Distinct regulatory role for *RFL*, the rice *LFY* homolog, in determining flowering time and plant architecture

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Activity of axillary meristems dictates the architecture of both vegetative and reproductive parts of a plant. In *Arabidopsis thaliana*, a model eudicot species, the transcription factor LFY confers a floral fate to new meristems arising from the periphery of the reproductive shoot apex. Diverse orthologous *LFY* genes regulate vegetative-to-reproductive phase transition when expressed in *Arabidopsis*, a property not shared by *RFL*, the homolog in the agronomically important grass, rice. We have characterized *RFL* by knockdown of its expression and by its ectopic overexpression in transgenic rice. We find that reduction in *RFL* expression causes a dramatic delay in transition to flowering, with the extreme phenotype being no flowering. Conversely, *RFL* overexpression triggers precocious flowering. In these transgenics, the expression levels of known flowering time genes reveal *RFL* as a regulator of *OsSOC1* (*OsMADS50*), an activator of flowering. Aside from facilitating a transition of the main growth axis to an inflorescence meristem, *RFL* expression status affects vegetative axillary meristems and therefore regulates tillering. The unique spatially and temporally regulated *RFL* expression during the development of vegetative axillary bud (tiller) primordia and inflorescence branch primordia is therefore required to produce tillers and panicle branches, respectively. Our data provide mechanistic insights into a unique role for *RFL* in determining the typical rice plant architecture by regulating distinct downstream pathways. These results offer a means to alter rice flowering time and plant architecture by manipulating RFL-mediated pathways.

axillary meristem | inflorescence branching | flowering transition | tillering

Arabidopsis thaliana LFY and its homologs encode an evolutionarily conserved land plant-specific transcription factor. Early studies on the expression pattern and phenotypes of loss-of-function mutations in *LFY* and *FLO*, homologs in two dicots *A. thaliana* and *Antirrhinum majus*, showed them to confer a floral fate to new meristems arising on the flanks of the shoot apex (1, 2). *LFY* homologs from species as diverse as gymnosperms, primitive land plants, and from many angiosperms retain the ability to at least partially complement *Arabidopsis lfy* mutants (3). These data show activation of floral meristem fate to be a conserved LFY function. Protein domains recognizable in all LFY homologs are an N-terminal proline-rich domain and a C-terminal domain; substitutions in these largely conserved DNA-binding domains are suggested to contribute to its potentially divergent functions (3). In fact, mutations in some *LFY* homologs show additional developmental roles (e.g., compound leaf development in pea and cell division in moss) (4, 5).

Unlike the simple inflorescence of *Arabidopsis*, grass inflorescences are striking in the multiple kinds of branch meristems made from the apical inflorescence meristem. In rice upon transition to reproductive phase, the vegetative apical meristem transforms to an inflorescence meristem. The latter terminates after making six to eight primary branch meristems. Primary branches produce two to four secondary branch meristems and terminate in a spikelet. Secondary branches also produce few spikelets. The branched inflorescence thus generated is called a panicle. Panicle-branching patterns in maize and wheat, two other crop plants of the grass family, differ from those in rice (6). Genetic loci that control panicle branching regulate spikelet (grain) number, an important yield trait. To unravel mechanisms regulating panicle architecture, approaches such as genetic analysis of inflorescence mutants, whole-genome microarray analysis, and understanding of gene interactions are required. These studies would enable the exploitation of inflorescence characteristics for improved yield (6).

Several lines of evidence implicate distinct functions for the rice *LFY* homolog, *RFL*. Examples are its inability to complement the phenotypes of *Arabidopsis lfy* mutants (7) and its deviant expression profile as compared with *LFY* or *LFY* orthologs from other grasses (8–11). *LFY* is expressed uniformly in floral meristem, but not in the apical inflorescence meristem (1). In contrast, *RFL* shows high-level and dynamic expression in apical inflorescence (panicle) meristem and is expressed in panicle branch primordia, but its expression is greatly diminished in the floral meristem (8, 10). This pattern also is distinct from maize *ZFL1* and *ZFL2*, which are expressed in branching spikelet meristems and floret meristems, but not the inflorescence apex (11). To unravel regulatory actions of *RFL* and to correlate this with its expression profile, we studied the phenotypic consequences of *RFL* knockdown and overexpression in rice. We coupled these analyses with the effects on global gene expression. Our studies show that *RFL* controls two important traits in rice: flowering time and plant architecture as a whole. These functions are executed by regulating the expression of distinct transcription factors and hormone-dependent-signaling pathways that implicate functions for RFL not predicted from studies of its other homologs.

