Table 1. Flowering times of transgenic and mutant plants.

Genotype*	Rosette leaves or leaves‖	SD	Range	*n*
	Experiment 1			
Wild type, LD	10.9	1.9	9–15	11
Wild type, SD	21.0	2.1	18–25	21
35S::FT, LD†‡	2.2	0.4	2–3	20
35S::FT, SD†	2.0	0.2	2–3	30
tfl1-17, LD	8.0	1.1	6–9	5
35S::FT; tfl1-17, LD‡	2.0	0	2	44
35S::TFL1, LD	18.5	2.7	12–25	63
35S::FT/−; 35S::TFL1/−, LD	4.0	0	4	3
	Experiment 2			
Wild type	11.3	1.9	8–15	61
co-1	19.9	2.4	15–25	43
35S::FT§	3.8	0.5	3–5	57
35S::FT; co-1§	3.4	0.6	2–4	70
	Experiment 3			
Wild type (L)	7.6	0.7	7–9	10
fwa-2/fwa-2 (L)	17.6	0.9	16–19	12
FWA+/FWA+; 35S::FT/− (L/C)	4.6	0.5	4–5	8
FWA+/fwa-2; 35S::FT/− (L/C)	10.1	1.0	9–12	7
	Experiment 4			
Wild type (L)	9.8	1.6	8–12	6
ft-3/ft-3 (L)	19.0	1.2	18–21	7
FT+/FT+; 35S::TFL1/− (L/C)	11.0	0.8	10–12	3
FT+/ft-3; 35S::TFL1/− (L/C)	17.7	0.5	17–18	3
ft-3/ft-3; 35S::TFL1/− (L/C)	38.0	3.7	32–42	4
	Experiment 5			
Wild type	10.4	1.0	9–12	20
35S::FT	5.3	0.5	5–6	43
Wild type (L)	10.9	1.3	8–13	29
35S::LFY (L)	7.1	0.8	6–8	7
35S::FT × wild type (L) F_1	5.8	0.7	5–7	6
35S::FT × *35S::LFY* (L) F_1	1.9	0.2	1–2	15

*Genetic background: L, Landsberg *er* (L*er*); L/C, F_1 between L*er* and Columbia (Col); otherwise, Col. Transgenic lines used were YK#11-1 (a strong line, experiments 1 and 5) and YK#1-5C (a weak line, experiments 2 and 3) of *35S::FT*, *35S::TFL1* (KG#9-5) and *35S::LFY* (DW151.2.5L). SD, 8 hours light/16 hours dark cycle; LD, 16 hours light/8 hours dark cycle; otherwise, continuous light conditions. In each experiment, there was a statistically significant difference (Student's *t* test, $P < 0.001$) among genotypes or conditions compared including the three pairs marked †, ‡, and §. ‖The number of rosette leaves (experiments 1 to 4) or leaves (experiment 5) as an indicator of flowering time (*16*).

deletion in the *FT-FAS1* region was identified. The bacterial artificial chromosome (BAC) clone F5I14 (GenBank accession number AC001229) was found to cover the deleted region. One candidate gene (*F5I14.3*) with similarity to *TFL1* (*4*) was examined in six *ft* alleles, including three new ones (*ft-4*, *ft-5*, and *ft-6* from ecotype Nossen) [this study and (*16*)], and a nucleotide substitution was found in all cases. The entire *F5I14.3* was deleted in vTAAT26C51. On the basis of these results, we concluded that *F5I14.3* is the *FT* gene. Sequences of cDNA were deposited in GenBank (accession numbers AB027504 and AB027505).

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5. Other members are: *TWIN SISTER OF FT* (*TSF*, GenBank accession number AB027506), *ARABIDOPSIS THALIANA CENTRORADIALIS* (*ATC*, GenBank accession number AB024715), *BROTHER OF FT AND TFL1* (*BFT* = MTG10.5, GenBank accession number AB016880), and *MOTHER OF FT AND TFL1* (*MFT*, GenBank accession number AF147721). The nomenclature was decided through agreement between D. Weigel's group and ours.
6. The putative orthologs are a *Citrus unshiu* expressed sequence tag clone for *CiFT* (GenBank accession number AB027456), and a rice BAC clone, nbxb0035E07r (GenBank accession number AQ289409), containing a part of *OsFT*.
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26. *35S::CO:GR; co-2* plants were grown on MS medium (3% sucrose, 0.8% agar) under LD (16 hours light/8 hours dark) conditions. On day 14, 5 ml of 10 μM dex solution was applied to the medium (25 ml) to give a final concentration of 1.7 μM.

