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Philippe Rieu, Moïra Arnoux-Courseaux, Gabrielle Tichtinsky, François Parcy. Thinking outside the F-box: how UFO controls angiosperm development. *New Phytologist*, 2023, 240 (3), pp.945-959. 10.1111/nph.19234 . hal-04237241

HAL Id: hal-04237241

<https://hal.science/hal-04237241v1>

Submitted on 11 Oct 2023

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Tansley review

Thinking outside the F-box: how UFO controls angiosperm development

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Received: 17 May 2023
Accepted: 19 July 2023

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New Phytologist (2023)
doi: 10.1111/nph.19234

Key words: evolution of development, flower and inflorescence development, LEAFY, transcriptional regulation, UFO.

Summary

The formation of inflorescences and flowers is essential for the successful reproduction of angiosperms. In the past few decades, genetic studies have identified the LEAFY transcription factor and the UNUSUAL FLORAL ORGANS (UFO) F-box protein as two major regulators of flower development in a broad range of angiosperm species. Recent research has revealed that UFO acts as a transcriptional cofactor, redirecting the LEAFY floral regulator to novel *cis*-elements. In this review, we summarize the various roles of UFO across species, analyze past results in light of new discoveries and highlight the key questions that remain to be solved.

I. Introduction

Plant meristems are tissues made of new cells that divide and feed the production of differentiated organs. During the vegetative development of angiosperms, the shoot apical meristem (SAM) produces vegetative structures such as leaves and shoots. Upon the perception of external and internal cues, flowering is initiated and flower meristems (FM) are formed: they can either replace the SAM itself (as in cymoses) or develop from its flanks following its conversion into an inflorescence meristem (IM; as in racemes). There are significant differences between IMs and FMs: the IM is indeterminate and produces FMs in a spiral arrangement, while the

FM is usually determinate and generates whorls of floral organs. Therefore, a deep genetic reprogramming is required to trigger precise spatiotemporal activation of floral genes during these developmental transitions. Through extensive research, many of the genetic pathways controlling flower development have been identified and several key regulatory proteins have been uncovered (Denay *et al.*, 2017; Thomson & Wellmer, 2019; Quiroz *et al.*, 2021).

One of these central regulators is the transcription factor (TF) LEAFY (LFY). Even if there are variations between species, LFY is essential for proper flower development. In Arabidopsis, LFY is expressed uniformly in nascent FMs where it orchestrates flower development by patterning the FM into the territories from which

each organ type will emerge. For this, LFY interacts with coregulators expressed locally to drive specific gene regulation (Moyroud *et al.*, 2010). *LFY* acts together with other floral meristem identity genes such as *APETALA1* (*API*), *APETALA2* or *CAULIFLOWER* to confer a floral identity to nascent meristems. It contributes to induce, in a whorl pattern, the *ABCE* genes that encode TFs specifying floral organ identities. The way the *ABCE* genes act in combination to confer the four types of organ identity is captured by the so-called 'quartet model' of floral development (Theissen *et al.*, 2016).

This review focuses on *UNUSUAL FLORAL ORGANS* (*UFO*), another key floral regulator in angiosperms that has long been proposed to act as a LFY cofactor (Lee *et al.*, 1997). Defects of *ufo* mutants were described early (Baur, 1930; Helm, 1951; Monti & Devreux, 1969), and molecular cloning of *UFO* was achieved in the 1990s in the snapdragon (*Antirrhinum majus*) and Arabidopsis (*Arabidopsis thaliana*) model plants (Simon *et al.*, 1994; Levin & Meyerowitz, 1995; Wilkinson & Haughn, 1995). Since then, *ufo* mutants have been described in numerous angiosperms. With the exception of a few species where *UFO* also controls the development of some vegetative structures (leaves, tendrils or shoots), the main defects of *ufo* mutants are observed in inflorescences and flowers. Such defects are often complex and reveal that this gene fulfills a variety of separable roles during plant reproductive development.

The goal of this review is to describe the developmental processes controlled by *UFO* in different angiosperm species and recapitulate current knowledge about how this protein performs its functions at the molecular level, notably as a LFY cofactor.

II. *UFO* regulates diverse developmental steps

In this section, we start by reviewing the different *UFO* functions based on the description of *UFO* loss- and gain-of-function phenotypes in various angiosperm species. To structure this descriptive part, we have classified *UFO* functions into three main categories, (1) the control of floral organ development and flower patterning, (2) the control of floral meristem identity (FMI) and (3) the control of the meristem activity *per se* (proliferation, meristem emergence and maintenance). We use this nonchronological order (when considering the developmental events) to start with the function that was first described in the literature and that is probably the most common and best documented in angiosperms.

There are obvious limitations of such grouping, and the three categories are not always perfectly distinct. In some species, it is also difficult to dissociate these functions. In particular, some late functions in floral organ development can remain cryptic when an early function (like the development of flowers itself) is strongly affected. In that case, the analysis of weak or partial mutants as well as gain-of-function experiments are needed to circumvent these limitations.

1. *UFO* regulates the development of floral organs

Controlling floral organ identities Flowers are characterized by a determinate number of specific organs (sepals, petals, stamens and

carpels) arranged in concentric whorls, as well as nectaries involved in rewarding pollinators. No matter the species examined, mutations in the *UFO* gene systematically lead to defects in the development of floral organs (Fig. 1). Yet, the nature of those defects varies between species.

In many eudicot species, the 2nd and 3rd floral whorls are affected by *ufo* mutations (Fig. 1). In strong *ufo* alleles, petals and stamens never properly develop and homeotic conversion of petals into sepals and stamens into carpels are observed. Moreover, extending the localized expression pattern of *UFO* (see below) in Arabidopsis or petunia (*Petunia hybrida*) triggers the conversion of sepals into petals and carpel into stamens, indicating that the spatially restricted expression pattern of *UFO* is limiting for petals and stamens development (Lee *et al.*, 1997; Souer *et al.*, 2008). *UFO* exerts this conserved function by upregulating *B* genes (namely *APETALA3* (*AP3*) and to a lesser extent *PISTILLATA* (*PI*) in Arabidopsis) (Simon *et al.*, 1994; Levin & Meyerowitz, 1995; Wilkinson & Haughn, 1995; Honma & Goto, 2000; Souer *et al.*, 2008; Sasaki *et al.*, 2012; Chen *et al.*, 2021). Overexpressing *AP3* in Arabidopsis *ufo* mutant is sufficient to restore petal and stamen development (Krizek & Meyerowitz, 1996), demonstrating that *AP3* is a major *UFO* target.

In some eudicot species, *ufo* mutations also affect the identity of the 4th floral whorl. It is the case in the snapdragon *fimbriata* (*fm*) mutant where the *C* gene *PLENA* (contributing to the 4th whorl identity) is strongly downregulated (Simon *et al.*, 1994; Ingram *et al.*, 1997). *UFO* also upregulates the *C* and *E* genes in petunia (Souer *et al.*, 2008) and *E* genes in cucumber (Chen *et al.*, 2021).

In monocots, mutations in the *ABERRANT PANICLE ORGANIZATION 1* (*APO1*) gene of rice (*Oryza sativa*) and its wheat (*Triticum aestivum*) ortholog *WHEAT ORTHOLOG FROM APO1* (*WAP01* or *WAP0-A1*) result in severe defects within the 3rd and 4th floral whorls (Ikeda *et al.*, 2005; Kuzay *et al.*, 2022). Specifically, stamens are either absent or converted into lodicules and carpels are formed indeterminately. The 2nd whorl lodicules are normal in the *apo1* mutant but display several abnormalities in *wap01* (Ikeda *et al.*, 2005; Kuzay *et al.*, 2022). These phenotypes correlate with downregulation of the *C* gene *OsMADS3* but not the *B* gene *SUPERWOMAN1* in the rice *apo1* mutant (Ikeda *et al.*, 2005) and downregulation of both *B* and *C* genes (but not *E* genes) in the wheat *wap01* mutant (Kuzay *et al.*, 2022). Therefore, in numerous angiosperms, *UFO* regulates the identity of floral organs (petals, stamens or carpels) by controlling the expression of specific homeotic genes.

Promoting petal growth In addition to its role in petal identity determination, *UFO* has been shown to promote the initiation and/or growth of petal primordia. While this function is not apparent in strong *ufo* mutants, it was proposed in Arabidopsis based on the analysis of weak *ufo* alleles and transient *UFO* activation (Durfee *et al.*, 2003; Laufs *et al.*, 2003).

