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REVIEW

The LEAFY Floral Regulators in Angiosperms: Conserved Proteins with Diverse Roles

Edwige Moyroud · Gabrielle Tichtinsky · François Parcy

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Abstract Genetic analyses in model angiosperms have shown that the LEAFY/FLORICAULA transcription factor plays a central role in flower development. In *Arabidopsis*, LEAFY (LFY) triggers the development of floral meristems and controls their patterning through the activation of floral organ identity genes. Several recent reports enlighten the structure and function of this conserved protein but also illustrate the variety of roles it plays in different angiosperms.

Keywords LEAFY · Angiosperms · Flower · Evolution · Architecture

Introduction

Flowering plants add to nature's beauty and supply many of the resources needed for human life. Molecular genetics of model angiosperms identified the *FLORICAULA/LEAFY* gene, thereafter named *LEAFY* (*LFY*), as a central regulator of floral development. The *LFY* gene is found throughout terrestrial plant genomes, including from groups such as mosses, ferns, or gymnosperms, predating the origin of flowering plants. Work in *Arabidopsis* and several other plants identified LFY as a novel type of transcription factor responsible for the regulation of genes controlling floral meristem and floral organs development (Benlloch et al. 2007; Blazquez et al. 2006; Parcy 2005). LFY possesses several intriguing features: (1) its sequence does not resemble any known transcription factor and its origin remains elusive; (2) LFY exhibits two domains with high levels of sequence conservation from mosses to angiosperms; (3) as opposed to most transcription factors, LFY did not form a multigene family and remained at very low copy number in the genome with no obvious signs of subfunctionalization.

Because of its essential function in flower development and its presence in plant genomes before the appearance of flowers, LFY stands at the center of several evolutionary scenarios attempting to explain the origin of angiosperms (Frohlich and Chase 2007; Theissen and Melzer 2007). In recent years, major progress has been made on our knowledge about the target genes of LFY, its structure, its mode of action, its interacting partners, and its roles in different species. In this review, we discuss these advances with a focus on the evolutionary implications of the properties and molecular activity of this peculiar protein in flowering plants.

Genetic Analysis in Model Angiosperms

Early Work in *Arabidopsis* and *Antirrhinum*

Two mutants (*floricaula* in *Antirrhinum majus* and *leafy* in *Arabidopsis thaliana*) provided the first genetic evidence of the involvement of the *FLO*/*LFY* gene in floral meristem identity (Carpenter and Coen 1990; Coen et al. 1990; Schultz and Haughn 1991; Weigel et al. 1992). In the snapdragon *flo* mutant, flowers are replaced by shoots

E. Moyroud · G. Tichtinsky · F. Parcy (✉)
Laboratoire Physiologie Cellulaire Végétale, UMR5168,
Centre National de la Recherche Scientifique, Commissariat à l'énergie atomique, Institut National de la Recherche Agronomique, University Joseph Fourier,
17 rue des Martyrs, bât. C2,
38054 Grenoble Cedex 9, France
e-mail: francois.parcy@cea.fr

(Carpenter and Coen 1990; Coen et al. 1990). In the *Arabidopsis lfy* mutant, the most basal flowers are also converted into shoots, but then, flower/shoot intermediates and abnormal flowers are formed at more apical positions (Huala and Sussex 1992; Schultz and Haughn 1991; Weigel et al. 1992). The cloning of *FLO* and *LFY* genes revealed their homology (Coen et al. 1990; Weigel et al. 1992). From now on, we will use *LFY* as a generic name for the *FLO*/*LFY* genes by simplicity, not underestimating the historical importance of *FLO*. Since *lfy* mutants display abnormal development of floral organs, the expression of the *ABC* floral organ identity genes (Lohmann and Weigel 2002) was analyzed in these backgrounds. *FLO* and *LFY* were found to be required for proper *B* and *C* genes regulation (Hantke et al. 1995; Weigel and Meyerowitz 1993) (Fig. 1). In *Arabidopsis*, *LFY* is also needed for the expression of the *APETALA1* (*AP1*) *A*-class gene in early floral meristems (Fig. 1), but *AP1* can also be activated in a *lfy*-independent manner (Ruiz-Garcia et al. 1997) which is also true for its snapdragon ortholog *SQUAMOSA* (*SQUA*) (Carpenter and Coen 1995). The regulation of *ABC* genes by LFY was further corroborated using various gain-of-function transgenic plants. The overexpression of *LFY* in transgenic *Arabidopsis* is sufficient to induce *AP1* in young leaves and the use of an inducible version of LFY (*35S:LFY-GR*) showed that *AP1* regulation by LFY is direct (Parcy et al. 1998; Wagner et al. 1999). Moreover, the expression of LFY fused to the VP16 activation domain demonstrated the capacity of LFY to regulate the *C* gene *AGAMOUS* (*AG*) and the concomitant overexpression of *LFY* and its *UFO* coregulator described later resulted in a precocious activation of the *B* gene *APETALA3* (*AP3*) (Parcy et al. 1998).

