through pathways that differ somewhat from those in dry fruited species like *Arabidopsis*. In tomato the overexpression of *AtmiR156* led to floral indeterminacy, the development of extra carpels, as well as to the development of fruits that contained ectopic meristematic structures. The *AtmiR156* overexpression lines displayed an up-regulation of *GOBLET* (a *NO APICAL MERISTEM/CUC-like* gene) and *TOMATO KNOTTED-LIKE2* (a class I *KNOX* gene) perhaps providing a mechanistic explanation for the presence of ectopic carpels and ectopic meristematic structures. Thus, although the *miR156-SBP* regulatory module appears to be important for gynoecial and fruit development in both tomato and *Arabidopsis*, the downstream targets of the module may be different in these two rather different fruit types.

### Auxin and auxin signaling

Auxin - A class of plant hormones originally defined by their ability to regulate growth via control of cell division and cell elongation. Recent reviews: <sup>18, 94, 95</sup>. Indole-3-acetic acid (IAA) is the most common plant auxin. The directional transport of auxins between the cells of developing organs can generate local auxin maxima or minima and thus support patterning events and patterns of organ growth. Two main types of auxin efflux transporters have been shown to participate in auxin-dependent patterning events: 1) *PINFORMED (PIN)* family members that are typically polarly-localized within a given cell and support directionally-biased or orientated flows of auxin within the organ primordium <sup>96</sup>; and 2) ABCB/PGP efflux transporters that are generally apolar localized within a cell, but may function in generating auxin patterns by regulating intracellular auxin concentration available to the PIN transporters <sup>41, 42</sup>.

1-N-naphtylphthalamic acid (NPA) – A polar auxin transport inhibitor. NPA binds to ABCB/PGP transporters, but not to PIN transporters <sup>39-41</sup>. However NPA inhibits, either directly or indirectly, the ability of both ABCB/PGP and PIN type reporters to transport auxin.

PINOID – a kinase that phosphorylates PIN family transporters and regulates their subcellular localization <sup>97, 98</sup>

DR5 reporter– a synthetic auxin response reporter in which a known auxin responsive element is fused to a reporter gene to allow monitoring of auxin response *in vivo* <sup>99</sup>. Note that this is a reporter of the transcriptional response to auxin, not strictly a reporter of intracellular auxin levels.

*TRYPTOPHAN AMINOTRANSFERASE OF ARABIDOPSIS1 (TAA1)/ TAA-RELATED 2 (TAR2)* and *YUCCA*-family genes - Genes that encode key enzymes in the auxin biosynthesis pathway <sup>100, 101</sup>.

*AMIDASE1 (AMI1)* - Enzyme that converts indole-3-acetamide (IAM) to IAA in an alternative pathway to the *TAA1/YUCCA* route <sup>102</sup>

### Cytokinin synthesis and signaling

Cytokinins: A class of adenine-derived plant hormones primarily associated with cell growth and differentiation during plant growth and development. Recent reviews: <sup>44, 103-105</sup>. Cytokinin response and signaling involves a set of two-component histidine kinases. Cytokinins bind to membrane bound cytokinin receptors, *ARABIDOPSIS HISTIDINE KINASES (AHKs)*, triggering a phosphor-relay ultimately causing the phosphorylation and activation of *ARABIDOPSIS RESPONSE REGULATORS (ARRs)*. Phosphorylated type B *ARRs* activate transcription of cytokinin-responsive genes, including the type A *ARRs*. The majority of type A *ARRs* act as inhibitors of cytokinin signaling. Cytokinin biosynthesis is catalyzed by enzymes encoded by members of the *ATP/ADP ISOPENTENYLTRANSFERASE (IPT)* gene family.

TCS (Two-Component-Output-Sensor) reporter: A synthetic cytokinin-response reporter that allows *in vivo* measurement of levels of cytokinin response in a given tissue or cell type. The reporter is

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3 driven by a promoter comprised of a concatenation of specific transcriptional elements that are  
4 responsive to cytokinin during immediate to early cytokinin response<sup>106</sup>.

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6 Benzylaminopurine (BAP) – A synthetic cytokinin analogue. The exogenous application of BAP allows  
7 the determination of the effects of increased levels of cytokinin on development.  
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10 Viral induced gene silencing (VIGS) – using a plant viral vector to deliver an interfering RNA (RNAi)  
11 construct that can target a specific gene within the host plant and reduce the ability of a specific  
12 RNA transcript to make a functional product.  
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## 14 15 16 Conclusion