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Activation Tagging of the Floral Inducer *FT*

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***FLOWERING LOCUS T* (*FT*), which acts in parallel with the meristem-identity gene *LEAFY* (*LFY*) to induce flowering of *Arabidopsis*, was isolated by activation tagging. Like *LFY*, *FT* acts partially downstream of *CONSTANS* (*CO*), which promotes flowering in response to long days. Unlike many other floral regulators, the deduced sequence of the FT protein does not suggest that it directly controls transcription or transcript processing. Instead, it is similar to the sequence of TERMINAL FLOWER 1 (TFL1), an inhibitor of flowering that also shares sequence similarity with membrane-associated mammalian proteins.**

The transition from the vegetative to the flowering phase of *Arabidopsis* is controlled by several genetic pathways that monitor the developmental state of the plant as well as environmental conditions (*1*). Despite the cloning of several *Arabidopsis* genes participating in these

pathways, substantial gaps remain in our knowledge of how the signals controlling flowering are transduced and integrated. To complement other approaches to the study of floral induction, we applied activation tagging to whole plants. Activation tagging is a random overexpression screen that was developed several years ago for isolated plant cells. In this scheme, transcriptional enhancers from the viral 35*S* promoter are randomly inserted in the genome with transferred DNA (T-DNA) of *Agrobacterium* (*2*). Using activation tagging, we identified a mutant, 1733, that flowered early, independently of day length (*3*). In addition, it had terminal flowers. Adjacent to the 1733 T-DNA insertion was an overexpressed gene that, when linked to either the original 35*S* enhancers (Fig. 1A) or the full 35*S* promoter and reintroduced into wild-type plants (*3*), recapitulated the 1733 phenotype (Table 1 and Fig. 2). Because the 1733 insertion mapped close to the flowering-time gene *FT*, we sequenced genomic DNA corresponding to the tagged gene from three

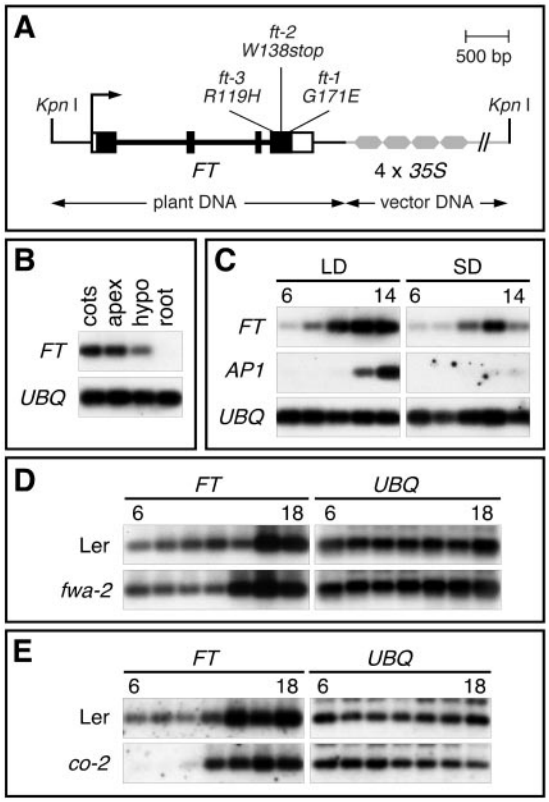

Fig. 1. Structure and expression of *FT*. (**A**) The K2 plasmid rescued from the 1733 mutant and reintroduced into plants. Boxes indicate exons; filled boxes indicate coding sequences. *ft* alleles are shown above. R119H, $Arg^{119} \rightarrow$ His; G171E, $Gly^{171} \rightarrow$ Glu; W138, Trp^{138}. (**B** to **E**) *FT* mRNA accumulation determined by RT-PCR, with *UBIQUITIN10* (*UBQ*) as control (*3*). (B) Ten-day-old, long-day–grown Columbia wild-type plants. cots, cotyledons; apex, shoot apex including young leaf primordia; hypo, hypocotyl. (C) Six- to 14-day-old Columbia plants, in long days (LD; 16 hours of light) or short days (SD; 9 hours of light). *AP1* expression is a marker for flower initiation (*23*). (D) Six- to 18-day-old Landsberg *erecta* (Ler) wild-type plants and *fwa-2* mutants in long days. (E) Six- to 18-day-old Landsberg *erecta* plants and *co-2* mutants in long days.

