PNAS
Proceedings of the National Academy of Sciences of the United States of America www.pnas.org

The wheat and barley vernalization gene VRN3 is an orthologue of FT

L. Yan, D. Fu, C. Li, A. Blechl, G. Tranquilli, M. Bonafede, A. Sanchez, M. Valarik, S. Yasuda, and J. Dubcovsky

PNAS published online Dec 8, 2006;
doi:10.1073/pnas.0607142103

This information is current as of December 2006.

Supplementary Material	Supplementary material can be found at: www.pnas.org/cgi/content/full/0607142103/DC1
	This article has been cited by other articles: www.pnas.org#otherarticles
E-mail Alerts	Receive free email alerts when new articles cite this article - sign up in the box at the top right corner of the article or click here.
Rights & Permissions	To reproduce this article in part (figures, tables) or in entirety, see: www.pnas.org/misc/rightperm.shtml
Reprints	To order reprints, see: www.pnas.org/misc/reprints.shtml

Notes:

The wheat and barley vernalization gene *VRN3* is an orthologue of *FT*

L. Yan*†, D. Fu*, C. Li*, A. Blechl‡, G. Tranquilli*§, M. Bonafede*§, A. Sanchez*, M. Valarik*, S. Yasuda¶, and J. Dubcovsky*‖

*Department of Plant Sciences, University of California, Davis, CA 95616; ‡U.S. Department of Agriculture–Agricultural Research Service, Western Regional Research Center, Albany, CA 94710; and ¶Research Institute for Bioresources, Okayama University, Kurashiki 710-0046, Japan

Edited by Richard M. Amasino, University of Wisconsin, Madison, WI, and approved October 30, 2006 (received for review August 16, 2006)

Winter wheat and barley varieties require an extended exposure to low temperatures to accelerate flowering (vernalization), whereas spring varieties do not have this requirement. In this study, we show that in these species, the vernalization gene *VRN3* is linked completely to a gene similar to *Arabidopsis FLOWERING LOCUS T* (*FT*). *FT* induction in the leaves results in a transmissible signal that promotes flowering. Transcript levels of the barley and wheat orthologues, designated as *HvFT* and *TaFT*, respectively, are significantly higher in plants homozygous for the dominant *Vrn3* alleles (early flowering) than in plants homozygous for the recessive *vrn3* alleles (late flowering). In wheat, the dominant *Vrn3* allele is associated with the insertion of a retroelement in the *TaFT* promoter, whereas in barley, mutations in the *HvFT* first intron differentiate plants with dominant and recessive *VRN3* alleles. Winter wheat plants transformed with the *TaFT* allele carrying the promoter retroelement insertion flowered significantly earlier than nontransgenic plants, supporting the identity between *TaFT* and *VRN-B3*. Statistical analyses of flowering times confirmed the presence of significant interactions between vernalization and *FT* allelic classes in both wheat and barley ($P < 0.0001$). These interactions were supported further by the observed up-regulation of *HvFT* transcript levels by vernalization in barley winter plants ($P = 0.002$). These results confirmed that the wheat and barley *FT* genes are responsible for natural allelic variation in vernalization requirement, providing additional sources of adaptive diversity to these economically important crops.

flowering | *Triticum aestivum* | *Flowering Locus T* | *Hordeum vulgare*

The propagation and survival of a plant species depends critically on its ability to precisely regulate the transition from vegetative to reproductive growth. Consequently, plants have evolved refined mechanisms capable of integrating photoperiod and vernalization (extended exposure to low temperatures) signals associated with seasonal variation to optimize flowering time and seed production.

The photoperiod pathway is relatively well conserved among flowering plants, with the gene *CONSTANS* (*CO*) playing a central regulatory role (1, 2). In *Arabidopsis*, a long-day (LD) plant, CO induces the transcription of the *FLOWERING LOCUS T* (*FT*) whereas in rice, a short-day (SD) plant, CO represses *FT* (referred to as *Hd1* and *Hd3a*, respectively, in rice) (2). Overexpression of *FT* in transgenic plants from several species is associated with early flowering (3–7), suggesting that this gene is a conserved promoter of flowering. *FT* induction in the leaves results in a transmissible signal that travels through the phloem to the apex, where it induces flowering (8–10).