### 17 18 **The medial and lateral gynoecial domains develop via different mechanistic relationships**

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20 One conclusion that is becoming increasingly clear is that the developmental programs within the  
21 medial domain and the lateral domains of the Arabidopsis gynoecium proceed by different  
22 trajectories and are likely under the control of different molecular mechanisms. Larsson *et al.* and  
23 Moubayidin and Ostergaard clearly demonstrate that the formation of the DR5 expression foci in the  
24 medial and lateral domains are mechanistically different<sup>21,22</sup>. Also the relationship between growth  
25 and auxin signaling appears substantially different in these two domains as well. Growth within the  
26 lateral domain appears to follow the existing paradigm for auxin flow and organ growth in lateral  
27 aerial organs: a reverse fountain flow pattern with an early apical maximum generates proximal to  
28 distal growth. What then is responsible for the growth of the medial domain if there are no early  
29 DR5 foci and vascular development is delayed in this domain? One possibility is that growth within  
30 the medial domain is coupled to lateral domain growth. Although some degree of coordination is  
31 perhaps likely, genetic evidence suggests that this is not absolute, as the growth of the medial and  
32 lateral domain can be genetically separated: *tousled ettin (arf3)* double mutants display a  
33 proliferation of medial tissues while lateral-derived valve tissues are greatly reduced<sup>107</sup>; and a  
34 somewhat complementary phenotype is observed in the *leunig ant* and *seuss ant* double mutants in  
35 which the valves form relatively normally in the lateral domains while the tissues from the medial  
36 domain are missing or are very reduced<sup>108,109</sup>.

### 37 38 **Relationship of auxin to medio/lateral, apical/basal and abaxial/adaxial patterning**

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40 Another unanswered question relates to the relationship between auxin and cytokinin signaling,  
41 domain specific growth and the assignment of positional identities. Evidence from Larsson *et al.*  
42 suggests that early patterning events along the medio-lateral axis are regulated by auxin transport<sup>21</sup>.  
43 Early NPA treatment flattens the DR5 response foci and engenders an overall higher, but more  
44 diffuse, auxin response. This is correlated with the expansion of medial fates and a reduction in the  
45 extent of lateral fates. At least two possible mechanisms are consistent with this observation. It may  
46 be that the overall higher levels of auxin response favor the medial fates. Alternatively, medial fates  
47 are favored, not because auxin responses are higher across the primordium, but rather because the  
48 tight auxin response foci are not formed in the lateral domains when treated with NPA, and these  
49 tight foci are required for lateral domain outgrowth. In this second model, the growth of the medial  
50 domain would be favored as it is presumed not to require a well-canalized auxin flow to maintain  
51 growth. Larsson *et al.* also suggest that these early patterning defects might impact the subsequent  
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3 elaboration of the apical-basal gynoecial structures. This is similar to previously proposed ideas that  
4 linked early abaxial-adaxial patterning or medio-lateral patterning events to the later elaboration of  
5 the apical-basal patterning of the gynoecium<sup>6, 18, 28</sup>. Hawkins and Liu (2014) recently proposed a  
6 model for auxin function during gynoecial development that invokes a role for auxin in patterning  
7 along the adaxial-abaxial extent of the early gynoecial primordium. They propose that the  
8 juxtaposition of adaxial and abaxial identities within the developing carpels is necessary for the  
9 upward growth of the valve domains<sup>28</sup>. This is based on similar models of growth of the leaf lamina  
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### 13 **Cross talk between cytokinin and auxin signaling pathways in the gynoecium**

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16 In a manner similar to that observed in the shoot apical meristem, cytokinins appear to promote cell  
17 proliferation within and prevent the differentiation of the meristematic adaxial portions of the  
18 medial domain. This action of cytokinin contributes to the ability of the meristematic tissues to  
19 generate the medial domain-derived structures, particularly the ovules. Many observations suggest  
20 that there is significant crosstalk between the auxin and cytokinin signaling pathways during  
21 gynoecial apical/basal patterning, medial domain development and ovule initiation. The  
22 mechanisms of cross talk in the gynoecium may be similar to those observed in other structures,  
23 however a clear definition of the molecular mechanisms of auxin and cytokinin cross talk during  
24 gynoecial development awaits further investigations. Given the importance of the cytokinin-  
25 responsive *ARR5* and *ARR17* regulators in mediating of auxin and cytokinin cross talk in the SAM<sup>59</sup> it  
26 will be interesting to further explore the role of these ARR proteins in medial domain and gynoecial  
27 development.  
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### 37 **Acknowledgements**

38 We thank Emma Larsson and anonymous reviewers for helpful comments on the manuscript.  
39 Funding for this work came from NSF IOS grant# 1355019 and NCARS grant #NC02463 to RGF. We  
40 apologize to authors of related work that could not be cited here due to space limitations.  
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### Figure captions

