Inflorescence Commitment and Architecture in Arabidopsis

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Flowering plants exhibit one of two types of inflorescence architecture: indeterminate, in which the inflorescence grows indefinitely, or determinate, in which a terminal flower is produced. The indeterminate condition is thought to have evolved from the determinate many times, independently. In two mutants in distantly related species, terminal flower 1 in Arabidopsis and centroradialis in Antirrhinum, inflorescences that are normally indeterminate are converted to a determinate architecture. The Antirrhinum gene CENTORADIALIS (CEN) and the Arabidopsis gene TERMINAL FLOWER 1 (TFL1) were shown to be homologous, which suggests that a common mechanism underlies indeterminacy in these plants. However, unlike CEN, TFL1 is also expressed during the vegetative phase, where it delays the commitment to inflorescence development and thus affects the timing of the formation of the inflorescence meristem as well as its identity.

The architecture of inflorescences depends on when and where flowers are generated (1–3). Most species have a vegetative phase of growth whereby the apical meristem generates leaf primordia on its periphery. Secondary meristems arise in the axils of leaf primordia and may lie dormant or grow out to form side shoots. Upon receiving the appropriate environmental and developmental signals, plants switch to the reproductive phase, giving rise to an inflorescence bearing flowers in a set pattern. Two basic types of inflorescence are found among flowering plants: indeterminate and determinate (1, 4). In determinate species, the inflorescence meristem is eventually converted to a floral identity, resulting in the production of a terminal flower. Indeterminate species produce an inflorescence meristem that only generates floral meristems from its periphery.

Comparisons of inflorescence architectures from a large range of species have suggested that the indeterminate pattern was derived from the determinate (5), and therefore a mechanism arose in determinate species to inhibit the production of the terminal flower. Moreover, the wide taxonomic distribution of species with indeterminate inflorescences suggests that this condition arose several times, independently. This raises the question of whether the mechanism for generating an indeterminate inflorescence is the same or different between distantly related species. We addressed this question by exploring the genetic control of inflorescence architecture in Arabidopsis and Antirrhinum. Recessive mutations in the CEN gene of Antirrhinum and the TFL1 gene of Arabidopsis result in the conversion of the normally indeterminate inflorescence to a determinate condition (Fig. 1A) (6–9). Here, we show that CEN and TFL1 are homologs and are expressed in a similar pattern in the inflorescence apex. This finding suggests that a common mechanism for preventing terminal flower formation arose very early in evolution and may have been lost or modified in some species with determinate inflorescences; alternatively, Arabidopsis and Antirrhinum may have independently recruited the same mechanism to promote indeterminate growth. However, the time to flowering is not affected in centroradialis (cen) mutants of Antirrhinum but is significantly reduced in terminal flower 1 (tfl1) mutants of Arabidopsis (10). We show that this additional TFL1 function correlates with its expression during the vegetative phase, during which it delays the commitment of plants to form an inflorescence.

During the vegetative growth phase of wild-type Arabidopsis, primordia arise in a spiral and give rise to leaves separated by short internodes, forming a compact rosette. The induction of flowering by appropriate environmental signals, such as long days (LD), results in the apical meristem acquiring an inflorescence identity and generating floral meristems from its periphery. In addition, the shoot elongates (bolts), bearing two or three leaves with secondary inflorescences (coflorescences) in their axils, above which flowers occur (Fig. 1B).

The tfl1 mutant of Arabidopsis has two key features that distinguish it from the wild type: (i) it bolts early (after producing fewer rosette leaves); and (ii) the inflorescence meristem eventually acquires floral identity, leading to the production of a terminal flower (Fig. 1) (8–11). Up to five floral meristems arise from the periphery of the inflorescence meristem before it acquires floral identity (11, 12). The structure of the terminal flower is often abnormal, displaying altered numbers, arrangements, and identities of organs relative to the wild type (12, 13). All of the above phenotypic effects, except for a marked change in flowering time, are also seen in cen mutants of Antirrhinum (7).

The similar effects of CEN and TFL1 on determinacy raised the possibility that they were homologs. We investigated this possibility by using a CEN cDNA at moderate stringency to probe a genomic library of Arabidopsis DNA, yielding one positive genomic clone, which was sequenced (14). In parallel, database searches with CEN revealed an Arabidopsis expressed sequence tag (EST), 129D7T7, that showed about 76% similarity over a 200–base pair (bp)
region of CEN (15). This clone was fully sequenced and was shown to be identical to four regions (exons) of the genomic clone. The EST predicted a large open reading frame (ORF) that had the potential to encode a protein of 20.2 kD (Fig. 2).

