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REVIEW PAPER

Auxin and the *Arabidopsis thaliana* gynoecium

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Abstract

Recent research is beginning to reveal how intricate networks of hormones and transcription factors coordinate the complex patterning of the gynoecium, the female reproductive structure of flowering plants. This review summarizes recent advances in understanding of how auxin biosynthesis, transport, and responses together generate specific gynoecial domains. This review also highlights areas where future research endeavours are likely to provide additional insight into the homeostatic molecular mechanisms by which auxin regulates gynoecium development.

Key words: *Arabidopsis*, auxin, development, female reproductive organ, gynoecium, patterning.

Introduction

The gynoecium is the female reproductive structure or structures of flowering plants (angiosperms), and is composed of one, two, or multiple carpels that are either fused or unfused. Its function, before developing into a fruit, is to harbour and protect one or several ovules, attract pollen to the apical stigma, induce pollen germination, and guide the pollen tubes through the transmitting tract towards the ovules. As these specialized tissues are critical for the fertilization of the female gametophyte, the gynoecial morphology can facilitate outcrossing-pollination or self-fertilization and provides a barrier to cross-species fertilization events. Thus, some of the genes that regulate gynoecial morphology are likely to be under selective pressure and may play a role in the evolution of new species (Alvarez-Buylla *et al*., 2000; Preston and Kellogg, 2006; Bartholmes *et al*., 2012). The gynoecium is also an agriculturally important structure in many crops in which the seeds or fruits are harvested.

Auxins are a class of plant hormones characterized originally by their growth modulating activities (Darwin, 1880; for a review, see Woodward and Bartel, 2005). Although there are several different biologically active auxins, this review uses the term 'auxin' to refer to indole-3-acetic acid (IAA), as this compound appears to be the most biologically important auxin within most plant species. It has been known for quite some time that auxin plays an important role for the gynoecial morphogenesis. Recent research findings provide an increased understanding of the complexity of auxin homeostatic mechanisms, the feed-forward and feed-back regulatory mechanisms that coordinate auxin biosynthesis, transport, inactivation, and signalling in plants. However, we are only just beginning to understand how the regulation of auxin homeostasis may provide positional information required for the proper specification of the different tissue types that constitute the gynoecium. Both the complexity of the gynoecial structure and the interconnectedness of the auxin homeostatic regulatory mechanisms have made the elucidation of the role of auxin in gynoecial development a challenging and interesting endeavour. Here the current status of efforts to understand auxin homeostatic mechanisms during development of the gynoecium in *Arabidopsis thaliana* are reviewed.

Gynoecial structure

The mature gynoecium is a complex structure with different organs, tissues, and cell types that are organized to function in concert to support female reproductive competence. Along

the apical–basal axis, the mature gynoecium is topped with stigmatic tissue, which is required for the reception and germination of pollen grains (Fig. 1A). Basal to the stigmatic tissue is the style, a section of the gynoecium that contains the apical portion of the transmitting tract, a specialized structure required for the growth of pollen tubes toward the internally located ovules. Basal to the style, and extending for the majority of the apical–basal extent of the gynoecium is the ovary, a hollow, tube-like section containing the ovules internally. Finally, the most basal portion of the gynoecium is the gynophore, a radially symmetrical internode structure that serves to connect the gynoecium to the base of the flower.

The ovary portion of the gynoecium is differentiated into medial and lateral domains that contain morphologically and functionally distinct structures (Fig. 1B, C). Additionally, both the medial and lateral domains of the ovary give rise to distinct structures or cell types from their adaxial and abaxial portions (Fig. 1B, C). The adaxial (inner) portion of the medial domain gives rise to the ovules, as well as the gynoecial septum that divides the ovary into two locules and contains the basal portion of the transmitting tract. The abaxial (outer) portion of the medial domain gives rise to the abaxial replum, an external structure that forms a narrow portion of the outer ovary wall. The lateral domains give rise to the valves that enclose the majority of the ovary chamber. The cells of the abaxial and adaxial sides of the valves can be distinguished both morphologically and based on differences in gene expression.