In contrast with the conserved photoperiod pathway, several aspects of the vernalization pathway vary between *Arabidopsis* and the temperate grasses (11). In *Arabidopsis*, the MADS-box gene *FLOWERING LOCUS C* (*FLC*) plays a central role in the vernalization pathway (12, 13). *FLC* delays flowering by repressing the production of *FT* in the leaves and *SOC1* in the meristems, where it prevents the up-regulation of the *FD* transcription factor, a partner to *FT* in the induction of flowering (9, 10, 14). Vernalization permanently down-regulates *FLC*, thereby releasing *FT* and *SOC1* repression to induce the transcription of *AP1*, which is responsible for the transition between the vegetative and reproductive meristem (12). *FLC* is positively regulated by *FRIGIDA* (*FRI*) and negatively regulated by genes in the *Arabidopsis* autonomous pathway (12, 13). Surprisingly, no clear homologues of *FRI* or *FLC* have been found in temperate grasses (e.g., wheat and barley).

The *VRN2* gene from temperate grasses (different from *Arabidopsis VRN2*; ref. 15) is a dominant repressor of flowering down-regulated by both vernalization (11) and SDs (16, 17). *VRN2* has no close homologues in *Arabidopsis*, but plays a role in vernalization similar to that of *FLC* (11). Reduction of *VRN2* transcript levels by RNA interference (RNAi) in hexaploid winter wheat variety Jagger significantly accelerates flowering (11). *VRN2* has a CCT domain (CO, CO-like, and TOC1) similar to that found in CO (11). Mutations within this domain or deletions of the complete *VRN2* gene result in recessive alleles for spring growth habit in diploid wheat and barley that eliminate the vernalization requirement (11, 18).

The effect of *VRN2* allelic variation on flowering time is reduced or eliminated by mutations in the promoter or first intron of the *VRN1* vernalization gene in both wheat and barley (18–21). This dominant promoter of flowering is orthologous to the *Arabidopsis* meristem identity gene *AP1* (22). *VRN1* transcripts are up-regulated by vernalization in winter wheat varieties (22), and its down-regulation by RNAi in transgenic wheat plants delays flowering (23).

Two additional vernalization genes have been reported in barley (*VRN-H3*) and wheat (*VRN-B4*). *VRN-H3* was tentatively assigned to chromosome 1H based on its loose linkage with the morphological marker *BLP* (24), whereas *VRN-B4* was mapped on the short arm of wheat chromosome 7B (25–28). We show here that the *VRN-H3* gene actually is located on barley chromosome arm 7HS and is orthologous to the wheat vernalization

Author contributions: L.Y., D.F., and C.L. contributed equally to this work; L.Y. and J.D. designed research; L.Y., D.F., C.L., A.B., G.T., M.B., and A.S. performed research; S.Y. contributed new reagents/analytic tools; L.Y., D.F., C.L., A.B., G.T., M.B., A.S., M.V., and J.D. analyzed data; and J.D. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS direct submission.

Data deposition: The sequences reported in this paper have been deposited in the GenBank database (accession nos. DQ890162, DQ890163, DQ890165, DQ898515–DQ898519, DQ899784, and DQ900685–DQ900687).

Abbreviations: LD, long day; QTL, quantitative trait loci; RFLP, restriction fragment length polymorphism; RSL, recombinant substitution line; SD, short day.

†Present address: Department of Plant and Soil Sciences, Oklahoma State University, Stillwater, OK 74078.

§Present address: Instituto de Recursos Biológicos, Instituto Nacional de Tecnología, Agropecuaria, Villa Udaondo, 1712 Castelar, Buenos Aires, Argentina.

‖To whom correspondence should be addressed. E-mail: jdubcovsky@ucdavis.edu.

This article contains supporting information online at www.pnas.org/cgi/content/full/0607142103/DC1.