In addition to its role in patterning the floral meristem by the local induction of the *A*, *B*, and *C* genes, the increasing LFY levels in leaves primordia prior to flower formation appear to contribute to the control of the flowering time (Blazquez et al. 1997; Weigel and Nilsson 1995).

Large-Scale Experiments Identified New Potential Target Genes

After the initial identification of a few target genes, large-scale experiments were performed that identified a battery of additional target genes. These experiments either used the LFY-GR inducible version (Wagner et al. 2004; William et al. 2004) or used shifts from noninductive short days to inductive long days in wild-type and mutant plants followed by expression analysis at the genomic scale (Maizel et al. 2005; Schmid et al. 2003). Many of these potential target genes still await further detailed analysis while the identity of a few of them guaranteed the functional relevance of their activation by LFY. For example, the *CAULIFLOWER* (*CAL*) gene, known to share meristem identity function with *AP1*, was shown to be directly activated by LFY. *CAL* is also regulated by another recently identified LFY target, *LATE MERISTEM IDENTITY1* (Saddic et al. 2006; William et al. 2004), revealing a regulatory mechanism by which LFY induces some primary targets that subsequently reinforce its role as a gene expression regulator (Fig. 1). Another example of functionally coherent targets for LFY are the *SEPALLATA* (*SEP*) genes (Robles and Pelaz 2005), which are responsible for the E function (Schmid et al. 2003; William et al. 2004). Experiments using the inducible LFY-GR fusion coupled to a translational inhibitor strongly suggested that LFY might directly regulate *SEP2* and *SEP3* (William et al. 2004) (Fig. 1).

Genes Downregulated by LFY

LFY not only activates gene expression but also represses genes controlling the identity of the vegetative or inflorescence meristem (Fig. 1), such as *EMF1* (Chen et al. 1997; Chou et al. 2001) and *TFL1* (Liljegren et al. 1999; Parcy et al. 2002; Ratcliffe et al. 1998) or *AGL24* (Yu et al. 2004; Yu et al. 2002). Genetic analyses showed that LFY is linked

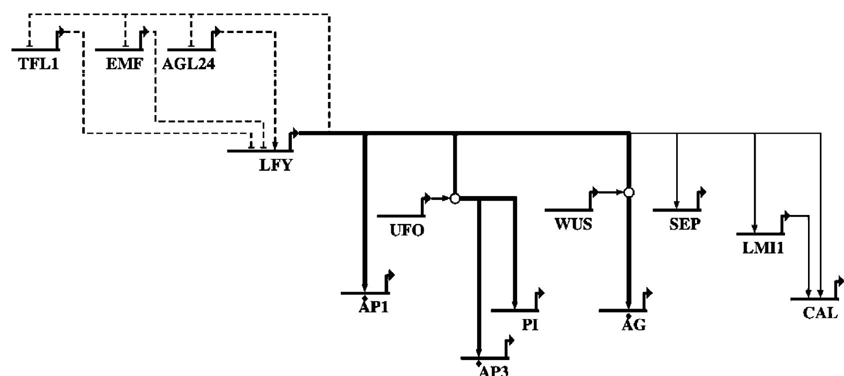

Fig. 1 *LFY* gene regulatory network in *Arabidopsis*. Positive regulations are indicated with *arrows* whereas *bars* indicate negative ones. *Diamonds* indicate the presence of LFY binding sites. Synergistic interactions between gene products are indicated with *bubbles*. For clarity, only the main regulatory relationships, mentioned in the text, are shown. This figure has been generated using the Biotapestry program (Longabaugh et al. 2009)

with these genes through mutual negative feedback loops, a general mechanism shown to be important for switches between different developmental fates. The precise nature of these regulations (direct or indirect) remains to be established.