Gynoecial development

Arabidopsis floral and gynoecial development has previously been described and reviewed (Smyth *et al.*, 1990; Sessions, 1997; Bowman *et al.*, 1999; Balanza *et al.*, 2006; Østergaard, 2009; Sundberg and Ferrandíz, 2009). Briefly, floral development starts as floral meristems arise from the inflorescence meristem. Subsequently, floral organ primordia initiate sequentially from the peripheral regions of the floral meristem. After the sepal, petal and stamen primordia are formed, the remaining dome of floral meristem cells gives rise to the gynoecial primordium, which at stage 6 of floral development (stages according to Smyth *et al.*, 1990), arises as an oval collar or ring of cells. It is generally believed that the gynoecium of *Arabidopsis* as well as all members of the *Brassicaceae* family is comprised of two carpel organs that arise congenitally fused at their margins in this ring-like structure (summarized by Sattler, 1973; however, see Saunders, 1929 or Eames and Wilson, 1930 for an alternative interpretation suggesting the gynoecium is comprised of four carpels, two fertile and two sterile). The evolutionary origin of the angiosperm carpel is still enigmatic. One theory is that the carpels, like the other floral organs, represent modified leaf structures (Honma and Goto, 2001; Pelaz *et al.*, 2001; Ditta *et al.*, 2004; Castillejo *et al.*, 2005; reviewed in Scutt *et al.*, 2006). An alternative hypothesis, based on fossil records, suggests that the carpel is composed of a subtending leaf, an axillary branch, and a fertile leaf on the axillary branch (Doyle, 2012).

The gynoecial ring continues to develop into a tube-like structure that is oval in cross-section. Early during gynoecial development, the medial and lateral ovary domains and the abaxial and adaxial positional identities can be distinguished, as marked by distinctive patterns of gene expression (Fig. 1C). The cells of the medial domain proliferate and two ridges of tissue (the medial ridges) begin to develop adaxially within the domain. Cells within the medial ridges retain a degree of meristematic potential and subsequently give rise to the ovules and the gynoecial septum (with associated transmitting tract) (Fig. 1B), and in addition likely contribute to the formation of the style and stigmatic tissue. The apical–basal patterning becomes apparent as stigmatic cells and style start to develop at the apical end of the cylinder, and the cylinder closes apically at floral stage 10. Cells within the abaxial portions of the medial domain give rise to the abaxial replum, while cells of the lateral domains give rise to the valves. Later in gynoecial development the cells of the valve margin develop into the valve dehiscence zone that is required for seed dispersal. At floral stage 12 (Fig. 1A), the gynoecia are fully mature and ready to accept pollen.

Studies have revealed that intricate molecular networks regulate the definition of different domains, and that auxin plays a central role in the coordination of these networks. Here, we will discuss recent work implicating the importance of auxin response, transport, and synthesis in regulating gynoecial development.