© 2006 by The National Academy of Sciences of the USA

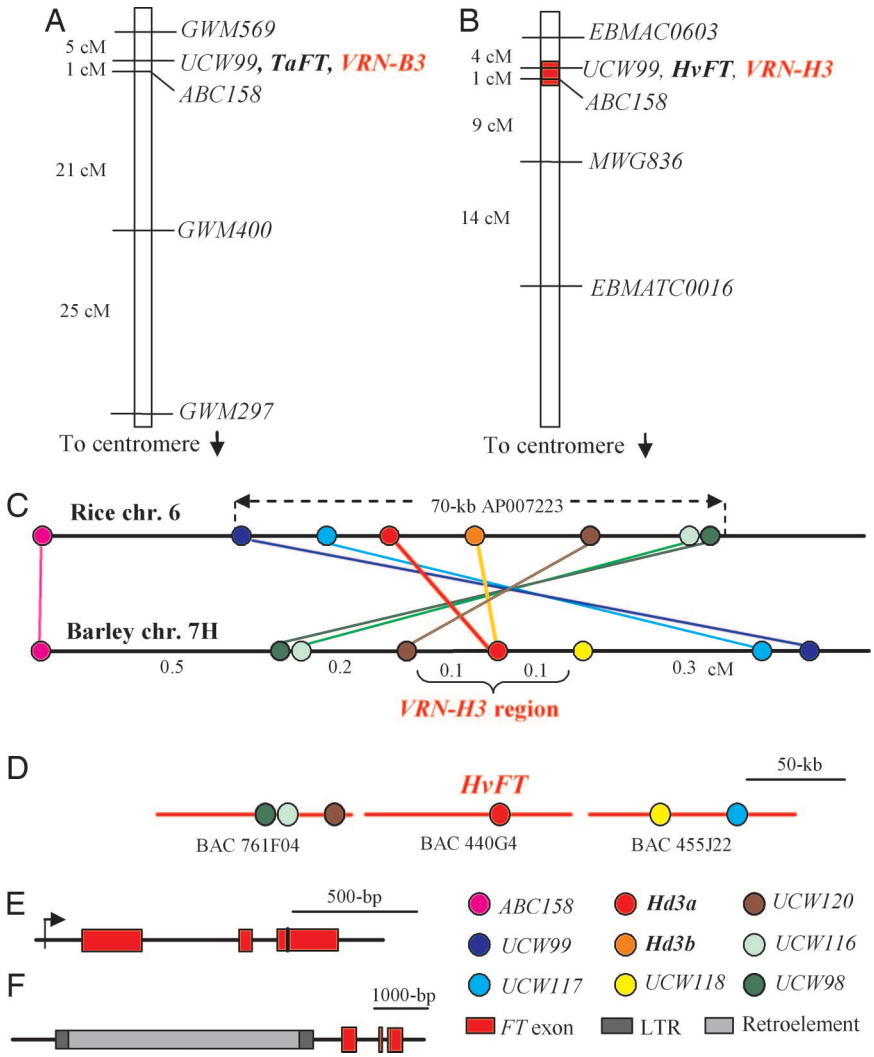

Fig. 1. VRN3 maps and gene structure. (*A*) Genetic map of wheat vernalization gene *VRN-B3* on chromosome arm 7BS. (*B*) Genetic map of barley *VRN-H3* on chromosome arm 7HS. The region in red is expanded in *C*. (*C*) High-density genetic map of *VRN-H3*. Note the 70-kb inversion in rice relative to the barley genetic map. Circles represent the different genes mapped in this study (SI Table 3). Orthologous barley and rice genes are presented in the same color. Duplicated rice genes *Hd3a* and *Hd3b* correspond to a single *HvFT* gene in barley. (*D*) Position of known genes within the three sequenced barley BACs (see SI Fig. 7 for more details). (*E*) *HvFT* gene structure. The arrow indicates the transcriptional start, the red rectangles represent exons, and the vertical line shows the fusion between the third and fourth exons relative to *Hd3a* and *Hd3b*. (*F*) Schematic representation of the Hope *TaFT* allele carrying a retrotransposon insertion in the promoter.

gene *VRN-B4*, which is referred hereafter as *VRN-B3*. We also show that *VRN3* is an orthologue of the *Arabidopsis FT* gene.