The Arabidopsis EST was mapped to the end of chromosome 5, in the region of the TFL1 locus (16). To determine whether this clone corresponded to TFL1, we sequenced the genomic region from the wild type and from four different tfl1 alleles that arose in the same Columbia background (17). Unique single point mutations were identified in each of the four tfl1 alleles: Gly → Ser in tfl1-1, Gly → Ser in tfl1-11, Glu → Lys in tfl1-13, and Thr → Ile in tfl1-14 (Fig. 2). The chance that four different mutations could have arisen in a locus other than TFL1, in each of the mutant plants, was negligible. These data, therefore, indicated that the CEN-like clone corresponded to the TFL1 gene.

The TFL1 and CEN genes are each composed of four exons that share high similarity throughout their length; the predicted proteins show ~70% identity and 80% similarity (Fig. 2). Database searches revealed two additional plant ESTs, both from rice (OSR29181A and OSS1946A), that predicted peptides similar to the exons of TFL1 and CEN (Fig. 2) (18). The CEN and TFL1 proteins have similarity to animal phosphatidylethanolamine-binding proteins (PBBs), which can associate with membrane protein complexes, but the biological function of these proteins is unclear (Fig. 2) (7, 19). All tfl1 alleles were affected in residues that were conserved between TFL1 and PBBs, even though these residues represented only 25% of the full sequence.

RNA in situ hybridizations were used to determine the pattern of TFL1 expression. Young inflorescences of wild-type Arabidopsis showed strong TFL1 expression in a group of cells lying just below the apical dome of inflorescence and coflorescence meristems (Fig. 3A). To confirm the identity of the region in which TFL1 RNA accumulated, we compared the expression domain of TFL1 with that of LFY, a gene required for floral meristem identity (20). Double labeling showed that although LFY was expressed in floral meristems emerging on the flanks of the apex, TFL1 was confined to a distinct domain below the dome of each inflorescence (Fig. 3B). In addition to its subapical expression, TFL1 RNA was also observed throughout the stem of the inflorescence (21). The expression of TFL1 was similar to that of CEN in Antirrhinum, although CEN RNA appears to be weaker in the stem (7).

Although expression of TFL1 in the inflorescence apex might account for its effect on indeterminate growth, it is less clear how TFL1 affects flowering time. One possibility is that tfl1 mutants are committed to flower at the same time as the wild type, but the initiation of floral development and bolting are accelerated. Alternatively, commitment to flowering may occur earlier in tfl1 mutants. To distinguish between these possibilities, we compared the commitment of tfl1 mutant plants with that of the wild type by transferring plants from conditions that induce flowering [long days (LD)] to noninductive conditions [short days (SD)] at daily intervals so as to reveal the number of LD required for plants to be committed to flower. Under continuous LD, wild-type plants made about eight rosette leaves whereas tfl1 mutant plants made about six; both made about 25 leaves under SD (Fig. 4A). On average, wild-type plants were committed to flower at about 7 LD, after which transfer to SD had little effect. In contrast, tfl1 mutant plants were committed to flower at about 5 LD. This difference of 2 days can account for the difference of two or three leaves, which suggests that the early flowering in tfl1 mutants is the result of an earlier commitment to form floral meristems.

To determine the developmental stage of plants at the time of commitment, we analyzed wild-type and tfl1 mutant plants by scanning electron microscopy (SEM). The first floral meristems appeared on about day 8 for tfl1 mutants, but not until day 9 or 10 for the wild type. Therefore, in both the wild type and the tfl1 mutants, morphological evidence of flowering was not visible until 3 days after the commitment to flower. By day 10, the tfl1 mutants had produced about three floral meristems and expression...
of LFY was detected throughout the apical dome, consistent with the dome having a floral identity (Figs. 3C and 5). One day later, the apex of the tfl1 mutants was more rounded than that of the wild type, and it had sepal primordia on its periphery that were associated with its conversion to a terminal floral meristem (Fig. 5). No more lateral flowers were made once the terminal flower had initiated, and the developmental stage of the terminal flower was similar to that of the oldest lateral flower (Fig. 5). The apical meristem appeared to be recruited at about stage 2 of development, similar to cen mutants in Antirrhinum; this may account for the abnormal morphology of the terminal flower (7–9).

The effect of TFL1 on commitment to flowering under LD suggested that it should be expressed during the vegetative phase, at or before day 5. To test this idea, we probed wild-type plants harvested at each LD time point with TFL1. Expression of TFL1 was detected from day 2 or 3, but it was weak up to the point of commitment in the wild type (day 7), after which the extent of TFL1 expression increased (Fig. 4B). Control sections also revealed that LFY expression was weak in leaf primordia from day 2 or 3 and appeared to increase after commitment, eventually becoming strong in floral meristems.