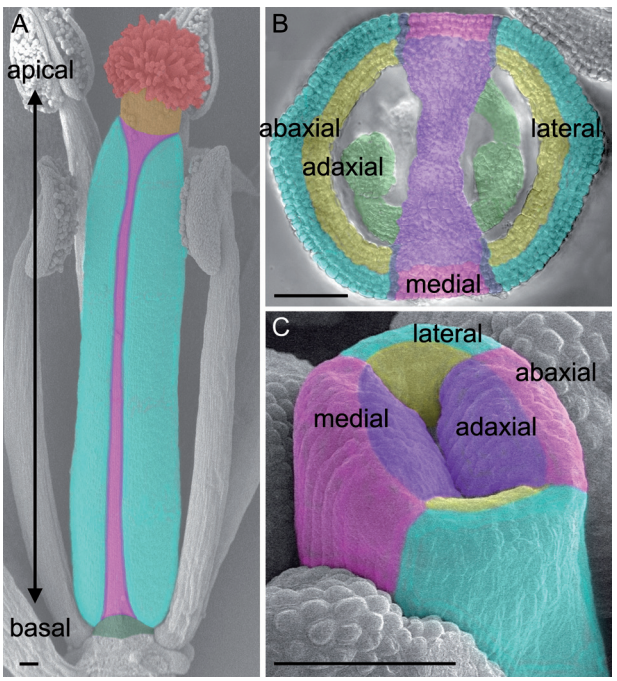

Fig. 1. Gynoecium development, axis formation, and tissue differentiation. (A) Mature gynoecium at floral stage 12; arrow indicates apical–basal axis. (B) Transverse section of a floral stage-11 gynoecium, indicating medial–lateral and abaxial–adaxial domains. (C) Young gynoecium at floral stage 7, indicating medial–lateral and abaxial–adaxial domains. (A) Red, stigma; mustard, style; blue and pink, ovary; green, gynophore. (B and C) Purple, medial adaxial domain; pink, medial abaxial domain; yellow, lateral adaxial domain; blue, lateral abaxial domain. (B) Green, ovules; purple, septum and transmitting tract. Bars, 50 μm.

Transcriptional response to auxin in the gynoecium

ARF and Aux/IAA protein families

The transcriptional response of *Arabidopsis* cells to auxin appears to be mediated by the nuclear TRANSPORT INHIBITOR RESISTANT1/AUXIN SIGNALLING F-BOX (TIR1/AFB) auxin receptors and their co-receptor AUXIN/INDOLE ACETIC ACID (Aux/IAA) family members (Dharmasari *et al.*, 2005; Kepinski and Leyser, 2005; Calderón Villalobos *et al.*, 2012). Auxin binding to a TIR1/AFB-Aux/IAA receptor complex induces proteasome-dependent degradation of the Aux/IAA, which results in the release of an AUXIN RESPONSE FACTOR (ARF) transcription factor otherwise bound to, and inhibited by Aux/IAA (Calderón Villalobos *et al.*, 2012; reviewed in Parry and Estelle, 2006). The *Arabidopsis* genome encodes six TIR1/AFB, 23 ARF and 29 Aux/IAA family members, enabling combinatorial interactions resulting in different auxin sensitivities, expression domains and responses. Loss-of-function mutants of the majority of the *ARF* and *Aux/IAA* genes do not condition a visible phenotype, or condition only relatively mild phenotypic disruptions (Woodward and Bartel, 2005). However, some members of the *ARF* gene family, notably *ARF3/ETTIN* (*ETT*) and *ARF5/MONOPTEROS* (*MP*), display obvious phenotypes when their activity is reduced or eliminated (Sessions and Zambryski, 1995; Przemeck *et al.*, 1996; Sessions *et al.*, 1997).

ARF3/ETT and ARF5/MP affect patterning along gynoecial positional axes

arf3/ett and *arf5/mp* single mutants display altered floral and gynoecial development. With respect to the structures along the apical–basal axis of the gynoecium, *arf3/ett* and *arf5/mp* mutants display a reduction of the valve tissue and expansion of stigmatic, abaxial stylar, and basal internode tissues (Sessions and Zambryski, 1995; Przemeck *et al.*, 1996; Sessions, 1997; Sessions *et al.*, 1997; Hardtke and Berleth, 1998). Although the role of *ARF5/MP* in early patterning of the embryonic axis is well established (Hardtke and Berleth, 1998), the *arf5/mp* gynoecial phenotypes have not yet been carefully described. However, studies of *arf3/ett* reveal that the expanded basal internode portions are unlikely to reflect true internodes, as they display characteristics of the abaxial style on their external surfaces (Sessions and Zambryski, 1995; Sessions, 1997). Additionally, *arf3/ett* mutant gynoecia display ectopic development of stigmatic and transmitting tract tissues on abaxial portions of the medial domain. As the transmitting tract is an adaxially localized tissue, this phenotype has been interpreted as the result of an adaxialization of the abaxial medial domain. The *arf3/ett* defects are partially mimicked by the application of the polar auxin transport inhibitor naphtylphtalamic acid (NPA), further supporting a role for auxin in the specification of positional identities during apical–basal and adaxial–abaxial gynoecial patterning (Nemhauser *et al.*, 2000).