Development of nectaries In Arabidopsis, *UFO* also controls the development of nectaries, small glands producing a sugar-rich liquid at the base of stamens (Baum *et al.*, 2001): nectaries are absent in the *ufo* mutant and ectopically present in the 4th whorl of

	Roles of UFO in...			Interaction with LFY	References
	Meristem identity determination	Development of floral organs	Other processes		
	○ Weak ○ Strong ■ FM identity ■ IM identity	Floral whorls affected in <i>ufo</i>		✓ Demonstrated physical interaction Functions: LFY-UFO UFO only LFY only	
<i>Oryza sativa</i> ABERRANT PANICLE ORGANIZATION 1 (APO1)	■ IM identity	3 4	• Negative effect on the number of leaves, branches and tillers • Cell division and meristem size homeostasis	✓ APO1 APO2	Ikeda <i>et al.</i> (2005, 2007, 2009) Ikeda-Kawakatsu <i>et al.</i> (2012)
<i>Triticum aestivum</i> WHEAT ORTHOLOG FROM APO1 (WAP01)	■ IM identity	2 3 4		Insufficient data	Kuzay <i>et al.</i> (2022) Wittern <i>et al.</i> (2022)
<i>Aquilegia coerulea</i> AqUFO1 and AqUFO2			Insufficient data		Sharma <i>et al.</i> (2019)
<i>Arabidopsis thaliana</i> UNUSUAL FLORAL ORGANS (UFO)	■ FM identity	2 3 + Petal growth + Nectary development	• Cell division and meristem size homeostasis • Establishment of boundaries within flower organs	✓ UFO LFY	Wilkinson and Haughn (1995) Levin and Meyerowitz (1995) Lee <i>et al.</i> (1997) Samach <i>et al.</i> (1999) Baum <i>et al.</i> (2001) Durfee <i>et al.</i> (2003) Laufs <i>et al.</i> (2003) Chae <i>et al.</i> (2008)
<i>Cucumis sativus</i> UNUSUAL FLOWER AND TENDRILS (UFT) or CsUFO	Not observed	2 3 4	• Tendril development	✓ CsUFO CsLFY	Chen <i>et al.</i> (2021) Zhao <i>et al.</i> (2017)
<i>Fragaria vesca</i> EXTRA FLORAL ORGANS (EFO) or FveUFO1			Insufficient data		Shahan <i>et al.</i> (2018) Zhang <i>et al.</i> (2023)
<i>Pisum sativum</i> STAMINA PISTILLOIDA (STP)	Not observed	2 3	• Development of compound leaves	Not tested STP UNI	Taylor <i>et al.</i> (2001)
<i>Lotus japonicus</i> PROLIFERATING FLORAL ORGANS (PFO)	Not observed	2 3 + 2 nd whorl determination	• Development of compound leaves	Not tested PFO LJLFY	Zhang <i>et al.</i> (2003) Dong <i>et al.</i> (2005)
<i>Gerbera hybrida</i> GhUFO	■ FM identity	2 3 4		✓ GhUFO GhLFY	Zhao <i>et al.</i> (2016)
<i>Cosmos bipinnatus</i> GREEN HEAD (GH) or CbUFO	■ FM identity		• Involucral bract repression	Insufficient data	Li <i>et al.</i> (2019)
<i>Antirrhinum majus</i> FIMBRIATA (FIM)	■ FM identity	2 3 4	• Cell division and meristem size homeostasis	Not tested FIM FLO	Simon <i>et al.</i> (1994) Ingram <i>et al.</i> (1997)
<i>Torenia foenigera</i> TUFO	Not observed	2 3		✓ Insufficient data	Sasaki <i>et al.</i> (2012)
<i>Petunia hybrida</i> DOUBLE TOP (DOT)	■ FM identity	2 3		✓ DOT ALF	Souer <i>et al.</i> (2008)
<i>Capsicum annuum</i> ANANTHA (CaAN)	■ FM identity		Insufficient data	Insufficient data	Lippman <i>et al.</i> (2008)
<i>Solanum lycopersicum</i> ANANTHA (AN)	■ FM identity	2 3		Not tested AN FA	Allen and Sussex (1996) Lippman <i>et al.</i> (2008) MacAlister <i>et al.</i> (2012)

Fig. 1 UNUSUAL FLORAL ORGANS (UFO) performs multiple functions during the development of angiosperms. Column 1 indicates species where *ufo* mutants have been reported and the *UFO* names in those species. Note that in some species (grey background) the only available *ufo* mutants are not knock-out mutants, and presented roles are only suggested. The *UFO* functions deduced from the analysis of *ufo* mutants are indicated in columns 2–4 and the interaction between *UFO* and *LEAFY* (*LFY*) in column 5. The Venn diagram aims at a qualitative and schematical representation of the relative importance of *UFO* and *LFY* functions (with overlapping functions in purple, *LFY*-specific ones in cyan and *UFO*-specific ones in red). The extent of the overlap is roughly represented with three different levels (complete as in petunia, large as in rice or medium as in *Arabidopsis*). FM, floral meristem; IM, inflorescence meristem.

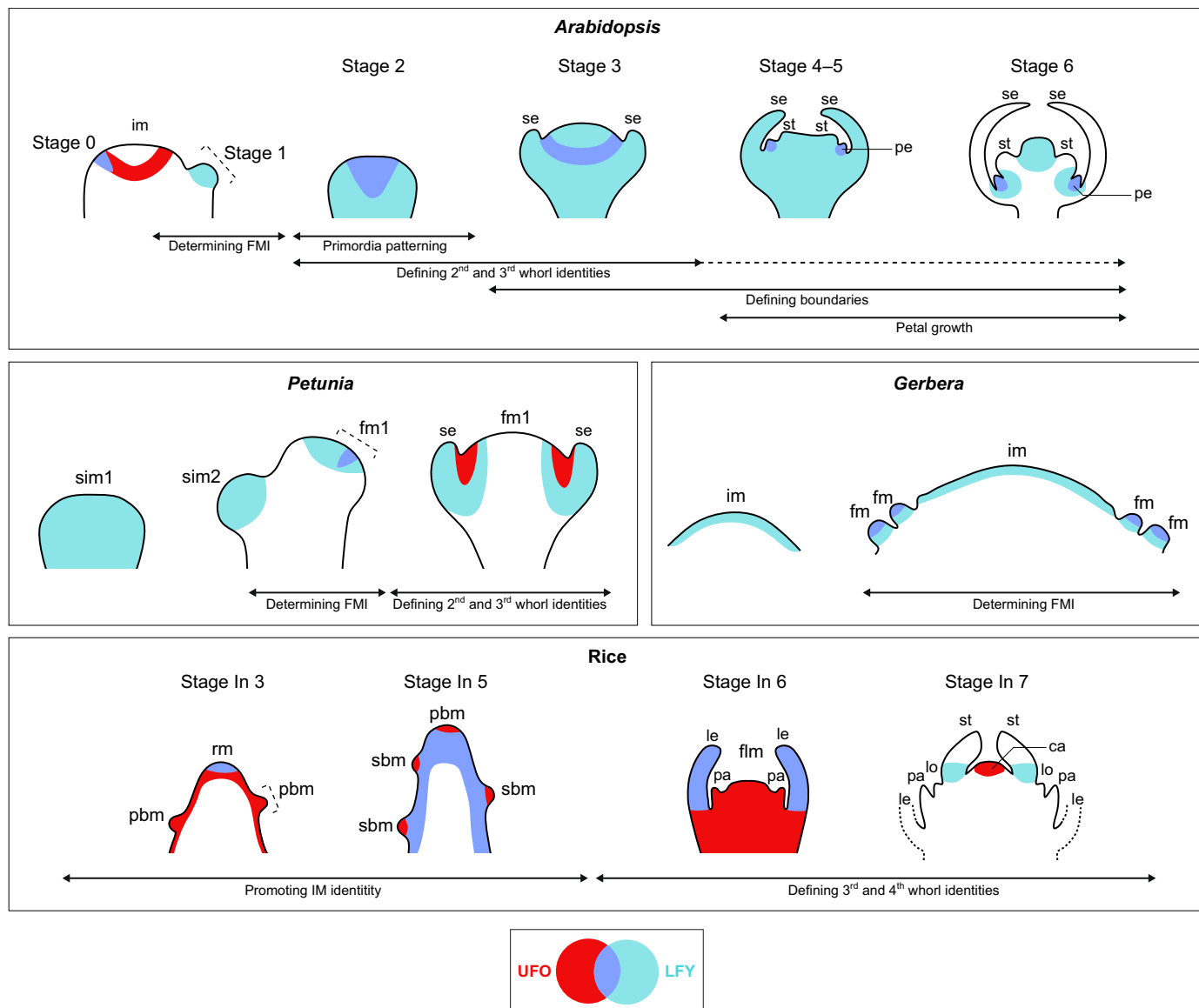


Fig. 2 UNUSUAL FLORAL ORGANS (*UFO*) has a dynamic expression pattern and separable roles during flower development. Expression domain of *UFO* (red) and *LEAFY* (cyan) in indicated species. Common expression domain is represented in dark blue. Segments indicate the structure represented in the following drawing. Flower development stages in *Arabidopsis* and rice are according to Smyth *et al.* (1990) and Ikeda *et al.* (2004), respectively. ca, carpel; flm, floret meristem; fm, floral meristem; FMI, floral meristem identity; im, IM, inflorescence meristem; le, lemma; lo, lodicule; pa, palea; pbm, primary branch meristem; pe, petal; rm, rachis meristem; sbm, secondary branch meristem; se, sepal; sim, sympodial inflorescence meristem; st, stamen. Patterns are based on Lee *et al.* (1997), Samach *et al.* (1999; *UFO*), Weigel *et al.* (1992; *LFY*), Zhao *et al.* (2016; gerbera), Souer *et al.* (2008; petunia), Miao *et al.* (2022), Ikeda *et al.* (2007) and Kyoizuka *et al.* (1998; rice).

