Auxin and Flower Development: A Blossoming Field

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The establishment of the species-specific floral organ body plan involves many coordinated spatiotemporal processes, which include the perception of positional information that specifies floral meristem and floral organ founder cells, coordinated organ outgrowth coupled with the generation and maintenance of inter-organ and inter-whorl boundaries, and the termination of meristem activity. Auxin is integrated within the gene regulatory networks that control these processes and plays instructive roles at the level of tissue-specific biosynthesis and polar transport to generate local maxima, perception, and signaling. Key features of auxin function in several floral contexts include cell nonautonomy, interaction with cytokinin gradients, and the central role of *MONOPTEROS* and *ETTIN* to regulate canonical and noncanonical auxin response pathways, respectively. *Arabidopsis* flowers are not representative of the enormous angiosperm floral diversity; therefore, comparative studies are required to understand how auxin underlies these developmental differences. It will be of great interest to compare the conservation of auxin pathways among flowering plants and to discuss the evolutionary role of auxin in floral development.

Angiosperm flowers display extraordinary diversity in their body plan and every aspect of morphology, including organ number and positioning, size, symmetry, color, and organogenesis. The study of select angiosperm species has elaborated the genetic basis of floral identity according to the coordinated expression of transcription factors. However, advances in auxin biology have demonstrated that auxin is a central developmental regulator of floral morphogenesis (Irish 2010; Wellmer et al. 2014).

Auxin is essential for the initiation of floral meristems (FMs) and organs and for terminating meristem growth; however, its role in deter-

mining positional information for floral organ initiation remains less mechanistically well understood than its function in organ outgrowth and development (Chandler 2011). Auxin can also function as a morphogen by generating response gradients, particularly in combination with those of cytokinins, which are instrumental in several aspects of floral organogenesis (Su et al. 2011).

This review summarizes how auxin coordinates diverse and complex regulatory gene networks in FM and organ initiation and development and focuses on the *Arabidopsis* model for which most is known. It also highlights the cen-

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tral role of canonical and noncanonical auxin response factors (ARFs) such as MONOPTEROS (MP) and ETTIN (ETT/ARF3), respectively.

AUXIN IS ESSENTIAL FOR FLORAL MERISTEM INITIATION

Floral organ primordia arise from FMs, which initiate as lateral organs at the inflorescence meristem (IM) periphery. The maintenance of the IM required a precise balance between the self-renewal of stem cells and cell differentiation. It has been proposed that the WUSCHEL (WUS) transcription factor achieves this balance by restricting auxin signaling in stem cells and simultaneously allowing low levels of auxin signaling output (Ma et al. 2019), although the precise mechanism of this rheostat function on select auxin signaling components remains to be elucidated.

Precise spatiotemporal local auxin biosynthesis within the IM and its polar transport and accumulation at specific sites from sources elsewhere in the plant are essential for FM initiation (Alvarez-Buylla et al. 2010; Yadav et al. 2019). This is evidenced by classical mutants, for example, in *PIN-FORMED1* (*PIN1*), which encodes a polar auxin efflux carrier (Gälweiler et al. 1998). Occasional flowers on *pin1* inflorescences lack sepals and have a variable number of abnormally shaped petals (Okada et al. 1991) and exogenous auxin application to *pin1-1* inflorescences restores flower formation at the point of application (Reinhardt et al. 2000). Mutation of the gene encoding the PINOID (PID) kinase that regulates PIN activity leads to flowers with supernumerary petals, few sepals and stamens, and mostly *pin*-like carpels (Bennett et al. 1995). Strong mutants of ARF5/MONOPTEROS (MP) produce naked inflorescences (Przemeck et al. 1996), although the hypomorphic *mps319* allele occasionally produces rudimentary flowers (Alonso et al. 2003; Cole et al. 2009). Loss-of-function of *MACCHI-BOU4* (*MAB4*)/*ENHANCER OF PINOID* (*ENP*)/*NAKED PINS IN YUC MUTANTS 1* (*NPY*), hereafter *MAB4*, which encode NONPHOTOTROPIC HYPOCOTYL 3-like proteins that regulate PIN endo-

cytosis, also causes *pin*-like inflorescences (Furutani et al. 2014), due to the abolition of auxin flow from external FM layers inward to create an auxin sink necessary for FM development after initiation.

Auxin response maxima prepattern sites of FM initiation at the IM periphery (Reinhardt et al. 2000), and in addition to the importance of the spatial distribution of auxin within the SAM as an instructive signal, recent high-resolution imaging of auxin response has revealed the importance of a dynamic temporal component to these auxin fluxes (Galvan-Ampudia et al. 2020). In this scenario, high-precision dynamic spatiotemporal auxin gradients within the IM periphery are coordinated with growth, to ensure that cells are exposed to a high level of auxin over time to activate organogenesis. The temporal aspect concerning the timing and duration of exposure of cells to high auxin is relevant because, according to phytomer theory, FMs initiate as lateral organs in the axils of bracts. *Arabidopsis* bracts are cryptic but are histologically and morphologically visible during early floral transition preceding FM initiation (Kwiatkowska 2008). Transcriptional reprogramming associated with bract initiation has been accessed in the *ap1 cal* mutant background by sorting cells that express the bract founder-cell marker *DORNROESCHEN-LIKE* (*DRNL*) (Frerichs et al. 2016). Minor transcriptional changes in genes in the auxin synthesis, transport, and response machinery suggest that its global activation might not be associated with bract initiation, but with FM initiation. This is consistent with spatially distinct domains of *DRNL* and *DR5* expression within the IM (Chandler et al. 2011) and that auxin response minima are required for the formation of axillary vegetative meristems in *Arabidopsis* and tomato (Wang et al. 2014a,b). Interacting concentration gradients of auxin and cytokinin establish the timing of FM outgrowth within the IM, which can be uncoupled from initiation (Besnard et al. 2014).

