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NEEDLY, a *Pinus radiata* ortholog of *FLORICAULA/LEAFY* genes, expressed in both reproductive and vegetative meristems

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ABSTRACT **The *LEAFY/FLORICAULA* genes from *Arabidopsis* and *Antirrhinum* are necessary for normal flower development and play a key role in diverse angiosperm species. A homologue of these flower meristem-identity genes, *NEEDLY* (*NLY*), has been identified in *Pinus radiata*. Although the NLY protein shares extensive sequence similarity with its angiosperm counterparts, it is lacking the proline-rich and acidic motifs thought to function as transcriptional activation domains. *NLY* already is expressed during vegetative development at least 5 years before the transition to the reproductive phase. Expression of *NLY* in transgenic *Arabidopsis* promotes floral fate, demonstrating that, despite its sequence divergence, *NLY* encodes a functional ortholog of the *FLORICAULA/LEAFY* genes of angiosperms. Expression of the *LFY*::*NLY* transgene can largely complement the defects in flower development caused by a severe *lfy* allele.**

Molecular and genetic studies have shown that the mechanisms controlling flower development largely are conserved across distantly related angiosperm plants (1). The first step in flower development is the switch from the vegetative phase, during which shoots and leaves are produced, to the reproductive phase, during which flowers are initiated. Once this switch has been made, flower meristem-identity genes promote the initiation of individual flowers. In *Arabidopsis*, these genes include *LEAFY* (*LFY*) (2), *APETALA1* (*AP1*) (3), *CAULIFLOWER* (*CAL*) (4), *APETALA2* (*AP2*) (5), and *UNUSUAL FLORAL ORGANS* (*UFO*) (6). At least two of these genes, *LFY* and *AP1*, not only are required for flower initiation but are also sufficient to induce flowering in lateral shoots when overexpressed in transgenic plants (7, 8).

The expression of *LFY* orthologs has been studied in detail in four angiosperm species. Of these, only expression of the snapdragon gene *FLORICAULA* (*FLO*) is specific to the reproductive phase (9), whereas the others are expressed, to varying degrees, during the vegetative phase as well. *LFY* expression during the vegetative phase is initially low but increases with the age of the plant. Expression levels are highest upon entering the reproductive phase, suggesting that *LFY* levels are critical for the transition to flowering. This point has been confirmed by demonstrating that increasing the copy number of endogenous *LFY* reduces the number of leaves produced before the first flower is formed (10). Both the *Nicotiana NFL* and the pea *PEAFLO* genes are expressed constitutively in emerging leaf primordia during the vegetative phase, as well as in floral organ primordia (11, 12). Although there is no evidence of a function of *LFY* and its ortholog in vegetative development of either *Arabidopsis* or *Nicotiana*, the situation in pea is different. Inactivation of *PEAFLO* in the pea mutant *unifoliata* (*uni*) not only causes a floral phenotype that is similar to that seen in *flo* or *lfy* mutants but also changes the morphology of the compound pea leaves (12).

Similar to many angiosperms, the "flowering" of *P. radiata* starts with the transformation of an indeterminate axillary apex into a determinate reproductive apex, which forms the strobili (cones) (13). A new long shoot terminal bud (LSTB) is formed at the tip of the rapidly elongating shoot during spring. The organogenic sequence of the apical meristem determines the fate of the shoot axis. The axillary apices that emerge on the sides of the apical meristem differentiate either as vegetative dwarf shoot buds (DSBs), reproductive pollen-cone buds (PCBs), or seed-cone buds (SCBs). SCBs become anatomically differentiated with the initiation of bracts (stage 1). Ovuliferous scale primordia are initiated from hypodermal cells on the adaxial base of bracts (stage 2). At stage 3, a fused bract-ovuliferous scale complex becomes displaced from the cone-bud axis. In differentiated PCBs, microsporangial initials appear in the peripheral zone of the axis (stage 1). During stage 2, microsporophyll initiation is complete and developed pollen mother cells are visible.

In contrast to angiosperms, our understanding of the molecular processes governing reproductive development in gymnosperms is very limited. A small family of MADS-box genes that is expressed in unisexual reproductive organs (and that shares similarity with floral organ-identity genes from angiosperms) has been isolated from two gymnosperms, Norway spruce (*Picea abies*) and *Pinus radiata*, and from their evolutionary ancestor, ferns (14–17). Here, we report the isolation and characterization of the first *P. radiata* gene belonging to the meristem-identity family of *FLO/LFY*-like genes. We show that the expression pattern of this gene, *NEEDLY* (*NLY*), is similar to that of these angiosperm genes, and we demonstrate with transgenic plants that it represents a true functional ortholog of the *Arabidopsis LFY* gene.