Results

***RFL* Promotes the Transition of the Vegetative Apical Meristem to an Inflorescence Meristem.** The functional relevance of *RFL* expression in the inflorescence meristem, from its inception and during branching, was investigated by knockdown and overexpression of *RFL*. Twenty-four independent transgenic lines expressing hairpin loop RNAs for *RFL* (Fig. 1*A*) showed a significant delay in flowering and had drastically reduced height (Fig. 1 *B* and *C*). The average time taken for flowering in these tissue culture-regenerated plants was 100 days at height ≈38 cm (Fig. 1*C*). Control wild-type-regenerated plants initiate panicles in ≈60 days at height ≈65 cm (Fig. 1 *B* and *C*). The weakest, yet statistically significant, *RFL* knockdown phenotype occurred in five lines where flowering took place ≈70 days after hardening

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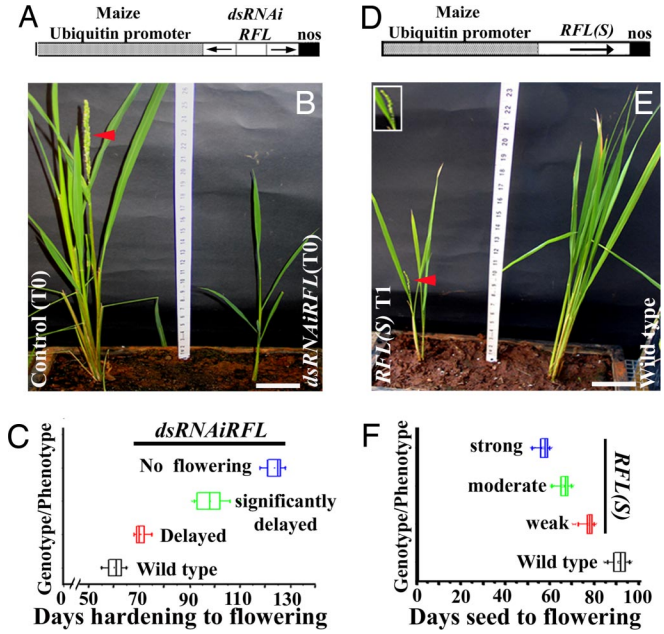

Fig. 1. Phenotypes of *RFL(S)* and *dsRNAiRFL* plants. (*A*) Schematic diagram of *dsRNAiRFL* transgene. The ubiquitin promoter transcribes hairpin loop RNAs for *RFL* exon 1 and exon 2 segments. (*B*) Morphology of a flowering wild-type plant (*Left*, red arrowhead) regenerated through tissue culture and a dwarf nonflowering *dsRNAiRFL* (*Right*) plant of same age. (*C*) Distribution of days to flowering in *dsRNAiRFL* T_0 plants. Flowering time (*x* axis) is plotted against phenotype (*y* axis). The statistical significance is $P < 0.0001$for all phenotypic groups. (*D*) Schematic diagram of *Ubi*::*RFL* transgene. (*E*) A *RFL(S)* plant (*Left*) with early panicle heading (*Inset*, red arrowhead with closeup), compared with a wild type of same age (*Right*). (*F*) Distribution of flowering time in *RFL(S)* T_1 plants showing strong, moderate, and weak phenotypes.

(Fig. 1*C*). Strikingly, six other lines did not produce an inflorescence meristem even after 120 days (Fig. 1*C*) and eventually died without forming a panicle. Together these data show a critical function for *RFL* in promoting transition of the vegetative growth apex to an inflorescence meristem. A 20-fold decrease in endogenous *RFL* transcript levels was achieved in young *dsRNAiRFL* panicles [supporting information (SI) Fig. 6*A*], implicating the severe reduction in *RFL* expression as causal in the extremely delayed flowering. Similarly, we find that knockdown of *RFL* through antisense RNAs, despite not being fully effective for knockdown, still delays flowering by ≈15 days in T_1 plants (data not shown). These results show that *RFL* expression in the panicle meristem is a critical determinant of its fate.

Importantly, we observe a complementary early flowering phenotype on *RFL* overexpression from the *Ubi*::*RFL* transgene (Fig. 1*D*). Flowering time in 35 independent *RFL(S)* T_1 lines was measured as days taken for the formation of young panicles. These plants flowered precociously with compromised vegetative growth (Fig. 1*E*). Ten lines with severe phenotypes made panicles (0.1–0.3 cm) in ≈54 days when plants were only ≈41 cm tall (Fig. 1 *E* and *F*). This contrasts with ≈90 days taken for wild-type plants to attain a similar developmental stage when the plants are ≈70 cm tall (Fig. 1 *E* and *F*). Eleven lines displayed moderate phenotypes; they flowered in 65 days at a height of ≈56 cm (Fig. 1*F*). Even the weakest phenotype (40% of the lines) was early flowering in 78 days (Fig. 1*F*). Ectopic expression of *RFL* in leaves and overexpression in young panicles of *RFL(S)* transgenics was quantitated (SI Fig. 6 *A* and *B*). Thus, we find that *RFL* overexpression triggers precocious flowering.