An early feature of FM initiation is inhibition of the pluripotency of stem cells that form FM primordia, by down-regulation of *SHOOT-MERISTEMLESS* (*STM*). Transcripts of *STM*

overaccumulate in the IM of *mpS319* and more so in an *mp arf3 arf4* triple mutant, resulting in complete loss of flower primordia. ETT directly binds to *STM* and silences its expression for FM fate acquisition. By contrast, MP does not bind *STM*, but directly activates expression of the repressor *FILAMENTOUS FLOWER (FIL)*, which further contributes to *STM* silencing. ETT and FIL also directly bind and repress expression of *BREVIPEDICELLUS (BP)*, which maintains meristematic identity. Although MP, ETTIN, and ARF4 repress *STM* and *BP* expression in parallel pathways, FIL physically interacts with ETTIN and ARF4 to create a complex that recruits chromatin modifiers such as histone deacetylase HDA19, which leads to target-gene silencing (Table 1; Fig. 1; Chung et al. 2019).

THE CENTRAL ROLE OF MP IN FM DEVELOPMENT

MONOPTEROS is the central regulator of FM development and translates a local auxin concentration maximum into a floral primordium by directly transcriptionally activating several transcription factors. These include the master regulator *LEAFY (LFY)*, which, together with its target *APETALA1*, regulates the IM-to-FM identity transition (Weigel et al. 1992). *LFY* expression in incipient flower primordia is enhanced by high auxin levels, via the canonical MP/IAA12 (BODENLOS) module (Yamaguchi et al. 2013). *LFY* expression only partially rescues *mp-S319* flower defects and MP also transcriptionally activates *AINTEGUMENTA (ANT)* and *AINTEGUMENTA-LIKE6 (AIL6)*, which function in parallel and redundantly with *LFY* in FM initiation. Because the *lfy-6 ant-4 ail6-2* triple mutant initiates some flowers, additional auxin-regulated factors probably contribute to FM production. *ANT* and *AIL6* also function upstream of *LFY* and regulate its expression in response to auxin and in parallel to MP. Accordingly, they are expressed earlier than *LFY* (Yamaguchi et al. 2016) and delayed *LFY* expression in *ant ail6* delays FM production. However, expression of *ANT* and *AIL6* in *mp* is reduced but not abolished (Yamaguchi et al. 2013), suggesting that they are also regulated

independently from MP, potentially via other ARFs.

In summary, MP triggers FM initiation and development by transcriptionally up-regulating *LFY*, *ANT*, and *AIL6* via two separate pathways in which *LFY* both targets *ANT/AIL6* and acts in parallel with them. In addition, *LFY* positively feeds back into the auxin pathway, to ensure maintenance of the auxin response network and to lock the auxin response in an “on” state that ensures commitment to flowering (Table 1; Fig. 1). Expression of *DR5er:GFP* positively correlates with *LFY* activity, and feedback regulation on auxin signaling by *LFY* occurs partly by up-regulation of *PID* expression (Li et al. 2013; Yamaguchi et al. 2013).

The reprogramming of MP targets in meristematic cells that develop into floral primordia involves chromatin remodeling via the SWI/SNF ATPases *BRAMA (BRM)* and *SPLAYED (SYD)*, which increase chromatin accessibility in early floral primordia. Initiation of fewer flower primordia and *pin*-like inflorescences in *mpS319 syd* double mutants and *mpS319* plants expressing an artificial microRNA responsible for *BRAHMA* down-regulation demonstrate the functional relevance of *BRM* and *SYD* in FM initiation. Auxin enhances MP binding to *BRM* and *SYD*, which also bind the direct MP targets *FIL*, *LFY*, *ANT*, and *TMO3* (Wu et al. 2015). Therefore, a chromatin switch model explains how binding of AUX/IAA proteins to MP at low auxin concentrations prevents the recruitment of SWI/SNF factors by MP, which instead binds to the TOPLESS (TPL) corepressor and HISTONE DEACETYLASE19 (HDA19), which promote a repressive chromatin state. Increasing auxin concentrations degrade AUX/IAA proteins, enabling MP to recruit SWI/SNF-containing complexes, which promote a permissive chromatin state that up-regulates MP targets (Table 1; Fig. 1; Wu et al. 2015).

FLORAL ORGAN INITIATION

Floral morphogenesis involves the perception of positional information within the FM by floral organ founder cells, to become specified, acquire a particular fate and outgrow. These processes

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Table 1. Summary of genes mentioned in the text

Gene	Protein function/relation with auxin	Floral developmental context	References
<i>PIN-FORMED1 (PIN1)</i>	Auxin efflux carrier	Flower initiation (FI), organ initiation (OI)	Okada et al. 1991
<i>PINOID (PID)</i>	Kinase that regulates PIN1 polarization	FI, OI	Bennett et al. 1995
<i>ARF5/MONOPTEROS</i>	Auxin response factor (ARF), transcription factor (TF)	FI, organ boundaries (OBs) OI, organ growth (OG)	Li et al. 2013; Yamaguchi et al. 2013
<i>MACCHI-BOU4 (MAB4)</i> <i>DORNROESCHEN-LIKE (DRNL)</i>	Regulates PIN1 polarization AP2/ETHYLENE RESPONSE FACTOR TF, a floral-organ founder-cell marker whose expression precedes DR5 and genetically interacts with <i>PID</i>	FI FI, petal initiation	Furutani et al. 2014 Chandler et al. 2011
<i>SHOOTMERISTEMLESS (STM)</i>	KNOX TF, <i>STM</i> is repressed by auxin	FI	Chung et al. 2019
<i>ARF3/ETTIN (ETT)</i>	ARF, TF	FI, gynoecium growth, megaspore mother cell (MMC), floral meristem determinacy (FMD)	Sessions et al. 1997; Chung et al. 2019
<i>ARF4</i>	ARF, TF	FI	Chung et al. 2019
<i>FILAMENTOUS FLOWER (FIL)</i>	TF, <i>FIL</i> is activated by auxin	FI	Chung et al. 2019
<i>BREVIPEDICELLUS (BP)</i>	KNOX TF, <i>BP</i> is repressed by auxin	FI	Chung et al. 2019
<i>HISTONE DEACETYLASE19 (HDA19)</i>	Chromatin remodeling factor, interacts with ETT and ARF4	FI	Chung et al. 2019
<i>LEAFY</i>	Transcriptional master regulator connected with auxin maximum maintenance, <i>LFY</i> is activated by MP	FI	Weigel et al. 1992; Li et al. 2013; Yamaguchi et al. 2013
<i>AINTEGUMENTA (ANT)</i>	AP2-domain TF transcriptionally up-regulated by MP	FI, OI	Yamaguchi et al. 2013)
<i>AINTEGUMENTA-LIKE6 (AIL6)</i>	AP2-domain TF transcriptionally up-regulated by MP	FI	Yamaguchi et al. 2013
<i>IAA12/BODENLOS</i>	Aux/IAA protein that interacts with MP	FI	Yamaguchi et al. 2013
<i>TOPLESS (TPL)</i>	Corepressor of MP and ETT targets	FI	Wu et al. 2015
<i>BRAMA (BRM)</i>	SWI/SNF ATPs chromatin remodeling interactor of MP	FI	Wu et al. 2015