plants overexpressing *UFO*. Ectopic expression of *B* genes in the *ufo* mutant fails to restore nectary development, showing that *UFO* does not control the development of these organs through the activation of *B* genes (Baum *et al.*, 2001). A possible *UFO* target explaining its implication in nectaries development is *CRABS CLAW* (Bowman & Smyth, 1999). This role is not described in other angiosperms.

Establishing FM patterning and boundaries Another role of *UFO* throughout flower development is to delineate territories and maintain boundaries between organs and whorls. Interwhorl mosaic organs and intrawhorl fused organs are observed in *ufo*

mutants of species such as *Arabidopsis*, snapdragon or wheat (Levin & Meyerowitz, 1995; Ingram *et al.*, 1997; Kuzay *et al.*, 2022). Thus, *UFO* contributes to specify boundaries between whorls and between organs throughout flower development. This cadastral role of *UFO* is likely linked to its precise expression pattern in the FM (Fig. 2).

2. *UFO* controls flower and inflorescence meristem fate

Whereas shoot meristems produce leaves and axillary meristems spaced by internodes in a spiral arrangement, FMs in most cases create floral organs in specific clusters called whorls. Additionally,

FMs usually produce a fixed number of floral organs whereas shoot meristems produce an indeterminate number of structures. In some species, axillary meristems are subtended by the leaf from the axil of which they originated whereas flowers are devoid of such leaf (bract repression). The acquisition of these FM traits is referred to as floral meristem identity (FMI) acquisition, and this process is regulated by *UFO* in numerous plant species that exhibit diverse inflorescence types (Fig. 1).

Cymose In cyme inflorescences, the apical meristem terminates by forming a flower and a new IM develops laterally to form the next unit, leading to a sympodial branching pattern. Petunia and tomato (*Solanum lycopersicum*) are two *Solanaceae* species with a cyme inflorescence where *UFO* homologs, respectively, *DOUBLE TOP (DOT)* and *ANANTHA (AN)*, play a key role (Allen & Sussex, 1996; Souer *et al.*, 2008). *DOT* and *AN* expressions are absent from the IM and strictly coincide with the emergence of FMs. Mutations in petunia *DOT* or tomato *AN* genes totally preclude the IM-to-FM conversion and lead to the absence of flowers. Instead, the IM continues to initiate lateral meristems indefinitely, producing hyper-branched inflorescences (Allen & Sussex, 1996; Lippman *et al.*, 2008; Souer *et al.*, 2008). The same phenotype was observed in the *an* mutant of pepper (*Capsicum annum*), a *Solanaceae* species that normally develops solitary flowers (Lippman *et al.*, 2008). Inversely, *DOT* overexpression or *AN* precocious expression triggers early flowering and induces inflorescence termination with a solitary flower (Souer *et al.*, 2008; MacAlister *et al.*, 2012). Thus, in these species with a cyme inflorescence, *UFO* stands as a major FMI determinant. In the cymose plant *Aquilegia coerulea*, the role of *UFO* is less clear since *AqUFO* downregulation appears to only delay the FMI acquisition without abolishing the production of FM (Sharma *et al.*, 2019).

Capitulum *UFO* deeply affects the FMI acquisition in species with a capitulum inflorescence (a flat disk covered by hundreds of FMs). In gerbera (*Gerbera hybrida*) or cosmos (*Cosmos bipinnatus*), the *ufo* mutation leads to a complete conversion of individual flowers into green structures made of an indeterminate number of leaves (Zhao *et al.*, 2016; Li *et al.*, 2019). Spectacularly, the ectopic expression of *GhUFO* throughout the gerbera capitulum turns it into a giant flower made of whorls of organs replacing individual flowers (Zhao *et al.*, 2016).

Raceme In species with a raceme inflorescence (such as Arabidopsis or snapdragon), the IM grows indefinitely and produces lateral meristems on its flank, giving secondary inflorescences at basal positions, and then flowers. Not all plants with a raceme-like inflorescence are equally affected by an *ufo* mutation. For example, in cucumber and Torenia, *ufo* mutants produce FMs showing no inflorescence-like features (Sasaki *et al.*, 2012; Chen *et al.*, 2021). The FMI function of *UFO* is more obvious in Arabidopsis and snapdragon. Arabidopsis *ufo* mutants show a slight increase in the number of secondary inflorescences and the most basal flowers are subtended by a bract or a filamentous structure, indicating a role of *UFO* in FMI

acquisition (Levin & Meyerowitz, 1995; Wilkinson & Haughn, 1995; Hepworth *et al.*, 2006). In snapdragon *fm* mutants, the spiral arrangement of some floral organs (typical of shoot meristem) also reveals a reduction of FMI (Simon *et al.*, 1994; Ingram *et al.*, 1997). However, the FMI role of *UFO* is best revealed by the study of double mutants: in contrast to single mutants, Arabidopsis *ufo ap1* or snapdragon *fm squamosa (squa, AP1* homolog) mutants do not produce flowers but only shoot-like structures with a spiral arrangement of leaves or sepals and a deformed gynoecium (Simon *et al.*, 1994; Levin & Meyerowitz, 1995; Wilkinson & Haughn, 1995), defects absent from *ap1* single mutants. Similarly, mutating both *FILAMENTOUS FLOWER (FIL)* and *UFO* in Arabidopsis leads to the absence of flowers, a phenotype not observed in each single mutant (Levin & Meyerowitz, 1995). Thus, in species like Arabidopsis or snapdragon, the implication of *UFO* in FMI acquisition is likely important but 'masked' by redundant pathways.

In legumes, *ufo* mutants are able to produce FMs; however, these structures display several defects. In the strong *pfö* mutant from lotus, stamens are absent and petals are systematically replaced by new FMs that produce a whorl of sepals and reiterate abnormal flower production (Zhang *et al.*, 2003; Dong *et al.*, 2005). In the pea *stamina pistilloida (stp)* mutant, additional flowers on a pedicel grow around the carpels (Taylor *et al.*, 2001). Also, ectopic flowers within flowers are observed in the strawberry (*Fragaria vesca*) *extra floral organs (efö)* mutant (Shahan *et al.*, 2018). The production of these ectopic flowers shows that, in these species, the FM retains some shoot meristem features and generates flowers in place of floral organs when *UFO* is not functional.

Panicle In several grass species such as rice, inflorescence is organized as a panicle, with spikelets, small branches containing flowers, attached to lateral branches. The IM produces primary branch meristems that give rise to secondary branches and spikelet meristems. Eventually, spikelet meristems give FMs. In rice, the *apo1* mutant has reduced inflorescence branching, with a strong decrease in the number of primary and secondary branches (Ikeda *et al.*, 2005). Hence, when *APO1* is not functional, IM and branch meristems change identity precociously and prematurely terminate into flowers. Conversely, dominant *apo1-D* mutants expressing *APO1* at higher levels have hyper-branched inflorescences (Ikeda *et al.*, 2007; Ikeda-Kawakatsu *et al.*, 2009). Similar results were found in wheat (a grass developing a spike inflorescence): the spikelet number per spike is reduced by a mutation in *WAP01* and increased when *WAP01* expression level is higher (Kuzay *et al.*, 2022; Wittern *et al.*, 2022). Thus, in contrast to what is observed in dicots, a major role of *UFO* in cereals is to promote IM/branch meristem identity. Since inflorescence architecture determines the number of spikelets, ultimately affecting the number of flowers and the grain yield, it is not surprising that *APO1* was identified in several QTL analyses in monocots crops (Ookawa *et al.*, 2010; Tsukahara *et al.*, 2015; Yano *et al.*, 2015; Muqaddasi *et al.*, 2019).