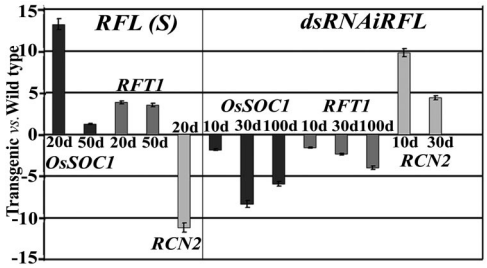

Fig. 2. Expression status of flowering activators and a repressor in *RFL(S)* and *dsRNAiRFL* plants. Quantitative RT-PCR showing fold change, with respect to wild type, in expression for *OsSOC1* and *RFT1* in leaves and *RCN2* in the culm of *RFL(S)* and *dsRNAiRFL* plants of various ages.

Relationship Between RFL and Activators and Repressors of Flowering. We have interrogated whether ubiquitous expression of *RFL* in *Ubi*::*RFL* transgenics promotes a change of the indeterminate apical vegetative meristem to determinate branched reproductive (panicle) meristem by affecting flowering time genes. Expression levels for some rice flowering time regulators were measured in transgenics with deregulated *RFL* expression. Transcript levels for *OsSOC1*/*OsMADS50*, a positive regulator of flowering, were measured in leaves of wild-type plants 20, 50, and 80 days after germination. Very low-level expression seen in 20-day-old leaves increases by day 50, as is known from previous work on *OsSOC1* (SI Fig. 7*A*) (12). In 20-day-old transgenics that ectopically overexpress *RFL*, we find *OsSOC1* transcripts levels are much higher than in wild-type plants of the same age (Fig. 2). This temporally early high-level *OsSOC1* expression achieved in *RFL* overexpression lines with extremely precocious flowering is not transient. Expression is maintained in 50-day-old *RFL(S)* transgenics that are near flowering, wherein transcript levels are marginally higher than in 50-day-old wild-type plants that are still to attain flowering (Fig. 2). Concordant with these results are the complementary effects seen on *RFL* knockdown through RNA interference (RNAi). Leaves of young tissue culture-regenerated *dsRNAiRFL* T0 plantlets, 10 and 30 days, after hardening show markedly reduced *OsSOC1* expression (Fig. 2), compared with control wild-type-regenerated plants of similar ages. We also analyzed the expression levels of *RFT1* encoding a predicted signaling factor closely related to *Hd3a* (13). This was taken up because *Hd3a* is not expressed in the variety used for transformation (SI Fig. 7*B*) as also is the case with other varieties with a reduced photoperiod response (14). *RFT1* is expressed in wild-type leaves, and *RFL* overexpression up-regulates *RFT1* expression, but to a lesser extent than *OsSOC1*. Knockdown of *RFL* reduces *RFT1* transcript levels with the effect persisting in 100-day-old plants, which is well beyond the time taken for flowering in control plants (Fig. 2).

In *Arabidopsis*, the mutually antagonistic relationship between repressors of flowering such as *TFL1* and activators of floral meristem fate (*LFY* and *AP1*) controls phase transition (15). The constitutive overexpression of the rice *TFL1* homologs, *RCN1* or *RCN2*, delays vegetative-to-reproductive phase transition (16), suggesting that *RCN* overexpression may extend the vegetative phase of the apical meristem. But whether this occurs through changes in expression of flowering activators is not known. We measured *RCN2* transcript levels in the vegetative shoot apex of regenerated wild-type plantlets (10 and 30 days after hardening) and compared the levels to those in *RFL* knockdown plantlets of similar age. We find that *RCN2* expression is up-regulated (Fig. 2) in young vegetative apices of *RFL* knockdown plants showing a reciprocal relationship between *RCN* and *RFL*, a promoter of panicle fate. Consistent with these data, *RCN2* expression is much reduced in shoot apices of young T_1 plants overexpressing