MATERIALS AND METHODS

***P. radiata* Samples.** Female, male, and vegetative LSTBs were collected from an adult tree (≈20 years of age and 30 m in height) in Victoria, Australia, from early March through June of 1996. Immature SCBs, PCBs, and DSBs were collected, placed on ice, dissected, and frozen in liquid nitrogen or fixed for *in situ* hybridization. Elongated needles also were collected

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Abbreviations: SCB, seed-cone bud; PCB, pollen-cone bud; DSB, dwarf shoot bud; LSTB, long shoot terminal bud; LD, long day; SD, short day.

Data deposition: The sequence reported in this paper has been deposited in the GenBank database (accession no. U76757).

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during this period of time, and tissues were dissected and frozen in liquid nitrogen. Roots and needles also were collected from 1-month-old pine seedlings.

Isolation of RNA. The total RNA from various tissues was isolated according to Chang *et al.* (18). Total RNA (5μg) from different organs were reverse transcribed by using the Ready-To-Go T-primed First Strand Kit (Pharmacia). RT-PCRs were performed by using the set of *NLY*-specific primers, amplifying the 308-bp fragment: 5′-AGCATCCTTTCATTGTCACG-3′ and 5′-CCAACATTTACTCCCCTCTCC-3′. As a control, a 520-bp PCR fragment of the rRNA was amplified by using a set of primers: 5′-AGTATAATCATGTTACTAATTCAT-3′ and 5′-GATCTCATCAGACTGAAGCGAGAA-3′. PCR fragments were run on a 1% agarose gel, blotted onto nylon membranes, and hybridized to ^{32}P-labeled *NLY* and rRNA cDNA clones.

cDNA Library Construction and Screening. A cDNA library was constructed [using a HybriZAP Two-Hybrid cDNA Cloning Kit (Stratagene)] from mRNA extracted from a mixture of SCBs (stages 1 and 2) and PCBs (stage 1). The cDNA library was screened at low stringency with a mixture of *FLO* and *LFY* cDNA clones from *Antirrhinum* and *Arabidopsis*.

***In Situ* Hybridization.** For *in situ* hybridization experiments, a 3′ untranslated sequence of a *NLY* cDNA clone was introduced into the pSPT18 vector (Boehringer Mannheim). Both the antisense and sense probes used in this study were single-stranded DIG-labeled RNA probes, derived from these constructs by using the SP6 and T7 RNA polymerases. The antisense probe for *NLY* was synthesized by using the *Eco*RI restriction enzyme (in the polylinker) and T7 polymerase. The control sense probe was synthesized by using the *Acc*I restriction enzyme and SP6 polymerase. *In situ* hybridization was prepared according to Jack *et al.* (19).

Construction of a Phylogenetic Tree. The complete amino acid sequences of the *FLO*/*LFY*-like genes were aligned by using CLUSTAL. Phylogenetic relationships among these genes were inferred by parsimony analysis (PAUP Ver. 3.1). A Branch and Bound search was used, and bootstrap analysis (100 replicates) was performed to assess the support of each branch.

Plasmids and *Arabidopsis* Transformation. Gene fusion of the CaMV ^{35}S promoter to the coding regions of the *NLY* gene was constructed in the *Agrobacterium* binary vector pBin 19. The *Xba*I–*Kpn*I *NLY* fragment, carrying the coding region of *NLY*, with its endogenous start and stop codons, was generated by PCR by using the set of primers: 5′-cgtctagaATGGATGCAGAGCACTTTCCT-3′ and 5′-cgggtaccCTATTGACACTCTTTGCTTCT-3′. An intermediate pBR-35S::GUS vector was prepared by cloning the *Hin*dIII fragment from pRT99GUS vector into pBR322. The *Xba*I–*Kpn*I PCR *NLY* fragment replaced the GUS gene in the pBR-35S::GUS vector. The *Sph*I fragment from the resultant pBR-35S::*NLY* vector was blunt-ended with DNA polymerase I (Klenow fragment) and cloned into the plant transformation vector Bin 19, which had been digested with *Sma*I (Bin-35S::*NLY*). For the construction of *LFY*::*NLY* gene fusion, the 2.3-kb *Bam*HI fragment, from the pDW132 vector containing the *LFY* promoter (10), replaced the 35S CaMV promoter in Bin-35S::*NLY*.

Arabidopsis plants (Columbia ecotype) transgenic for pBin19 derivatives were generated by using *Agrobacterium tumefaciens*-mediated *in vitro* root transformation, as described by Cardon *et al.* (20). Transformants were selected on medium containing kanamycin at 50 μg/ml. Homozygous (kanamycin-resistant) lines were created by selfing. The segregation ratio of km^R:km^S was used to estimate the number of transformed T-DNA loci. T_2 lines, homozygous for the 35S::*NLY* and *LFY*::*NLY* T-DNA loci, were identified by plating 200–300 T_2 seeds, derived from different T_1 plants on kanamycin plates. For analysis of floral phenotype and flowering time, seeds were kept for 5 days at 4°C on wet filter paper before sowing. Plants were grown in growth chambers at 25°C under illumination with fluorescent lights: long day (LD) conditions (16 h of light/8 h of dark) or short day (SD) conditions (8 h of light/16 h of dark).