Results

Genetic Mapping of Wheat *VRN-B3*. We mapped *VRN-B3* on the short arm of chromosome 7B, 1 cM distal to marker *ABC158* and 5 cM proximal to microsatellite marker *GWM569* (Fig. 1*A*) by using 82 recombinant substitution lines (RSLs) from a cross between Chinese Spring (CS) and chromosome substitution line CS(Hope7B) [supporting information (SI) *Appendix* 1]. The *ABC158* sequence (L43928) is 90% identical to a DNA sequence on rice chromosome 6 coding for protein BAD69198. This sequence is 50 kb proximal to *Hd3a*, a rice gene responsible for significant differences in flowering time and orthologous to the *Arabidopsis FT* gene (6).

Because *FT* was a potential candidate gene for *VRN-B3*, we developed a marker for the orthologous *Triticum aestivum* L. gene (*TaFT*) by using the published sequence of the barley orthologue (*HvFT*, DQ100327) (29). We developed a second marker from a rice gene located between *ABC158* and *Hd3a* (AK121981), which was designated *UCW99* (SI Table 1). Using these markers, we mapped *TaFT* completely linked to *VRN-B3* and *UCW99* and 1 cM distal to *ABC158* (Fig. 1*A*). Complete linkage between *VRN-B3* and *TaFT* was confirmed by analyzing flowering time** for 10–11 plants from each critical RSLs with recombination events between flanking markers *ABC158* and *GWM569* (SI Table 2).

Genetic Mapping of Barley *VRN-H3*. We mapped *VRN-H3* in an F_2 population from the cross between BGS213 (spring, *Vrn-H3*) and *Hordeum vulgare* subsp. *spontaneum* (C. Koch) Thell. (winter, *vrn-H3*). The 3:1 ratio between spring and winter plants found before in this population (30) confirmed segregation for a single dominant gene. This gene was mapped on chromosome 7H linked to microsatellite loci *EBMAC0603* and *EBMATC0016* (Fig. 1*B*). To explore the relationship between *VRN-H3* and the vernalization gene mapped on the homoeologous chromosome

**Throughout this study flowering time refers to the time of complete emergence of the spike from the leaf sheath, which is usually referred by cereal scientists as heading time.

7B in wheat, we developed barley markers for the same genes mapped in wheat (SI Table 3). Markers *UCW99* and *HvFT* were completely linked to each other and to *VRN-H3* and 1 cM distal to *ABC158* (Fig. 1*B*).

Plants carrying the BGS213 *UCW99/HvFT* allele flowered 36 to 50 days after sowing, whereas those carrying the *H. vulgare* subsp. *spontaneum UCW99/HvFT* allele flowered 85 to 111 days after sowing, facilitating the precise mapping of *VRN-H3*. The complete linkage between *HvFT* and *VRN-H3* was confirmed by using a second barley mapping population from a cross between the spring genetic stock BGS213 and the winter barley variety "Igri" (*SI Appendix*, section I).

To rule out the possibility of an error in the BGS213 genetic stock, we tested two additional sets of *VRN-H3* isogenic lines in which the spring growth habit from Tammi (*Vrn-H3*) was introgressed into winter varieties Hayakiso 2 and Dairokkaku 1 by 11 backcrosses. Using the molecular markers developed for *HvFT* (SI Table 3), we confirmed that the two spring *Vrn-H3* isogenic lines have the Tammi allele (same as BGS213), whereas the recurrent winter parents have a different *HvFT* allele. Our results indicate that *Vrn-H3* is on chromosome 7H and linked to *HvFT*, and not on chromosome 1H as initially suggested by its loose linkage to *BLP* (24).