Mechanisms of Target Gene Regulation by LFY

Identification of DNA Binding Sites for *Arabidopsis* LFY

The FLO and LFY protein sequences did not immediately reveal their function because these proteins did not show any similarity to other known regulators. In 1998, the LFY protein from *Arabidopsis* was demonstrated to be nuclear and to bind DNA elements present in the *AP1* promoter both in vitro and by yeast one-hybrid assays (Parcy et al. 1998). Later, additional binding sites were also identified in the *AG* regulatory intron and the *AP3* promoter (Busch et al. 1999; Lamb et al. 2002; Lohmann et al. 2001). These data identified LFY as a novel type of transcription factor. To understand its mode of action, it is important to note that LFY was able to activate transcription in yeast only when fused to a heterologous activation domain (Parcy et al. 1998), suggesting that it may lack an intrinsic capacity for transcriptional activation. It might, however, not be the case for all LFY proteins, as suggested by FLO sequence analysis and by the phenotype of *Arabidopsis* plants expressing the rice *LFY* ortholog, *RFL* (Chujo et al. 2003; Coen et al. 1990).

The alignment of the binding sites present in *AP1* and *AG* regulatory regions identified the pseudopalindromic CCANTGG/T sequence as the consensus recognized by *Arabidopsis* LFY, although the binding sites found in the *AP3* promoter match poorly with this motif (they display only CCNNNG) (Lamb et al. 2002). Thus, the current definition of the LFY binding site has thus little predictive value and more work is needed to establish a position weight matrix capable of accurately predicting the presence of LFY binding sites in a given DNA stretch.

Structural Analysis of LFY DNA Binding Domain

Once the LFY capacity to bind specific DNA sequences was established, the question of the nature and the origin of this novel transcription factor became more acute. The answer was obtained with the crystallographic structure of LFY DNA binding domain (LFY-DBD) in complex with *AP1* or *AG* binding sites (Hames et al. 2008). This structure revealed a novel protein fold, made of seven alpha helices, which is not found in any other protein structures. Within the seven helices, three form a helix-turn-helix (HTH) motif, frequently found in proteins interacting with nucleic acids (Aravind et al. 2005). Interestingly, structural comparisons showed that LFY possesses similarities with DNA binding proteins such as the Tc3 transposase, paired, or homeodomain transcription factors (Hames et al. 2008). Like these proteins, LFY interacts with both DNA grooves: the HTH contacts conserved bases in the major groove, whereas a N-terminal extension with an arginine residue enters into the minor groove. The contacts between LFY and DNA extend farther than anticipated from the consensus *cis*-element.

The DNA binding mode of LFY was also elucidated (Hames et al. 2008). Consistent with the semipalindromic nature of the LFY binding site, LFY-DBD was found to bind DNA as a dimer. However, LFY-DBD appears to be monomeric in the absence of DNA and to dimerize upon DNA binding following a cooperative mode of DNA binding: the binding of the first monomer to DNA favors the binding of the second one. At the atomic level, the cooperativity was explained by the presence of several H bonds between both monomers. This cooperative binding mechanism was proposed to contribute to the sharp induction of flowering.

Further analysis of the LFY protein should determine whether this mechanism is valid for the entire protein and how the presence of the conserved N-terminal domain contributes to LFY functional properties.

Interaction of LFY with Coregulators

As indicated previously, LFY is thought to be a neutral transcription factor, at least in *Arabidopsis*, requiring coactivators to activate the transcription of its target genes in different domains. Two of these coregulators have been identified: WUSCHEL (WUS) in the case of *AG* activation and UNUSUAL FLORAL ORGANS (UFO) for *AP3*. WUSCHEL is a homeodomain transcription factor required for meristem homeostasis (Laux et al. 1996) and expressed in the center of shoot and flower meristems. WUS binding sites have been identified in close proximity to LFY binding sites on *AG* regulatory intron, and yeast assays demonstrated the capacity of these proteins to synergistically activate transcription when coexpressed (Lohmann et al. 2001). However, the complex containing LFY and WUS bound together to DNA has never been observed in vitro, and the recent crystallographic structure suggests that a LFY dimer might not fit together with WUS on *AG* binding sites. It is, therefore, not clear whether LFY and WUS could form a heterodimer or whether they need to bind alternatively to recruit complementary members of the transcription machinery. Moreover, the expression domain of *WUS* overlaps only partially with that of *AG*, suggesting either that WUS capacity to act at a distance widely extends its action domain or that other proteins (such as members of

the WOX family) (Breuninger et al. 2008) might also contribute to *AG* regulation together with LFY.