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Table 1. Continued

Gene	Protein function/relation with auxin	Floral developmental context	References
<i>SPLAYED (SYD)</i>	SWI/SNF ATPs chromatin remodeling interactor of MP	FI	Wu et al. 2015
<i>TARGET OF MONOPTEROS3 (TMO3)</i>	AP2 TF transcriptionally up-regulated by MP	FI, ovules	Wu et al. 2015; Cucinotta et al. 2016
<i>YUCCA (YUC)</i>	Flavin-monooxygenases that catalyze IAA biosynthesis	FI, OI	Cheng et al. 2006
<i>DORNROESCHEN (DRN)</i>	AP2/ETHYLENE RESPONSE FACTOR that genetically interacts with <i>PIN</i>	FI, OI	Chandler and Werr 2014
<i>PUCHI</i>	AP2/ETHYLENE RESPONSE FACTOR that genetically interacts with <i>PID</i>	FI, OI	Chandler and Werr 2014
<i>PERIANTHIA (PAN)</i>	bZIP TF that regulates <i>YUC1</i> and <i>YUC4</i>	OB, petal initiation	Maier et al. 2011
<i>PRESSED FLOWER (PRS)</i>	WUSCHEL-RELATED HOMEODOMAIN, <i>PRS</i> expression is induced by auxin	Petal initiation	Chandler and Werr 2014; Caggiano et al. 2017
<i>ABC1/19</i>	Auxin efflux carrier	FI, anther growth, pollen	Zhao et al. 2013; Cecchetti et al. 2015
<i>CUP-SHAPED COTYLEDON (CUC)</i>	NAC-domain TFs regulated by auxin, <i>CUC</i> function is connected with polar auxin transport (<i>PAT</i>)	OB, ovules	Laufs et al. 2004; Mallory et al. 2005; Galbiati et al. 2013
<i>RABBIT EARS (RBE)</i>	C2H2 zinc finger protein connection with <i>PAT</i>	OB, petal growth	Lampugnani et al. 2013
<i>HANABA TARANU (HAN)</i>	GATA3-type transcriptional repressor with a putative role in <i>PAT</i> regulation	OB	Zhang et al. 2013
<i>PETAL LOSS (PTL)</i>	Trihelix TF connected with <i>PAT</i>	OB, petal growth	Lampugnani et al. 2013
<i>AUX1</i>	Auxin influx carrier	Petal growth	Lampugnani et al. 2013
<i>SUPERMAN (SUP)</i>	C2H2 zinc-finger protein that represses <i>YUC</i> expression, <i>SUP</i> is regulated by <i>LFY</i>	OB	Sakai et al. 1995; Xu et al. 2018
<i>DEVELOPMENT-RELATED MYB-LIKE1 (DRMY1)</i>	MYB-like TF that regulates auxin response	Sepal outgrowth	Zhu et al. 2020
<i>JAGGED (JAG)</i>	Zinc-finger protein that regulates auxin response	Petal growth	Dinnyen et al. 2004; Sauret-Güeto et al. 2013
<i>ARGOS</i>	Auxin-inducible gene that regulates <i>ANT</i>	Petal growth	Hu et al. 2003

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Table 1. *Continued*

Gene	Protein function/relation with auxin	Floral developmental context	References
<i>BIGPETALp</i>	bHLH TF that interacts with ARF8	Petal growth	Varaud et al. 2011
ARF8	ARF, TF	Petal growth, stamen growth	Nagpal et al. 2005; Varaud et al. 2011
ARF8.4	ARF, TF	Stamen growth	Ghelli et al. 2018
ARF6	ARF, TF	Stamen growth	Nagpal et al. 2005
<i>SHORT INTERNODES (SHI)</i>	Zinc-finger TFs that regulate auxin biosynthesis	Anther growth	Estornell et al. 2018
<i>STYLISH1/2 (STY1/2)</i>	Zinc-finger TFs that regulate auxin biosynthesis	Anther growth, gynoecium growth	Trigueros et al. 2009; Estornell et al. 2018
TIR1/AFB	Nuclear auxin receptor	Pollen, female gametophyte (FG)	Cecchetti et al. 2008, 2017
<i>DEFECTIVE IN ANTHER DEHISCENCE1 (DAD1)</i>	Enzyme for JA biosynthesis transcriptionally up-regulated by ARF6/ARF8	Anther dehiscence	Nagpal et al. 2005; Tabata et al. 2010
<i>MYB26</i>	TF, <i>MYB26</i> is induced by ARF6/ARF8	Anther dehiscence	Yang et al. 2017
ARF17	ARF, TF	Anther dehiscence	Xu et al. 2019
<i>MYB108</i>	TF, <i>MYB108</i> is transcriptionally up-regulated by ARF17	Anther dehiscence	Xu et al. 2019
IAA19	Aux/IAA protein	Anther dehiscence	Tashiro et al. 2009; Cecchetti et al. 2017
<i>AGAMOUS (AG)</i>	MADS-box TF, regulates <i>YUC4</i>	FMD	Liu et al. 2011, 2014; Yamaguchi et al. 2018
<i>WUSCHEL (WUS)</i>	Homeobox TF that controls the stem-cell pool, regulated by ETT	FMD	Sun et al. 2009; Liu et al. 2011; Huang et al. 2017
<i>APETALA 2 (AP2)</i>	AP2/EREBP TF, negatively regulates <i>ETT</i>	FMD	Liu et al. 2014
<i>CRABS CLAW (CBR)</i>	YABBY TF that transcriptionally up-regulates <i>TRN</i> and <i>YUC4</i>	FMD	Yamaguchi et al. 2018
<i>TORNADO (TRN2)</i>	Tetraspanin protein that regulates auxin homeostasis	FMD	Yamaguchi et al. 2018
<i>CHROMATIN REMODELING 11/17 (CHR11/17)</i>	Recruited by AG to activate <i>YUC</i>	FMD	Yamaguchi et al. 2018
<i>KANADII/4 (KAN1/4)</i>	GARP TF that interacts with ETT	Gynoecium growth	Pekker et al. 2005
<i>SPATULA (SPT)</i>	bHLH TF that regulates PAT	Gynoecium growth	Moubayidin and Ostergaard 2014