Rescue Experiments and Genotyping. LFY::NLY transformants in the Columbia ecotype were crossed to the strong *lfy-26* mutant allele in the Landsberg *erecta* background (21). To genotype F_2 plants at the *LFY* locus, CAPS (Cleared Amplified Polymorphic Sequences) markers that distinguished between Columbia and Landsberg were used (URL: http://www.salk.edu/LABS/pbio-w/caps.html).

RESULTS

Cloning of NLY. The 1,620-bp *NLY* cDNA clone (GenBank accession no. U76757) has a 1,212-bp ORF predicted to encode a 404-amino acid, 46-kDa protein. The predicted NLY protein aligns well with the sequences of other FLO/LFY-like proteins (Fig. 1). Sequence comparison revealed two large conserved regions (c1 and c2) and two shorter regions of lower similarity (variable regions v1 and v2). A proline-rich region near the amino terminus and an acidic central region are found in the variable regions of all angiosperm FLO/LFY-like proteins. Both of these domains are typical for transcriptional activators and may be important for the function of FLO/LFY-like proteins (2, 22). Unlike angiosperm proteins, NLY, as well as PrFLL, a product of another *FLO*/*LFY*-like gene from *P. radiata* (accession number U92008), does not contain either of these domains.

To more closely determine the evolutionary relationship between FLO/LFY-like proteins, a phylogenetic tree was constructed (Fig. 2). This tree showed that the topology of these genes seems to be concordant with the topology of the species phylogeny and suggests that *NLY* is a gymnosperm ortholog of *FLO*/*LFY*-like genes.

Expression of *NLY*. The expression patterns of the *NLY* gene first were analyzed by RT-PCR. Cone sizes selected for this experiment approximately represent three stages of SCB and two stages of PCB development. The 308-bp *NLY* fragment was amplified from the RNA isolated from differentiated SCBs and PCBs (Fig. 3). *NLY* expression also was detected in vegetative organs, such as DSBs and needles, from the adult tree as well as from 1-month-old seedlings. No expression was detected in roots.

In situ hybridization was used to localize *NLY* transcripts during the early stages of LSTB development and within differentiating vegetative SCBs and reproductive PCBs and DSBs (Fig. 4). *NLY* transcript first was detected during early stages of LSTB development, in the first apices emerging on the side of the apical meristem. These axillary apices could later differentiate as DSBs, PCBs, or SCBs. Fig. 4*a* shows a female LSTB with cone buds at different stages of development. *NLY* was expressed uniformly in the early, undifferentiated cone buds located at the top part of the LSTB. In the progressively more developed, although still not differentiated, cone buds located at the base of the LSTB, *NLY* no longer accumulated uniformly but preferentially in the peripheral zones of the cone buds. No expression was observed in the apical meristem region nor in the fertile cataphylls surrounding the axillary apices.

Differentiation of the SCBs usually began in the most basal axillary apices and proceeded acropetally. In the large apex of differentiated SCBs, *NLY* transcripts initially were concentrated in numerous, regularly spaced groups of dividing cells in the peripheral zone (Fig. 4*b*). The hybridization signal extended 8–10 cells deep into the peripheral zone. Continued periclinal division of the cells in these basal pockets led to the formation of bract primordia. The number of cells expressing *NLY* increased in more developed bract primordia and was concentrated in their middle parts and adaxial sides (stage 1, Fig. 4*c*). This *NLY* expression pattern was transient. During

