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A Role for Auxin in Flower Development

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Abstract

Auxin has long been implicated in many aspects of plant growth and development including flower development. However, the exact roles of auxin in flower development have not been well defined until the recent identification of auxin biosynthesis mutants. Auxin is necessary for the initiation of floral primordia, and the disruption of auxin biosynthesis, polar auxin transport or auxin signaling leads to the failure of flower formation. Auxin also plays an essential role in specifying the number and identity of floral organs. Further analysis of the relationship between the auxin pathways and the known flower development genes will provide critical information regarding mechanisms of organogenesis and pattern formation in plants.

Key words: auxin; development; flower.

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Auxin is an essential hormone that has been implicated in many aspects of plant growth and development (Woodward and Bartel 2005). In the past few years, tremendous progress has been made in understanding various aspects of auxin biology including auxin biosynthesis (Zhao et al. 2001; Tobena-Santamaria et al. 2002; Cheng et al. 2006), polar auxin transport (Galweiler et al. 1998; Petrasek et al. 2006; Wisniewska et al. 2006), and auxin signal transduction (Dharmasiri et al. 2005; Kepinski and Leyser 2005). Identification of the molecular machinery of auxin pathways and the availability of various auxin mutants have made it possible to investigate the exact roles of auxin in developmental processes. In the study, we focus on the role of auxin in flower development.

Flower development has served as an excellent model for elucidating molecular mechanisms that govern the formation of complex structures in plants (Krizek and Fletcher 2005). Molecular genetics studies in *Arabidopsis* and other model systems have led to the identification of many components that control flower formation and patterning (Krizek and Fletcher 2005). Transcription factors responsible for floral meristem and floral organ identities have been isolated and the genetic interactions between various floral genes are well documented. Therefore, flower development provides a great system to analyze the mechanisms by which auxin regulates plant organogenesis and pattern formation.

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Auxin is Necessary for the Formation of Flowers

Auxin has long been postulated as a key regulator for flower development, but the exact role of auxin in flower development was not well defined until recently (Nemhauser et al. 1998). A link between auxin and flower development was first established when the auxin transport mutant *pin1* was isolated and characterized (Okada et al. 1991; Galweiler et al. 1998). The *pin1* mutant displayed pleiotropic defects throughout almost all stages of development, but the most striking phenotype is that the inflorescence of *pin1* often does not have any flowers (Figure 1), suggesting that auxin plays an essential role in initiating flower primordia. PIN1 encodes a transmembrane protein that has recently been shown to localize with polarity in cells and to participate in auxin efflux (Galweiler et al. 1998; Petrasek et al. 2006; Wisniewska et al. 2006). Interestingly, the disruption of auxin polar transport appears not to prevent the transition from vegetative growth to reproductive growth as

Figure 1. Auxin mutants lack the ability to initiate flower primordia. From left to right: wild-type, *pin1*, *pinoid*, and *mp.*

pin1 still forms an inflorescence. However, converting an inflorescence meristem to a flower meristem apparently requires normal polar auxin transport. The essential role of polar auxin transport in the formation of flower primordia was further supported by the observations that treatment of plants with polar auxin transport inhibitors such as NPA also leads to the formation of pin-shaped inflorescences (Okada et al. 1991). In spite of the obvious defects in the *pin1* mutants, it was not understood why disruption of polar auxin transport leads to the failure of developing floral primordia. It is possible that inhibition of auxin transport leads to accumulation of auxin in meristems to levels that are inhibitory for the formation of new primordia. Alternatively, blocking of auxin transport may also lead to depletion of auxin in the meristem depending on where auxin is synthesized. Furthermore, disruption of auxin transport may not change the overall auxin levels; rather the phenotypes may be caused by a change in the local auxin gradients, which are presumably essential for organogenesis. It is intriguing that local application of exogenous auxin to the naked inflorescence of *pin1* or to the NPA-induced pins of tomato can induce the formation of floral primordia or the initiation of leaves, respectively (Reinhardt et al. 2000).