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Table 1. *Continued*

Gene	Protein function/relation with auxin	Floral developmental context	References
<i>INDEHISCENT (IND)</i>	bHLH TF that regulates PAT	Gynoecium growth	Moubayidin and Ostergaard 2014; Kuhn et al. 2019
<i>PIN-FORMED3 (PIN3)</i>	Auxin efflux carrier	Gynoecium growth	Gremski et al. 2007
<i>NGATHA (NGA3)</i>	RAV family TF that regulates YUCs	Gynoecium growth	Trigueros, et al. 2009
<i>TOPLESS-RELATED2 (*TPR2)</i>	Corepressor of ETT targets	Gynoecium growth	Benjamins et al. 2001; Gremski et al. 2007
<i>HECATE (HEC)</i>	bHLH TF that regulates PAT	Gynoecium growth	Gremski et al. 2007

The gene or protein function and relationship to auxin is described in the second column, and the third column shows the specific developmental context within flower development with which these genes are involved. Genes whose proteins have a direct molecular link with auxin are shown in bold.

are associated with the establishment and maintenance of organ boundaries.

Expression of the synthetic auxin response reporters DR5::GFP and DII (van Mourik et al. 2012; Goldental-Cohen et al. 2017) demonstrate that auxin response maxima colocalize with sites of organ initiation. However, it is unclear whether auxin specifies floral organ founder cells, or merely activates outgrowth. Modeling of auxin responses (van Mourik et al. 2012) and the ABCE model of floral organ identity acquisition propose that concentric organ whorls are specified centripetally from outer to inner (Theissen and Saedler 2001). However, *Arabidopsis* floral body plan establishment is more complex and the *DRNL* founder-cell marker reveals biphasic development in which sepals initiate unidirectionally from the center of the IM, petals and lateral stamens arise from adjacent domains, and medial stamens initiate from a ring of *DRNL* prepatterning (Chandler and Werr 2014). Expression of *DRNL* precedes auxin response maxima for some floral organs (Chandler et al. 2011) and the response of *DRNL* to auxin remains enigmatic (Comelli et al. 2020). However, *DRNL* and its close relatives *DRN* and *PUCHI* redundantly determine numbers of all floral organs at the founder-cell level, partly cell-nonautonomously (Table 1; Fig. 1; Chandler and Werr 2017). All three proteins integrate into auxin pathways: *DRN* genetically interacts

with *pin1*, *DRNL* with *pid*, *yuc1* and *yuc4* (Chandler et al. 2011), and *PUCHI* with *pid* (Chandler and Werr 2014). *PERIANTHIA* (*PAN*) regulates the number of *Arabidopsis* perianth organs by targeting YUC-mediated auxin biosynthesis (Maier et al. 2011). The radially symmetrical arrangement of five perianth-whorl organs in *pan* (Running and Meyerowitz 1996) derives from bifurcations of sepal founder-cell populations at abaxial and adaxial positions and is partially dependent on *PRESSED FLOWER* (*PRS*) activity (Chandler and Werr 2014), which promotes cell proliferation in lateral floral primordium domains (Matsumoto 2001). In leaf primordia, auxin up-regulates *PRS* and its paralogue *WOX1* (Caggiano et al. 2017) via MP (Guan et al. 2017). Increased expression of DR5::GFP in the leaf base of *prs wox1* and the increased sensitivity of *prs* leaves to auxin transport inhibitors implies a role for *PRS* in auxin transport (Nakata et al. 2018). By analogy, positive feedback between auxin and *PRS* might exist during sepal initiation. Mutation of *ETT* also leads to one supernumerary petal and sepal (Sessions et al. 1997; Pekker et al. 2005).

FLORAL ORGAN BOUNDARY ESTABLISHMENT

Appropriate spatial positioning of floral organs requires the establishment and maintenance of

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Figure 1. The gene regulatory networks involving auxin that regulate early floral meristem (FM) initiation and development. Schematic diagram of gene regulatory networks superimposed onto an image of an *Arabidopsis* FM emerging from the flank of the inflorescence meristem (IM), to illustrate how auxin coordinates FM initiation and identity and floral organ initiation (see text for details). (ANT) AINTEGUMENTA, (AIL6) AINTEGUMENTA-LIKE6, (ARF4) AUXIN RESPONSE FACTOR4, (BDL) BODENLOS, (BP) BREVIPEDICELLUS, (BRM) BRAHMA, (CUC) CUP-SHAPED COTYLEDON, (DRN) DORN RÖSCHEN, (DRNL) DORN RÖSCHEN-LIKE, (ETT) ETTIN, (FIL) FILAMENTOUS FLOWER, (HDA19) HISTONE DEACETYLASE19, (MAB4) MACCHI-BOU4, (PID) PINOID, (PIN1) PIN-FORMED1, (RBE) RABBIT EARS, (PTL) PETAL LOSS, (PYD) SPLAYED, (STM) SHOOTMERISTEMLESS, (SUP) SUPERMAN, (TPL) TOPLESS, (YUC1) YUCCA1, (YUC4) YUCCA4.