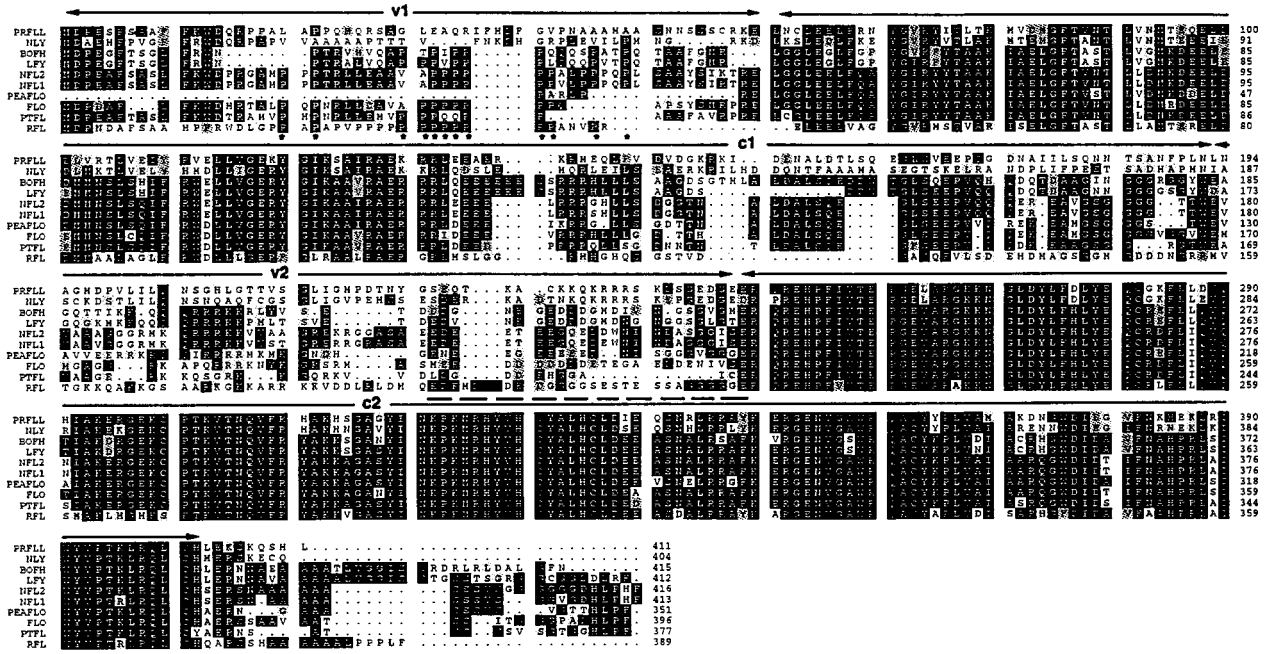

FIG. 1. Sequence comparison of FLO/LFY-like proteins (accession numbers in parentheses): PrFLL from *P. radiata* (U92008); NLY from *P. radiata* (U76757); BOFH from *Brassica oleracea* (718362); LFY from *Arabidopsis thaliana* (M91208); NFL1 and NFL2 from *Nicotiana tabacum* (U16172 and U16174, respectively); PEAFLO from *Pisum sativum* (AF010190); FLO from *Antirrhinum majus* (M55525); PtFL from *Populus balsamifera* (U931 96); and RFL from *Oryza sativa* (AB005620). Black boxes indicate identical amino acids, shaded boxes indicate amino acids with similar properties, and dots indicate gaps introduced to optimize alignment. c1 and c2, conserved regions; v1 and v2, variable regions. Positions of the proline residues within the proline-rich region are indicated by asterisks. Acidic domain indicated by dashed line.

stage 2 of development, the number of *NLY* positive cells within adaxial side bract primordia decreased, with their subsequent concentration in a group of hypodermal cells (6–8 cells deep) at the adaxial side of the bract primordia (Fig. 4*d*). Regulated division of the cells expressing *NLY* led to ovuliferous scale initiation. During stage 3, *NLY* expression was almost uniform within the ovuliferous scale primordia of the fused bract-ovuliferous scale complex, whereas *NLY* levels in bracts dramatically decreased (Fig. 4*e*).

Accumulation of *NLY* in differentiated PCBs is shown in Fig. 4 *f* and *g*. During stage 1, *NLY* was expressed at low levels within microsporophyll primordia in a group of cells that gave rise to sporogenous tissue. A much higher level of expression was detected in developed pollen mother cells during stage 2, when microsporophyll initiation was complete (Fig. 4*g*). Expression of *NLY* in undifferentiated axillary buds that emerged within vegetative LSTB is shown in Fig. 4*h*. In differentiated DSBs, *NLY* transcripts were found in groups of cells within the peripheral zone on the side of the small DSB apex (Fig. 4*i*). Continued periclinal division of these cells indicated the initiation of needle primordia (arrowheads). Uniform and strong accumulation of *NLY* transcripts in two of the three to five needle primordia surrounding the apex is shown in Fig. 4*j*. Hybridization with control, sense probe, in all analyzed sections did not show any detectable signals (not shown).

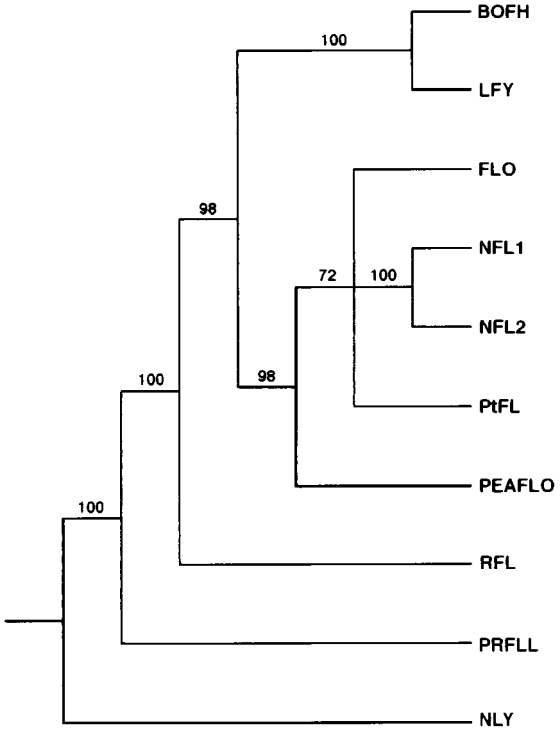

FIG. 2. Phylogenetic tree of the *FLO/LFY* gene family. A single most parsimonious tree was obtained (consistency index = 0.917; retention index = 0.760). Bootstrap values for 100 replicates are shown above each branch.