In conclusion, *UFO* controls various facets of FMI acquisition in many angiosperm species.

3. UFO controls meristematic traits

In various species, *ufo* mutants also show a diversity of defects indicating a role of UFO on cell division or meristem activity (distinct from its role in FMI acquisition). Depending on species and organs examined, UFO appears to have either a positive or a negative effect on cell division/meristem size.

In rice, the size of the IM and the expression of cell division markers are higher in *APO1* overexpressing plants and lower in *apo1* mutant (Ikeda *et al.*, 2005; Ikeda-Kawakatsu *et al.*, 2009). Moreover, the reduced panicle branching observed in *apo1* was linked to the decrease in meristematic cell proliferation (Ikeda-Kawakatsu *et al.*, 2009). Thus, *APO1* appears to stimulate cell division in the IM. This could also be true in wheat where strong branching defects are reported in *wapo1* mutants (Kuzay *et al.*, 2022; Wittern *et al.*, 2022). However, *APO1* expression in the vegetative SAM negatively affects the number of leaves and branches produced (Ikeda *et al.*, 2005, 2007; Ikeda-Kawakatsu *et al.*, 2009). *APO1* is also involved in repressing tillers as their number is increased in the *apo1* mutant (Ikeda *et al.*, 2005) and strongly decreased when *APO1* is overexpressed (Ikeda-Kawakatsu *et al.*, 2009; Ookawa *et al.*, 2010). A similar phenomenon occurs in wheat *WAP01* overexpressors (Wittern *et al.*, 2022). Therefore, UFO appears to repress vegetative meristems in monocots and thus have opposite effects between vegetative and inflorescence meristems.

In snapdragon and Arabidopsis, it was proposed that UFO/FIM represses cell division in some regions of early meristems and in boundaries (Ingram *et al.*, 1997; Samach *et al.*, 1999). Other data indicate that UFO rather stimulates cell division: some Arabidopsis *ufo* mutant flowers are empty (developing a single whorl of sepals), and *ufo fil* double mutant flowers are replaced by filaments (short-lived determinate structure; Levin & Meyerowitz, 1995; Wilkinson & Haughn, 1995). These later observations suggest that UFO is required for the proper establishment of the pool of meristem cells required to form a FM. Moreover, the overexpression of an activated form of UFO (UFO-VP16, a translational fusion between UFO and the activation domain from the viral transcription factor VP16) in Arabidopsis, tobacco (*Nicotiana tabacum*) or rapeseed (*Brassica napus*) induces the remarkable formation of ectopic FMs directly on leaves (Risseuw *et al.*, 2013). This shows that UFO-VP16 triggers the emergence of meristems from the leaf surface (and subsequently converts them into flowers). Along the same line, in some legumes species, UFO promotes the development of compound leaves: in pea, the *stp* mutant has simple leaves instead of compound and the lotus *pf0* mutant has leaves with a reduced number of leaflets (Taylor *et al.*, 2001; Dong *et al.*, 2005). Since leaflet development requires the reactivation of meristematic genes within the leaf primordia (Mo *et al.*, 2022), it shows that UFO in some legumes is needed for this reactivation. Altogether, such evidence suggests that UFO is able to stimulate cell proliferation and/or the establishment of meristem traits in some plant species. These data indicate that UFO can act positively or negatively on cell proliferation/meristem traits, but the underlying mechanism is not yet understood.

4. The spatiotemporal expression pattern of UFO

UFO expression is tightly regulated but its protein expression domain in flowers is poorly described Consistent with its major functions, *UFO* expression is mostly found in floral tissues. However, *UFO* is also expressed in the compound leaves of some legumes and in the stem of cucumber, with a role in both territories (Taylor *et al.*, 2001; Chen *et al.*, 2021). In some other species, *UFO* is expressed in vegetative tissues but without any observed role (Lee *et al.*, 1997; Pouteau *et al.*, 1998). For example, in Arabidopsis, *UFO* RNA is first detected at the heart stage of embryo development (Long & Barton, 1998; Reddy, 2008), and it remains present in the SAM periphery (Lee *et al.*, 1997).

During reproductive development, *UFO* expression pattern in meristems is very well delineated and is linked to the different functions UFO performs (Fig. 2). In species like petunia, gerbera or tomato where UFO is the main FM determinant, its precise expression pattern is crucial to trigger the onset of flower development (Lippman *et al.*, 2008; Souer *et al.*, 2008; MacAlister *et al.*, 2012; Zhao *et al.*, 2016). The expression pattern of *UFO* is also well delineated in the early-stage FMs, allowing activation of homeotic genes in precise territories. However, *UFO* expression pattern was mostly described with RNA *in situ* hybridization and the protein localization in inflorescences and floral tissues is still missing. In snapdragon, it was shown using periclinal chimeras that FIM activates genes in regions where it is not transcribed (Schultz *et al.*, 2001). Similarly, UFO negatively affects bract development in Arabidopsis without being expressed in this tissue (Hepworth *et al.*, 2006). The UFO protein is thus possibly mobile and active outside of its expression domain.

Little is known about the regulation of UFO expression It is known that the tight control of *UFO* expression involves its promoter. Indeed, this promoter is sufficient to recapitulate the divergence of *UFO* expression patterns in diverse species (Kusters *et al.*, 2015), and several *ufo* mutant phenotypes are caused by alterations in its promoter sequence (Ingram *et al.*, 1997; Durfee *et al.*, 2003). However, few *cis*-elements have been described in the *UFO* promoter, and the sequence comparison between promoters from related species only identified conserved blocks (Kusters *et al.*, 2015).

Only a few *UFO* upstream regulators have been proposed to date. In Arabidopsis, *SHOOT MERISTEMLESS (STM)* is a direct *UFO* activator throughout development (Long & Barton, 1998; Roth *et al.*, 2018). In tomato, *AN* is regulated by the ALOG gene *TERMINATING FLOWER (TMF)* (MacAlister *et al.*, 2012). *TMF* represses *AN* and allows its gradual activation upon flowering. A mechanism involving *TMF* phase separation on the *AN* promoter was recently proposed (X. Huang *et al.*, 2021, 2022). In petunia, a crucial *DOT* activator is the WOX-protein EVERGREEN, but this activation is indirect and may implicate other unknown factor(s) (Rebocho *et al.*, 2008). In some species, *LFY* is an *UFO* activator (see below). Then, during flower development, *AP3* and *PETAL LOSS* positively regulate *UFO* expression in Arabidopsis (Wuest *et al.*, 2012; Takeda *et al.*, 2022), and *AP1* is required for late *UFO* expression (Lee *et al.*, 1997; Ng & Yanofsky, 2001). Similarly, the

AP1 ortholog SQUA likely activates *FIM* in snapdragon (Simon *et al.*, 1994). Overall, precisely understanding the regulation of *UFO* in time and space will require more work and the identification of additional regulators.

III. UFO is an F-box protein

Unlike many other developmental regulators, *UFO* does not encode a TF (Samach *et al.*, 1999). Its protein sequence contains two conserved domains: an N-terminal F-box domain (Samach *et al.*, 1999) that assigns *UFO* to the large F-box gene family, comprising over 700 genes in Arabidopsis (Gagne *et al.*, 2002), and a C-terminal Kelch-repeat β -propeller domain, predicted to be an interface for interactions with other proteins.

1. Formation and functionality of the SCF^{UFO} complex

At the molecular level, F-box proteins are part of Skp1–Cullin–F-box (SCF) complexes. First described in yeast, SCF complexes act as ubiquitin E3 ligase, targeting proteins for proteasome-dependent degradation (Stefanowicz *et al.*, 2015). The F-box domain is required for the interaction with ARABIDOPSIS SKP1-LIKE (ASK) proteins, core SCF subunits. F-box proteins confer specificity to SCF complexes by recruiting target proteins thanks to the large variety of domains they harbor in addition to the F-box domain (Zhang *et al.*, 2019).