inter-organ boundary zones within floral whorls. In addition to separating organs, boundary domains act as organizing centers to developmentally pattern adjacent tissues. Many transcription factors have boundary functions that relate to auxin signaling (Yu and Huang 2016). A boundary between the IM and FM ensures that the FM buttress separates from the IM. It involves polar auxin transport by ATP-BINDING-CASSETTE B19 (ABCB19) and in *abcb19* mutants, auxin levels increase within the boundary (Zhao et al. 2013). Boundaries are zones of low auxin signaling (Wang et al. 2016), which repress cell division and expansion (Žádníková and Simon 2014; Wang et al. 2016).

The *CUP-SHAPED COTYLEDON* (*CUC*) genes *CUC1*, *CUC2*, and *CUC3* are expressed in different floral organ boundaries (Takada et al. 2001; Hibara et al. 2006; Xu et al. 2008). Their combinatorial mutation leads to fused flo-

ral organs (Laufs et al. 2004; Mallory et al. 2005) and *CUC1* and *CUC2* promote carpel margin meristem (CMM) initiation and positioning (Kamiuchi et al. 2014). Auxin represses *CUC2* expression (Furutani et al. 2004), consistent with boundary domains having low auxin levels, high *CUC* levels, and repressed cell division. Developmental contexts such as cotyledon initiation, leaf serration formation, and ovule primordium initiation have suggested how *CUC2* activate floral organ initiation in adjacent whorls, by up-regulating *PIN1* expression to promote an auxin convergence point, which creates an auxin maximum where organ founder cells are specified (Vernoux et al. 2000; Furutani et al. 2004; Heisler et al. 2005; Bilsborough et al. 2011; Galbiati et al. 2013). The auxin maximum then represses *CUC2* expression and restricts it to the boundary in a negative feedback loop (Vernoux et al. 2000; Bilsborough et al. 2011).

Posttranscriptional regulation of *CUC1* and *CUC2* by three *miRNA164* isoforms and mutation of *MIR164c* (also named *EARLY EXTRA PETALS1 [EEP1]*), leads to supernumerary petals and is associated with additional boundary domains in the second whorl (Baker et al. 2005). After petal primordium formation, *MIR164c/EEP1* expression is repressed in part by RABBIT EARS (RBE) (Huang et al. 2012), which also regulates sepal boundaries cell-non-autonomously. RBE is also regulated by PETAL LOSS (PTL) in the intersepal zone, which establishes sepal boundaries concertedly with *CUC1* and *CUC2* (Lampugnani et al. 2013). *HANABA TARANU (HAN)* is expressed at organ boundaries and *han* mutants display fused sepals and fewer floral organs, which might be caused by impaired polar auxin transport by analogy to embryogenic HAN function, which regulates auxin flux to the hypophysis (Nawy et al. 2010; Zhang et al. 2013).

Auxin response within the intersepal boundary zone is controlled by PTL (Brewer 2004), which synergistically regulates petal initiation with RBE and PTL, illustrating that cell-non-autonomy is another important facet of auxin function (Griffith et al. 1999; Brewer 2004; Takeda et al. 2004; Krizek et al. 2006). In *ptl* mutants, petals arise sporadically and are absent in later-arising flowers (Griffith et al. 1999) and sites of petal initiation are restored by directing auxin synthesis within the intersepal zone (Lampugnani et al. 2013). Thus, auxin confers positional information for petal initiation by functioning as a mobile inter-whorl signal. RBE and PTL both function within the same pathway, with RBE downstream of PTL. The petal defects of *ptl* and *rbe* mutants are enhanced by mutation of the auxin influx carrier AUX1 (Lampugnani et al. 2013). Disruption of auxin efflux via PID and PIN1 in the petal initiation zone generally increases petal number, dependent on PTL function. RBE also defines the second-whorl domain independently of PTL, by regulating several boundary genes (Fig. 2).

The SUPERMAN (SUP) C2H2 transcription factor, which is closely related to RBE (Sakai et al. 1995), limits *APETALA3* expression in

whorl 4, and *sup* mutants produce supernumerary stamens (Bowman et al. 1992). *SUP* expression spans the whorl 3/4 boundary (Prunet et al. 2017) and reduces local auxin biosynthesis by directly binding to *YUC1* and *YUC4* and recruiting Polycomb-repressive complex 2 to repress their expression (Table 1; Fig. 2; Xu et al. 2018). This up-regulation of auxin biosynthesis in *sup* both expands the stem-cell domain between whorls 3 and 4 cell-non-autonomously and prolongs its activity (Xu et al. 2018), leading to supernumerary stamens as the fate of cells in whorl 4 changes from female to male (Prunet et al. 2017). In conclusion, the fine-tuning of auxin synthesis or activity in inter-organ or inter-whorl boundaries is one mechanism for controlling cell divisions and stem-cell availability for organ differentiation.

FLORAL ORGAN OUTGROWTH

The concentration of free auxin, indicated by *DR5::GUS*, is high in the tips of floral organ primordia in all whorls (Aloni et al. 2006), which drives rapid organ outgrowth. After the specification of sepal founder cells in a stereotypic order (Chandler and Werr 2014), sepal outgrowth in *Arabidopsis* is coordinated by DEVELOPMENT-RELATED MYB-LIKE1 (DRMY1) (Zhu et al. 2020). Coordinated auxin and cytokinin signaling regulates sepal outgrowth, analogous to the temporal regulation of FM outgrowth (Besnard et al. 2014). *drmy1* mutants display variable sepal sizes due to delayed outgrowth, which results from diffuse auxin and cytokinin response domains in sepal primordia. Wild-type DRMY1 signaling involves initial auxin responses in the outer primordium cell layer that encroach into the underlying cytokinin signaling domain. These localized and focused auxin and cytokinin signaling domains define regions of competency for outgrowth, in which *DR5::GFP* accumulates at the sepal tips and TCS at the base. In tomato, the MADS-box gene *SIMBP21* negatively regulates sepal cell expansion, probably partly via auxin-related pathways.