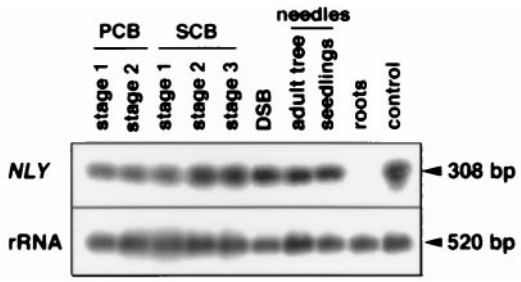

FIG. 3. RT-PCR analyses of *NLY* transcripts levels in PCB (stages 1–2), SCB (stages 1–3), DSB from an adult tree and 1-month-old seedlings and roots. PCR products were blotted onto nylon membranes and hybridized to ^{32}P-labeled *NLY* cDNA clone. The rRNA fragment was amplified from the same RNA samples as a positive control.

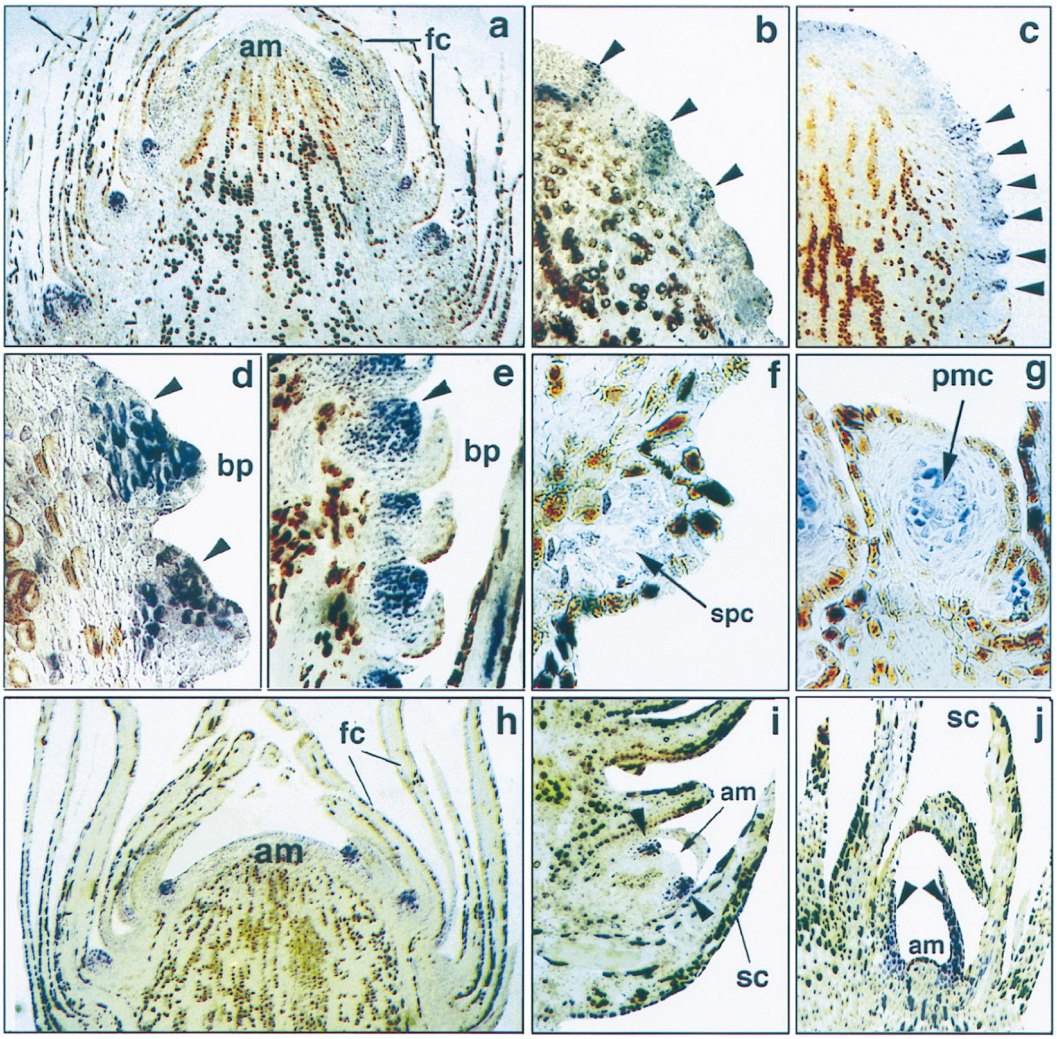

FIG. 4. *In situ* localization of *NLY* transcripts in: (*a*) female LSTB during initiation of axillary apices on the side of apical meristem. (×40.); (*b*) differentiating SCB with initiating bract primordia (arrowheads). (×80.); (*c*) SCB with developed bract primordia (arrowheads) (stage 1). (×50.); (*d*) SCB with initiating ovuliferous scale primordia (arrowheads) (stage 2). (×200.); (*e*) SCB with developed fused bract-ovuliferous scale primordia complex (ovuliferous scale primordia indicated with arrowheads) (stage 3). (×100.); (*f*) PCB with initiating microsporophylls (stage 1). (×200.); (*g*) PCB after completion of microsporophyll initiation (stage 2). (×60.); (*h*) vegetative LSTB during initiation of undifferentiated axillary apices. (×40.); (*i*) DSB with initiating needle primordia (arrowheads). (×90.); (*j*) DSB with developing needle primordia (arrowheads). (×45.) am, apical meristem; bp, bract primordia; fc, fertile cataphylls; pmc, pollen mother cells; sc, sterile cataphylls; spc, sporogenous cells

Constitutive *NLY* Expression Converts *Arabidopsis* Shoot Meristems into Floral Meristems. To determine whether *NLY* was a functional ortholog of the *FLO/LFY*-class of genes, we generated transgenic *Arabidopsis* (Columbia ecotype) in which the *NLY* gene was expressed under the control of the constitutive 35S promoter from cauliflower mosaic virus. In contrast to the wild-type, most of the transgenic 35S::*NLY* lines showed early flowering. In 35S::*NLY-1, -3, -7*, and *-10* plants grown under LD conditions, floral buds were visible after an average of 13–15 days, with 9–10 rosette leaves (40–60 plants were analyzed for each line). The earliest line, 