The roles for TFL1 in commitment and indeterminacy correlate with two patterns of expression: weak expression during early development delays commitment to flowering, whereas increased expression of TFL1 at later stages maintains inflorescence meristem identity. In Antirrhinum, CEN expression appears to be limited to the later inflorescence phase, consistent with CEN controlling only indeterminacy (7). It remains unclear which role of TFL1 is more ancestral; either TFL1 has gained a role during the evolution of Arabidopsis, or CEN has lost a role during the evolution of Antirrhinum. This question may be resolved by analyzing the roles of CEN and TFL1 homologs in other species. Phylogenetic studies have suggested that the determinate condition may have been ancestral and that the indeterminate condition arose several times in many species (5). It is possible that Arabidopsis and Antirrhinum have independently recruited the same genes, or that indeterminacy arose very early in flowering plants and has been lost in some determinate species (3).

**REFERENCES AND NOTES**

10. All tfl1 alleles in the Columbia background show significant reduction in the time to flowering, under both LD (16 or more hours of light per day) and SD (10 or fewer hours of light per day) at ~20° to 25°C (8, 11). A weaker effect was reported for tfl1 alleles in the Landsberg erecta background (9). The flowering time phenotype is semidominant (8, 11).
12. In plants carrying strong tfl1 alleles, one to five lateral flowers are generated below the terminal flower when plants are grown under LD, whereas more than 20 flowers are generated under SD (8, 9). Increasing the growth temperature results in fewer flowers being generated before the terminal flower (9). Plants carrying weak tfl1 alleles can produce more than 60 flowers before the terminal flower, although this number is markedly reduced when...
plants grow at a higher temperature (9).

13. The terminal flowers of tf1 mutant plants often vary in their organ numbers and arrangement relative to wild-type flowers (Fig. 1A). Wild-type flowers are composed of four whorls of organs: four sepals on the outermost, four petals, six stamens, and two central, fused carpels. In tf1-1 mutants, the terminal flower and two flowers generated below may be partially united at the apex. Organ primordia may arise in a mix of whorls and spirals, with some organs apparently fused together. Mosaic organs may occur, with patches of one floral organ type mixed with another. The number of each organ type is often less than in the wild type, though carpels are usually normal.

14. An Arabidopsis genomic clone was obtained by searching a library [G. C. Whitelam et al., Plant Cell 5, 757 (1993)] with the CEN cDNA (7). About 80,000 recombinants were screened at 60°C and washed at 60°C with 0.1 x SSC and 0.5% SDS, as described (22). Of five positives, one yielded a 14-kb Xba I fragment that was subcloned into Bluescript KS+ vector (Stratagene) to give pJAM2043. A 240-bp Eco R1-Xba I fragment of pJAM2043 contained all of the CEN-hybridizing signal and was subcloned as pJAM2044.


16. The Arabidopsis EST was mapped to the top of chromosome 5, above the restriction fragment length polymorphism marker 447 (R. Schmidt, personal communication), in agreement with previous mapping (8, 9).

17. Wild-type Arabidopsis (Columbia) and plants carrying alleles tf1-1, tf1-11, tf1-13, or tf1-14 were grown on soil under LD. Seeds carrying tf1 alleles were obtained from the Arabidopsis Biological Resource Center at Ohio State University. Genomic DNA was isolated from wild-type and mutant plants by means of a miniprep method (R. Simon, personal communication). Leaf tissue was homogenized while frozen, buffered [50 mM EDTA, 0.1 M Tris-HCl (pH 8), and 1% SDS] was added, and the sample was thawed at 85°C for 2 min. DNA was extracted with phenol, phenol/chlorof orm (1:1), and chloroform, and precipitated with isopropanol and sodium acetate. DNA was resuspended in tris-EDTA containing ribonuclease. Oligonucleotide primers were designed to sequences ~160 bp upstream of the ATG and 120 bp downstream of the stop codon. To avoid polymerase chain reaction (PCR) artifacts, we carried out three separate PCRs on each DNA preparation and cloned one PCR product from each into pGEM-T vector (Promega). Each clone of ~1.3 kb was sequenced using the ABI Prism system (Perkin-Elmer), and only base changes present in all three PCR products for any one allele were considered genuine.