The formation of a SCF^{UFO} complex was demonstrated *in vitro* and *in planta*. Interaction between *UFO* F-box domain and ASK proteins was confirmed genetically (Zhao *et al.*, 1999, 2001; Ni *et al.*, 2004) and/or biochemically in several species (Ingram *et al.*, 1997; Samach *et al.*, 1999; Souer *et al.*, 2008). The ASK family comprises several members (21 in Arabidopsis), and *UFO* preferentially interacts with specific ones (like ASK1, ASK2 or ASK11 in Arabidopsis; Gagne *et al.*, 2002). *UFO* also interacts genetically with other SCF core subunits like CULLIN1 (CUL1), and CUL1 is co-immunoprecipitated with *UFO* *in planta* (Wang *et al.*, 2003). Several lines of evidence show that the SCF^{UFO} complex is functional *in vivo*. Arabidopsis mutants (notably *ask1* mutants) or RNAi lines targeting any core subunits of the SCF complex exhibit altered floral organs in 2nd and 3rd whorls similar to those observed in *ufo* mutants (Zhao *et al.*, 1999; Liu *et al.*, 2004; Ni *et al.*, 2004). Finally, a decrease in COP9 SIGNALOSOME (CSN) complex activity (a regulator of SCF complexes) in Arabidopsis impairs some *UFO* functions like *AP3* activation, revealing that CSN likely regulates a functional SCF^{UFO} complex (Wang *et al.*, 2003). Thus, SCF^{UFO} probably targets proteins for ubiquitination, but these targets are unknown. LFY was an obvious candidate, and the possibility of a SCF^{UFO}-dependent LFY ubiquitination is discussed below. Other proteins may be targeted for ubiquitination, and some were proposed in snapdragon but were never characterized in detail (Wilkinson *et al.*, 2000). Obtaining a complete *UFO* interactome would help to identify putative targets, but yeast-two-hybrid (Y2H) screens performed with *FIM*, *UFO* or *DOT* as bait yielded nearly only ASK or LFY proteins as interacting proteins (Ingram *et al.*, 1997; Samach *et al.*, 1999; Souer *et al.*, 2008).

2. The role of *UFO* within an E3 ligase complex remains unclear

In contrast to a previous study (Risseuw *et al.*, 2013), it was recently found that overexpression of a truncated *UFO* version lacking the F-box domain (i.e. unable to participate in a SCF complex) largely complemented a strong Arabidopsis *ufo* allele, restoring petal and stamen development (Rieu *et al.*, 2023). Hence, the connection of *UFO* to an E3 ligase complex appears partially dispensable, at least in Arabidopsis.

Still, in the absence of the F-box domain, complemented *ufo* plants retained some mutant defects such as missing or misshapen petals. This observation together with the conservation of the *UFO* F-box domain across species suggests that the F-box domain (and thus the connection to the SCF complex) has a function *in planta*. One possibility is that *UFO* acts redundantly with other F-box proteins in ubiquitination pathways. A *ufo* enhancer screen in Arabidopsis identified mutations in three genes named *FUSED FLORAL ORGANS 1 to 3* (*FFO1-3*; Levin *et al.*, 1998). The *ufo ffo1* double mutant (but not single mutants) does not produce flowers but only filamentous structures. *FFO1* corresponds to *HAWAIIAN SKIRT* (*HWS*), a close paralogous gene of *UFO* (González-Carranza *et al.*, 2007, 2017). *HWS* is implicated in the microRNA function (biogenesis, action or degradation) and, for this, acts through an E3 ligase complex (González-Carranza *et al.*, 2017; Zhang *et al.*, 2017). *HWS* targets are yet unknown (Lang *et al.*, 2018), and *HWS* and *UFO* could work redundantly in ubiquitination pathways. Thus, redundancy between *UFO* and other F-box proteins could explain why *UFO*'s role as an E3 ligase is not easily discernible in Arabidopsis. While the role of *UFO* within an E3 ligase complex remains unclear, a ubiquitination-independent function was proposed for *UFO* that explains how it regulates transcription together with LFY.

IV. *UFO* acts as a LFY transcriptional cofactor

1. *UFO* and LFY interact genetically and physically in multiple species

Initial genetic studies in Arabidopsis, snapdragon and pea noticed that *ufo* and *lfy* mutants share many similarities (Simon *et al.*, 1994; Levin & Meyerowitz, 1995; Taylor *et al.*, 2001). Also, in rice, a mutant was named *apo2* due to its phenotypic similarity with the *apo1* (*ufo*) mutant and was later found to affect the rice homolog of LFY (also called *RICE FLORICAULA LEAFY* (*RFL*); Ikeda-Kawakatsu *et al.*, 2012; Kyojuka *et al.*, 1998). The severe phenotype enhancement observed by combining weak alleles of *ufo* and *lfy* as well as the epistasis of LFY over *UFO* revealed a strong genetic interaction between the two genes in many species (Fig. 1).

A first hypothesis to explain such observations would be that one gene transcriptionally regulates the other. LFY was indeed reported to regulate *UFO* expression in snapdragon (Simon *et al.*, 1994), tomato (Lippman *et al.*, 2008), cucumber (Zhao *et al.*, 2018) and rice (Ikeda-Kawakatsu *et al.*, 2012). However, such transcriptional regulation is not sufficient to explain the epistasis between the two genes observed in all species.

Instead, it was shown in all plant species examined that UFO and LFY physically interact, indicating that epistatic relationships could be explained by UFO and LFY acting together within the same protein complex (Fig. 1). Some of the missense mutations in *LFY* (Selva *et al.*, 2021) or *UFO* (Sasaki *et al.*, 2012) that induce a phenotype *in planta* precisely impair the UFO-LFY interaction, revealing its importance *in vivo*. Mapping of the interaction domains showed that in some species like *Arabidopsis* or gerbera, interaction implies LFY C-terminal DNA-Binding-Domain (DBD) and UFO Kelch-repeat β -propeller domain (Chae *et al.*, 2008; Zhao *et al.*, 2016). However, in *petunia*, Y2H assays suggested that DOT interacts with ABERRANT LEAF AND FLOWER (ALF, the LFY homolog in *petunia*) N-terminal sterile alpha motif (SAM) domain instead (Souer *et al.*, 2008). This result is surprising given the high level of homology between ALF-LFY and DOT-UFO. Hence, even if the interaction is conserved, the mode of interaction may have evolved and this could account for differences in activity. The interaction between UFO and LFY is likely important for them to jointly regulate gene expression. In fact, *Arabidopsis* or *petunia* plants overexpressing both *UFO* and *LFY* (but not single genes) do not survive beyond the seedling stage and ectopically express several flower-specific genes (Parcy *et al.*, 1998; Souer *et al.*, 2008). These experiments showed that UFO and LFY act synergistically when expressed together.

As UFO physically interacts with LFY, it was long thought that SCF^{UFO} regulates LFY activity through ubiquitination. In fact, TF regulation through ubiquitination is a well-described mechanism (Kodadek *et al.*, 2006; Geng *et al.*, 2012) and proteasome-mediated degradation was shown to be required for UFO-LFY activity in seedlings (Chae *et al.*, 2008). However, LFY protein level is globally unaltered in a strong *ufo* mutant (Chae *et al.*, 2008) and specific LFY mono- or poly-ubiquitination is insufficiently described in *Arabidopsis* or *petunia* (Chae *et al.*, 2008; Souer *et al.*, 2008) even if UFO-mediated degradation of LFY was observed in the cytoplasm in *Arabidopsis* (Dolde *et al.*, 2023). The role of a SCF^{UFO}-dependent LFY ubiquitination requires further investigations.

2. UFO and LFY form a transcriptional complex binding specific *cis*-elements

Initial studies showed that UFO might be acting within a transcriptional complex: adding an activation or a repression domain to UFO changes its activity in *Arabidopsis*, suggesting a role for UFO in close vicinity of DNA (Chae *et al.*, 2008; Risseuw *et al.*, 2013). Moreover, it was shown that *Arabidopsis* UFO is recruited on DNA in a LFY-dependent manner (Chae *et al.*, 2008). However, the nature of the *cis*-element needed to recruit UFO and LFY had remained unknown. Recently, *Arabidopsis* UFO and LFY were found to form a transcriptional complex able to bind novel DNA sequences (named UFO-LFY binding sites or LUBS) that LFY or UFO poorly accesses on their own (Rieu *et al.*, 2023). Whereas LFY normally binds as a dimer to pseudo palindromic DNA sites called canonical LFY binding sites (LFYBS), LUBS are composed of a low-affinity canonical LFYBS (that can also be a half LFYBS bound by a single LFY monomer) located at a fixed distance

from a UFO recruiting motif (URM). The presence of those two features allows DNA binding by a UFO-LFY complex. Analysis of this complex using cryo-electron microscopy revealed that UFO Kelch-repeat β -propeller domain contacts both DNA (at the URM) and LFY-DBD, explaining its role as a LFY cofactor (Fig. 3). This characteristic of the Kelch-repeat β -propeller domain is particularly interesting as this domain is highly represented among plant F-box proteins (Gagne *et al.*, 2002).