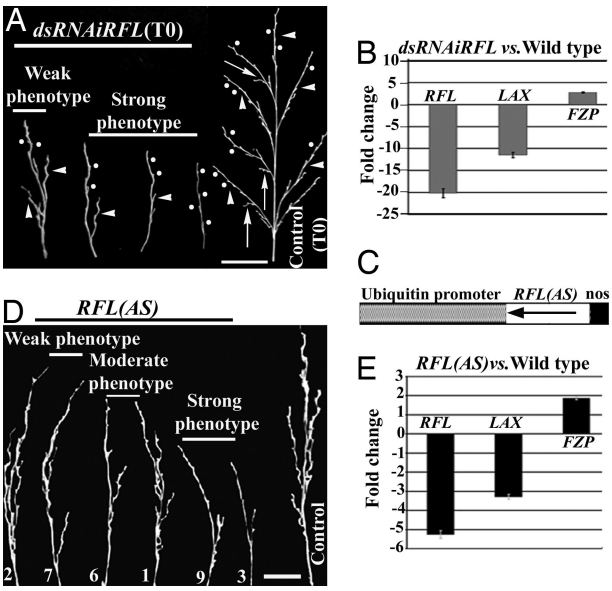

Fig. 3. Panicle growth and branching in *RFL* knockdown plants. (*A*) Mature deseeded wild-type and *dsRNAiRFL* panicles displayed for rachis length and branching. The primary branches (arrowheads), secondary branches (arrows), and spikelet pedicels (solid dots) are marked at representative positions. (*B*) Fold change in expression of branching regulators in *dsRNAiRFL* panicles compared with wild-type panicles determined by quantitative RT-PCR. (*C*) Schematic diagram of *Ubi*::*RFL(AS)* transgene. (*D*) Progressive reduction in the panicle branching and no secondary branches in these plants (line numbers at the bottom of each panicle). (*E*) Normalized fold change in the expression of branching regulators in *RFL(AS)* panicles. (Scale bars: 1.0 cm.)

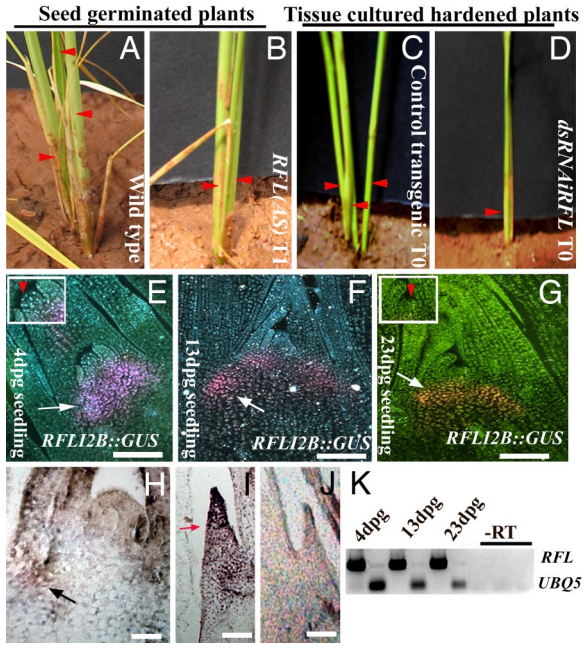

Fig. 4. Tiller development in *RFL* knockdown plants. (*A*) Basal portion of a wild-type plant with tillers (red arrowheads). (*B*) *RFL(AS)* plant with few tillers. (*C*) Basal part of the tissue culture regenerated wild-type plant. (*D*) *dsRNAiRFL* plant with no side tillers. (*E–G*) Histochemical distribution of GUS activity in vegetative axillary meristems. Pink-orange fluorescence at sites of axillary/tiller bud initials shows reporter activity. Basal nodes (*F* and *G*, arrow) and internodes (*E*, arrow) of transgenics culms with *RFL* promoter::*GUS* fusions. (*E* and *G Insets*) Shoot apical meristem. (*H–I*) *RFL* mRNA localization in wild-type 23-day-old culms. (*H* and *I*) RNA expression at leaf axils (*H*, arrow) and in a young tiller bud (*I*, arrow). (*J*) Culm with a tiller bud probed with sense RNA. (Scale bars: *E–H*, 50 μm; *I* and *J*, 20 μm.) (*K*) Semiquantitative RT-PCR of *RFL* transcripts in 4-, 13-, and 23-day-old culms (*Upper*) and control *UBQ5* transcripts (*Lower*).

RFL (Fig. 2). These data indicate that the antagonistic interaction between RFL and this flowering repressor is conserved with regard to transition from the vegetative to the reproductive phase.

Functions for RFL in Panicle Development. Transgenic plants with near-complete knockdown of *RFL* expression in the panicle were extremely delayed for flowering or did not flower at all. Further, panicles when produced were severely compromised for growth and branching (Fig. 3*A*). Less than two primary branches were made, if at all, instead of the six to eight branches in wild-type panicles. In these severely affected *dsRNAiRFL* panicles, the stunted main rachis or the stunted primary branches bear a few spikelets (Fig. 3*A* and SI Table 1). Further secondary branches are not produced in any panicles severely knocked down for *RFL*.

The effects on panicle architecture also were analyzed in transgenic lines expressing *RFL* antisense RNAs (Fig. 3 *C* and *D*) because we could examine the effects of varying degrees of *RFL* knockdown. Twenty-two independent transgenics have been characterized over three generations (T_0, T_1, and T_2). Based on the degree of branching defects, these lines can be classified as strong, moderate, and weak (Fig. 3*D*). The graded effects on branching correlate with the level of antisense RNAs expressed in these plants (data not shown); progressively stronger phenotypes occur with increased antisense RNAs. Panicles in plants with strong and moderate phenotypes have no secondary branches (Fig. 3*D* and SI Table 2), but primary branches are made with a few fertile spikelets. The extent of endogenous *RFL* down-regulation was measured in T2-generation plants by quantitative RT-PCR where a marked 5-fold down-regulation was seen (Fig. 3*E*) in lines with the strongest phenotypes. The down-regulation is lesser than that attained through RNAi (Fig. 3 *B* vs. *E*). Importantly, the most severe panicle-branching phenotypes observed in the antisense *RFL* transgenic lines were nearly identical to the moderate branching defects of some *RFL* knockdown lines. These data confirm that the expression of endogenous *RFL* transcripts in the incipient branch primordia is a prerequisite for their formation. Surprisingly, we also observed poor panicle branching in transgenic lines overexpressing *RFL*, the indications of which are in *Discussion* section 2.