Nemhauser and Sessions models of gynoecial patterning

Based on the observations of the *arf3/ett* mutant phenotype, and the morphological disruptions of gynoecial patterning that were seen upon NPA application to developing inflorescences, Nemhauser and *et al.* (2000) proposed a model for the role of auxin in patterning along the apical–basal axis of the gynoecium. The model suggests that auxin produced in the apical part of the gynoecia is transported basally, generating a morphogenic gradient that is used to pattern the gynoecium along the apical–basal axis. In this model, high auxin levels result in style and stigma development, intermediate levels result in ovary formation, while low levels promote gynophore development. Although widely cited, the key tenets of this model have yet to be confirmed.

The Nemhauser model is a modification of the earlier model proposed by Sessions (1997). The Sessions model also proposes the patterning of the gynoecial primordia through the specification of two boundaries. However, Sessions suggests that these boundaries are specified very early, during stage 6, when the gynoecial primordium is a radially symmetric dome of cells. As the early stage-6 gynoecium has yet to extend significantly in the apical–basal direction, the apical–basal and abaxial–adaxial identities are linked at this early stage. Thus what can be considered an abaxial–adaxial or central–peripheral pre-pattern is later elaborated into an apical–basal pattern in subsequent developmental stages. As the Sessions model was proposed before the identification of *ARF3/ETT* as an auxin response factor family member, the model does not specifically include auxin as a morphogen. However, it appears prudent to consider that an auxin gradient or some differential auxin signalling mechanism along the abaxial–adaxial or central-to-peripheral axis in the stage-6 gynoecium is required for proper specification of positional information along the apical to basal axis.

ARF3/ETT, KANADI, and abaxial fate specification

Further support for this latter assumption comes from the work revealing that the short valve and basally expanded style phenotypes of the *arf3/ett* mutant are also observed when activity of members of the *KANADI* (*KAN*) gene family is compromised (Pekker *et al.*, 2005). *KAN* genes encode GARP transcription factor proteins that play a key role in the specification of abaxial identity in lateral organs (including the gynoecium), the embryo and the vasculature (Eshed *et al.*, 2001, 2004; Kerstetter *et al.*, 2001; Ilegems *et al.*, 2010). The similarity between the gynoecial patterning defects of *arf3/ett* and higher-order *kan* mutants suggests that these phenotypes may be associated with some degree of loss of abaxial identity. The patterning defects, dramatically enhanced in *arf3/ett arf4* double mutants, with a near complete loss of valve tissue and with ovules developing at the apical tip (Pekker *et al.*, 2005), are also very similar to that reported for *kan1 kan2* double mutants (Eshed *et al.*, 2001). Additionally, mutations in *arf3/ett* enhance the phenotypes of *kan* loss-of-function alleles and suppress the phenotypes of ectopic *KAN*

overexpression, suggesting that these gene families work together to regulate adaxial–abaxial identities (Pekker *et al.*, 2005). Indeed, Kelley *et al.* (2012) demonstrated that ARF3/ETT physically interacts with the KAN1 and KAN4 proteins. The *kan4* mutants, also known as *aberrant testa shape* (*ats*), generate ovules with fused outer and inner integuments, a phenotype which again is similar to that of *arf3/ett* (Leon-Kloosterziel *et al.*, 1994; McAbee *et al.*, 2006; Kelley *et al.*, 2012). The expression patterns of *ARF3/ETT* and *KAN4/ATS* overlap within the abaxial portions of the inner integument and it has been suggested that the ARF3/ETT–KAN complex may be a conserved developmental module that functions in several developmental events (i.e. during embryogenesis, integument development and the regulation of laminar growth in the leaves; Kelley *et al.*, 2012). As *ARF3/ETT* and *KAN1* expression overlaps in the abaxial medial domain during early gynoecium development (Sessions *et al.*, 1997; Kerstetter *et al.*, 2001), they may act as a complex regulating early gynoecial patterning events.