In the case of *AP3* activation, the UFO protein was shown to be required as LFY coregulator. UFO is not a transcription factor but an F-BOX protein involved in protein ubiquitination through an SCF complex (SKP1, CULLIN, F-Box). Recently, two independent studies in *Arabidopsis* and *Petunia hybrida* demonstrated a direct interaction between UFO and LFY (DOT and ALF in petunia) (Chae et al. 2008; Souer et al. 2008). Although interaction data are not entirely consistent between the two studies (interaction seems to occur through LFY N terminus in petunia and C terminus in *Arabidopsis*), the evidence supporting this interaction is very convincing. The fusion between UFO and the EAR repression domain, triggering a LFY loss-of-function phenotype, nicely demonstrates that UFO is expected to be recruited in LFY regulatory complexes not only at *AP3* promoter (Chae et al. 2008). Based on work on several transcription factors, different models have been proposed to explain how ubiquitination might promote transcription factor activity (Conaway et al. 2002; Kodadek et al. 2006; Lipford and Deshaies 2003). Until now, examples of such regulation in the plant kingdom are rare and it will be interesting to investigate how LFY fits with the models arising from other kingdoms.

Coregulators and precise mechanisms for other LFY-dependent genes such as *SEP* or *TFL1* genes have not been identified. Furthermore, despite the wealth of data regarding *ABC* genes activation, the reason why each gene is induced in a specific spatiotemporal expression domain has not been elucidated. Whether this information lies in the LFY binding site themselves, in their vicinity, or in the chromatin environment is still a matter of investigation.

Evolution of the Protein

Origin

The origin of the ancestral *LFY* gene is unknown. *LFY* is found in all terrestrial plants but has not so far been identified in algae. The DNA binding domains of LFY and the Tc3 transposase show some structural similarity (Hames et al. 2008) suggesting that, as many other transcription factors, LFY might be derived from a transposon (Breitling and Gerber 2000; Feschotte 2008). LFY could have been brought to plants early on by viral or bacterial transfer and would have drifted until acquiring a new essential function. Unfortunately, sequence similarity with Tc3 transposase is too weak to suggest a common origin and confirm this attractive hypothesis. The sequencing of new genomes might help answering the question in the future.

Why not a family

As opposed to most developmental regulators in angiosperms, *LFY* is not part of an extended gene family (e.g., Bharathan et al. 1999; Martinez-Castilla and Alvarez-Buylla 2003). *LFY* homologues have been cloned in more than 200 species and *LFY* is mostly found as a single copy gene. It is clear that *LFY* experienced duplication as any other genes since there are traces of copies being eliminated (Aagaard et al. 2006; Baum et al. 2005; Bomblies and Doebley 2005; Bomblies and Doebley 2006; Southerton et al. 1998). Moreover, several species exhibit two or three *LEAFY*-like genes but the phylogeny studies demonstrate that the paralogs are recent copies (Archambault and Bruneau 2004; Shu et al. 2000; Southerton et al. 1998; Wada et al. 2002; Wang et al. 2008; Yoon and Baum 2004). They either result from polyploidy, as in *Nicotiana tabaccum*, or from smaller-scale duplication events, as the two paralogs did not persist long enough to be inherited by multiple species (Baum et al. 2005; Kelly et al. 1995). There are only two documented cases (Maize and Lamiales) where a second copy seems to have been kept unusually long (Aagaard et al. 2006; Bomblies et al. 2003). The reason why *LFY* copies are not more often maintained is not understood.

It has been suggested that extra copies might be detrimental (Baum et al. 2005; Cronk 2001). For instance, increased *LFY* expression might affect plant architecture and reduce the number of progeny, as *35S:LFY* does in *Arabidopsis* (Weigel and Nilsson 1995). It has also been proposed that hub proteins, which contain several distinct interaction surfaces with coregulators, are less prone to form extended families (Kim et al. 2006). This could apply to LFY, although only two interaction surfaces have thus far been identified (for dimerization and interaction with DNA). Testing this hypothesis will thus require some more experimental evidence.