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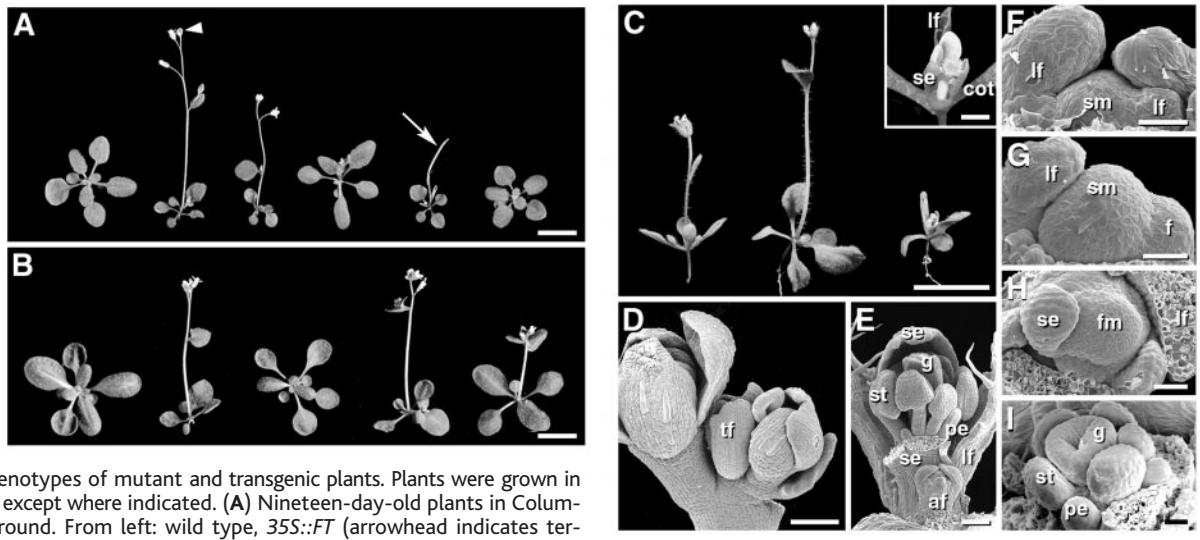

Fig. 2. Phenotypes of mutant and transgenic plants. Plants were grown in long days, except where indicated. (**A**) Nineteen-day-old plants in Columbia background. From left: wild type, *35S::FT* (arrowhead indicates terminal flower), *35S::FT* (short days), *35S::FT 35S::TFL1*, *35S::FT tfl1-1* (arrow indicates a silique formed by the single terminal flower), and *tfl1-1*. (**B**) Nineteen-day-old plants in Landsberg *erecta*. From left: wild type, *35S::FT*, *35S::FT fwa-2*, *35S::FT 35S::CO*, and *35S::CO*. (**C**) Fourteen-day-old plants in Columbia. From left: *35S::FT 35S::AP1*, *35S::FT*, and *35S::FT 35S::LFY*. Inset shows close-up of another 12-day-old *35S::FT 35S::LFY* plant, with a slightly more severe phenotype than the one in the main panel. The first whorl of the terminal flower includes two sepals (se) and two true leaves (lf). cot, cotyledon. (**D** to **I**) Scanning electron micrographs of shoot apices. (D) Twelve-day-old *35S::FT* plant. The shoot apical meristem has been consumed by the formation of a terminal flower (tf). (E) Nine-day-old *35S::FT 35S::LFY* plant. A cotyledon and a sepal have been removed. An abnormal flower (af) has formed in the axil of a cotyledon, which has been removed. st, stamen; pe, petal; g, gynoecium. (F to I) Six-day-old plants. (F) In Columbia wild type, the shoot meristem (sm) is vegetative and produces leaves (lf). (G) In *35S::FT*, the shoot apical meristem is domed and has produced the first lateral flower primordium (f). (H) In *35S::FT tfl1-1*, the apical meristem has been transformed into a floral meristem (fm) that has begun to produce sepals (se). (I) In *35S::FT 35S::LFY*, the apical meristem has been replaced by a flower, in which development of sepal, petal (pe), stamen (st), and gynoecium (g) primordia is advanced. Two leaves and two sepals have been removed. Scale bars, 1 cm in (A) to (C), 100 μm in (D), (E), and the inset in (C), and 20 μm in (F) to (I).

ethylmethane sulfonate (EMS)–induced *ft* alleles (*4*). All three contained mutations in the open reading frame of the tagged gene (Fig. 1A) (*3*), indicating that the 1733 mutant carried a dominant, early flowering allele of *FT*, whose recessive alleles cause late flowering (*4*).