Auxin contributes to patterning petal shape and size, which depend on cell division during early development and cell expansion during

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Figure 2. The gene regulatory networks involving auxin that orchestrate development of the different flower organs. A representation of an *Arabidopsis* flower linked to schematic diagrams to show how auxin integrates into the gene regulatory networks that coordinate gynoecium, stamen, petal, and sepal initiation and growth. An additional diagram shows the gene regulatory network involving auxin that relates to floral meristem (FM) determinacy and termination (see text for details). (AG) AGAMOUS, (ARF8/ARF17) AUXIN RESPONSE FACTOR7/17, (AUX1) AUXIN EFFLUX CARRIER1, (Aux/IAA19) AUX/IAA PROTEIN19, (AXR4) AUXIN-RESISTANT4, (CRC) CRABS CLAW, (DRMY1) DEVELOPMENT-RELATED MYB-LIKE1, (HAN) HANABA TARANU, (HEC1/HEC2/HEC3) HECATE1/2/3, (JAG) JAGGED, (KNU) KNUCKLES, (MYB26/MYB108) MYB-DOMAIN PROTEIN26/08, (SPT) SPATULA, (TMO3) TARGET OF MONOPTEROS3, (TRN) TORNADO, (WUS) WUSCHEL (see additional abbreviations in Fig. 1).

late development (Smyth et al. 1990). Many genes directly involved with auxin contribute to petal growth, including *JAGGED* (*JAG*), *ARGOS*, *ANT*, *BIGPETALp* (*BPEp*), and *ARF8*. *JAG* regulates growth by modulating the switch from cell proliferation to cell expansion (Sauret-Güeto et al. 2013). *jag* petals are narrow with a reduced distal region and a jagged or serrated margin (Dinneny et al. 2004). In *jag*, *DR5::GFP* expression is reduced, but is broader when *JAG* is ectopically expressed. Moreover, *JAG* directly represses *PTL* (Sauret-Güeto et al. 2013).

Overexpression of the auxin-inducible gene *ARGOS* increases petal size by increasing the number and size of petal epidermal cells. This can be blocked by loss of *ANT* function, suggesting that *ARGOS* positively regulates *ANT* during petal organogenesis (Hu et al. 2003). Conversely, *BPEp* and *ARF8* together regulate petal size by negatively affecting mitotic growth and restricting cell expansion during early and late petal development, respectively (Varaud et al. 2011), and *arf8* and *bpe* mutants are associated with the up-regulation of early auxin-responsive genes. Notably, interaction between *BPEp* and *ARF8* occurs via the *BPEp* carboxy-terminal domain, which shares sequence similarity with motif III of *ARF* and *AUXIN/INDOLE-3-ACETIC ACID* proteins (Varaud et al. 2011).

The stereotypic conical shape of cells in the *Arabidopsis* adaxial petal epidermis requires auxin signaling and apoplastic pH changes (Dang et al. 2020). Decreased auxin concentration, transport, or signaling abolishes cell-wall acidification and affects the height and shape of conical cells; notably, *arf6-2 arf8-3* displays severe defects in conical cell shape and outgrowth (Tabata et al. 2010).

In *Arabidopsis*, stamens develop asynchronously: primordia of the four long medial stamens arise contemporaneously with petals during stage 5 of flower development, whereas the two short lateral stamens develop slightly later (Bowman et al. 1989). Reduction in auxin biosynthesis, especially by *yuc2* and *yuc6* or polar transport (*pin1* and *pid*), reduces stamen number. The fewer-stamen phenotype of *ett* is enhanced by inhibiting auxin transport, leading to third-whorl organs that often consist of filamen-

tous structures that lack anthers. Moreover, *ABCB/PGP* genes that encode auxin efflux carriers are expressed in young stamen primordia, as well as in tapetum and pollen mother cells (PMCs) entering meiosis, and *abcb1* and *abcb19* show unsynchronized and precocious meiosis (Cecchetti et al. 2015).

The role of auxin during premeiotic phases of stamen development is poorly understood, partly because *DR5* expression is only detected after microspore release (Cheng et al. 2006; Feng et al. 2006; Cecchetti et al. 2008, 2015). The R2D2 auxin reporter revealed that auxin-mediated DII depletion occurs in the locules early in anther development and overlaps with *YUC1* and *YUC4* activity (Cheng et al. 2006; Ståldal et al. 2012). Auxin biosynthesis is positively regulated by *SHI/STY* transcription factors, which are active throughout anther development and affect early anther patterning (Estornell et al. 2018).

Late stamen development involves the coordination of rapid filament elongation with anther dehiscence and pollen maturation and finally terminates with mature pollen-grain release. Anthers of *abcb1 abcb19* overproliferate tapetum cells and show enhanced septum lignification, suggesting that auxin transport regulates cell division and lignification (Cecchetti et al. 2015). Inhibition of auxin efflux (Cecchetti et al. 2017) suggests that auxin flows from the tapetum to the middle layer, where auxin response maxima are important for coordinated pollen maturation and anther dehiscence.

