Supplementary Methods (Ahn et al.)

Chimeric genes

Oligonucleotide primers spanning the exon (JH1061-JH1076) and segment (JH1125-JH1134) boundaries within the fourth exon were used to amplify each fragment. Chimeric genes were generated by PCR using the hot-start method with *Pfu* Taq polymerase (Bassam & Caetano-Anolles, 1993). In the first round of PCR, appropriate fragments from *FT* or *TFL1* cDNA were generated. Following gel-purification of PCR fragments, a second round of PCR was performed with the appropriate combinations of fragments as template and two primers flanking the ends of the final chimeric constructs of the desired configuration. The primers designed to amplify the final forms of the chimeras contained a synthetic *Kpn* I restriction site before the translational start codon (JH1089 for *FT* and JH1091 for *TFL1*) and an *Xba* I site after the translational stop codon (JH1090 for *FT* and JH1092 for *TFL1*). Thus, no additional sequences, other than the coding region of *FT* or *TFL1,* were included after *Kpn* I*/Xba* I double digestion. Each chimeric construct was sequenced, and correct chimeras were subcloned into pCHF3, a plant transformation vector that contains a cauliflower mosaic virus 35S promoter and an *rbc*S terminator (Jarvis *et al*, 1998).

The segment-swapping chimeras within the fourth exon were based on the pJA1055 construct (Figure 2), whose fourth exon was from *TFL1*, with the remaining exons from *FT*. Similar procedures as used in constructing the exon-swapping chimeras were applied, except that segment B was directly synthesized as complementary oligonucleotides. The chimeric sequences were fused to the first three exons of *FT* (encoding amino acids 1-101) and subcloned into pCHF3 (Jarvis *et al*, 1998).

Oligonucleotide sequences are given in Supplementary Table S4.

RNA and protein blot analysis

Ten micrograms of total RNA extracted from transgenic plants carrying the chimeric genes was fractionated and hybridized with a mixture of radio-labeled *FT* and *TFL1* cDNA as a

probe. Glutathione-*S*-transferase (GST)-tagged bacterial FT protein was expressed and purified from *E. coli*. Rat antisera were raised against the purified bacterial FT antigen. Protein samples were prepared by mixing ground leaf tissues in a sample buffer (50 mM Tris [pH7.5], 250 µM PMSF) and boiling the homogenate for 5 minutes. After centrifugation, supernatants were separated on a denaturing 12% polyacrylamide gel and blotted onto Immobilon polyvinylidene difluoride (PVDF) membranes (Millipore). After electrotransfer, membranes were stained with Ponceau S to determine the amount of protein that had been loaded. The blot was probed with anti-FT antibody as described (Schumacher *et al*, 1999), and immunoreactive proteins were detected with a chemiluminescence kit (Pierce).

Expression of recombinant proteins for crystallization studies

TFL1 and FT expression was induced with 0.2 mM isopropyl-β-D-thiogalactopyranoside (IPTG). TFL1 was isolated from insoluble inclusion bodies, using a Ni^{2+} affinity column in the presence of 8 M urea, refolded by rapid 50 fold dilution to remove the urea. The refolded protein was concentrated by a further pass through the Ni^{2+} column prior to removal of the 6-histidine tag by thrombin digestion. Undigested TFL1 was removed by anion exchange chromatography with a MonoS column. FT was isolated from the soluble fraction of the cell lysate using a Ni^{2+} column, the N-terminal 6-histidine tag removed by overnight digestion with thrombin, followed by a further pass over the Ni^{2+} column to remove undigested protein, which was further purified by gel filtration chromatography using Superdex-75.

Bassam BJ, Caetano-Anolles G (1993) Automated "hot start" PCR using mineral oil and paraffin wax. *Biotechniques* **14**: 30-34.

Jarvis P, Chen LJ, Li H, Peto CA, Fankhauser C, Chory J (1998) An *Arabidopsis* mutant defective in the plastid general protein import apparatus. *Science* **282**: 100-103.

Schumacher K, Vafeados D, McCarthy M, Sze H, Wilkins T, Chory J (1999) The Arabidopsis det3 mutant reveals a central role for the vacuolar H(+)-ATPase in plant growth and development. *Genes Dev* **13**: 3259-3270.