35S::*NLY-7*, flowered with 7.9 ± 0.4 rosette leaves (compared with 12.1 ± 0.3 leaves in wild-type Col plants). The reduction of flowering time was most dramatic when 35S::*NLY* plants were grown under SD conditions. Flowering time was reduced to 4–5 weeks after germination, with a much lower number of rosette leaves (22.8 ± 1.8) than control, wild-type plants (40.8 ± 1.8 rosette leaves).

In addition to early flowering, 35S::*NLY* plants showed the conversion of shoots into flowers. Although the severity and details of the phenotype varied among different lines, the most common 35S::*NLY* phenotype was a conversion of all lateral shoots into solitary flowers (and termination of the primary inflorescence shoot with an apical flower) in plants grown under either LD or SD conditions (Fig. 5 *a-d*). This phenotype was found in 12 35S::*NLY* lines among 36 kanamycin-resistant lines. In contrast, in wild-type *Arabidopsis*, lateral shoots developed in the axils of cauline leaves (bracts) and produced clusters of flowers (Fig. 5 *a* and *e*). In three lines (35S::*NLY-1, -3* and *-10*), development of the primary shoot under LD conditions ceased prematurely with a terminal flower, resulting in a very short inflorescence (middle plant in Fig. 5*a*). One extreme line (35S::*NLY-7*) formed a terminal flower immediately above the rosette (Fig. 5*c*).

Control of Flowering Time by the Level of *NLY* Expression. The gain-of-function experiments presented above show that constitutive *NLY* expression can cause early flowering in transgenic *Arabidopsis* plants. To confirm that this effect indicated a specific function of *NLY* in controlling meristem identity, another set of transgenic plants was created in which *NLY* was expressed in a more restricted fashion. A transgene was constructed in which the *NLY* cDNA was expressed under the control of 2.3 kb of *LFY* 5′ upstream sequences. This promoter fragment confers levels of *LFY* expression that are sufficient to support normal flower development, and its activity mimics that of the endogenous *LFY* gene during the vegetative phase (10).