Effects of *RFL* Knockdown on Regulators of Panicle Branching. Mutations in the bHLH transcription factor *LAX* abrogate secondary branch formation without affecting the establishment and growth of the main panicle axis (17). Conversely, mutations in the transcription factor *FZP* promote the formation of supernumerary axillary meristems, causing excessive panicle branches without any spikelet meristems (18). During panicle development, the expression of *RFL* precedes that of either *LAX* or *FZP*, which are spatially restricted, to distinct but small sets of cells. Therefore, we examined the relationship between *RFL* expression levels and these genetic regulators of panicle branching. Quantitative RT-PCR was used to measure *LAX* and *FZP* transcripts in RNA from both *dsRNAiRFL* and *RFL(AS)* transgenics (Fig. 3 *B* and *E*). A clear and reproducible down-regulation of *LAX* and an up-regulation of *FZP* occur in transgenics with strong panicle-branching phenotypes.

Down-Regulation of *RFL* Affects Vegetative Axillary Meristems. In rice, axillary meristems normally develop from the basal nodes of the plant to form tillers that generate the typical bushy plant architecture (Fig. 4*A*). The complete knockdown of *RFL* abol-

ishes tiller development (Fig. 4 *C* vs. *D*), and tiller numbers are reduced on partial knockdown (Fig. 4 *A* vs. *B*). Closer inspection of the dissected culm in *dsRNAiRFL* plants show that, despite the normal number of nodes, tiller outgrowth is severely compromised. In contrast to the five to six buds seen in wild-type plants, the *dsRNAiRFL* transgenics of similar age either do not initiate tiller buds or, in some instances, generate one to two buds that fail to grow further (SI Fig. 8 *D–F*). Expression of *RFL* in tiller bud was not investigated in previous studies (8, 10). To ascertain any role for *RFL* in vegetative axillary meristem development, we have reexamined *RFL* expression at sites of tiller bud formation and in developing tiller buds using *RFL* promoter::*GUS* transcriptional fusions (10) and RNA *in situ* hybridization. The reporter constructs chosen (*RFLI2B*::*GUS* and its derivatives) were those that drive the normal spatially and temporally regulated *RFL* expression in the developing panicle. We note GUS expression at sites of new tiller primordia formation in young plants of various ages (Fig. 4 *E–G* and SI Fig. 8 *A* and *B*). The shoot apical meristem (SAM) in these plants does not express *RFL* (Fig. 4 *E–G* and *Insets*) as previously shown for SAM of yet older plants (8, 10). In addition to expression in incipient tiller primordia, we observe robust reporter expression at basal unelongated nodes and internodes of very young plants (Fig. 4*E* and SI Fig. 8*A*). RNA *in situ* hybridization confirms the presence of *RFL* transcripts in the axils of leaves that are sites for future tiller primordia (Fig. 4*H*) and in very young tiller primordia (Fig. 4*I*). Although the promoter::GUS fusions recapitulate some aspects of the *RFL* RNA expression patterns, it is perhaps insufficient to confer the entire profile in vegetative tissues. Further, semiquantitative RT-PCR also confirms *RFL* expression in the main culm enclosing the SAM of 4-, 13-, and 20-day-old plants (Fig. 4*J*). Together these expression analyses account for the phenotypes of poor or no tiller development upon *RFL* knockdown.