KAN family members have been suggested to regulate patterning during embryonic development by altering auxin fluxes via regulation of the PIN-FORMED1 (PIN1) auxin transport facilitator (Izhaki and Bowman, 2007). Similarly, studies of vascular development in *kan* loss-of-function and gain-of-function alleles suggest that *KAN* genes act to reduce the expression and polarization of PIN1 (Ilegems *et al.*, 2010). In these experiments, the effects of ectopic KAN expression on vascular development could be rescued by the expression of PIN1 under the control of the *35S* promoter. Additionally, an inducible KAN activity was able to reduce the accumulation of *PIN1*, *PIN3*, and *PIN4* transcripts within 80 minutes after induction, suggesting regulation at least in part at the level of mRNA accumulation (Ilegems *et al.*, 2010). Kelley *et al.* (2012) suggest that the loss of *ARF3/ETT* and *KAN4/ATS* activity in the ovule results in altered *PIN1* expression, disrupting auxin homeostasis and resulting in the failure to properly specify the boundary between the inner and outer integuments. It would be interesting to study whether this module also affects PIN1 activity and localization during positional axis determination of the developing gynoecia.

Auxin response maxima or gradients during early gynoecia development

The auxin DR5 transcriptional reporter and the DII auxin sensor do not support the Nemhauser model

Although spatiotemporal auxin responses have been assessed for the last 15 years by analysing the expression of the synthetic auxin response promoter *DR5* (Ulmasov *et al.*, 1997; Friml *et al.*, 2003), a detailed description of its activity during early gynoecia development (earlier than stage 9) has not been presented. Benkova *et al.* (2003) showed that *DR5rev:GFP* is active in the stage-7 gynoecium primordium and that the signal was stronger towards the apex. In addition, Girin *et al.* (2011) reported a ring of strong *DR5rev:GFP* activity in the apical end of stage-9 gynoecia, confirming the results by Aloni *et al.* (2006), who saw a conspicuous but transient *DR5:GUS* expression in the developing style at approximately the same stage. The *DR5:GUS* expression later reappeared strongly in the developing stigma right before pollen maturation. After pollination the signal decreased.

In order to examine early auxin responses during gynoecia development, we have studied the *DR5rev:GFP* activity at stages 7–12 (Fig. 2). There was strong expression in two laterally localized foci in the apical tips of the lateral (valve) domains at stage 7 (Fig. 2A; Larsson *et al.*, unpublished). Two weaker foci of expression were detected in the apical tips of the medial domains starting at stage 8 (Fig. 2B; Larsson *et al.*, unpublished). *DR5rev* was also active in the presumptive provasculature cells, in both medial and lateral domains, most strongly in the basal and apical portions of the gynoecia. At late stage 8 or early stage 9 (Fig. 2C), the four *DR5rev:GFP* response foci observed in the apices of the lateral and medial domains began to broaden until *DR5rev:GFP* signal was seen as a ring encircling the apex of the gynoecium by stage 9. Girin *et al.* (2011) have suggested that auxin response in this domain may be required for the formation of style and stigma. Interestingly, no *DR5rev* activity could be detected in the developing style (stages 11 and 12) (Fig. 2D).

Together, these results indicate that local auxin maxima or local auxin responses may play important roles in establishing polarity, growth, or differentiation, although the expression pattern of the DR5 reporter does not strongly support the gradient hypothesis proposed by Nemhauser *et al.* (2000). It should be kept in mind that DR5 reports the transcriptional activity of a synthetic array of auxin response elements and may be influenced by cellular factors independent of the level of free auxin. As such, the DR5 reporter may not be a reliable reporter of all auxin responses in all tissues.

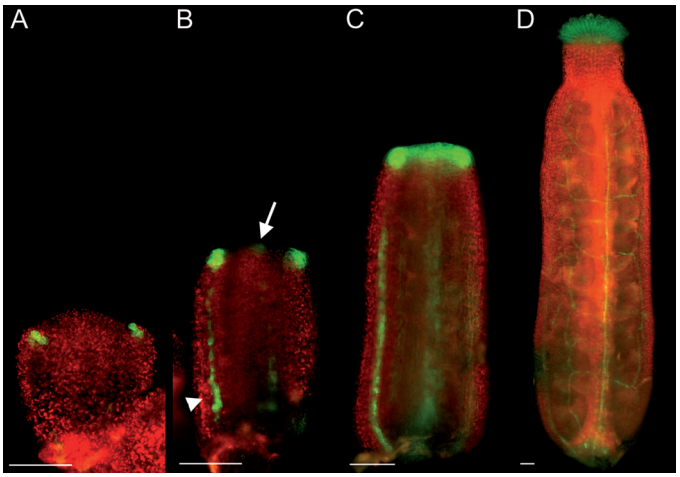

Fig. 2. *DR5rev:GFP* expression during gynoecia development. (A) *DR5rev* is expressed apically in two lateral foci in a stage-7 gynoecium. (B) Arrow indicates weak expression in apical medial tip, arrowhead indicate expression in pro-vasculature of a stage-8 gynoecium. (C) *DR5rev* expression encircles the whole apical part of a stage-9 gynoecium. (D) *DR5rev* is expressed in vasculature and in stigmatic papillae of mature stage-12 gynoecia. Bars, 50 μm.

