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 *CENTRORADIALIS* (*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)

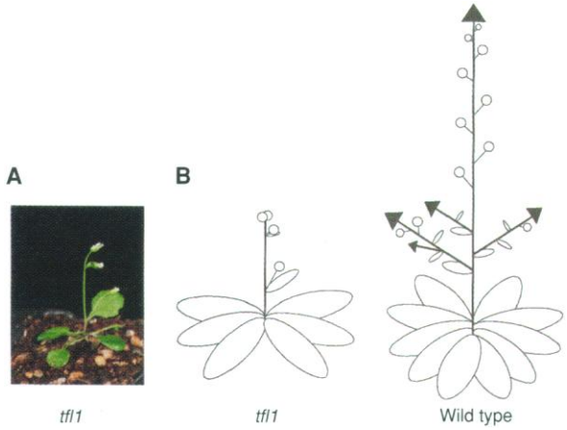

Fig. 1. The *tfl1* mutant of *Arabidopsis*. (**A**) Photograph of the *tfl1-1* mutant, grown under LD. The apical meristem of *tfl1* mutants first produces a basal rosette of leaves before bolting and forming the inflorescence. Bolting occurs earlier (after fewer leaves) in *tfl1* plants than in the wild type, and the inflorescence meristem generates only a few flowers before it is converted to a floral meristem. (**B**) Cartoons of *tfl1* and wild-type plants grown under LD. In the wild type, the inflorescence grows indefinitely, and flowers (circles) are generated from the periphery of indeterminate inflorescence meristems (arrowheads). Secondary inflorescences (coflorescences) arise in the axils of stem leaves. In *tfl1* plants, these secondary inflorescences are often replaced by a single, terminal flower.

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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 → Asp 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 (PBPs), 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 PBPs, 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

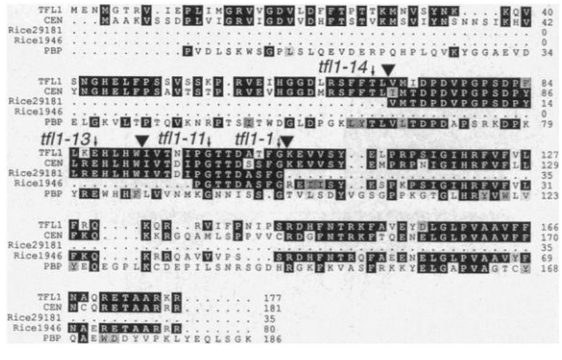

Fig. 2. Sequence comparison of the deduced amino acid sequences for TFL1 (*Arabidopsis*), CEN (*Antirrhinum*), two rice ESTs, and PBP from rats (*7*, *19*, *20*, *23*). The *TFL1* cDNA sequence was obtained from the *Arabidopsis* EST (*15*) and will be deposited in GenBank (accession number U77674). Abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr. The predicted longest ORF is shown, and point mutations detected in the *tfl1* alleles indicated have the predicted changes: G → D in *tfl1-1* (codon: ggc → gac); G → S in *tfl1-11* (codon: ggc → agc); E → K in *tfl1-13* (codon: gaa → aaa); and T → I in *tfl1-14* (codon: act → att). Both rice clones appeared to be derived from unspliced transcripts or genomic DNA; only the putative exons with similarity are shown. The OSS1946A rice clone was fully sequenced but only gave the two predicted 3′ exons shown. Conserved intron positions are marked by filled triangles for TFL1, CEN, and the rice clones. The amino acids altered in the *tfl1* alleles are conserved between all species. Identical and similar amino acid residues are indicated by black and gray backgrounds, respectively.

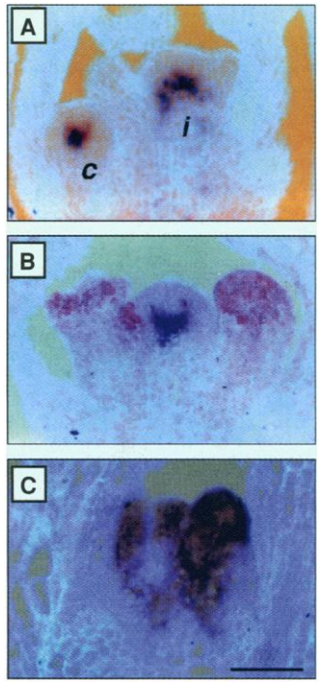

Fig. 3. Expression of *TFL1* and *LFY* in the inflorescence. Longitudinal sections of wild-type *Arabidopsis* were probed with digoxigenin-labeled antisense *TFL1* or *LFY* RNA (*24*). Plants were harvested when the apical meristem had been converted to an inflorescence meristem. RNA signal was detected as a purple or red color on a white tissue background when viewed under a light field. (**A**) Expression of *TFL1* (purple) just below the dome of the inflorescence (*i*). A secondary inflorescence or coflorescence (*c*), subtended by a leaf, arises from the inflorescence. (**B**) Double labeling reveals *TFL1* (purple) in a subapical region and *LFY* (red) in young floral meristems arising from the flanks of the inflorescence. (**C**) Ectopic expression of *LFY* (purple) in the apical dome of a *tfl1* mutant apex after 10 LD. Scale bar, 50 μm.

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*).