Evolution of the Sequence

LFY contains two domains of high conservation (Maizel et al. 2005). Recent structural data showed that amino acids from the C terminus with side chains interior to the protein or facing the DNA are extremely conserved whereas there is more variation on the protein surface opposite to DNA (Fig. 2). According to this structural model, there is thus no reason to imagine major changes in the DNA recognition in angiosperms (with the exception of the *Brownea* genus where several amino acids in direct contact with DNA are modified; Hames et al. 2008). This prediction is consistent with the complementation experiments showing that *LFY* from several angiosperms partially complement the *Arabidopsis lfy* mutant phenotype. However, careful experiments

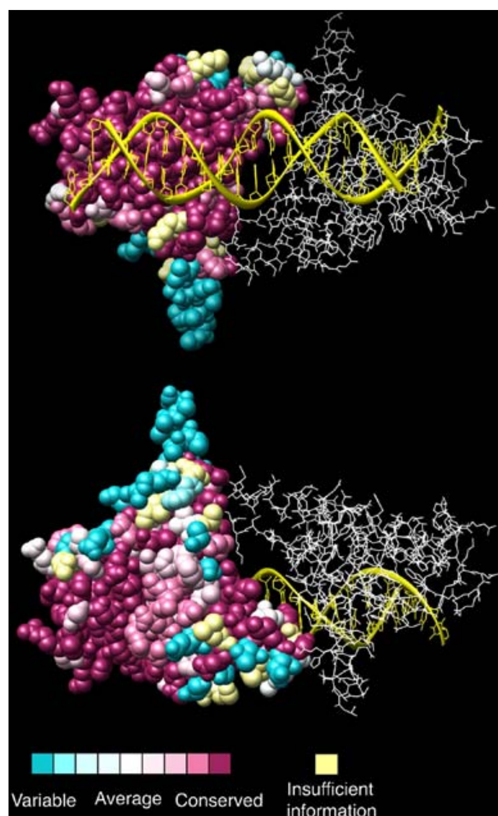

Fig. 2 Conservation of LFY amino acid sequence in angiosperms. Two LFY monomers are shown bound to the DNA (in *yellow*). One monomer is shown in *white sticks* whereas the other one is shown as *spheres* and color-coded according to amino acid sequence conservation using the consurf program (Landau et al. 2005). Conservation color scale is indicated

using microarrays clearly showed that an apparent phenotypic complementation does not guarantee that gene expression is fully restored (Maizel et al. 2005).

Evolution of the Role

The studies in the model plant *Arabidopsis* established that *LFY*'s expression level is critical to trigger flowering and that LFY subsequently controls the development and patterning of newly formed floral meristems. LFY loss-of-function mutants (or transgenic plants) are available for an increasing number of angiosperms species [snapdragon (*A. majus*), *Arabidopsis (A. thaliana*), tomato (*Lycopersicon esculentum*), tobacco (*N. tabaccum*), petunia (*P. hybrida*), *Lotus japonicus*, pea (*Pisum sativum*), *Medicago truncatula*, maize (*Zea mays*), and rice (*Oryza sativa*)]. It thus becomes possible to investigate the evolution of *LFY* function during flowering plants history. As we will elaborate below, with the data available from this reduced number of species, at least one conclusion emerges: in most cases, *LFY* is required for a normal flower development but does not necessarily have the role established in *Arabidopsis* and *Antirrhinum* (Fig. 3).

In tomato, the two *lfy* mutants available [*falsiflora* (*fa*) and *leafy inflorescence* (*lfi*)] flower late and exhibit a complete conversion of flowers to shoots (Kato et al. 2005; Molinero-Rosales et al. 1999). In maize, the plants mutant for the two *LFY* homologs, known as *ZFL* genes, are late flowering and display a loss of floral meristem identity (Bomblies and Doebley 2005; Bomblies and Doebley 2006; Bomblies et al. 2003), demonstrating that these *LFY* functions are relevant outside of the dicots. Both *FA* from tomato and *ZFL* from maize are expressed in all floral primordia and appear to regulate the expression of *ABC* genes: *FA* promotes the induction of *TDR6* (group *B* gene) and *TAG1* (group *C* gene) and the few flowers that develop in maize double mutants are highly modified with sterile carpels or stamens, suggesting that at least B and C functions are altered when ZFL is not active (Bomblies et al. 2003). There is, however, no clear evidence that LFY controls the A function gene in these species.