Based on the known colinearity between barley and wheat chromosomes (31) and the close linkage between both barley *VRN-H3* and wheat *VRN-B4* with the same three molecular markers located on homoeologous group 7 (Fig. 1 *A* and *B*), we conclude that these two genes are orthologous and propose to rename the wheat vernalization gene as *VRN-B3*.

High-Density Genetic Map and Physical Map of Barley *VRN-H3*. We selected the barley-mapping population BGS213 × *H. vulgare* subsp. *spontaneum* for the *VRN3* high-density mapping because of its simpler diploid inheritance and higher level of polymorphism relative to the wheat population. To generate additional markers in the region, we developed several *UCW* markers corresponding to the rice genes flanking *Hd3a* (Fig. 1*C* and SI Table 3). Despite a 70-kb inversion detected between barley and rice in this region, the colinearity of the genes within the inversion facilitated the development of barley markers tightly linked to *HvFT* (Fig. 1*C*).

We first used *HvFT* flanking markers *UCW98-UCW99* to screen 1,600 gametes from this population and found 12 lines with recombination events within the targeted region (SI Fig. 5). Progeny tests of these 12 lines were used to map *VRN-H3* 0.3 cM distal to *UCW98*, 0.4 cM proximal to *UCW99*, and completely linked to *HvFT* (Fig. 1*C*).

Barley probes for *HvFT* and its flanking markers *UCW98*, *UCW99*, *UCW116*, and *UCW117* (Fig. 1*C*) were used to screen a "Morex" barley BAC library (32). Nineteen BACs were recovered and assembled via fingerprinting and hybridization into three contigs separated by two gaps (SI Fig. 6). The sequencing of barley BACs 440G4 (DQ900686), 761F4 (DQ900685), and 455J22 (DQ900687) revealed the presence of the noncolinear barley gene *UCW118* (Fig. 1*D*) and of the putative gene *UCW120* in both rice and barley (SI Fig. 7). The mapping of these two barley markers further delimited the location of *VRN-H3* to a 0.2-cM interval flanked by *UCW120* and *UCW118* (Fig. 1*C* and SI Table 3). The only annotated genes (excluding hypothetical genes and repetitive elements) found in the colinear 28-kb region in rice were *Hd3a* and *Hd3b*, the rice orthologues of *FT*.

Similarly, no other known gene was found between *UCW120* and *UCW118* in the three barley BACs except for *HvFT* (Fig. 1*D*). To test whether additional genes were closely linked to *FT*, we also sequenced *Aegilops tauschii* Coss. BAC HI41I11 (DQ899784), which includes an orthologue of *HvFT* (coverage 2.8× at PHRED ≥20). Eighty percent of this 170-kb BAC showed similarity to repetitive elements, whereas the rest showed no similarity to known genes outside of *FT* (data not shown). Based on these results, *HvFT* is our only candidate gene for *VRN-H3*.

Southern blot analyses by using *HvFT* as a probe resulted in a single hybridization band with the barley genomic DNA, suggesting that the *Hd3a-Hd3b* duplication on rice chromosome 6 occurred after the divergence with the *Triticeae*, a hypothesis also supported by the phylogenetic analysis of *FT*-like genes in wheat, barley, rice, and *Arabidopsis* (SI Fig. 8).

***FT* Allelic Differences.** The *TaFT* and *HvFT* genes have three exons encoding for a protein of 177 aa (Fig. 1 *E* and *F*). In contrast, all other *FT* and *FT*-like genes included in the phylogenetic analysis (SI Fig. 8) have four exons. This difference was generated by the fusion of exons 3 and 4 in *TaFT* and *HvFT*.

Wheat. We subcloned and sequenced the wheat *TaFT* genes and their flanking 5′ and 3′ regions from CS(Hope7B) (DQ890165) and CS (DQ890162). The CS(Hope7B) allele associated with early flowering (*Vrn-B3*) has a 5,295-bp repetitive element inserted 591-bp upstream from the start codon, an insertion that is absent in the CS allele associated with late flowering (*vrn-B3*) (Fig. 1*F*). Six additional SNPs were detected in the promoter region and three within a foldback element present in intron 1. No differences were detected between the two *TaFT* alleles in the coding region or in the first 628 bp downstream from the stop codon.

