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DOT/UFO Emerges as a Key Factor in Inflorescence Patterning

Flowering plants exhibit an astonishing variety of floral forms, which arise from two basic patterns of floral meristem initiation: determinate and indeterminate. Many indeterminate inflorescences show some variation of a racemose pattern, which maintains an apical meristem and produces flowers laterally along the length of the peduncle such that the oldest flowers are at the base. By contrast, a common determinate pattern is cymose, in which the apical meristem terminates in a flower and lateral inflorescence meristems subsequently emerge below or to the side and repeat this pattern, such that the oldest flower is at the apex (see figure). Typical racemose inflorescences include those of the butterfly bush (*Buddleja*), lupin, (*Lupinus*), snapdragon (*Antirrhinum*), and *Arabidopsis*. Common cymose inflorescences are *Geranium*, tomato (*Solanum lycopersicum*), *Petunia*, and numerous other members of the Solanaceae and Malvaceae.

There are many variations on these basic themes, and compound inflorescences can sport complex arrangements of racemose and/or cymose patterns. For example, many grasses produce panicles that are branched collections of multiple racemes. Lilac and horse chestnut produce a compound inflorescence known as a thyrse, in which the main axis is racemose and the branches are cymose. The highly complex compound inflorescences of the Asteraceae, called heads or capitulae, also can have both cymose and racemose components. Understanding what controls these patterns of floral meristem initiation is central to understanding the evolution of inflorescence architecture.

Genetic factors controlling the transition to flowering and floral meristem identity are well characterized in *Arabidopsis*. Central among these is LEAFY (LFY), a plant-specific transcription factor, which promotes floral meristem identity by activating expression of the MADS box transcription factor APETALA1 (AP1), as well as other

homeotic floral organ identity genes (reviewed in Krizek and Fletcher, 2005). A current model suggests that the evolution of inflorescence types has been driven by alterations in the spatio-temporal expression patterns of *LFY* and *TERMINAL FLOWER1* (*TFL1*), considered to be key genes promoting floral versus vegetative meristem identity, respectively (Prusinkiewicz et al., 2007). This is based on genetic evidence from *Arabidopsis*, where *LFY* and *AP1* are activated in lateral floral meristems but repressed in the apical meristem, resulting in the racemose inflorescence. Constitutive expression of *LFY* and/or *AP1* is able to convert the racemose inflorescence into a solitary flower. While these studies are well advanced in the racemose species *Arabidopsis*, it is important to test the generality of the findings by examining other species, especially those with cymose flowering shoots.

In this issue of *The Plant Cell*, **Souer et al. (pages 2033–2048)** show that the *Petunia* F-box protein DOUBLE TOP (DOT) plays a major role in regulating the cymose inflorescence meristem pattern in petunia. DOT is a homolog of *Arabidopsis* UNUSUAL FLORAL ORGANS (UFO), which appears to play a minor role in conferring floral meristem identity in this species. Levin and Meyerowitz (1995) characterized *Arabidopsis ufo* mutants and concluded that *UFO* and *LFY* likely function in the same processes and may act together. However, in *Arabidopsis*, *UFO* appears to be important only for specific events, such as maintaining boundaries between floral organs, whereas *LFY* is a key global regulator that promotes floral development. The *LFY* homolog in petunia is known as *ABERRANT LEAF AND FLOWER* (*ALF*). Souer et al. show that, in petunia, DOT acts together with ALF, but unlike the situation in *Arabidopsis*, it is the localization of *DOT* expression that is the key factor controlling when and where flowers are made.

First, Souer et al. found that overexpression of *35S:ALF* has a completely different

outcome in petunia versus *Arabidopsis*. In *Arabidopsis*, *35S:ALF* (similar to *35S:LFY*) caused precocious flowering and conversion of primary and secondary inflorescences to terminal flowers, demonstrating that ALF and LFY have similar functions. However, *35S:ALF* expressed in petunia produced no apparent phenotypic effect, suggesting that floral meristem identity is controlled by another factor in this species. Through mutant analysis, the authors identified *DOT*, a homolog of *Arabidopsis UFO*, as a key regulator of floral meristem identity in petunia. In the petunia *dot* mutant, which is indistinguishable from the *alf* single mutant as well as the *alf dot* double mutant, the apical floral meristems were converted into inflorescence meristems that do not produce flowers. Expression of *353:DOT* in the wild type produced a dramatic phenotype characterized by precocious flowering and transformation of the cymose inflorescence to a solitary flower. Interestingly, the authors also show that tomato *ANANTHA* encodes a *DOT* homolog that appears to have the same function in tomato as *DOT* in petunia (both members of the Solanaceae).

Next, the authors show that DOT interacts directly with ALF. Chae et al. (2008) previously showed through yeast two-hybrid analysis that UFO and LFY are capable of direct interaction, and they provided some evidence that UFO triggers polyubiquitination of LFY. Souer et al. extended these observations, first, by showing that the interaction occurs in petunia as well as *Arabidopsis*, and second, with the use of an in vivo split-YFP assay, showing that the interaction occurs in planta and promotes ALF/LFY function. Souer et al. also show that the interaction between DOT and ALF is quite specific, as they screened a petunia inflorescence cDNA library for DOT-interacting partners, whereas Chae et al. (2008) tested only the interaction between UFO and LFY. Souer et al. identified 71 clones encoding DOT-interacting proteins; 70 of these corresponded to four distinct proteins in petunia having similarity to yeast

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Examples of Racemose and Cymose Inflorescences.

The racemose inflorescence of *Buddleja davidii* (top panel) shows oldest flowers at the base and new buds emerging from the indeterminate apical meristem. By contrast, in the cymose inflorescence of *Malva sylvestris* (bottom panel), the apical meristem was converted to a floral meristem that terminated in a flower, and new floral meristems have emerged laterally and beneath the apex.