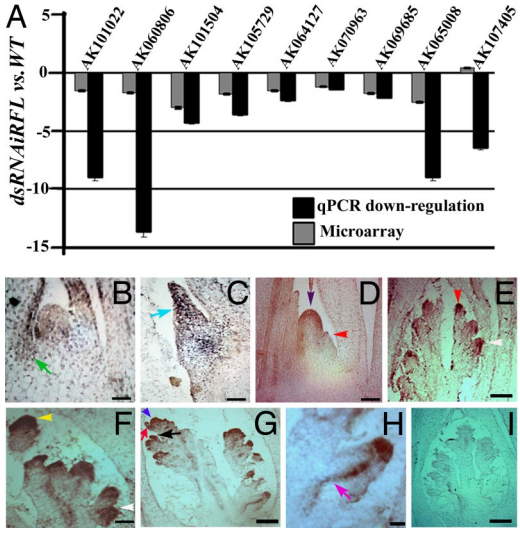

Fig. 5. Genome-wide expression analysis of genes regulated by *RFL*. (*A*) Comparison of fold change in expression levels in wild-type versus dsRNAiRFL panicles for nine representative transcripts chosen from microarray data (SI Table 3). Data from microarrays are compared with that from quantitative RT-PCR analysis. (*B–I*) *In situ* RNA hybridization of an *RFL*-regulated transcript, AK101504 (*PIN3*-like). Transcripts at leaf axils (*B*, green arrow) and in a young tiller bud (*C*, cyan arrow) are indicated. (*D*) Transcripts in the panicle apex (purple arrowhead) and initiating primary branch (red arrowhead). (*E*) Expression at the apical end of a primary branch (red arrowhead) and in emerging secondary branches (white arrowhead). (*F*) Uniform expression in a young spikelet meristem (yellow arrowhead). (*G*) Transcripts in the emerging lemma (black arrow), palea (red arrow), and carpel anlagen (blue arrowhead) of spikelets with differentiating organs. (*H*) Expression in the vascular strands of an emerging primary branch (pink arrow). (*I*) Panicle probed with sense AK101504 RNA. (Scale bars: *B–D*, *F*, and *I*, 20 μm; *E* and *G*, 50 μm; *H*, 10 μm.)

Global Expression Profiling Shows *RFL* as a Master Regulatory Transcription Factor. Functions for *RFL* as a regulator of meristem fate particularly during formation of the branched inflorescence and spikelet was explored through global gene expression profile analysis. RNA pools from young panicles that were wild type or knocked down for *RFL* were compared in rice Agilent 22,000 arrays. These competitive hybridizations were performed with two independent RNA pools from *dsRNAiRFL* and *RFL(AS)* transgenics and matched wild-type panicles. Briefly, 522 genes deregulated in both experimental hybridizations and in their reverse-labeling hybridizations were studied further. These genes were manually inspected and categorized based on the occurrence of predicted protein domains to assign them to functional categories (SI Table 3). We note a preponderance of transcription factors (9.4%) and signaling molecules (10.36%) among the transcripts affected on *RFL* knockdown (SI Fig. 9). In addition, genes involved in various aspects of metabolism (37%) are deregulated, significant among them are genes that may contribute to the synthesis or catabolism of plant hormones or metabolites (e.g., cytokinin oxidase, GA oxidase, cytochrome P450s, etc.). Nine representative candidate downstream genes were validated for their down-regulation in panicle RNAs of *RFL* knockdown transgenics (Fig. 5*A*) by quantitative RT-PCR. These data place *RFL* as a regulator of several unique genes encoding transcription factors; ethylene signaling factors (*EIL3*), auxin efflux facilitator (*PIN3*-like), and perhaps even hormone biogenesis/catabolism (Fig. 5*A* and SI Table 3). The latter is suggested from the down-regulation of many cytochrome P450s that may be involved in gibberellin, carotenoid, or brassinosteroid biogenesis and from the down-regulation of an ethylene biosynthesis enzyme (ACC synthase) and an enzyme in cytokinin metabolism (cytokinin oxidase) (Fig. 5*A* and SI Table 3). The data also hint at roles for factors affecting meristem function and emergence of lateral organs (19). One of the 18 rice *AGO*-like genes is down-regulated on *RFL* knockdown. The expression of this gene in the early stages of panicle and spikelet development (20) is consistent with a plausible role for *RFL* in regulating this AGO family member (Fig. 5*A* and SI Table 3). Notably, most of the predicted rice homologs of genes regulated by *LFY* in *Arabidopsis* inflorescence apices or genes regulated by the ectopic expression of LFY::GR in young *Arabidopsis* plants are not found in our dataset of genes affected on *RFL* knockdown (SI Table 4) (3, 21). This finding suggests that the global architecture of RFL regulatory action is different from its *Arabidopsis* counterpart and that RFL executes its functions through distinct pathways.

Discussion

An Effect of *RFL* on Flowering Time Genes Places RFL as a Regulator of Vegetative to Inflorescence Meristem Transition. Unlike other grass *LFY* genes, such as *Lolium LtLFY* and maize *ZFL1* and *ZFL2*, rice *RFL* shows robust expression in the early reproductive shoot (panicle) apex, but not in the vegetative apical meristem (8–11). The drastic effects on flowering time that we see on the deregulation of *RFL* are concordant with a role for the unique expression profile of this gene. These flowering time effects are far more pronounced than the mild flowering delay of the maize *zfl1* and *zfl2* mutants (11). The flowering time phenotypes, seen on perturbations in *RFL* expression, are similar to the precocious flowering triggered by *LFY* overexpression in *Arabidopsis* or other species, such as aspen (22).