A new complementary Aux/IAA-based auxin sensor, DII-VENUS, has recently been used to characterize auxin levels in the developing plant (Brunoud *et al*., 2012). The DII-VENUS sensor acts upstream of the DR5 reporter in the auxin signalling pathway and is likely to be more sensitive and respond more quickly to dynamic changes in auxin levels (Brunoud *et al*., 2012). DII-VENUS signals in the developing gynoecium suggest that auxin responses are higher in the whole medial domain, than they are in the lateral domain (valves), but, like those from the DR5 reporter, the DII-VENUS signals do not suggest an apical–basal auxin response gradient (Larsson *et al.*, unpublished).

Considering the complexity of the gynoecial structure, the best way to comprehensively map auxin levels may be to do thorough tissue-specific auxin measurements from isolated protoplast populations, similar to those made in roots (Petersson *et al*., 2009).

PIN-dependent auxin transport during gynoecial development

Benkova *et al*. (2003) suggested that early gynoecial primordium development is, similar to all other aboveground developing primordia, dependent on apical auxin transport mediated by PIN1. In support of this, *pin1* (Okada *et al*., 1991) as well as *pin3 pin7* (Benkova *et al*., 2003) mutants have severely distorted gynoecia. The valve-length of these mutants is reduced concomitantly with enlarged style, stigma, and gynophore (Bennett *et al*., 1995; Sohlberg *et al*., 2006). Similar gynoecia defects have been described when *PINOID*, which encodes an auxin-inducible serine–threonine protein kinase regulating the polarity of PIN1 localization, is compromised (Christensen *et al*., 2000; Benjamins *et al*., 2001; Friml *et al*., 2004; Sohlberg *et al*., 2006). However, floral meristem initiation is largely dependent on proper polar auxin transport (PAT), and, since the gynoecia start to differentiate late during flower development, it is hard to know whether the severe mutant phenotypes are caused by disruptions to early PAT before meristem initiation or later PAT disturbances in the developing gynoecia.

As mentioned above, blocking auxin transport by spraying inflorescence meristems with the PAT inhibitor NPA results in similar gynoecium defects as when *PIN* or *PINOID* gene activities are compromised; these results founded the Nemhauser gradient hypothesis (Nemhauser *et al*., 2000). However, the treatment also affects the amount of medial versus lateral tissues (Nemhauser *et al*., 2000; Nole-Wilson *et al*., 2010), an observation that the gradient hypothesis does not take into account. An alternative hypothesis would be that lateral and medial tissues experience different levels or responses to auxin upon the block of PAT and that this results in less growth of the lateral domain-derived valves, while the growth of the medial-derived style and other tissues is enhanced. In addition, in contrast to what may be expected according to the apical–basal auxin gradient hypothesis, Sorefan *et al*. (2009) detected apically localized PIN1-GFP in the valve epidermis of stage-10 gynoecia. Although Alvarez *et al*. (2009) could show that *PIN1* is strongly expressed in stage-7 and stage-8 gynoecia, we are still missing detailed descriptions of PIN protein localizations at these and earlier stages, when auxin maxima important for axis formation may be established. Thus, it is too early to draw any conclusions about where these are localized.