In petunia, LFY activity is required for normal flower development but LFY does not seem to be the limiting factor in floral initiation. A mutation in *LFY*'s ortholog *ABERRANT LEAF AND FLOWER* (*ALF*) causes a leafy shoot to form instead of flowers. However, *ALF* is already strongly expressed in the organogenetic zone of the shoot apical meristem long before the transition to flowering and overexpressing *ALF* (or *Arabidopsis LFY*) in petunia produces no apparent phenotypic effect (Souer et al. 1998, 2008). The limiting factor in this species has been identified as *DOUBLE TOP* (*DOT*), the homolog of *UFO*: the *DOT* loss-of-function leads to the same phenotype as an *alf* mutation and *DOT* overexpression leads to precocious flowering and transformation of inflorescence to a solitary flower. These results both suggest that *ALF* is not the key factor controlling where and when flowers are produced. It might also be the case in other species such as impatiens (*Impatiens balsamifera*) or tobacco where *LFY* expression is already detected in the vegetative apical meristem and does not increase upon flowering (Kelly et al. 1995; Pouteau et al. 1997). Along the same line, the over-expression of *LFY*'s orthologs in tobacco and poplar does not accelerate flowering in these species as opposed to what is observed in *Arabidopsis* (Ahearn et al. 2001; Rottmann et al. 2000).

In Fabaceae (*P. sativum*, *L. japonicus*, and *Medicago sativa*), *lfy* mutant plants do develop flowers but they are highly modified and display indeterminate growth: sepals and abnormal carpels form, but the region that normally generates stamens and petals initiates new abnormal flowers instead. In *L. japonicus lfy* mutants, *ABC* genes expression is strongly affected but the expression of the *C* gene initiates almost normally in the center of the meristem