Arabidopsis flowering time genes that promote transition of the vegetative apical meristem to an inflorescence meristem act through multiple pathways that are integrated by transcriptional up-regulation of *FT* and *SOC1*. The latter activate floral mer-

istem genes on new lateral primordia (23). The rice *SOC1* ortholog, *OsSOC1*/*OsMADS50*, can accelerate flowering in rice upon overexpression, and its knockdown delays flowering (12). Our data of precocious transcriptional up-regulation of *OsSOC1* upon *RFL* overexpression and of its delayed activation on *RFL* knockdown strongly support a new role for RFL as a regulator of *OsSOC1*. *OsSOC1* is thought to act downstream of or function parallel to other rice flowering time genes, such as *Hd1* (*CO* ortholog) and *OsGI*, to eventually activate expression of the *FT*-like gene *Hd3a* (12). FT is a potent photoperiod-dependent mobile activator of flowering in both *Arabidopsis* and rice (23). In addition to *Hd3a*, nine other *FT*-like rice genes are known (13). *Hd3a* is expressed at very low levels even in inductive conditions in varieties like Taichung65 that are mutant for *Hd1* and *EHD1* and show poor photoperiod response (14). The variety Taipei TP309, used in our studies, does not express *Hd3a* (SI Fig. 7*B*), whereas *RFT1* (an *FT*-like gene) is expressed in these growth conditions. We find that *RFT1* expression is regulated by RFL, but to a lesser extent than *OsSOC1*. These data indicate that changing *RFL* levels alters *OsSOC1* expression whose effects on flowering time may be mediated by other members of the rice FT family. Unlike *LFY*, which functions downstream of *SOC1* (23), our data show that *RFL* acts upstream of *OsSOC1* and *RFT1* to promote flowering in a photoperiod-insensitive variety. Establishing a regulatory and possibly even a feedback relationship between *RFL* and *OsSOC1* awaits the analysis of overexpression of *OsSOC1* in *RFL* knockdown lines and vice versa.

Dynamic *RFL* Expression Profile, Unlike That in Other Species, Regulates Plant Architecture. The diversity of inflorescence branching patterns and vegetative axillary shoot development seen in grasses (6) presents an interesting hypothesis that changes in inflorescence architecture and plant form may arise, at least in part, from changes in expression pattern of conserved regulators. We now demonstrate that *RFL* is expressed at sites of vegetative axillary meristems and in very young tiller buds, which is required for their outgrowth. Expression in axillary buds, or even the initials of axillary meristems, is not known for *LFY* or its other homologs, except pea *UNI* and tobacco *NFL*, which are expressed in developing lateral shoot primordia (4, 24). Interestingly, the expression of *RFL* in leaf axils is similar to that of *STM* (25) and may relate to a role in maintaining a zone of meristematic cells.

RFL expression in the branching panicle is dynamic, with the expression in incipient lateral branch primordia being high but transient (8, 10). The dynamic pattern of *RFL* expression, in the panicle, bears similarity to rice *KN1*-type and *Arabidopsis STM* homeodomain transcription factor genes (25, 26). By comparison of *RFL* overexpression and knockdown phenotypes, we infer that this profile, in the panicle, may first support a meristematic state and act later for the formation of inflorescence branches. These inferences agree with a recent study on the evolution of inflorescence forms (27). They predict that in panicles all lateral meristems are first in a transient vegetative state, where brief *LFY* expression is followed by its repression, thereby resulting in branch meristems. Subsequently, these meristems are fully committed to form flowers by entering a different state. The expression levels of critical regulators such as *LFY* and *TFL* and their mutual interactions determine these two meristem states (27). The phenotypic effects of poor inflorescence branching that occur on changes in *RFL* expression are consistent with their model as argued below. *RFL* knockdown fails to provide the initial high-level expression required to maintain meristems, and the overexpression fails to repress *RFL* needed for the transition to another meristem state. Our study clearly demonstrates how a diverged *RFL* expression pattern in incipient vegetative and reproductive lateral branch primordia, which is unlike other *LFY*-like genes, regulates their initiation and growth, thereby controlling the architecture of entire rice plant.

RFL Regulates Panicle-Branching Regulators. The graded phenotypic effects on panicle branching seen on the gradual reduction in *RFL* expression levels, together with the failure of inflorescence meristem specification and growth on complete *RFL* knockdown, indicate critical functions for *RFL* in determining panicle morphology. Our data are consistent with *RFL* promoting panicle branch primordia by activating positively acting branching regulators such as *LAX*. *LAX* expression is restricted in the inflorescence meristem to boundary cells adjacent to sites of new lateral meristems (17). This profile overlaps with the broader expression of *RFL* in the branching inflorescence. Excessive panicle branching occurs in *fzp* mutants, where supernumerary axillary meristems are formed in axils of bracts in young spikelet meristems (18). Our data of *FZP* overexpression and lack of axillary meristems in *RFL* knockdown panicles agree with the hypothesis that *FZP* represses axillary meristem formation (18). These data attribute an upstream position for *RFL* in the genetic network controlling panicle architecture. Regulators of rice panicle architecture are conserved in maize, where *BRANCHED SILKLESS1* (*BD1*) is the homolog for *FZP* and *BA1* is the homolog of *LAX*. However, their relationship to maize *LFY* genes *ZFL1* and *ZFL2* is unknown (28).