Late-flowering mutants have been functionally grouped by their environmental responses and their genetic interactions (*4*, *5*). One class, which flowers much later than wild type in long days, includes the recessive *co* and *ft* mutants and the dominant *fwa* mutants. In contrast to *co* mutants, *ft* and *fwa* not only flower late in long days but are also moderately delayed in short days. The two groups, *FT/FWA* and *CO*, also interact differently with the meristem-identity gene *LFY* because only *co* mutations affect transcriptional induction of *LFY* (*6*, *7*). In addition, *ft lfy* and *fwa lfy* but not *co lfy* double mutants have a phenotype that is associated with loss of expression of the meristem-identity gene *APETALA1* (*AP1*), indicating that *FT* and *FWA* act redundantly with *LFY* to regulate *AP1* (*8*, *9*). *AP1* was expressed precociously in *35S::FT* plants, but in contrast to *35S::LFY*, *AP1* expression was confined to floral primordia (Fig. 3, A and D), suggesting that *FT* regulates *AP1* expression less directly than the LFY transcription factor (*10*).

Table 1. Flowering times determined by total leaf number on the main shoot (*3*). Measurements are in long days except where indicated. Each group represents plants that are from the same genetic background and are grown at the same time.

Genotype	Leaves	SD	Range	*n*
Columbia wild type	14.3	1.2	13–16	16
35S::FT	3.9	0.7	3–5	26
35S::FT (short days)*	4.1	0.3	4–5	32
35S::FT tfl1-1	3.2	0.4	3–4	13
35S::FT tfl1-1 (short days)*	3.3	0.5	3–4	16
tfl1-1	9.3	1.0	8–11	21
35S::FT 35S::TFL1	5.2	0.7	4–6	9
35S::TFL1	37.7	3.0	34–43	10
Landsberg *erecta* wild type	10.5	0.7	9–12	25
ft-3/+	13.2	0.7	12–15	23
Landsberg *erecta* wild type	10.9	0.8	10–13	35
35S::FT	4.0	0.4	3–5	24
*35S::FT 35S::CO**	4.0	0.0	4	9
35S::CO	5.5	0.5	5–6	21
35S::FT fwa-2	17.6	1.5	15–20	16
fwa-2	23.0	1.4	21–26	23
Landsberg *erecta* wild type	10.6	0.7	9–12	40
35S::FT	4.0	0.5	3–5	25
*35S::FT co-2**	4.0	0.0	4	19
co-2	31.9	2.1	28–36	21
Columbia wild type	16.5	1.2	14–19	35
35S::FT	4.3	0.5	3–5	28
35S::FT 35S::LFY	2.0	0.0	2	17
35S::LFY	11.4	2.1	8–15	13
35S::FT 35S::AP1	3.4	0.5	3–4	9
35S::AP1	6.9	1.7	5–10	7
Landsberg *erecta* wild type	9.8	1.2	8–12	16
*ap1-1**	10.6	1.0	9–12	20
35S::FT ap1-1	4.2	0.5	3–5	24
*35S::FT**	4.0	0.5	3–5	24

*Indicates no statistically significant difference to genotype above; otherwise, all genotypes within each group are significantly different (Student's *t* test, $P < 0.005$).