3. Revisiting UFO and LFY targets in light of the UFO-LFY transcriptional complex

Revisiting UFO-LFY targets regulating floral organ development The role of UFO-LFY on floral organ development is established in most angiosperm species, and several target genes have been identified (see above). In *Arabidopsis*, such targets include the *B* genes *AP3* for which functional LUBS were identified in their promoter sequence, explaining their regulation by the UFO-LFY complex (Rieu *et al.*, 2023). Functional LUBS sites are expected to be found in the regulatory sequences of genes under the control of the UFO-LFY complex in many other plants, in particular *B*, *C* or *E* homeotic genes.

RABBIT EARS (*RBE*), another gene under the transcriptional control of UFO, is likely a target of the UFO-LFY complex thanks to a single LUBS present in its promoter (Krizek *et al.*, 2006; Rieu *et al.*, 2023). *RBE* controls petal development: in *rbe* mutants, petals are deformed, replaced by filaments or even absent (Takeda *et al.*, 2004). The absence of petals in both *rbe* and some *ufo* weak alleles is explained by the ectopic expression of *AGAMOUS* (*AG*), a gene normally repressed from the second whorl by *RBE* (Krizek *et al.*, 2006). Hence, UFO-LFY has a cadastral function in the flower through the induction of *RBE* that prevents *AG* expression in the second whorl.

It is worth noting that the different UFO-LFY targets (like *AP3* and *RBE* in *Arabidopsis*) have very distinct expression patterns, indicating that other proteins must act with UFO and LFY to regulate their spatiotemporal expression. For example, in *Arabidopsis*, UFO acts in parallel to *SEPALLATA3* to activate *AP3* (Castillejo *et al.*, 2005). A detailed mechanism for *AP3* promoter activation is presented in Box 1. In *petunia*, ALF-DOT likely associates with the *WUSCHEL* (*WUS*) homolog *TERMINATOR* to regulate *C* gene expression (Souer *et al.*, 2008). Hence, a future challenge will be to characterize interactors of the UFO-LFY complex. In particular, it could be relevant to test the physical interaction between UFO and MADS-box TFs (Box 1).

Revisiting UFO-LFY targets regulating meristem identities UFO and LFY together regulate FMI acquisition in many species, suggesting that the UFO-LFY complex is also at work on the regulatory elements of FMI genes. However, key target genes involved in processes like the control of determinacy, the whorled arrangement of floral organs, the absence of internodes or the suppression of bracts largely need to be unraveled.

Still, some targets have been reported and others can be proposed. In *petunia*, UFO-LFY targets determining FMI likely include the MADS-box genes *FLORAL BINDING PROTEIN9* (*FBP9*) and *FBP23* (belonging to the *SEP* clade). Indeed, these two

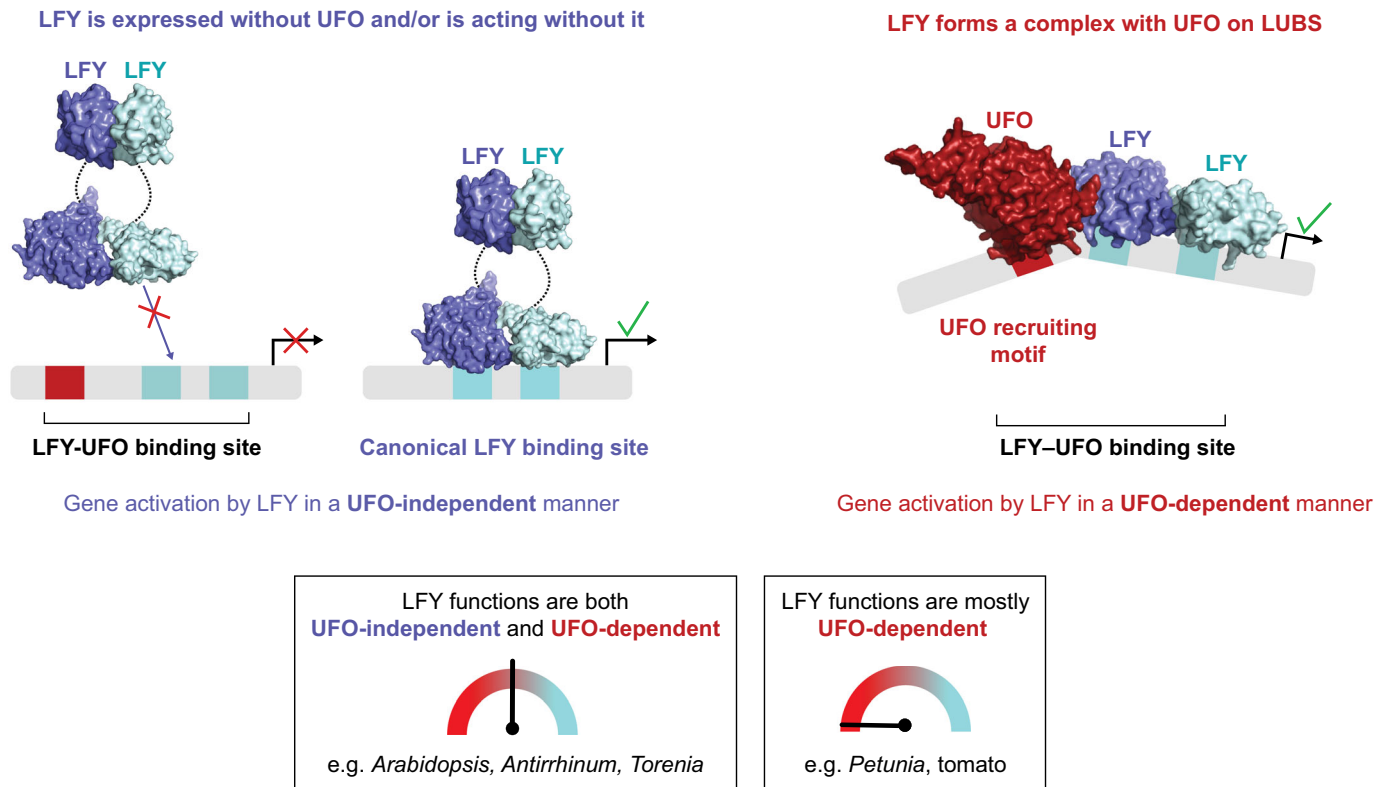


Fig. 3 LEAFY (LFY) acts through diverse molecular mechanisms and the importance of UNUSUAL FLORAL ORGANS (UFO) as a LFY cofactor varies depending on species. LFY can bind DNA alone or with UFO (upper). In the absence of UFO, LFY (dark or light blue, sterile alpha motif (SAM) domain and DNA-binding domain (DBD) as surface) cannot bind LFY-UFO binding sites (LUBS) but binds canonical LFY binding sites as a dimer (Hamès *et al.*, 2008) and activates LFY-specific genes. When UFO and LFY are present together, they form a complex on LUBS where UFO (red) binds the UFO recruiting motif and LFY binds the low-affinity canonical LFY binding sites (Rieu *et al.*, 2023). Together UFO and LFY regulate specific genes. Depending on species, the relative importance of LFY UFO-dependent and -independent functions vary (lower).

genes are induced by the coexpression of ALF (LFY) and DOT (UFO) in seedlings (Souer *et al.*, 2008) and the phenotype of the *fbp9 fbp23 fbp4* triple mutant strongly resembles *alf* and *dot* mutants (Morel *et al.*, 2019). A similar mechanism might be present in tomato for the induction of crucial MADS-box genes controlling FMI acquisition (Soyk *et al.*, 2017). In rice, transcriptomic analysis in *APO1*-overexpressing lines also identified potential targets from the SEP clade like *OsMADS5* and *34* that could mediate the action of *APO1* in branching and IM identity (Yano *et al.*, 2015; Zhu *et al.*, 2022).

In *Arabidopsis*, the implication of UFO in the determination of FMI could be explained by the upregulation of *FD* or *SQUAMOSA PROMOTER BINDING PROTEIN-LIKE 5* (*SPL5*; Goslin *et al.*, 2017; Rieu *et al.*, 2023), two proteins cooperating for the activation of *API1*, *FRUITFULL* and *LFY* within the early FM (Jung *et al.*, 2016). In rice also, genes like *SPL14* and the GATA TF *NECKLEAF1* were shown to be regulated by *APO2* (LFY), but are possibly under the regulation of the *APO1*-*APO2* complex (Miao *et al.*, 2022).