**Figure 1. Structural components along the apical/basal and medial/lateral axes of the Arabidopsis gynoecium.**

A) A scanning electron microscopic image of a mature Arabidopsis gynoecium. The stigma (stg), style (sty), carpel valve (cv), abaxial replum (abr), gynophore (gn), ovary (ovy) and valve margin/dehiscence zone (dz) are indicated. B and C) False-colored confocal gynoecial cross sections B) A stage 11 cross section. A plane of symmetry (indicated with a dashed line) divides the gynoecium into two component carpels. Ovules (ov) and septum (s) are indicated. C) A stage 8 cross section. Medial and lateral domains are indicated. The carpel margin meristem/medial ridge (mr) is false colored pink. D) A scanning electron microscopic image of a developing gynoecium from a stage 7 flower. Dashed line indicates plane of symmetry that bisects the medial domain. E and F) Stage 7 floral cross sections from *in situ* hybridization experiments. E) *CRC* expression (brown product) is detected in the two lateral domains. F) *STM* expression is detected in the medial domain. Panels A, B and C are adapted from Azhakanandam et al.<sup>109</sup> with permission. Panel D is adapted from Wynn et al.<sup>111</sup> with permission.

**Figure 2. Diagrammatic representation of proposed patterns of auxin transport in the lateral and medial domains of a stage 7 gynoecium.**

A) In the lateral domains auxin is transported predominantly via the PIN1 transporter and follows the “reverse fountain” model. Auxin flow in the abaxial and adaxial epidermal cell layers is toward the apex. Green circles indicate the lateral domain DR5 response foci. B) In the medial domain the flow of auxin in the abaxial epidermis is similarly toward the apex. However, in the adaxial epidermis and in subepidermal cells auxin transport is less polarized resulting in a less canalized (channelled) flow of auxin and in weaker and later-forming DR5 foci (smaller green dots). Note: the confocal longitudinal gynoecium images in A and B are for diagrammatic purposes and are both images of an oblique lateral plane section. C and D) A diagram of a cross-sectional view of a stage 7 gynoecium. The dotted line in A and B indicates the plane of section for which auxin flows are represented in panels A and B, respectively.

**Figure 3 – A model of ovule formation from the meristematic medial domain as proposed by Galbiati et al.<sup>73</sup>**

See text for details. Figure 3 is reproduced from Galbiati et al.<sup>73</sup> with permission.

**Related Articles**

Subtopic	Article title
e.g., Fertilization to Gastrulation	e.g., Maternal mRNA breakdown

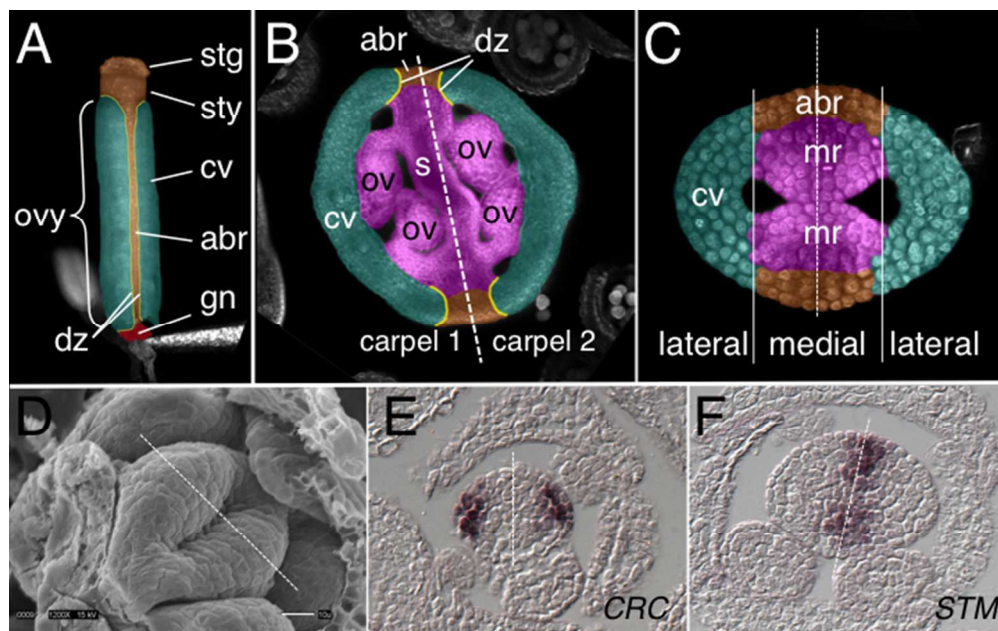


Figure 1. Structural components along the apical/basal and medial/lateral axes of the Arabidopsis gynoecium.