The retrotransposon inserted in the *TaFT* promoter has identical LTRs, suggesting a recent insertion. This is further supported by the low frequency of this insertion in the wheat germplasm. With the exception of the variety Hope, we did not find this retrotransposon insertion in a collection of 19 tetraploid spring wheats, 29 hexaploid winter wheats, and 77 hexaploid spring wheats (SI Table 4). These results indicate that this mutation has not yet been used extensively in commercial varieties and, therefore, represents a potentially valuable source of genetic diversity to modulate wheat flowering time.

Barley. The *HvFT* allele from BGS213 (DQ898515) associated with *Vrn-H3* differs from both Igri (DQ898517) and *H. vulgare* subsp. *spontaneum* (DQ898516) alleles associated with *vrn-H3* by nine linked polymorphisms (seven SNPs and two indels) in the first 550 bp upstream from the start codon and two linked polymorphisms in the first intron (SI Fig. 9).

We sequenced *HvFT* from seven additional winter varieties (recessive *vrn-H3*) and found heterogeneity for the promoter haplotypes. The promoter haplotypes were similar to Igri in two varieties and similar to BGS213 in the other five varieties (SI Fig. 9). These results indicate that the BGS213 promoter haplotype is not sufficient to determine a dominant spring growth habit. However, we cannot rule out an effect of this promoter polymorphism on flowering time when alleles for spring growth habit from the other vernalization genes are present. A germplasm survey at Okayama University showed that all of the varieties carrying the *Vrn-H3* allele also have the dominant *Vrn-H1* allele (33).

The haplotypes found in the first intron were more consistent with the observed differences in growth habit. All of the winter varieties showed the same haplotype in the first intron as Igri and *H. vulgare* subsp. *spontaneum*, which was different from the one observed in the varieties carrying the dominant *Vrn-H3* allele (SI Fig. 9). These results suggest that regions in the first intron may play an important role in the regulation of *HvFT* by vernalization (SI Fig. 9). This possibility is also supported by previous reports indicating that *FLC* binds a region within *FT* first intron, which is critical for the regulation of this gene in *Arabidopsis* (14). We are developing several segregating populations to assess the roles of the *HvFT* promoter and first-intron polymorphisms in determining flowering time in different barley genetic backgrounds.