Revisiting UFO-LFY targets regulating meristematic traits The effect of UFO on meristematic traits is species-dependent. As described before, it is documented in legumes and rice but it is less evident in other species. The meristematic function proposed for LFY

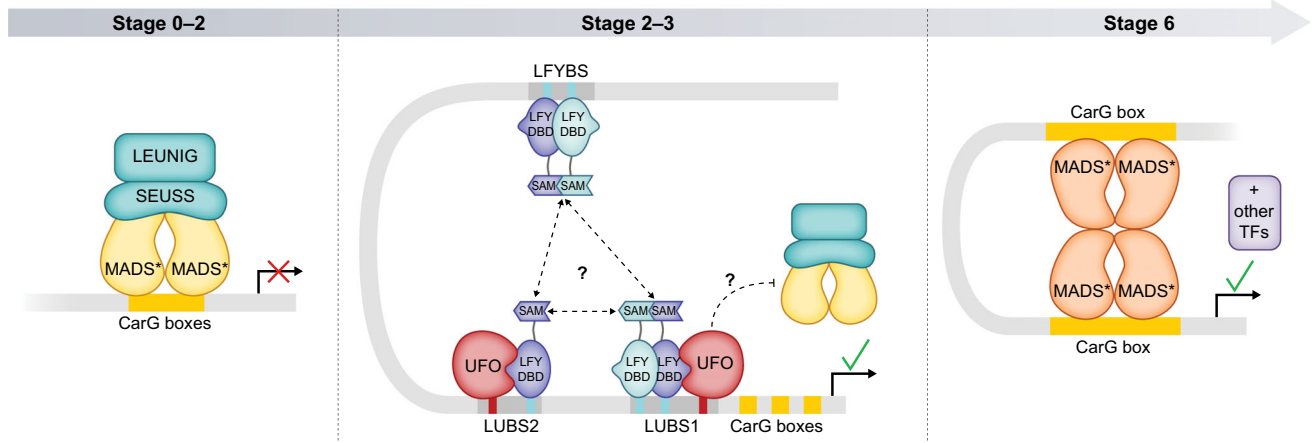
(Moyroud *et al.*, 2010; Chahtane *et al.*, 2013; Yamaguchi *et al.*, 2013) could actually be a UFO-LFY function and involve binding of the UFO-LFY complex on regulatory sequences of genes involved in cell division. Genes such as *CYTOKININ OXIDASE 5* (involved in cytokinin degradation; Werner *et al.*, 2003) or *CYCLIN D3;1* (involved in cell division; Menges *et al.*, 2006) in *Arabidopsis* and the *APO2* targets *CYCLIN-DEPENDENT KINASE-LIKE1* (*CKL1*) or *KIP-RELATED PROTEIN2* (*KRP2*) are obvious candidates to link UFO-LFY and cell proliferation (Miao *et al.*, 2022; Rieu *et al.*, 2023). Alternatively, the role of UFO on cell division could be explained not only by UFO transcriptional activity but also by its E3 ligase activity, with the degradation of specific cell cycle regulators.

Thus, UFO targets are still to be discovered in many species, and LFY targets will also have to be reanalyzed as potential UFO-LFY targets.

V. UFO-LFY interplay: an old roller-coaster romance?

1. UFO always needs LFY but LFY can fly solo

The conservation of the UFO-LFY interaction and the available phenotypes of *ufo* and *lfy* mutants in numerous species offer a unique opportunity to examine the interdependence between UFO and LFY in a wide diversity of flowering plants.

Box 1 Different steps of *APETALA3* promoter regulation in Arabidopsis.

APETALA3 promoter (*pAP3*) is the best-characterized target of UNUSUAL FLORAL ORGANS-LEAFY (UFO-LFY). During vegetative and early flower development, the *AP3* locus is kept silent thanks to repressive epigenetic marks (Goodrich *et al.*, 1997; Gómez-Mena *et al.*, 2001). Release of this repression is regulated by several mechanisms (Carles & Fletcher, 2009; Sacharowski *et al.*, 2015). During very early flowering stages (stage 0–2), *pAP3* CarG boxes (notably CarG3; Tilly *et al.*, 1998) are bound by a repressive complex comprising CarG-bound MADS transcription factors (TFs) like SHORT VEGETATIVE PHASE (SVP), AGAMOUS-LIKE 24 (AGL24) or *APETALA1* (AP1) in complex with LEUNIG and SEUSS repressors (Liu & Meyerowitz, 1995; Franks *et al.*, 2002; Gregis *et al.*, 2006, 2009). From stage 2, UFO and LFY activate *pAP3* by binding LUBS. It is not known whether UFO associates with a LFY monomer or a LFY dimer *in planta*. *pAP3* also contains a relatively high-score canonical LFYBS that acts only as an enhancer element (Lamb *et al.*, 2002). The different LFY-containing complexes may interact, notably through LFY sterile alpha motif (SAM) oligomerization domain (Sayou *et al.*, 2016). The repressive complex is removed from CarG boxes at this stage, and we speculate that UFO may help to degrade specific subunits through the Skp1–Cullin–F-box^{UFO} complex. Later on, CarG boxes are bound by positive MADS regulators like AP1 (Ng & Yanofsky, 2001), SEPALLATA3 (Castillejo *et al.*, 2005), AP3 and PISTILLATA (positive feedback loop; Jack *et al.*, 1994). In addition to UFO-LFY, other TFs like AINTEGUMENTA also regulate *AP3* (Krizek, 2009). DBD, DNA-binding domain; SAM, sterile alpha motif; LFYBS, LFY binding site; URM, UFO recruiting motif; LUBS, UFO-LFY binding site.

In all angiosperm species examined to date, the phenotypes described for *ufo* mutants are also present in *lfy* mutants. Moreover, the gain-of-function phenotypes of Arabidopsis, petunia or rice plants overexpressing *UFO* are lost in a *lfy* mutant background (Lee *et al.*, 1997; Souer *et al.*, 2008; Ikeda-Kawakatsu *et al.*, 2012; Risseuw *et al.*, 2013). Both observations indicate that, for most of its functions in reproductive and vegetative development, UFO acts in a LFY-dependent manner (and thus likely within the UFO-LFY transcriptional complex). This is compatible with the poor ability of UFO to bind DNA on its own (Chae *et al.*, 2008; Rieu *et al.*, 2023) and the likely absence of other UFO-interacting TFs. The only apparent exception to this rule is the tendril defects observed in the cucumber *csufo* mutant but not in plants where *CsLFY* expression is downregulated (Zhao *et al.*, 2018; Chen *et al.*, 2021). Hence, it is possible that in some rare cases UFO has LFY-independent functions, but this remains to be formally demonstrated.

On the opposite, LFY has both UFO-dependent and -independent functions, and their relative importance varies between species. In petunia or in legumes (pea and lotus), *ufo* and *lfy* phenotypes are very similar, suggesting that both proteins mostly function together. Accordingly, overexpressing *ALF* or *LFY* in petunia does not induce any ectopic flower development, showing that UFO is limiting for LFY action (Souer *et al.*, 2008).

Conversely, in other species, a variety of phenotypes are observed in *lfy* but not in *ufo* mutants. For example, in rice, *apo1* (*ufo*) and *apo2* (*lfy*) mutants share the same inflorescence phenotype (reduced branching), but flowers are less affected in *apo1* than in *apo2* where lodicule formation is also impaired (Ikeda-Kawakatsu *et al.*, 2012). In gerbera, both GhLFY and GhUFO similarly control FMI but only GhLFY controls ray flower development and capitulum indeterminacy (Zhao *et al.*, 2016). In Arabidopsis and snapdragon, FMI defects of *lfy* mutant are much more severe than *ufo*'s, showing that LFY performs part of FMI determination independently of UFO. In tomato, the flower development is arrested earlier in *falsiflora* (*fa*, *lfy*) than in the *an* (*ufo*) mutant as FA first represses bracts and induces AN before working together with the AN protein (Allen & Sussex, 1996; Lippman *et al.*, 2008). The fact that LFY performs some of its functions in a UFO-independent manner is well understandable at the molecular level. For example, AP1 and AG inductions by LFY in Arabidopsis involve direct binding by a LFY homodimer to canonical LFYBS present in the regulatory elements of these genes, independently of UFO (Parcy *et al.*, 1998; Busch *et al.*, 1999; Wagner *et al.*, 1999).

Hence, it appears that the UFO-LFY complex exists in all studied angiosperm species, and the mechanism unraveled for *B* gene activation in Arabidopsis could be valid for other UFO functions and in many other angiosperms. However, the relative roles of UFO-LFY vs LFY-LFY have varied during evolution (Fig. 1). In

several cases, LFY has a wider role than UFO and the localized *UFO* expression is leveraged by LFY to exert its effects selectively, within specific regions of the developing structures.