18. The rice clone S19461A was obtained from the National Institute of Agrobiological Resource Rice Genome Resource Project (RGP), Ibaraki, Japan, and was isolated from Oryza sativa [GenBank accession number D24998]. A partial sequence of the rice clone R29181A (GenBank accession number D40166) was made by M. Yuzo and S. Takuji (RGP, personal communication). The sequence alignment programs used the GCG package (University of Wisconsin).


23. Sequence alignment programs used the GCG package (University of Wisconsin).

24. Wild-type plants of Arabidopsis thaliana ecotype Columbia were grown under 16 hours light/8 hours dark and harvested just as plants showed signs of bolting. Methods for digoxigenin labeling of RNA probes, tissue preparation, and in situ hybridization were as described (22). Double labeling first involved digoxigenin-labeled antisense TFL1 RNA and purple color detection, followed by fluorescein isothiocyanate-labeled antisense LFY RNA and red color (F. R. Fobert, E. S. Coen, G. J. P. Murphy, J. H. Dooman, EMBO J. 13, 616 (1994)). The TFL1 probe was made with the plasmid pJAM2045. This plasmid contained an internal fragment of ~500 bp of TFL1, generated by PCR and subcloned into pGEM-T vector (Promega). The LFY probe was made from the plasmid pDW122 as described (20).

25. Wild-type and tf1-1 mutant plants were imbibed at 4°C for 5 days in the dark, before sowing on soil under LD (16 hours light/8 hours dark) or SD (8 hours light/16 hours dark). Plants were transferred at daily intervals from LD to SD and scored when plants had bolted. Assigning leaves to the basal, primary rosette was difficult for plants exhibiting the SD phenotype as secondary shoots developed. This variation was reflected in greater standard errors. SEM analysis confirmed the scoring of plants exhibiting a LD phenotype.


27. We thank P. Boivin and D. Barker for help in sequencing the tf1 alleles; D. Weigel for plasmid pDW122; G. Ingram and R. Simon for advice on manipulation of Arabidopsis; R. Schmidt and C. Dean for mapping of the Arabidopsis EST; the Arabidopsis Biological Resource Center at Ohio State University (Columbus, OH) and T. Sasaki et al. of the Rice Genome Resource Project (Ibaraki, Japan) for clones; E. Schultz for helpful discussions on TFL1, and I. Amaya, P. Cubas, and S. Doyle for comments. Supported by grants to E.C. and R.C. from the UK Biotechnology and Biological Sciences Research Council (BBSRC) PM82 and Stem Cell Programmes, the European Economic Community AMICA program, and Gatsby Foundation. D.B. was also supported by a BBSRC Fellowship and the Sainsbury Laboratory.

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Common Neural Substrates for the Addictive Properties of Nicotine and Cocaine

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Regional brain activation was assessed by mapping of Fos-related protein expression in rats trained to self-administration of intravenous nicotine and cocaine. Both drugs produced specific overlapping patterns of activation in the shell and the core of the nucleus accumbens, medial prefrontal cortex, and medial caudate areas, but not in the amygdala. Thus, the reinforcing properties of cocaine and nicotine map on selected structures of the terminal fields of the mesocorticollimbic dopamine system, supporting the idea that common substrates for these addictive drugs exist.

Nicotine is critical in the maintenance of tobacco smoking (1). Recent observations indicate that nicotine, like cocaine, activates the mesocorticollimbic dopamine (DA) system (2). This suggests similarities between the neuroactive properties of cocaine and nicotine but does not show whether the reinforcing properties of these two drugs involve similar neural substrates.

Experiments with animals that voluntarily press a lever to receive cocaine infusions strongly indicate that the mesocorticollimbic DA system is also a key neuroanatomical substrate for drug-seeking behavior itself (3). Because nicotine is intravenously self-administered in rats (4, 5), a study was designed to investigate whether the same set of neurons, a target of the mesocorticollimbic DA system, is activated by self-administration of nicotine and of cocaine. Overlaps in brain activation maps between cocaine and nicotine self-administration might identify a common substrate for cocaine and nicotine addiction.

Neuronal activation of the rat brain can be measured by mapping the expression of the immediate-early gene c-fos (6). Acute injection of cocaine and nicotine is known to produce transient increases of the expression of c-fos protein (Fos) and other Fos-related antigens (FRAs) in the nucleus accumbens and caudate region (7, 8). Newly synthesized Fos and FRAs heterodimerize with members of theJun family to form the activating protein–1 (AP–1) complexes, which are important transcriptional regulators in neurons (6–9). Some FRAs, such as the 35-kD component, do not behave as immediate-early genes but their products, once induced, may last for several days (9).

Here, a computer-based detailed re-