Two other *Arabidopsis* mutants with inflorescence phenotypes similar to that of *pin1* are *pinoid* (Bennett et al. 1995) and *monopteros* (*mp*) (Przemeck et al. 1996) (Figure 1). Both *mp* and *pinoid* often fail to initiate floral buds (Figure 1) and have pin-shaped inflorescences (Figure 1). PINOID encodes a serine/threonine protein kinase and is suggested to participate in auxin signaling and polar auxin transport (Christensen et al. 2000; Benjamins et al. 2001; Friml et al. 2004), further supporting the theory that auxin plays an essential role in the formation of floral buds. MP/ARF5 is a member of a large family of transcription factors called auxin response factors (ARF) (Hardtke and Berleth 1998). MP and other ARFs are considered key components responsible for auxin-mediated signaling and gene regulation (Quint and Gray 2006). The founding member of the ARF family, ARF1, was isolated biochemically as a transcription factor that binds to the conserved auxin response elements (Ulmasov et al. 1997). ARF proteins are believed to have the capacity to form homodimers or heterodimers with other ARFs or AUX/IAA proteins. The AUX/IAA proteins also belong to a family of transcription factors that play a negative regulatory role in auxin signaling. In the absence of auxin, formation of the heterodimers between ARFs and the AUX/IAA proteins prevents ARFs from binding to the auxin response elements and thus transcription is off. In the presence of auxin, the AUX/IAA proteins are targeted for degradation by the SCF^{TIR1} pathway (Dharmasiri et al. 2005; Kepinski and Leyser 2005). Because of the striking phenotypic similarities between *pin1*, *pinoid*, and *mp* (Figure 1), it is likely that these genes are involved in some aspects of auxin biology during flower development. However, the underlying biochemical and genetic mechanisms of why disruption of the genes can cause similar phenotypes are not understood. It is conceivable that expression and proper localization of PIN proteins requires functional MP and PINOID, which would be consistent with recent findings that PINOID affects the polarity of the PINs (Friml et al. 2004).

Although there is overwhelming evidence that implicates auxin playing essential roles in floral development, there has been no direct evidence until recently that auxin is essential for the formation of flowers and other developmental processes. A main difficulty in definitively deciphering the roles for auxin in any development processes including flower development is the lack of understanding of auxin biosynthesis in plants. Without knowledge of auxin biosynthesis, it is difficult to know with certainty what the consequences are if a plant does not make sufficient auxin and whether auxin is necessary for certain processes. Recent studies on roles of the YUCCA (YUC) family of flavin monooxygenases in auxin biosynthesis provide unambiguous evidence that auxin is necessary for the formation of flowers (Zhao et al. 2001; Tobena-Santamaria et al. 2002; Cheng et al. 2006).

The *YUC1* gene was initially isolated in a screen for auxin overproduction mutants by activation tagging (Zhao et al. 2001). Overexpression of *YUC1*, which encodes a flavin monooxygenase, leads to auxin overproduction. Physiological, genetic, and biochemical studies indicate that YUC1 catalyzes a rate-limiting step in auxin biosynthesis (Zhao et al. 2001). YUC1 belongs to a flavin monooxygenase family that has eleven members in the *Arabidopsis* genome (Cheng et al. 2006). The *YUC* genes have overlapping expression patterns and overlapping functions, which is consistent with the observations that inactivation of a single *YUC* gene does not cause any obvious defects (Cheng et al. 2006). However, the *yuc1yuc2yuc4yuc6* quadruple mutants displayed pleiotropic defects and produced inflorescence structures without flower primordia (Cheng et al. 2006). The cylinder-like structures in the inflorescence apex of the quadruple mutant resembled pin-like structures seen in *pin1*, although it is not clear whether the cylinder structure is related to the pin structure in *pin1* mutants. If they are structurally similar, it would suggest that the naked pin structure probably results from auxin deficiency in certain cells, which would also be consistent with findings that locally applied auxin on pin-like structures induced floral and leaf primordia formation (Reinhardt et al. 2000). It will be interesting to investigate whether expression and localization of the PINs are altered in the various *yuc* mutant backgrounds. It will also be informative to study the genetic interactions between the *yuc* mutants and the *pin* mutants.