A) A scanning electron microscopic image of a mature Arabidopsis gynoecium. The stigma (stg), style (sty), carpel valve (cv), abaxial replum (abr), gynophore (gn), ovary (ovy) and valve margin/dehiscence zone (dz) are indicated. B and C) False-colored confocal gynoecial cross sections B) A stage 11 cross section. A plane of symmetry (indicated with a dashed line) divides the gynoecium into two component carpels. Ovules (ov) and septum (s) are indicated. C) A stage 8 cross section. Medial and lateral domains are indicated. The carpel margin meristem/medial ridge (mr) is false colored pink. D) A scanning electron microscopic image of a developing gynoecium from a stage 7 flower. Dashed line indicates plane of symmetry that bisects the medial domain. E and F) Stage 7 floral cross sections from in situ hybridization experiments. E) CRC expression (brown product) is detected in the two lateral domains. F) STM expression is detected in the medial domain. Panels A, B and C are adapted from Azhakanandam et al. with permission. Panel D is adapted from Wynn et al. with permission.

254x159mm (72 x 72 DPI)



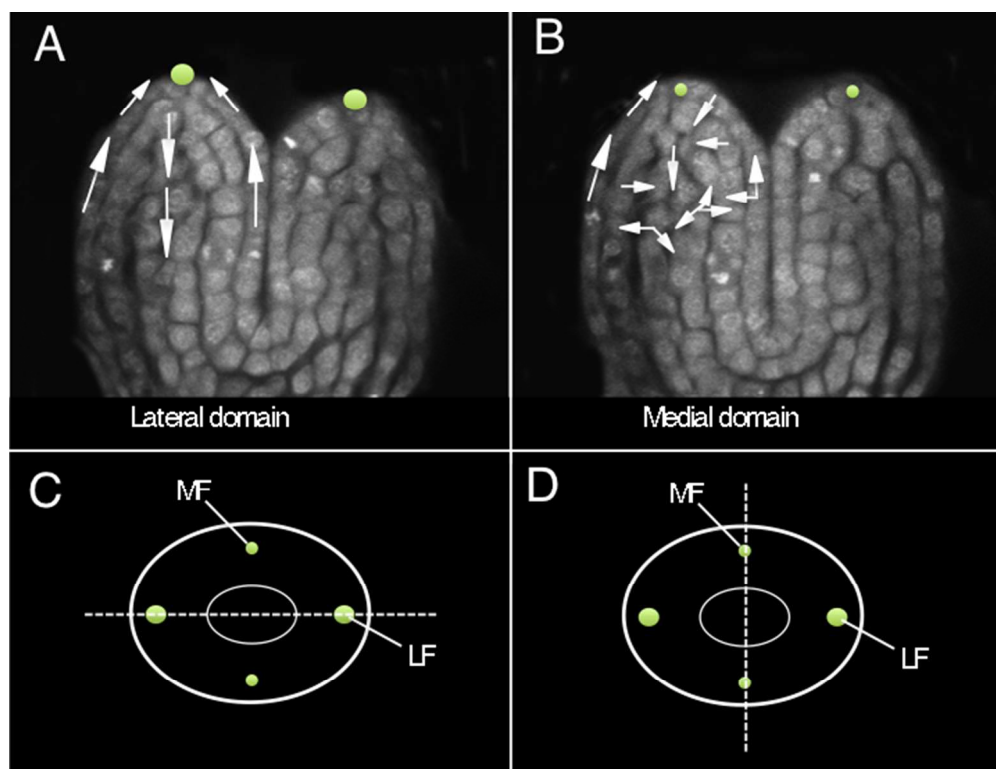


Figure 2. Diagrammatic representation of proposed patterns of auxin transport in the lateral and medial domains of a stage 7 gynoecium.

A) In the lateral domains auxin is transported predominantly via the PIN1 transporter and follows the “reverse fountain” model. Auxin flow in the abaxial and adaxial epidermal cell layers is toward the apex. Green circles indicate the lateral domain DR5 response foci. B) In the medial domain the flow of auxin in the abaxial epidermis is similarly toward the apex. However, in the adaxial epidermis and in subepidermal cells auxin transport is less polarized resulting in a less canalized (channelled) flow of auxin and in weaker and later-forming DR5 foci (smaller green dots). Note: the confocal longitudinal gynoecium images in A and B are for diagrammatic purposes and are both images of an oblique lateral plane section. C and D) A diagram of a cross-sectional view of a stage 7 gynoecium. The dotted line in A and B indicates the plane of section for which auxin flows are represented in panels A and B, respectively.

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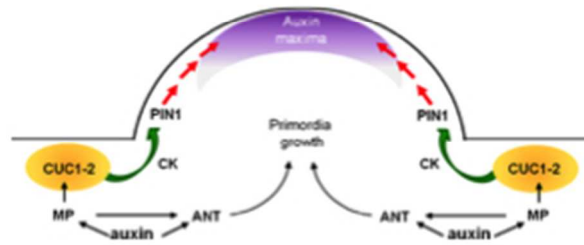


Figure 3 – A model of ovule formation from the meristematic medial domain as proposed by Galbiati et al.

See text for details. Figure 3 is reproduced from Galbiati et al. with permission.

127x84mm (72 x 72 DPI)