It has recently been suggested that the basic helix–loop–helix proteins SPATULA and INDEHISCENT collectively affect PAT by inducing and repressing the WAG2 and PINOID kinases respectively, which in turn determine the polar localization of the PINs (Girin *et al*., 2011). *SPATULA* is expressed in medial tissues (Heisler *et al*., 2001), and *spatula* gynoecia are often split open at the apex and display reduced development of the medial tissues and the style (Alvarez and Smyth, 1999). Induction of ectopically expressed *INDEHISCENT* resulted in PIN relocalization (Sorefan *et al*., 2009), and in *spt* mutants the strong circular *DR5:GFP* expression in the apical end of stage-9 gynoecia is perturbed (Girin *et al*., 2011). Interestingly, NPA treatment can rescue the *spt* split-style phenotype (Nemhauser *et al*., 2000; Ståldal *et al*., 2008), which together with the above-mentioned results suggest that *SPATULA* and *INDEHISCENT* are involved in maintaining a high auxin level in the apical end of stage-9 gynoecia by inhibiting PAT. However, a thorough analysis of the spatiotemporal localization of PIN proteins during early gynoecium development is required before a well-supported hypothesis about auxin transport and its relationship to overall gynoecium morphogenesis can be put forward.

What role does local auxin biosynthesis play during gynoecia development?

It has recently been suggested that the auxin biosynthesis pathway via tryptophan and indole-3-pyruvate involves the activity of both the *TRYPTOPHAN AMINOTRANSFERASE OF ARABIDOPSIS1* (*TAA1*)/*TRYPTOPHAN AMINOTRANSFERASE RELATED* (*TAR*) and the *YUCCA* (*YUC*) family genes (Mashiguchi *et al*., 2011; Stepanova *et al*., 2011; Won *et al*., 2011). Members of these gene families are expressed in different locations or domains during early gynoecia development, suggesting that they play a central role in auxin-regulated gynoecia morphogenesis. *TAA1* is expressed in the apical medial domain already when the gynoecium emerges and later expands basally to cover two cell files along the medial meristematic ridge, whereas *TAR2* is expressed throughout the gynoecium primordium, and later becomes restricted to the outer cell layers that give rise to the valves (Stepanova *et al*., 2008). *YUC1* is expressed in the base of young gynoecia, as is also *YUC4*. Later, *YUC4* is highly expressed in the apical end, whereas *YUC2* is expressed in the valves and earlier in a wider domain (Cheng *et al*., 2006), suggesting that auxin is produced in several regions during gynoecium development, and not only apically as suggested by the Nemhauser model.

The expression domains of all members have not yet been described, thus a complete picture of auxin biosynthesis during gynoecium development cannot yet be established. However, it appears as if there is some redundancy or compensatory pathways involved, as none of the single mutants

studied shows a gynoecial phenotype deviating from wild type. Within these multigene families, it might be informative to look for compensatory changes in the expression patterns of paralogous family members when the activity of one member of the gene family is eliminated. Compensatory expression of members of the *PIN* gene family has been observed in roots and is likely to support the redundant functioning of members of this gene family (Blilou *et al*., 2005). Multiple mutant combinations in *TAA*/*TAR* and *YUC* genes, on the other hand, develop severely distorted gynoecia with abnormal apical parts and complete lack of valve tissue (Cheng *et al*., 2006; Stepanova *et al*., 2008). Still, these mutant phenotypes may not clarify the role of local auxin synthesis within the developing gynoecium as loss of function of these biosynthesis genes also may limit the availability of incoming auxin synthesized via these pathways in tissues outside of the gynoecium. To fully understand the role of spatiotemporally regulated auxin synthesis in the gynoecia, constructs or mutants allowing for downregulation of the biosynthesis genes only at one specific expression site within the gynoecia are needed.