Role of Auxin in Floral Organ Patterning

Auxin not only determines whether flower primordia are formed, it also plays an essential role in specifying floral organs and determining the pattern formation within a floral organ. Several *Arabidopsis* mutants were initially isolated from genetic screens for mutants with abnormal flowers, but later found to be involved in aspects of auxin pathways. Wild-type *Arabidopsis* flowers usually have four sepals, four petals, six stamens, and two fused carpels (Figure 2). Along the apical/basal axis, wild-type gynoecium can be divided into three regions with distinguished features: an apical stigma, a style, and a basal ovary. The *ettin* mutations affect the development of all four types of floral organs, but the most dramatic defect is in gynoecium patterning (Sessions and Zambryski 1995; Sessions et al. 1997). Mutations in the *ETTIN* gene strongly affect all parts of gynoecium including reduction in the ovary size and mispositioning of stylar and stigmatic tissues (Sessions and Zambryski 1995; Sessions et al. 1997). The strong *ettin* alleles often lack valves and are sterile. *ETTIN* also encodes an ARF (ARF3), suggesting that auxin plays a critical role in floral patterning. The pleiotropic nature of the *ettin* phenotypes indicates that ETTIN affects flower meristem, floral organ initiation, and the boundaries between floral organs. The phenotypes are consistent with the expression patterns of *ETTIN* (Sessions and Zambryski 1995; Sessions et al. 1997). The *ettin* phenotypes are further enhanced by disruption of the closest ETTIN homolog ARF4. In addition to the dramatic gynoecium patterning defects observed in *ettin-1*, all other floral organs are misshapen in *ett-1arf4* double mutants (Pekker et al. 2005). Other members of the ARF family in *Arabidopsis* including ARF1, ARF2, ARF6, and ARF8 recently have also been implicated in some aspects of flower development (Ellis et al. 2005; Nagpal et al. 2005; Goetz et al. 2006; Schruff et al. 2006).

The *pin1* mutant occasionally produces a few abnormal flowers (Okada et al. 1991). The *pin1* flowers often have no stamens and have petals with abnormal shapes or fused petals. The number of petals is also variable in the *pin1* mutants. In some cases, a pistil-like structure without any sepals, petals, or stamens is formed at the top of the flower. In strong alleles of *pin1,* flowers are totally sterile (Okada et al. 1991). The *pinoid* mutant often does not produce any flowers (Figure 1), but occasionally, *pinoid* inflorescences give rise to a limited number of flower primordia that eventually develop into abnormal flowers. Both floral organ numbers and shapes are

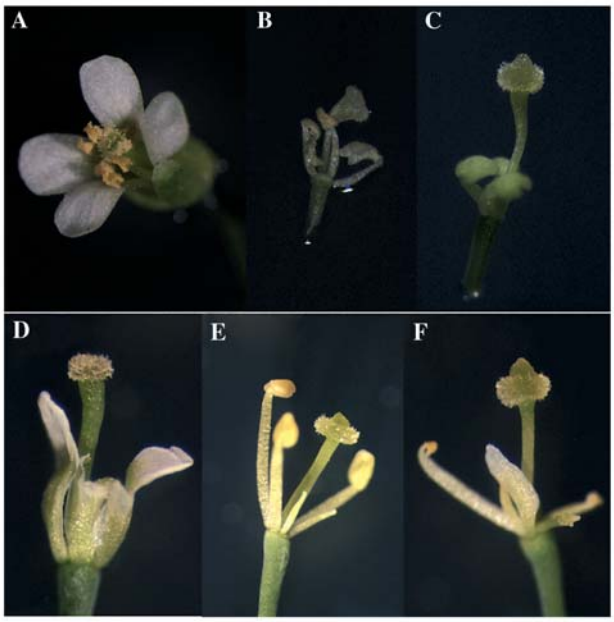

Figure 2. Disruption of auxin pathways profoundly affects flower development.