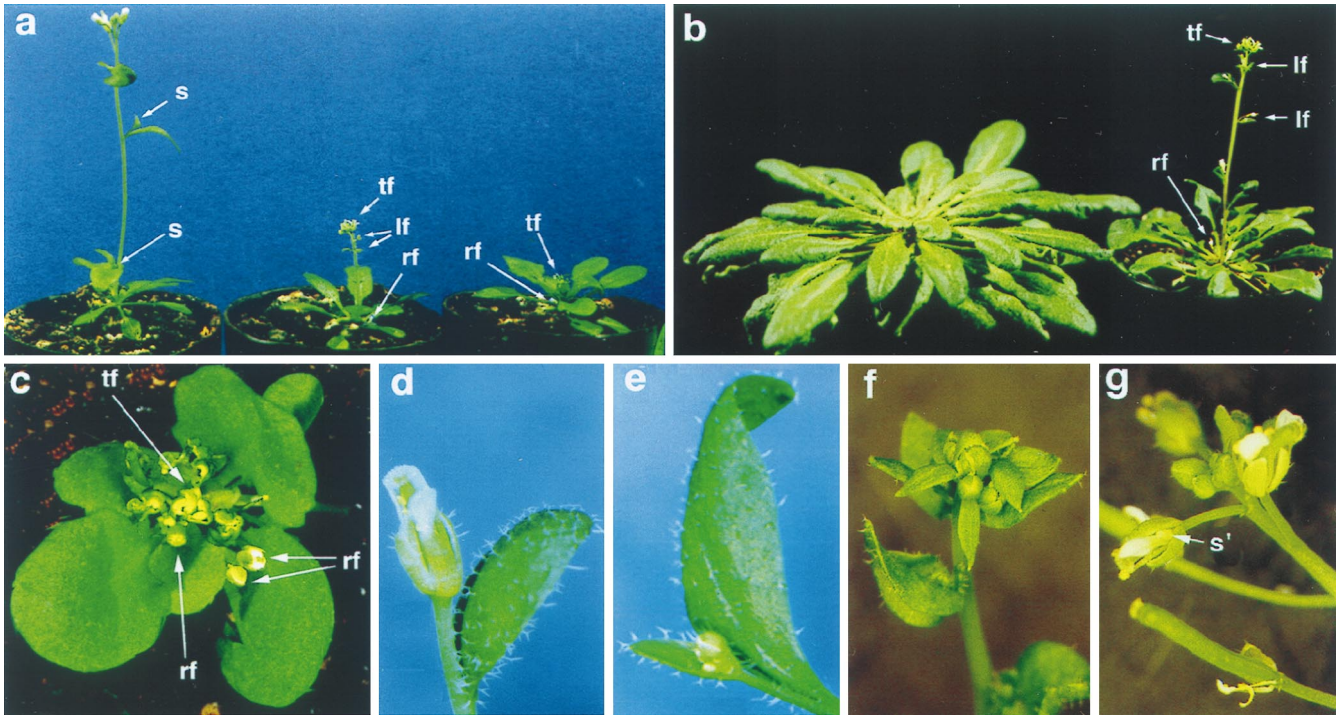

FIG. 5. Phenotypic effects of *NLY* expression in *Arabidopsis*. (*a*) Plants (4 weeks old) grown under LD conditions. (*Left*) Wild-type plant (Columbia ecotype) with developing lateral shoots (s); (*Center*) 35S::*NLY-1* plant; and (*Right*) 35S::*NLY-7* plants with shorter primary shoots terminating with terminal flowers (tf) and solitary flowers arising in the axils of rosette leaves (rf) and bracts (if). (*b*) Plants (9 weeks old) grown under SD conditions. (*Left*) Wild-type plant (Columbia ecotype). (*Right*) 35S::*NLY-1* plant, which has already produced a flowering shoot. (*c*) Top view of extreme phenotype observed in 35S::*NLY-7* line, with terminal flower (tf) immediately above the rosette. Secondary shoots in the axils of rosette leaves have been transformed into solitary flowers (rf). (*d*) Solitary flower in the axil of a bract, replacing a lateral shoot in a 35S::*NLY* plant. (*e*) Lateral shoot of wild-type *Arabidopsis* showing clusters of developing flowers. (*f*) Main shoot of a *lfy-26* mutant; (*g*) Main shoot of a *lfy-26* *LFY*::*NLY-6* plant. s', petaloid stamen with shortened filament.

Among 28 independent transgenic lines, statistically significant differences with wild-type plants were observed in seven *LFY*::*NLY* lines. These lines flowered earlier than wild-type plants under both LD and SD growth conditions. The most severe phenotype was observed in lines *LFY*::*NLY-4* and *-7*. The average numbers of rosette leaves on *LFY*::*NLY-4* and *-7* lines grown under LD conditions were 10.2 ± 0.3 and 10.7 ± 0.4, respectively, compared with 12.6 ± 0.2 in wild-type plants (50 plants were analyzed for each line). Acceleration of flowering was more dramatic under SD conditions, where the average numbers of rosette leaves for *LFY*::*NLY-4* and *-7* lines were 28 ± 0.3 and 25 ± 0.7, respectively.