2. Why does LFY require UFO in some cases but not in others?

An interesting question arising from these genetic data is: why can LFY bypass the need of UFO in some species but not in others? Several answers can be proposed.

Cis-element variations A first explanation could be that the UFO-LFY complexes from different species always act in the same way but target different genes because *cis*-elements have varied. For example, in Arabidopsis, the initial induction of *API* by LFY involves a canonical LFYBS and is independent of UFO (Benlloch *et al.*, 2011) while in petunia the induction of MADS-box genes from the *API* clade depends on both UFO and LFY (Souer *et al.*, 2008). Consistently, LFYBS in *API* promoter are not widely conserved (Minguet *et al.*, 2015) and it is possible that they are functionally replaced by LUBS in several species.

Because LUBS contains a low-quality LFYBS, it may easily become a canonical LFYBS if mutations increase its affinity for LFY. Conversely, if an URM appears next to a LFYBS, this later site may then lose its affinity for LFY to become a LUBS, bound and regulated by UFO-LFY only. Such conversions might explain that, depending on species, FMI and homeotic genes are regulated by the LFY homodimer or by the UFO-LFY complex. Comparing the occurrence of those *cis*-elements using binding site models within various species will allow the characterization of such evolution (Lai *et al.*, 2019).

UFO might be more than a cofactor bringing LFY to LUBS In the previous model, we assume that UFO is only required for LFY to access LUBS. UFO could also be actively participating in the transcriptional co-activation by other mechanisms. For example, UFO could degrade repressors present in the vicinity of LFY or recruit other proteins needed for the transcription initiation. The interspecific variations of LFY ability to bypass the need of UFO could be explained by variations in the capacity of LFY to activate transcription without UFO. Consistent with this hypothesis, the rice APO2 behaves like an activated version of LFY when expressed in Arabidopsis (Chujo *et al.*, 2003). Such nonexclusive scenarios remain very hypothetical and will require further investigations.

3. Many molecular properties of the UFO-LFY complex are yet to be discovered

Several features of the UFO-LFY complex remain unknown (Rieu *et al.*, 2023). The low resolution of the published complex structure prevents the identification of the residues involved in UFO-DNA or UFO-LFY contacts. Moreover, this structure was obtained with the LFY-DBD only and the role of the N-terminal LFY sterile alpha motif (SAM) domain within the UFO-LFY complex is not determined. Finally, it was reported that UFO induces a strong DNA bending but whether this plays a regulatory role (e.g. through promoter looping) has not been examined.

It also remains to be explored whether the functionality of the UFO-LFY complex is regulated by other factors. In rice, it was shown that the HECT-domain E3 ligase LARGE2 stabilizes the APO1-APO2 complex and positively regulates its activity (L. Huang *et al.*, 2021). In Arabidopsis, the interaction between LFY and WUS was proposed early but it remains unclear and the formation of a transcriptional complex is not demonstrated (Lohmann *et al.*, 2001). Data from petunia suggest an interplay between LFY, UFO and WUS (Souer *et al.*, 2008), and it would be relevant to test the formation of a ternary complex.

Another major question not yet tackled is the relation between UFO-LFY and chromatin regulation. It was recently proposed that LFY possesses pioneer TF properties, that is, the capacity to bind to and open closed chromatin regions during early flower development (Jin *et al.*, 2021; Lai *et al.*, 2021). However, whether UFO plays a role in this process by helping LFY to reshape the chromatin status of some floral genes is unknown. The position of UFO on the same side as LFY would be compatible with UFO-LFY binding of DNA wrapped around a nucleosome.

Finally, the dual role of UFO as an E3 ligase and as a transcriptional cofactor is very intriguing. Genetic experiments described above (with *ask1* mutant notably; Liu *et al.*, 2004; Ni *et al.*, 2004; Wang *et al.*, 2003) clearly established a role for UFO within a SCF complex. Also, UFO was recently found to regulate LFY level through ubiquitination and degradation in the cytoplasm (Dolde *et al.*, 2023). This is in opposition with an exclusive role of UFO as a transcriptional cofactor, and the relation between these two functions remains to be determined. It is also possible that UFO works as an E3 ligase in the context of the transcriptional complex, for example, to ubiquitinate coregulators or even histones at precise locations in the genome.

VI. UFO-LFY partnership within and outside flowering plants

1. UFO-LFY in flowering plants

The description of the UFO-LFY-DNA complex in Arabidopsis raises questions of its origins and the evolution of its functions. This complex has been observed in monocots and core eudicot species, indicating that it is likely ancient. However, it has not yet been reported in early-diverging flowering plants such as magnoliids or ANA (Amborellales, Nymphaeales and Austrobaileyales) grade species. The examination of the UFO-LFY functions during angiosperm evolution (Fig. 1) does not give any immediate clue regarding how the UFO-LFY functions have evolved during flowering plant history. Apart from obvious similarities between related species (wheat and rice, or tomato and petunia), there is no specific pattern emerging with functions associated to specific branches. This suggests that plants have rather used the UFO-LFY complex in a plastic manner across evolution. Still, it is worth noting that the absolute requirement for LFY (with or without UFO) for making flower meristem varies between species: whereas LFY is absolutely required in plants such as snapdragon, tomato, Arabidopsis and gerbera, it is dispensable in cereals and legumes where MADS-box TF play key roles, and LFY or LFY-UFO are

rather needed for flower organs development. Hence, in most angiosperms, the same actors are involved (LFY-LFY, LFY-UFO and MADS-TF from the AP1 and SEP clades) but with different relative importance and regulatory links between them.

2. UFO-LFY before flowering plants

The fact that LFY orthologs from gymnosperms and ferns functionally interact with Arabidopsis UFO in a transient protoplast assay and *in vitro* (Rieu *et al.*, 2023) suggests that the UFO-LFY complex might even have arisen before the origin of flowers, in vascular plants last common ancestor. Hence, a future goal is to identify new UFO proteins in nonflowering vascular plants and characterize the formation and the biochemical properties of UFO-LFY complex (its DNA-binding specificity in particular). Reverse genetics in bryophytes or ferns could help widening the collection of *ufo* mutants and shed light on UFO ancestral functions. Specifically, it will be interesting to examine whether the LFY functions described in nonangiosperm species could rather be UFO-LFY functions. This applies to gymnosperms such as *Welwitschia mirabilis* where LFY was proposed to regulate *B* and *C*-like genes during cone development or *Picea abies* in which LFY could play a role in areas of active cell division within reproductive structures (Carlsbecker *et al.*, 2013; Moyroud *et al.*, 2017). The same question exists for LFY functions in cell division or meristem maintenance described in the moss *Physcomitrium patens* or in the fern *Ceratopteris richardii* (Tanahashi *et al.*, 2005; Plackett *et al.*, 2018). Whether such functions are actually performed by the UFO-LFY complex will await the identification and the genetic analysis of UFO genes in such species. An interesting scenario would be that the UFO-LFY complex played a role in meristem control before being later coopted for the development of flowers, with a diversification of its functions later during angiosperm evolution.

VII. Conclusion and perspectives

The making of flowers requires profound meristem reprogramming in order to produce whorls of floral organs. In all angiosperms examined to date, UFO regulates crucial steps of this process. The analysis of floral gene induction in Arabidopsis revealed that UFO acts as a LFY transcriptional cofactor, bringing LFY to loci where it cannot bind on its own. Future experiments will tell whether such mode of action is also valid for the multiple functions that UFO plays in various plant species. Undoubtedly, many other properties of UFO are yet to be discovered, notably its role within an E3 ligase complex.

Deciphering the evolution of the UFO-LFY complex is another major question for the years to come. Available genetic and biochemical data suggest a conserved mode of action for the UFO-LFY complex, but the nature and the importance of its role is variable from one species to another. Hence, it appears that evolution has diversified the functions of this complex while preserving the actors involved. The characterization of the molecular function of the UFO-LFY complex in Arabidopsis offers the opportunity to better understand its role during the evolutionary history of floral

development. In the future, describing different UFO-LFY complexes from several species (notably their structure, the motif they bind and the genes they regulate) should unveil key data about the evolution of gene regulatory networks controlling flower development in angiosperms and maybe also meristem functions in nonflowering land plants.

Acknowledgements

We thank Chloe Zubieta for commenting on a draft version of this article. This work was supported by the CEA (PhD fellowship to PR), by the University Grenoble Alpes (PhD fellowship to MA-C), by the ANR-17-CE20-0014-01 Ubiflor project to FP and the GRAL Labex financed within the University Grenoble Alpes graduate school (Ecoles Universitaires de Recherche) CBH-EUR-GS (ANR-17-EURE-0003).

Competing interests

None declared.

Author contributions

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