(A) Wild-type.
(B, C) *yuc1yuc4*.
(D–F) *mp.*

affected in the *pinoid* mutants (Bennett et al. 1995).

The auxin biosynthetic *YUC* genes have profound effects on flower development and affect all aspects of flower development (Cheng et al. 2006). The *YUC* family members have unique yet overlapping roles in flower development. Analysis of a series of *yuc* mutants has shown that localized auxin biosynthesis provide key information for floral organ initiation and maturation (Cheng et al. 2006). For example, both *yuc2yuc6* and *yuc1yuc4* are essentially sterile, but the infertile phenotypes in the two mutants are caused by defects in different parts of the flowers. All four types of organs were present in *yuc2yuc6* flowers, but their stamens were shorter than those of wild-type and the anthers of *yuc2yuc6* matured later than normal and rarely produced any pollen (Cheng et al. 2006). The floral defects in *yuc1yuc4* were much more severe than those in *yuc2yuc6*. Although all of the flowers on *yuc1yuc4* double mutants appeared abnormal, the severity of the defects was highly variable (Figure 2). Some flowers had sepal-like organs while other flowers totally lacked such sepal-like tissue. The gynoecium is also variable and often do not have any ovules. The *yuc1yuc4* flowers are also totally sterile. When more *YUC* genes are inactivated in the *yuc1ytuc4* background, the flower phenotypes become more severe. In general fewer flowers are formed and the formed flowers are smaller when more *YUC* genes are compromised. In the *yuc1yuc2yuc4yuc6* quadruple mutant, often no flowers are produced from inflorescences (Cheng et al. 2006).

Auxin and Flower Indeterminacy

Upon the transition to the reproductive growth, the shoot apical meristem turns into an inflorescence meristem that initiates floral meristems. Floral meristems are determinant, suggesting that meristematic activities eventually cease to allow the differentiation of a pistil, the formation of gametes, and the production of seeds. Inactivation of floral meristem identity genes such as *AGAMOUS* leads to repeated flower initiations within a flower (Yanofsky et al. 1990). Auxin also plays a role in flower indeterminacy. In wild type flowers, the apical region of the gynoecium is covered with stigmatic tissue, however, the stigma-like tissue of *yuc1yuc4* flowers often forms a ring with a protrusion of meristem-like tissue in the center (Cheng et al. 2006) (Figure 2). Occasionally, a new gynoecium is produced on the top of an existing gynoecium, suggesting that auxin not only plays a critical role in specifying floral organ identities, but also regulates floral meristemic cell proliferation (Cheng et al. 2006). Similar indeterminacy phenotypes are observed in *pinoid* and *mp* mutants (Figure 2). Interestingly, disruption of auxin biosynthesis, or polar transport, or signaling prevents the inflorescence meristems from turning into floral meristems (Figure 1). Paradoxically, auxin biosynthesis, auxin signaling, and polar auxin transport are required to terminate the meristematic activities of floral meristems.

General features of floral defects in auxin mutants

Mutations in any aspects of auxin pathways including biosynthesis, polar transport, and signaling cause profound defects in flower development. Several features of flower defects in the auxin mutants are different from those of floral organ identity or flower meristem identity mutants. (i) The number of floral organs in a flower of an auxin mutant is dramatically altered without homeotic transformations. For example, mutations in the *YUC* genes in general lead to fewer floral organs whereas disruption of PIN1 or PINOID often leads to more petals. It is also reported that *ettin* mutants have increased perianth organ numbers. However, the organ number changes are not due to the inter-conversion of floral organs. (ii) Disruption of auxin pathways affects all four types of floral organs. As shown in the *yuc*, *pin1*, and *pinoid* mutants, all four types of floral organs are affected. (iii) There are huge variations from flower to flower in the auxin mutants. In *yuc1yuc4* double mutants, all of the flowers are abnormal, but each flower is different with different numbers of floral organs and different types of floral organs (Figure 2). This is also true for *pin1*, *pinoid*, and *mp* mutants (Figure 2). It is currently not understood why the flower phenotypes vary so much in the auxin mutants. However, one explanation is that formation of the floral organs relies on local auxin gradient that is created and maintained by coordinated auxin biosynthesis and auxin polar transport. When the auxin polar transport system is compromised, the normal auxin distribution is disrupted and various floral organs are initiated according to the transient local auxin concentrations that may vary from one floral initial to another. Therefore, variations in flower defects are observed in *pin1* and *pinoid* mutants. In contrast, the residual auxin levels in the auxin biosynthetic *yuc* mutants were different among various floral initials, thus different patterns of floral organ initiation occurred. One indication that a local auxin gradient is required for the formation of floral buds or floral organs is that the *yuc* mutants cannot be rescued by exogenously applied auxin, but can be rescued by tissue specific expression of the bacterial auxin biosynthesis gene *iaaM* (Cheng et al. 2006).