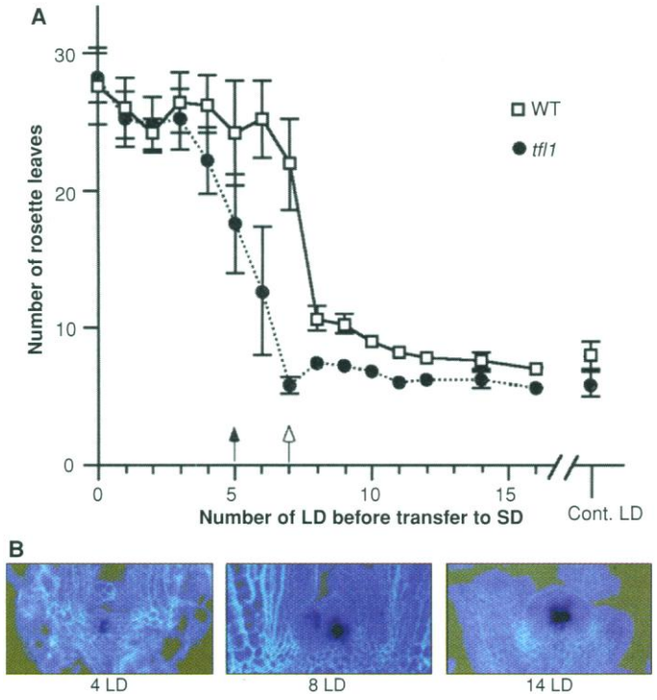

Fig. 4. Time course of *TFL1* expression and the commitment to flower. (**A**) Commitment to flower in wild-type and *tfl1* mutant plants. Plants were grown in LD and transferred to SD at the time points shown, or grown in continuous (cont.) LD or SD as controls (*25*). Numbers of rosette leaves were counted for 10 to 20 plants for each time point. The commitment points (arrows) were defined as the days when ~50% of plants still flowered after the same number of rosette leaves as plants grown in continuous LD. The error bars indicate standard error of the mean with 95% confidence limits. (**B**) Sections of wild-type plants were harvested after 4, 8, or 14 LD and probed for *TFL1* expression (*24, 25*).

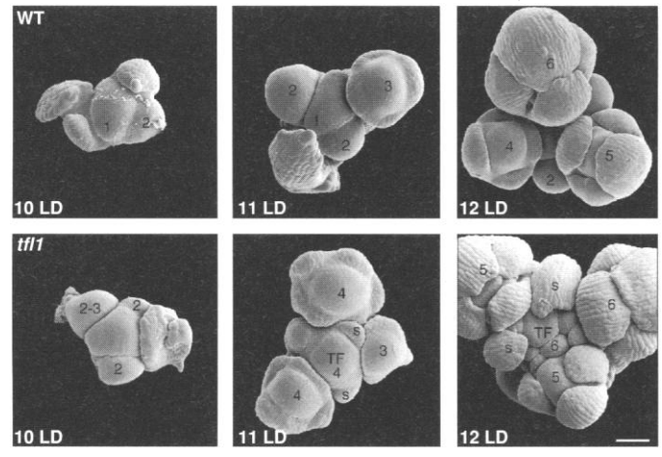

Fig. 5. Inflorescence development in the *tfl1* mutant. Wild-type and *tfl1* mutant plants grown under LD were analyzed by SEM. Plants were harvested after 10, 11, or 12 LD, dissected, and prepared for SEM (*26*). After 11 or 12 LD, *tfl1* mutants had a terminal flower (TF) bordered by sepals (s) and three lateral flowers. Stages of development are indicated for floral meristems. These first appear on the periphery of the inflorescence apex (stage 1) and become separated from the apex by a groove (stage 2); sepal primordia appear (stage 3); the sepal primordia develop (stage 4) and grow over the meristem (stage 5) before they cover the meristem, wherein petal and stamen primordia have initiated (stage 6) (*26*). Scale bar, 50 μm. All of the image below each inflorescence was blacked out using Adobe Photoshop, with the remainder unaltered.

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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*).
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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 *tfl1* mutant plants often vary in their organ numbers and arrangement relative to wild-type flowers (*8, 9, 11*). Wild-type flowers are composed of four whorls of organs: four sepals outermost, four petals, six stamens, and two central, fused carpels. In *tfl-1* mutants, the terminal flower and one or 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 screening a Landsberg *erecta* 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 0.4× 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 2-kb Eco RI–Xba I fragment of pJAM2043 contained all of the *CEN*-hybridizing signal and was subcloned as pJAM2044.
15. Database searches involved BLAST [S. F. Altschul, W. Gish, W. Miller, E. W. Myers, D. J. Lipman, *J. Mol. Biol.* **215**, 403 (1990)] and FASTA [W. R. Pearson and D. J. Lipman, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 2444 (1988)]. The *Arabidopsis* clone 129D7T7 was obtained from the Arabidopsis Biological Resource Center at Ohio State University (Columbus, OH) and was originally isolated from *Arabidopsis thaliana* ecotype Columbia; see T. Newman *et al.* at Michigan State University (East Lansing, MI) (accession number T44654).
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 *tfl1-1, tfl1-11, tfl1-13,* or *tfl1-14* were grown on soil under LD. Seeds carrying *tfl1* 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, buffer [50 mM EDTA, 0.1 M tris-HCl (pH 8), and 1% SDS] was added, and the sample was thawed at 65°C for 2 min. DNA was extracted with phenol, phenol/chloroform (1:1), and chloroform, and precipitated with isopropanol and sodium acetate. After an ethanol wash, 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 D40166). The partial sequence of the rice clone R29181A (GenBank accession number D24998) was made by M. Yuzo and S. Takuji (RGP, Ibaraki, Japan) and was obtained from the databases (*15*).
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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 [see (*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 [P. R. Fobert, E. S. Coen, G. J. P. Murphy, J. H. Doonan, *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 *tfl1-1* 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.
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27. We thank P. Bovill and D. Barker for help in sequencing the *tfl1* 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) PMB2 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.

15 October 1996; accepted 12 November 1996

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 mesocorticolimbic 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 mesocorticolimbic 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 mesocorticolimbic 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 mesocorticolimbic 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 synthetized Fos and FRAs heterodimerize with members of the Jun 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-

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