RFL Targets Are Putative Hormone Signaling, Metabolic, and Transcription Factors. The effects of RFL on panicle regulators LAX and FZP implicate a likely mechanism of RFL action during inflorescence branching. Our global microarray analysis of gene expression profiles, in the branching panicle, provides further mechanistic insights into RFL regulatory action. A large proportion of the genes deregulated in the absence of RFL are predicted signaling molecules and transcription factors. In addition, we anticipate that many deregulated genes currently hypothesized to perform metabolic roles may influence the levels of signaling molecules. Our attempt to understand downstream signaling molecules also is motivated by recent studies showing the STM and KNOX homeodomain factors to orchestrate meristem function by simultaneously activating cytokinin and repressing gibberellin biosynthetic pathways in *Arabidopsis* (29). Besides the down-regulation of molecules involved in hormone biogenesis/catabolism, a notable finding is the down-regulation of AK101504, which is a predicted homolog of *Arabidopsis PIN3*, an auxin efflux facilitator. The spatial distribution of AK101504 transcripts overlaps with *RFL* in the very young panicle apex (Fig. 5*D*) (8, 10). In branch meristems, its expression persists in domains that overlap with *RFL* and in adjacent cells that do not (Fig. 5 *E–H*) (8, 10), hinting at signaling-mediated interactions. Strikingly, AK101504 transcripts also are expressed in leaf axils and in young tiller buds (Fig. 5 *B* and *C*), as is *RFL*. This raises the possibility that the AK101504 gene could contribute to some extent to the panicle-branching and axillary meristem defects of *RFL* knockdown plants. Our hypothesis agrees with the critical role played by PIN-dependent auxin transport during axillary meristem initiation in *Arabidopsis* (30). Recently, one of the rice *PIN1*-like genes has been implicated in tiller bud outgrowth and adventitious root initiation (31), but its contributions to inflorescence structure are not known. Furthermore, the maize *BIF2* (co-ortholog of PINOID-like serine/threonine kinase) regulates the initiation of axillary meristem and lateral primordia (32), underscoring the importance of auxin signaling for primordia emergence in grasses. Our data provide starting points for further investigations on RFL mechanism of action. Altogether, we demonstrate functions for RFL as a regulator of plant architecture through its effects on apical and axillary meristems throughout the growth of the rice plant.

Materials and Methods

Transgenic Plants. Transformation of rice calli was carried out as in ref. 10. The construction of pUbi::RFL, pUbi::RFL(AS), and pUbi::dsRNAiRFL plasmids is given in *SI Materials and Methods*.

Flowering Time Measurements. *RFL(S)* and wild-type 8-day-old aseptically grown T_1 seedlings were moved to clay, and the date when panicles (0.1–0.3 cm) were formed was recorded. For *dsRNAiRFL* and control transgenics, tissue-cultured plantlets that regenerated at about the same time were hardened together in soilrite and moved to clay. The time taken from hardening to make panicles (0.1–0.3 cm) was noted.

RT-PCRs. Quantitative RT-PCR analysis of specified transcripts was done as in ref. 33. Panicle RNAs isolated with a plant RNeasy minikit (Qiagen) or RNA from main culm or leaf lamina extracted with TriReagent was used for reverse transcription with SuperScript III or cloned AMV enzymes (Invitrogen). Then 25 to 150 ng of the cDNA was taken for each quantitative PCR with SYBR green kit (Finnzymes) and detected in an ABI prism 7000 system. Transcript levels normalized to *UBQ5*, in three to six PCRs from two biological samples, were used to determine the difference in the cT values between transgenic and wild-type RNAs. This was used to compute mean and standard deviation for fold change in gene expression. Primer sequences are detailed in SI Table 5.

Expression Profiling Using DNA Microarrays. The *RFL(AS)* (0.1–0.3 cm) and *dsRNAiRFL* (0.1–0.5 cm) panicle RNA pools isolated with the RNeasy plant minikit (Qiagen) were compared with two matched pools of wild-type RNAs. For microarray analysis, Agilent Technologies custom rice (22,000) arrays were hybridized with Cy3- and Cy5-labeled cRNAs in dye-swap experiments according to the manufacturer's instructions. The data (GEO database accession no. GSE-10098) were analyzed by using Genespring GX. An average ratio of the mutant to wild type of <0.5 for a given gene was taken as the criterion for its differential expression.

GUS Assays and *in Situ* Hybridizations. Briefly, 4-, 13-, and 23-day-old culms with shoot apices were processed for GUS assays as in ref. 10. Then 10-μm paraffin longitudinal sections were observed in dark field illumination (Axioscop2 microscope; Zeiss). RNA *in situ* hybridization was performed according to ref. 33. Riboprobes nucleotides $+1$ to $+764$ for *RFL* or $+1,777$ to $+1,982$ for AK101504 were prepared from cDNA clones.

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