Future perspectives

Auxin clearly plays an essential role in various aspects of flower development, but the underlying mechanisms are just beginning to unfold. Apparently, auxin is a key regulator for the fate of several founder cells for flower primordia. Hypothetic

auxin concentration differences that may be generated by polar auxin transport and/or local auxin synthesis between the founder cells and the surrounding cells may provide the necessary positional information for the initiation of a primordium. Determination of the relative contributions from auxin transport and auxin biosynthesis to local auxin gradient formation will be informative. It will also be interesting to test whether artificially created auxin gradients are sufficient to induce primordia formation in the *yuc* mutant background. So far, the relationship between the auxin pathways and the various flower development genes has not been understood. It is not known how auxin might regulate the floral meristem and floral organ identity genes. One difficulty in elucidating such relationships is that the interactions may be highly cell type specific. With the understanding of auxin pathways, it is now possible to address such problems with auxin mutants and tissue specific inducible systems. Further analysis of the mechanisms of auxin in flower development will provide clues for understanding general developmental mechanisms.

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References

Benjamins R, Quint A, **Weijers D, Hooykaas P, Offringa R** (2001). The PINOID protein kinase regulates organ development in Arabidopsis by enhancing polar auxin transport. *Development* **128**, 4057–4067.

Bennett R, Alvarez J, Bossinger G, Smyth D (1995). Morphogenesis in *pinoid* mutants of *Arabidopsis thaliana*. *Plant J* **8**, 505–520.

Cheng Y, Dai X, Zhao Y (2006). Auxin biosynthesis by the YUCCA flavin monooxygenases controls the formation of floral organs and vascular tissues in *Arabidopsis*. *Genes Dev* **20**, 1790–1799.

Christensen SK, Dagenais N, Chory J, Weigel D (2000). Regulation of auxin response by the protein kinase PINOID. *Cell* **100**, 469–478.

Dharmasiri N, Dharmasiri S, Estelle M (2005). The F-box protein TIR1 is an auxin receptor. *Nature* **435**, 441–445.

Ellis CM, Nagpal P, Young JC, Hagen G, Guilfoyle TJ, Reed JW (2005). AUXIN RESPONSE FACTOR1 and AUXIN RESPONSE FACTOR2 regulate senescence and floral organ abscission in *Arabidopsis thaliana*. *Development* **132**, 4563–4574.

Friml J, Yang X, Michniewicz M, Weijers D, Quint A, Tietz O et al. (2004). A PINOID-dependent binary switch in apical-basal PIN polar targeting directs auxin efflux. *Science* **306**, 862–865.

Galweiler L, Guan C, Muller A, Wisman E, Mendgen K, Yephremov A et al. (1998). Regulation of polar auxin transport by AtPIN1 in *Arabidopsis* vascular tissue. *Science* **282**, 2226–2230.

Goetz M, Vivian-Smith A, Johnson SD, Koltunow AM (2006). AUXIN RESPONSE FACTOR8 is a negative regulator of fruit initiation in *Arabidopsis*. *Plant Cell* **18**, 1873–1886.