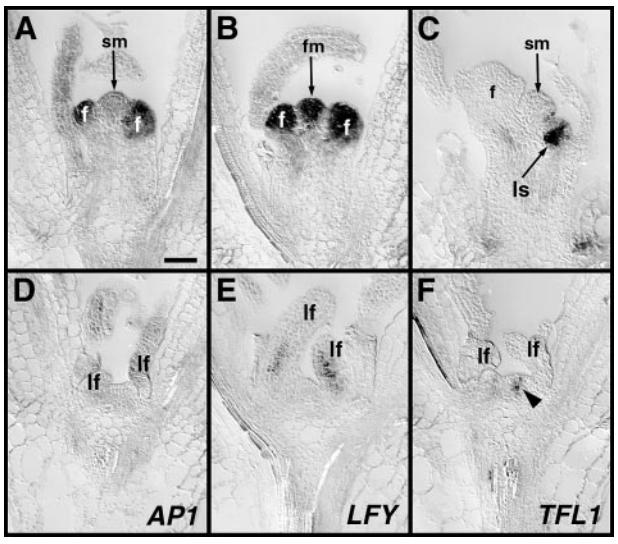

Fig. 3. Expression of meristem-identity genes in 8-day-old *35S::FT* (top) and Columbia wild-type plants (bottom) determined by in situ hybridization (*3*). (**A**) *AP1* mRNA is apparent in the flower primordia (f) that have formed on the flanks of the shoot apical meristem (sm). (**B**) *LFY* mRNA is apparent in lateral flower primordia. The shoot apical meristem of this plant has already undergone the transition to a floral meristem (fm), which also expresses *LFY*. (**C**) *TFL1* expression in *35S::FT* is transient, similar to what is seen in *tfl1* mutants (*24*). In this plant, *TFL1* expression was already reduced in the primary shoot apical meristem and only apparent in the adjacent section. Strong *TFL1* expression is, however, still seen in the lateral shoot (ls). (**D**) No *AP1* mRNA is detected in wild type. (**E**) Weak *LFY* expression is observed in leaf primordia (lf). (**F**) The vegetative wild-type apex expresses *TFL1* weakly (arrowhead) (*15*). Scale bar in (A), 50 μm. All panels are at the same magnification.

Because changes in *FT* levels affected flowering, as deduced both from the *35S::FT* phenotype and the semidominant nature of *ft* mutants (Table 1), we determined whether *CO* or *FWA* regulates *FT* mRNA accumulation. In both long and short days, *FT* levels in wild type increased from young seedlings to older plants, with higher overall levels in long days (Fig. 1C) (*3*). Whereas *FT* expression profiles were similar in wild-type and *fwa-2* plants grown in long days, *FT* expression was reduced in *co-2* seedlings, rising to wild-type levels in older plants (Fig. 1, D and E). These data suggest that *CO* functions partially upstream of *FT* and that *FWA* acts downstream of or in parallel with *FT* (*3*).

We complemented the expression studies by testing how constitutive *FT* expression affected the *co-2* or *fwa-2* mutant phenotypes. Even though *co* mutants have a more severe phenotype in long days than *ft* mutants, *35S::FT* could completely suppress the *co-2* phenotype. *35S::FT* also masked the effects of *CO* overexpression (*6*), confirming that changing *CO* activity had no effect in a *35S::FT* background (Table 1 and Fig. 2B). Although these interactions would normally suggest that *FT* is the only downstream effector of *CO*, *FT* and *CO* interact differently with *LFY* (*6*–*9*), which argues against a simple linear hierarchy from *CO* through *FT* to flowering. A possible explanation is that increased activity of the *FT*-dependent pathway can compensate for reduced activity of a parallel, normally *FT*-independent pathway in *co-2* mutants. Consistent with such a scenario, *35S::FT* caused precocious induction of *LFY* mRNA (Fig. 3, B and E), even though *FT* is not normally required for *LFY* expression (*7*, *8*). Unlike *35S::FT co-2* plants, *35S::FT fwa-2* plants flowered much later than wild type (Table 1 and Fig. 2B), consistent with *FWA* affecting events downstream of *FT* (*3*).

These and other findings indicate that *FT* and *LFY* have parallel functions downstream of

the long-day–dependent and –independent pathways of floral induction (*1*, *7*, *8*, *11*). Indeed, in contrast to plants that overexpressed only *FT* or *LFY* (*12*), the vegetative phase was bypassed in *35S::FT 35S::LFY* plants, which produced a terminal flower immediately upon germination. The only leaves produced by these plants were the first two leaves, which are already initiated in the embryo (Fig. 2, C, E, and I, and Table 1). A less marked effect was seen in *35S::FT 35S::AP1* plants (Fig. 2C and Table 1), even though *35S::AP1* plants on their own flowered considerably earlier than did *35S::LFY* plants (*7*, *12*–*14*) (Table 1). The *35S::FT 35S::LFY* phenotype was also more severe than that of *35S::AP1 35S::LFY* plants (*14*), indicating that *FT* does not only induce *AP1*, which is confirmed by a failure of an *ap1* mutation to suppress early flowering of *35S::FT* plants (Table 1).