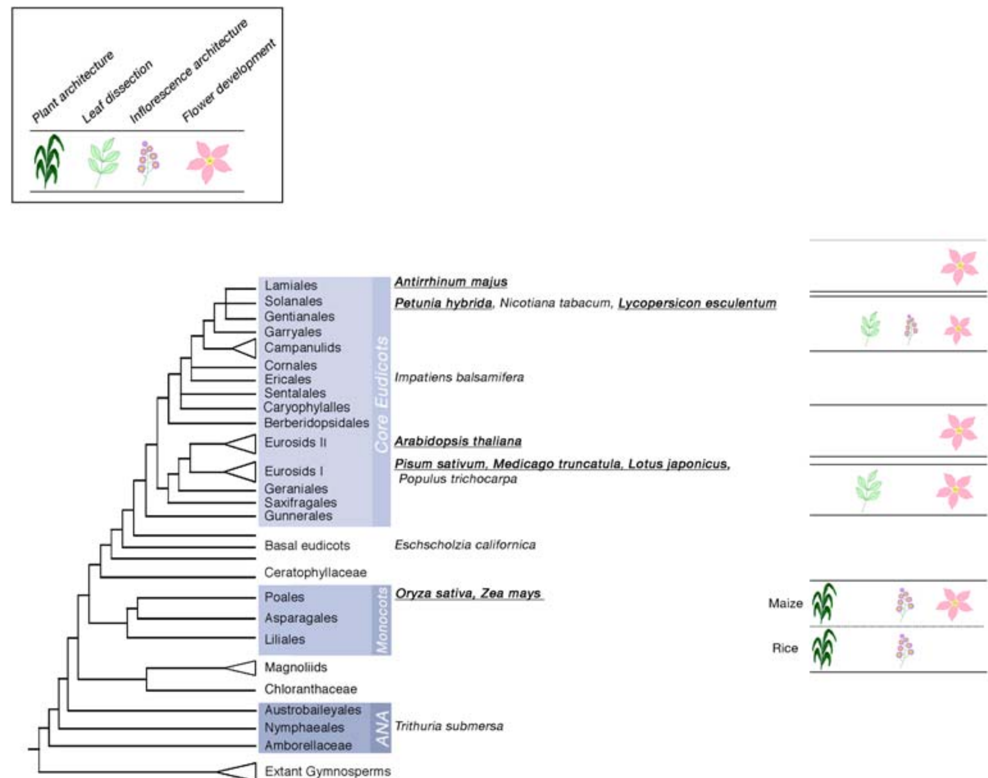

Fig. 3 The variety of roles fulfilled by LFY homologs in angiosperms. The summary phylogenetic tree of major lineages of angiosperms is based on the analyses of Jansen et al. (2007) and Moore et al. (2007). The species named in the text are *listed*, and those where *lfy* mutant or loss-of-function have been described are *underlined*. The processes involving a LFY homolog in these species are indicated on the *right*

indicating a partially *LFY*-independent regulation (Dong et al. 2005). That *C* gene expression could be *LFY*-independent is also suggested by the analysis of several species: in impatiens, tobacco, or the basal eudicot Californian poppy (*Eschscholzia californica*), LFY's expression pattern does not coincide with *C* gene expression and is absent from the center of the floral meristem (Busch and Gleissberg 2003; Kempin et al. 1993; Ordidge et al. 2005). In the absence of *lfy* mutant in these species, a distant action of LFY cannot be excluded (Sessions et al. 2000) but it is also possible that LFY's ability to regulate C activity was acquired long after the appearance of the angiosperms.

A Role in Promoting Indeterminacy

The Fabaceae species are particularly interesting because they show that LFY can also control leaf shape. In wild-type pea plants, for example, the leaves are compound; their limb is divided into leaflet and tendrils, combined together to form a typical dissected leaf. In contrast, mutants in the *UNIFOLIATA (UNI)* gene, the *LFY* homolog, generate simple leaves, with no tendrils and a reduced number of visible leaflets. Similar defects are also observed in two other species of the family, *Lotus* and *Medicago*, but also, to a lesser extent, in tomato, a member of the Solanaceae (Dong et al. 2005; Molinero-Rosales et al. 1999; Wang et al. 2008). The expression pattern studies confirmed that *UNI* is expressed at the margin of the leaves during leaflet formation and it has been suggested that UNI may maintain cells in a transient indeterminate state to facilitate the formation of a compound leaf (Hofer et al. 1997).

Maintaining an indeterminate state is a function often fulfilled by genes of the *KNOTTED* family (Blein et al. 2008) and appears difficult to reconcile with the LFY function in *Arabidopsis* that rather consists in promoting a determinate differentiation of cells on the flanks of the apical meristem. Such a role might actually not be restricted to compound leaf development as indicated by a very interesting recent study in rice (Rao et al. 2008). In this worldwide-cultivated cereal, the *RFL* gene (*LFY* from rice) plays a clear role in flowering time as plants with a compromised *RFL* level show a strong flowering delay. However, once flowering occurs, the architecture of the inflorescence (panicle) of these plants is deeply modified with a reduced number of branches demonstrating that *RFL* plays a role in the generation of outgrowths from the inflorescence meristem and the maintenance of its indeterminacy (Rao et al. 2008). Surprisingly, the few flowers produced in these plants have a normal structure and are fertile, suggesting that LFY does not regulate floral organ identity in rice (even if *RFL* does so when expressed in *Arabidopsis*).

Focusing mostly on the small number of plants where *lfy* mutants or loss-of-function are available already reveals that the highly conserved LFY protein plays a variety of roles in different angiosperms. It is a difficult task,

therefore, to propose an ancestral role for LFY in such a context. A recent study in *Trithuria submersa* (Rudall et al. 2009), a species from one of the earliest extant angiosperm lineages (ANA grade; Fig. 3), clearly demonstrates that the LFY protein is mainly localized in the reproductive organs (stamens and carpels primordia) of this early divergent flowering plant. However, functional studies have not yet been carried out within the ANA grade, and there is an urgent need to develop tools offering us the possibility to unveil the role of *LFY* in significant groups. Indeed, given the high level of conservation, complementation in heterologous systems (such as *Arabidopsis*) is a nice way to test the conservation of biochemical properties, but is not very informative regarding the role of the protein in its own species. An integrative analysis of a few basal and gymnosperm plants will be key to understanding the evolution of LFY's role and may, in turn, shed light on the mysterious origin of flowering plants.

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