***LFY*::*NLY* Complements a Strong *lfy* Mutation in *Arabidopsis*.** To determine whether the sequence similarity between *NLY* and *LFY* reflected conservation of function, the transgenic *LFY*::*NLY-4* line was crossed to plants carrying the strong allele *lfy-26*. Phenotypes were analyzed in the F_2 progeny. In *lfy-26* plants, the early arising (basal) flowers are replaced by bracts with secondary inflorescence shoots, whereas later arising flowers were replaced by small bracts, in whose axils abnormal flowers developed (2). Under our conditions, these abnormal flowers contained 8.5 ± 0.6 sepals and 4.9 ± 0.3 carpels (n = 60) but no petals or stamens (Fig. 5*f*). In contrast, wild-type flowers typically contain four sepals, four petals, six stamens, and two carpels. The *lfy*-26 floral phenotype largely was complemented by the *LFY*::*NLY* transgene. The main shoot of these plants developed flowers in both basal and apical positions, and most of these contained all four floral organ types [4.6 ± 0.6 sepals, 2.3 ± 0.2 petals, 4.9 ± 0.4 stamens, and 2.6 ± 0.3 carpels (n = 93); Fig. 5*g*].

DISCUSSION

The analysis of the protein structures showed that NLY, as well as PrFLL, are distinct from their angiosperm homologues. The products of *FLO*/*LFY*-like genes have been proposed to be transcription factors, based on their structure and their nuclear localization (22, 23). Among their characteristics are a proline-rich domain at the amino terminus and an acidic region in the middle part of the proteins. Surprisingly, these domains are missing in NLY and PrFLL. Because the proline-rich and acidic domains are located within the variable regions, they may be subject to evolutionary changes after the separation of the angiosperm and gymnosperm lineages. The common ancestor of FLO/LFY-like proteins might have had (*i*) gymnosperm-type structure, and separation and evolution of the angiosperm lineage were associated with elaboration of their gene structure; (*ii*) angiosperm-type structure, and separation was associated with loss of certain protein domains; or (*iii*) an intermediate structure that was elaborated during angiosperm evolution and simplified in the evolution of gymnosperms.

The radiata pine genome contains two *FLO*/*LFY*-like genes. Surprisingly, the NLY and PrFLL proteins are less closely related to each other (50–55%) than the *FLO* and *LFY* gene products from the distantly related angiosperm species *Arabidopsis* and *Antirrhinum* (70%). However, divergence between the two pine genes apparently has not occurred at an equal rate throughout the coding sequences. Within the conserved c2 domain, NLY and PrFLL share several nonconservative changes, such as H>D, D/E>K, and Y>H, suggesting that they diverged more rapidly from each other within c1, as well as v1 and v2, than angiosperm proteins did after the gymnosperm/angiosperm split.

***NLY* Can Function as a Flower Meristem-Identity Gene in an Angiosperm.** The expression pattern of *NLY* in young emerging primordia, on both vegetative and reproductive apices, is very similar to that of its angiosperm homologues. We have demonstrated that *NLY* is likely to act in a conserved

network of regulatory genes; it functions in transgenic *Arabidopsis* in a very similar manner to the endogenous *LFY* gene.

Strikingly similar to overexpression of *LFY*, overexpression of *NLY* caused the conversion of lateral shoots into solitary flowers, as well as the truncation of the main shoot with a terminal flower. Moreover, similar to *LFY*, the effect of ectopic *NLY* activity differs for primary and secondary shoot meristems. Secondary meristems of transgenic plants produced only a solitary flower, whereas primary meristems produced leaves before they switched to the formation of bractless lateral flowers and finally a terminal flower. The final aspect that *NLY* shares with *LFY* is its ability to induce early flowering, most dramatically under SD conditions. Recently, Blázquez and colleagues (10) demonstrated that *LFY* not only has properties of a flower meristem-identity gene but also of a flowering-time gene. Thus, changing the copy number of wild-type *LFY* affects the number of leaves produced before the first bractless flower was initiated. This fact confirms that transcriptional regulation of *LFY* is an important determinant of flowering time. Similarly, we found that expression of *NLY* under control of the endogenous *LFY* promoter reduces flowering time, without ectopic transformation of shoot into flower meristems. The effect of *NLY* on early flowering, when expressed from a physiologically relevant promoter, confirms that the *in vivo* activity of *NLY* is indeed very similar to that of *LFY*. The observation that the *LFY*::*NLY* transgene can largely complement the defects in flower development caused by a severe *lfy* allele strongly supports the conclusion that *NLY* is a pine ortholog of *LFY*.

Apart from their role in flower development, *FLO*/*LFY*-like genes in angiosperms can have other functions, as demonstrated by the leaf phenotype of *uni* mutants in pea. The latter observation suggests that the more generalized function of angiosperm *FLO*/*LFY* genes is in maintaining a transient phase of indeterminacy before lateral derivatives of an apical meristem are specified. Although this seems less likely in perennials such as pine trees, which have a prolonged vegetative phase of many years, functional studies of *NLY* in pine are needed to assess its function in the development of vegetative and reproductive primordia.

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