Auxin signaling also represses precocious entry into subsequent developmental steps in several anther cell layers, and anthers of mutants in genes encoding the four TIR/AFB nuclear auxin receptors show premature dehiscence and pollen release compared to wild-type (Cecchetti et al. 2008, 2017). Mutations in *ARF1*, *ARF2* (Ellis et al. 2005), *ETT* (Sessions et al. 1997), *ARF4* (Pekker et al. 2005), *ARF5* (Przemek et al. 1996), *ARF17* (Xu et al. 2019), *ARF6*, and *ARF8* (Nagpal et al. 2005) all cause defects in male reproductive tissues. Stamens of *arf6 arf8* are short with indehiscent anthers due to reduced jasmonic acid (JA) biosynthesis (Nagpal et al. 2005; Tabata et al. 2010). Furthermore, restriction of *ARF6* and *ARF8* domains by

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miR167 cleavage is required for anther development and fertility (Wu et al. 2006).

Several flower-specific, intron-retaining splice variants of *ARF8* affect stamen development in *Arabidopsis* (Ghelli et al. 2018). *ARF8.4* transcriptionally activates *MYB26*, which regulates secondary thickening and anther dehiscence (Yang et al. 2007, 2017), and *ARF8.2* regulates JA biosynthesis by activating *DAD1* to promote the final steps of anther dehiscence. The *ARF17*–*MYB108* complex also regulates anther dehiscence in parallel with the *ARF8.4*–*MYB26* pathway (Xu et al. 2019). The stamen-specific AUX/IAA gene *IAA19* controls filament elongation (Tashiro et al. 2009) and is regulated by auxin (Cecchetti et al. 2017). *IAA19* is also regulated by *ARF8.4*, but is hardly affected by *ARF8.2* and *ARF8.1*, highlighting the specificity of *ARF8* isoforms during early stamen development (Table 1; Fig. 2; Ghelli et al. 2018).

FLORAL MERISTEM DETERMINACY AND GYNOCECIUM DEVELOPMENT

The final floral development stage involves termination of FM growth following realization of the species-specific floral body plan. In *Arabidopsis*, the master regulator of FM determinacy, AGAMOUS (AG), represses the stem-cell marker *WUS* by recruiting Polycomb group (PcG) proteins to the *WUS* locus and in parallel, activating the expression of the *WUS* repressor *KNUCKLES* (*KNU*) (Sun et al. 2009; Liu et al. 2011). *APETALA2* (*AP2*) antagonizes AG function by activating *WUS* expression (Huang et al. 2017). AG directly activates *CRABS CLAW* (*CRC*), which represses *TORNADO* (*TRN2*), a regulator of auxin homeostasis, leading to a shift from meristem maintenance to organ differentiation (Yamaguchi et al. 2017). AG and *CRC* both activate the *YUC4* promoter and AG recruits CHROMATIN REMODELING FACTOR11 and 17 (*CHR11/17*), which increases chromatin accessibility (Yamaguchi et al. 2018). Therefore, the regulation of auxin homeostasis by multiple pathways is an important component of FM determinacy (Table 1; Fig. 2).

The AG–*AP2* FM determinacy pathway is also mediated by *ETT*. *ETT* and AG coordinate-

ly repress *WUS* expression in the FM and combined loss of *ETT* and AG strongly enhances defects in FM indeterminacy. Moreover, *ETT* expression is indirectly positively regulated by AG. Binding of *ETT* to *WUS* is enhanced by AG activity, but independently from the *WUS* repression pathway mediated by *KNU*. In addition, *ETT* function in FM determinacy is negatively regulated by *AP2* (Liu et al. 2014).

The appropriate termination of FM growth relates to gynoecium initiation in the FM center, which depletes the meristematic cell pool. The absence of a gynoecium in *ag* mutants indicates that FM determinacy is essential for female organ differentiation (Marsch-Martínez and de Folter 2016; Yamaguchi et al. 2017).

Gynoecium development involves the establishment of apical–basal and adaxial–abaxial axes. Mutation of *ETT* perturbs gynoecium organogenesis along the apical–basal axis, leading to reduced valve development and the ectopic expansion of transmitting tract and stigma (Sessions and Zambryski 1995; Alvarez and Smyth 1998). A long-standing model for gynoecium development along the apical–basal axis derived from observations that inhibition of auxin transport and auxin biosynthesis or transport mutants all generate a valveless gynoecium topped with stigmatic tissue. This suggests that a high local auxin biosynthesis at the gynoecium tip establishes a basipetally decreasing auxin morphogenetic gradient that specifies style, ovary, and gynophore (Nemhauser et al. 2000). However, *DR5rev::GFP* reports no auxin gradient, only auxin response maxima in carpel founder cells at stage 7, the provasculture at stage 8, apical tissue at stage 9, and the vasculature and stigmatic papillae at stage 12 (Larsson et al. 2013). Additionally, specification of lateral and medial gynoecium domains, including valve outgrowth, depends on *PIN1*-mediated lateral auxin maxima (Larsson et al. 2014). A refined, so-called early action model (Hawkins and Liu 2014) based on early polarity establishment in lateral organs such as leaves, proposes that abaxial–adaxial polarity in early carpel primordia is critical for subsequent development. This is consistent with *YUC1* and *YUC4* activity at the start of organ initiation (Cheng et al. 2006),

which potentially contributes to establishing an adaxial–abaxial boundary. Adaxial–abaxial axis formation also involves protein interactions between KANADI (KAN)1, KAN4, and ETT (Pekker et al. 2005). Therefore, gynoecium patterning involves two distinct phases: the early establishment of abaxial–adaxial identity within the mediolateral axis domains by coordinated auxin synthesis, transport, and signaling. Subsequently, this lateromedial domain is a prerequisite for establishing an apical–basal axis (Hawkins and Liu 2014).

During late gynoecium development, two additional foci of auxin response maxima initiate in the medial domain in addition to two in the lateral domain in the gynoecium apex. Immediately preceding style development, these four foci fuse into a ring due to SPATULA (SPT)/INDEHISCENT (IND)-dependent rearrangement of PIN proteins, which involves the direct repression of *PID* and leads to the acquisition of radial symmetry (Moubayidin and Østergaard 2014). During style and gynoecium development, auxin promotes the function of an ETT–IND complex that also leads to *PID* repression and finely regulates PIN localization and auxin flux (Moubayidin and Østergaard 2017; Kuhn et al. 2019). As well as interaction with IND, SPT associates with HECATE to regulate *PIN1* and *PIN3* expression and block cytokinin signaling (Gremski et al. 2007).