To this end, mutations in the *YUC4* transcriptional regulators encoded by the *SHORT INTERNODES*/*STYLISH* (*SHI*/*STY*) and *NGATHA* (*NGA*) gene families can be informative concerning the function of the *YUC4*-mediated apical auxin biosynthesis. Members of the *SHI*/*STY* and *NGA* gene families are expressed in the apical part of the gynoecia from stage 6 and this remains the major expression site until stages 9 or 10 (Kuusk *et al*., 2002; Alvarez *et al*., 2009; Trigueros *et al*., 2009). Inactivation of STY1 leads to subtle style defects, but this effect is dramatically enhanced, including an unfused apical end, when additional SHI/STY members are compromised (Kuusk *et al*., 2006). Knocking down the activity of multiple members of the *NGA* gene family results in similar apical defects (Alvarez *et al*., 2009; Trigueros *et al*., 2009) and it has been suggested that SHI/STYs and NGAs act together (Trigueros *et al*., 2009). It has been shown that STY1 binds directly to and activates the *YUC4* gene, suggesting that the early apical expression of *YUC4* in the *STY1* expression domain is mediated by STY1 activity (Sohlberg *et al*., 2006; Eklund *et al*., 2010). In addition, Trigueros *et al*. (2009) could show that when NGA protein formation is down-tuned via the activity of an artificial micro-RNA-NGA construct, expression of *YUC4:GUS* was blocked only in the apical end of gynoecia. This indicates that auxin biosynthesis in the apical end is important for the postgenital fusion of the apical end and for style morphogenesis. Interestingly, the apical style defect of *SHI*/*STY* mutants can be rescued by local addition of auxin to the very tip of developing gynoecia (Ståldal *et al*., 2008), suggesting that it is indeed auxin that is lacking when *YUC4* apical expression is compromised in *shi*/*sty* or *nga* mutant lines. Worth noting is that the septum also is formed during a postgenital fusion event at the positions of strong *TAA1* expression. However, whether a lack of this distinct expression pattern result in aberrant postgenital fusion of the medial domain or not remains to be investigated.

It would be interesting if similar approaches were to be used to reduce the expression of auxin biosynthesis genes in, for example, the medial domain (*TAA1* expression site) or the lateral domain (*TAR2*/*YUC2*) using tissue-specific promoters driving artificial micro-RNA constructs against the auxin-biosynthesis genes.

The role of auxin during gynoecia development in other species

The *Arabidopsis* gynoecium is a rather complex structure compared to the gynoecia of several other angiosperm species. Plant species possessing a single free carpel, such as the members of the family *Leguminosae*, would therefore serve as better models of the angiosperm gynoecium. However, studies of other species besides *Arabidopsis* are lagging behind and only very limited data from experimental studies of auxin responsiveness during carpel development in legumes (e.g. *Pisum sativum* and *Medicago truncatula*) is available. Interestingly, when the *M. truncatula MtPIN10* gene is compromised, two carpels form in some flowers (5/60) (Peng and Chen, 2011), suggesting that auxin may be involved in defining the species-specific carpel number. Pea and *M. truncatula* plants transformed with the *DR5* promoter driving GUS or GFP, revealed *DR5* activity in all incipient organ primordia of young floral meristems, including the central part that will become the carpel (DeMason and Polowick, 2009; Zhou *et al*., 2011). Later during gynoecium development, DeMason and Polowick (2009) observed the strongest *DR5::GUS* expression at the tip and base of the pea carpel, which is similar to the *DR5* activity peaks in the *Arabidopsis* gynoecium. However, it is not clear if the basal expression in pea was in the vasculature, as we suggest for *Arabidopsis*. Interestingly, in contrast to *Arabidopsis*, pea displays a strong *DR5:GUS* expression in the style, but lacks expression in the stigma. Further studies are needed to study the possible biological significance of these differences.

Conclusion

It is evident from the reviewed work that auxin plays a central role in gynoecium development and pattern formation. However, although a lot of great work has been done in this area, it is clear that we still are only in the beginning of the journey to fully understand when, where, and how auxin is involved in defining tissue domains, or determining tissue differentiation and growth. One conclusion in this direction that can be drawn from the published work is that differential auxin concentrations and/or auxin responses along the abaxial–adaxial or central–peripheral axis appear to also affect patterning along the apical–basal axis. However, whether auxin affects these developmental decisions before the gynoecium emerges from the central dome of the flower or during later gynoecium development remains to be investigated.

Considering the relative complexity of the gynoecium, it may not be surprising that we still have a lot to learn. It is clear that additional spatiotemporal mapping of auxin response, content, transport, and biosynthesis as well as conjugation and catabolism is required. In order to connect this information to

the developmental decisions during early gynoecia development, cell- or tissue-specific manipulations of auxin content, responses, or directions of auxin transport, using for example amiRNAs expressed from tissue-specific promoters or inducible constructs would be extremely valuable.

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