The deduced FT protein belongs to a small family of *Arabidopsis* proteins, which includes the TFL1 protein, whose amino acid sequence is more than 50% identical to that of FT (*3*, *15*). *FT* and *TFL1* have opposite effects on flowering. Loss of *FT* function causes late flowering (*4*), whereas loss of *TFL1* causes early flowering along with the formation of terminal flowers (*16*). However, *FT* and *TFL1* effects are not entirely mirror images of each other, because *35S::FT* plants flower much earlier than *tfl1* loss-of-function mutants, particularly under short days, and *35S::TFL1* plants not only flower late, as do *ft* loss-of-function mutants, but they also show transformation of individual flowers into shootlike structures (*17*).

To clarify the relation between *FT* and *TFL1*, we tested whether *FT* promotes flowering by eliminating *TFL1* activity. *35S::FT tfl1-1* plants flowered even earlier than *35S::FT* plants and often formed only a single, terminal flower on the main shoot, indicating that *TFL1* is still active in *35S::FT* (Table 1 and Fig. 2, A and H). Consistent with this finding, *TFL1* was expressed in *35S::FT* plants (Fig. 3, C and F). Independent action of *FT* and *TFL1* was likewise evident from the fact that *35S::TFL1* attenuated the early flowering of *35S::FT*, even though the attenuation was modest (Table 1 and Fig. 2A). Together, these observations suggest that *FT* and *TFL1* act at least partially in parallel.

TFL1 mRNA is highly expressed in a small group of shoot meristem cells (*15*) (Fig. 3F). Using reverse transcriptase polymerase chain reaction (RT-PCR), we detected *FT* mRNA throughout the aerial part of the plant (Fig. 1B). In situ hybridization revealed no specific concentration of *FT* transcripts at the shoot apex, suggesting that *FT* and *TFL1* do not have to be expressed in the same pattern to antagonize each other's effects.

FT and TFL1 are related to a membrane-associated mammalian protein that can bind hydrophobic ligands (*18*). This protein also gives rise to hippocampal cholinergic neurostimulating peptide (HNCP), which is generated from its precursor by cleavage after amino acid 12 (*19*). Comparison of the FT and TFL1 sequences (*3*) with the crystal structure (*20*, *21*) of HCNP precursor, also called phosphatidylethanolamine binding protein (PEBP), revealed several interesting features. The conserved residue Arg^{119} has been proposed to activate the bond between Leu^{12} and Ser^{13} for cleavage of HCNP (*20*). Arg^{119} is important for FT function as well, because this residue was changed to histidine in the strong *ft-3* allele (Fig. 1A). It has also been proposed that access to the PEBP ligand-binding site is regulated by a COOH-terminal α helix (*20*, *21*). A missense mutation in *ft-1* close to the COOH-terminus indicates that this region is critical for FT as well (Fig. 1A).

In summary, *FT* and *TFL1* encode related proteins with opposite effects on flowering. Similarly to *FT*, its antagonist *TFL1* is positively regulated by *CO* (*6*), suggesting that the balance between *FT* and *TFL1* activity serves to fine tune the response to floral inductive signals (*3*). It remains to be determined how far the sequence similarity between FT, TFL1, and mammalian PEBP reflects similar biochemical modes of action.

Note added in proof: Human PEBP was recently shown to be identical to RKIP (Raf kinase inhibitor protein), which regulates the activity of the RAF/MEK/ERK signal transduction pathway (*22*).

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Organogenic Role of B Lymphocytes in Mucosal Immunity

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Follicle-associated epithelium (FAE) in the intestinal Peyer's patches contains M cells that deliver pathogens to organized lymphoid tissue. Development of Peyer's patches, FAE, and M cells was found to be impaired in mice that had no B cells. Transgenic expression of membrane-bound immunoglobulin M restored B cells and FAE development. The lack of M cells abrogated infection with a milk-borne retrovirus. Thus, in addition to secretion of antibodies and presentation of antigens, B cells are important for organogenesis of the mucosal immune barriers.

The gut-associated lymphoid tissue (GALT) consists of highly organized Peyer's patches (PPs) in the small intestine and intraepithelial lymphocytes (IELs) found throughout the length of the gastrointestinal tract. The intestinal surface of PPs is characterized by the presence of FAE-covering "domes," regions free of intestinal villi (*1*). M cells are found in these domes, scattered among enterocytes (*2*). M cells lack microvilli on their apical