Because auxin biosynthesis mutants still differentiate stigmatic tissue, late gynoecium development is probably independent from the early action model and dependent on local auxin synthesis via *YUC* expression activated by STYLISH1/2 and NGA3 transcription factors in the gynoecium tip (Sohlberg et al. 2006; Trigueros et al. 2009; Eklund et al. 2011). SQUAMOSA PROMOTER-BINDING PROTEIN-LIKE transcription factors also contribute to style, septum, and transmitting tract development, probably upstream of *YUC4* and by interactions with CRC, ETT, SPT, and STY1, which are all involved in auxin homeostasis or responsiveness (Xing et al. 2013).

The direct binding of auxin to the carboxy-terminal domain of ETT (Kuhn et al. 2020) reveals an additional layer of auxin regulation that

triggers a rapid and reversible switch of chromatin states during gynoecium development. ETT cannot interact with Aux/IAA proteins or mediate canonical auxin signaling because it lacks a PB1 domain. In low-auxin conditions, the ETT EAR repressor motif physically interacts with members of the TPL and TPL-RELATED (TPR) family of corepressors, which recruit histone deacetylase HDA19 to maintain chromatin of ETT targets in the gynoecium, such as *PID* (Benjamins et al. 2001) and *HECATE1* (*HEC1*) (Gremski et al. 2007) in a repressed state. At higher auxin levels, binding of IAA to ETT causes its dissociation from TPL/TPR2, which prevents HDA19 from deacetylating histones at ETT target loci and leads to their transcriptional activation.

AUXIN AND OVULE DEVELOPMENT

Auxin maintains the meristematic activity of the placenta and determines the sites of ovule initiation and ovule identity. In most auxin-related mutants, defects in CMM formation cause the reduction or absence of placental tissue and ovules (Cucinotta et al. 2014; Reyes-Olalde and de Folter 2019). In particular, auxin concentration maxima generated by PIN1 in the placenta determine sites of ovule primordium initiation (Benková et al. 2003; Ceccato et al. 2013; Galbiati et al. 2013). Placental PIN1 expression is promoted by cytokinins via transcription of the direct MP targets *CYTOKININ RESPONSE FACTOR2/TARGET OF MONOPTEROS3* and *CUC1/CUC2* (Galbiati et al. 2013; Cucinotta et al. 2016, 2020), and a lower PIN1 level in *crf2* and *cuc2-1 CUC1_RNAi* mutants results in fewer ovule primordia. After ovule initiation, ANT promotes primordium cell proliferation and integument growth. Correct ANT expression is also controlled by auxin via MP and AR-GOS (Hu et al. 2003; Galbiati et al. 2013).

Auxin is critical for the somatic-to-germline fate transition, and for downstream events of megasporogenesis and female gametophyte (FG) development. PIN1 also drives an auxin maximum in the nucellus at the ovule tip in the L1 surrounding the megaspore mother cell (MMC) (Benková et al. 2003; Ceccato et al.

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2013). ETT is a negative regulator of MMC identity and is expressed in central ovule regions, where it restricts *WUS* expression, similar to its role in the FM. In the nucellus, *ETT* expression is tightly regulated by TAS3 tasiRNA, and deregulation of its expression causes supernumerary MMCs (Su et al. 2017).

After MMC enlargement, meiosis generates four haploid megaspores, but only the functional megaspore undergoes three mitoses to form an FG consisting of seven cells of four distinct cell types. During megagametogenesis, auxin response is localized to the chalazal pole in the sporophytic tissues surrounding the FG (Lituiev et al. 2013). This suggested a model in which the auxin cell-non-autonomously indirectly affects cell specification within the gametophyte by functioning in sporophytic tissues (Lituiev et al. 2013). Auxin helps to pattern the micropylar pole of the FG where egg and synergid cells are located (Skinner and Sundaresan 2018), and loss of synergid identity and the occasional acquisition of egg identity results when TIR1 and ARFs are down-regulated or inactivated in the young FG (Panoli et al. 2015; Liu et al. 2018). Conversely, ectopic *YUC* expression shifts micropylar cell fates toward the chalazal end of the gametophyte, conferring synergid and egg-cell marker expression to the central cell and antipodal cells (Panoli et al. 2015). It remains unknown whether auxin influences mitosis and cell identity within the TM, which could be addressed by live imaging.

CONCLUDING REMARKS

Despite detailed understanding of how auxin integrates into the gene networks that regulate floral development, and major advances in characterizing components of the auxin response machinery, higher-order layers of complexity involved in auxin response continue to emerge. However, understanding how auxin confers positional information for organ initiation requires knowledge concerning spatiotemporal auxin responses at single-cell resolution. Despite the central role of MP in coordinating auxin responses, the complete genome-wide repertoire of direct ARF targets has not been captured and

paradigmatic canonical auxin response signaling might involve as-yet-unknown ARF-binding sequences.

Most importantly, although *Arabidopsis* is the best-studied plant development model, its flowers are hardly representative of angiosperm floral diversity and the molecular-genetic basis of many features of floral development that underlie differences in the floral body plan and morphogenesis in other species have not been considered; some examples include the unidirectional organ initiation, the existence of overlapping temporal organ initiation in different whorl, and common primordia in many legumes (Ferrandiz et al. 1999; Tucker 2003; Benlloch et al. 2015) or flowers that show centripetal, centrifugal (or basipetal), or more chaotic sequences of organ development within a single flower or whorl (Rudall 2010). One recurring theme in floral development is how auxin gradients orchestrate aspects of floral development in concert with those in cytokinin. Despite the characterization of auxin signaling components in comparative species, this needs to be combined with studies on comparative floral morphology at a high spatiotemporal resolution, to identify evolutionarily conserved aspects of the regulation of floral development by auxin to be identified, and to elucidate further levels of complexity not evident in *Arabidopsis*.

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