SKP1, known to be a core component of SCF ubiquitin ligases, and the remaining clone was found to encode ALF. Furthermore, expression of *35S:DOT* in the *alf* mutant background produced no phenotypic differences from that of *alf*, supporting the conclusion that ALF is the major target of DOT.

The outcome and precise details of the interaction between DOT/UFO and ALF/LFY are unclear. A number of studies have shown that UFO is an F-box protein that forms part of an SCF complex associated with the COP9 signalosome, which is known to be involved in proteasome-mediated protein degradation. Therefore it was postulated that the substrate of SCF^{UFO} is an unidentified inhibitor of floral meristem initiation, which would be targeted for degradation by interaction with UFO (Lohmann and Weigel, 2002). However, Souer et al. provide strong evidence that ALF is a direct target of DOT, and this interaction leads to activation, rather than degradation, of ALF, resulting in the promotion of floral meristem initiation. Their results suggest that ALF/LFY and DOT/UFO functions are fully interdependent: ALF/LFY is required to specify floral meristem identity and DOT/UFO is required for activation of ALF/LFY. The absence of a severe floral meristem initiation phenotype in the *Arabidopsis ufo* mutant suggests that the role of UFO in the activation of LFY is partially redundant with another factor or factors in *Arabidopsis*, but this remains to be demonstrated.

The idea that an F-box protein could be involved in direct activation of a transcription factor is not without precedent. The ubiquitin-proteasome system has been shown to stimulate the activity of a number of transcription factors in yeast through a variety of mechanisms. These include processing to an active form (involving cleavage of a ubiquitylated domain or interacting partner, resulting in direct activation and/or transportation of the transcription factor into the nucleus), and intriguingly, an unknown mechanism whereby ubiquitylation causes an initial (transient) direct activation of the transcription activation domain followed by subsequent degradation (reviewed in Conaway et al., 2002). Interestingly, Souer et al. mapped the interaction of

the full-length DOT protein to the ALF N terminus and also detected weak transcription activation activity in the N terminus of both ALF and LFY. This differed from the results of Chae et al. (2008), who mapped the interaction of a truncated UFO protein and LFY to the UFO C terminus. The difference is perhaps due to the use of the full-length DOT protein by Souer et al.

The key finding of Souer et al. is that, unlike the situation in *Arabidopsis*, DOT/ UFO plays a major role in floral meristem identity in the Solanaceous species petunia and tomato. The authors show that the main difference in the functions of *DOT/ UFO* and *ALF/LFY* in *Arabidopsis* and petunia appears to lie in their patterns of expression. In *Arabidopsis*, *UFO* is expressed in the apical meristem throughout the vegetative phase, whereas *LFY* is expressed only at the end of the vegetative phase. Thus, constitutive transcription of *LFY* triggers the precocious formation of (terminal) flowers. By contrast, in petunia, *ALF* is expressed in the apex during the vegetative phase, and *DOT* is inactive. Hence, in petunia the transcriptional activation of *DOT* is necessary and sufficient to induce flowering.

How might these differences in expression pattern contribute to the development of a cymose inflorescence in petunia versus the racemose architecture in *Arabidopsis*, given that the proteins show similar interaction in both species and DOT/UFO is directly involved in the activation of ALF/ LFY? The expression of *LFY* is excluded from the apical meristem in *Arabidopsis* racemes, whereas in petunia, the *LFY* homolog *ALF* in expressed in the apical meristem, which is then converted to a floral meristem only upon activation of *DOT* expression. Thus, it is the transcription pattern of *DOT* in the apex, together with *ALF*, which restricts floral identity to the apical meristem and specifies the cymose architecture.

Interestingly, Allen and Sussex (1996) conducted an analysis of tomato that suggested that the inflorescence is indeter-minate rather than determinate and hence shows more of a racemose than cymose character. Welty et al. (2007) recently conducted a detailed scanning electron microscopy study of the inflorescence of tomato and concluded that it indeed has a cymose pattern, in that the apical meristem is converted to an inflorescence meristem, but the inflorescence meristem in fact maintains an indeterminate rather than determinate growth pattern, in that it bifurcates to produce a terminal floral meristem and another inflorescence meristem that repeats this patten. These authors noted that there are many examples of inflorescences that cannot be classified easily into determinate cymose versus indeterminate racemose. The work of Souer et al. might help to explain these complex patterns, as diverging patterns of expression of *DOT/UFO* and *ALF/LFY* into specific subdomains of the apical meristem and lateral inflorescence meristems might be predicted to give rise to numerous different determinate versus indeterminate and cymose versus racemose outcomes.

The results of Souer et al. provide important experimental evidence for the model of evolution of inflorescence architecture proposed by Prusinkiewicz et al. (2007), constructed from computer simulation and genetic evidence from *Arabidopsis*, that a simple genetic mechanism based on alterations in meristem identity gene expression can account for the divergence of floral forms. The results might also add grist to the mill in the debate over the significance of mutations in *cis*-elements versus coding sequence in the evolution of adaptive features (see Pennisi, 2008). The results of Souer et al. suggest that the evolution of functional differences in ALF/ LFY and DOT/UFO in different species might be explained entirely by alterations in their *cis*-regulatory promoter regions. The current work shows only that the expression patterns have changed, and additional experiments are needed to determine whether this is due to mutations in the *DOT* versus *UFO* promoter regions.

Further investigation into *DOT/UFO* and *ALF/LFY* expression patterns and their *cis*-regulatory sequences related to their function in species with more complex inflorescence types may prove instructive in this debate.

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