Hardtke CS, Berleth T (1998). The *Arabidopsis* gene *MONOPTEROS* encodes a transcription factor mediating embryo axis formation and vascular development. *EMBO J* **17**, 1405–1411.

Kepinski S, Leyser O (2005). The *Arabidopsis* F-box protein TIR1 is an auxin receptor. *Nature* **435**, 446–451.

Krizek BA, Fletcher JC (2005). Molecular mechanisms of flower development: An armchair guide. *Nat Rev Genet* **6**, 688–698.

Nagpal P, Ellis CM, Weber H, Ploense SE, Barkawi LS, Guilfoyle TJ et al. (2005). Auxin response factors ARF6 and ARF8 promote jasmonic acid production and flower maturation. *Development* **132**, 4107–4118.

Nemhauser JL, Zambryski PC, Roe JL (1998). Auxin signaling in *Arabidopsis* flower development? *Curr Opin Plant Biol* **1**, 531–535.

Okada K, Ueda J, Komaki MK, Bell CJ, Shimura Y (1991). Requirement of the auxin polar transport system in early stages of *Arabidopsis* floral bud formation. *Plant Cell* **3**, 677–684.

Pekker I, Alvarez JP, Eshed Y (2005). Auxin response factors mediate *Arabidopsis* organ asymmetry via modulation of KANADI activity. *Plant Cell* **17**, 2899–2910.

Petrasek J, Mravec J, Bouchard R, Blakeslee JJ, Abas M, Seifertova D et al. (2006). PIN proteins perform a rate-limiting function in cellular auxin efflux. *Science* **312**, 914–918.

Przemeck GK, Mattsson J, Hardtke CS, Sung ZR, Berleth T (1996). Studies on the role of the *Arabidopsis* gene *MONOPTEROS* in vascular development and plant cell axialization. *Planta* **200**, 229–237.

Quint M, Gray WM (2006). Auxin signaling. *Curr Opin Plant Biol* **9**, 448–453

Reinhardt D, Mandel T, Kuhlemeier C (2000). Auxin regulates the initiation and radial position of plant lateral organs. *Plant Cell* **12**, 507–518.

Schruff MC, Spielman M, Tiwari S, Adams S, Fenby N, Scott RJ (2006). The *AUXIN RESPONSE FACTOR 2* gene of *Arabidopsis* links auxin signalling, cell division, and the size of seeds and other organs. *Development* **133**, 251–261.

Sessions A, Nemhauser JL, McColl A, Roe JL, Feldmann KA, Zambryski PC (1997). ETTIN patterns the *Arabidopsis* floral

meristem and reproductive organs. *Development* **124**, 4481–4491.

Sessions RA, Zambryski PC (1995). *Arabidopsis* gynoecium structure in the wild and in ettin mutants. *Development* **121**, 1519–1532.

Tobena-Santamaria R, Bliek M, Ljung K, Sandberg G, Mol JN, Souer E et al. (2002). FLOOZY of petunia is a flavin monooxygenase-like protein required for the specification of leaf and flower architecture. *Genes Dev* **16**, 753–763.

Ulmasov T, Hagen G, Guilfoyle TJ (1997). ARF1, a transcription factor that binds to auxin response elements. *Science* **276**, 1865–1868.

Wisniewska J, Xu J, Seifertova D, Brewer PB, Ruzicka K, Blilou I et al. (2006). Polar PIN localization directs auxin flow in plants. *Science* **312**, 883.

Woodward AW, Bartel B (2005). Auxin: Regulation, action, and interaction. *Ann Bot* (*Lond*) **95**, 707–735.

Yanofsky MF, Ma H, Bowman JL, Drews GN, Feldmann KA, Meyerowitz EM (1990). The protein encoded by the *Arabidopsis* homeotic gene *agamous* resembles transcription factors. *Nature* **346**, 35–39.

Zhao Y, Christensen SK, Fankhauser C, Cashman JR, Cohen JD, Weigel D et al. (2001). A role for flavin monooxygenase-like enzymes in auxin biosynthesis. *Science* **291**, 306–309.

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