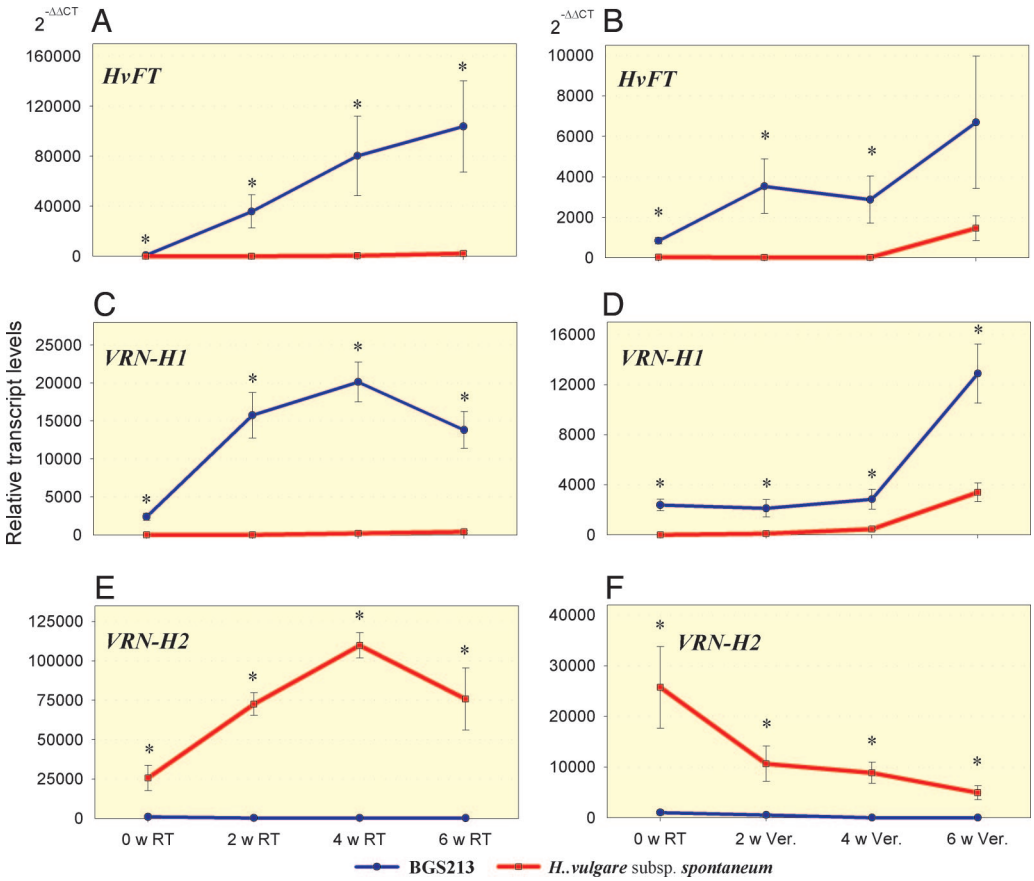

Fig. 2. Transcript levels of *HvFT*, *VRN-H1*, and *VRN-H2* in F_3 plants homozygous for the *HvFT H. vulgare subsp. spontaneum* allele (red, late flowering) or BGS213 allele (blue, early flowering). Plants at the three-leaf stage grown at room temperature (RT = 20–24°C) under LD (16 h of light) were either maintained at the same nonvernalizing condition (*A*, *C*, and *E*) or transferred to a cold room at 4°C under LD (*B*, *D*, and *F*; Ver., vernalization). Time is indicated in weeks (w) after the transfer to the separate chambers (0 w RT). Unvernalized plants carrying the BGS213 allele were flowering at the time of the 6-w RT sampling. Values are the means of 10 independent $F_{2:3}$ plants, and bars are SE of the means. The $2^{-\Delta\Delta C_T}$ method (38) was used to normalize and calibrate transcript values relative to the *ACTIN* endogenous control. Scales can be compared within the same genes but not across genes. Asterisks indicate significant differences ($P < 0.05$).

***FT* Expression Profiles.** *HvFT* transcript levels were analyzed by quantitative PCR (SI Table 5) in 20 different F_3 plants selected from the mapping population, 10 homozygous for the BGS213 *HvFT* allele and 10 homozygous for the *H. vulgare* subsp. *spontaneum* allele. Plants at the three-leaf stage carrying the BGS213 *HvFT* allele showed significantly higher *HvFT* transcript levels ($P < 0.01$) relative to those carrying the *H. vulgare* subsp. *spontaneum* allele [Fig. 2 *A* and *B*, 0 weeks (w)], a difference that persisted for the next weeks in both vernalized and unvernalized plants (Fig. 2 *A* and *B*).

A similar result was observed in wheat. RSLs carrying the Hope *TaFT* allele with the retrotransposon insertion showed significantly higher *TaFT* transcript levels ($P < 0.01$) than the RSLs carrying the CS allele (SI Fig. 10). Because *FT* induces flowering in other plant species, these results suggest that the earlier flowering of the lines carrying the dominant *Vrn3* alleles might be related to their higher *FT* transcript levels relative to lines carrying the *vrn3* allele.

Spring plants homozygous for the dominant *Vrn-H3* allele showed a rapid increase of *HvFT* transcripts with development (Fig. 2 *A* and *B*). However, winter plants carrying the recessive *vrn-H3* exhibited low *HvFT* transcript levels in the absence of vernalization (Fig. 2*A*). After six weeks of vernalization, *HvFT* transcript levels in the vernalized winter plants were significantly higher than in the nonvernalized plants (Fig. 2*B*, 6w, vs. Fig. 2*A*, 6w; $P = 0.002$). Up-regulation of *FT* transcript levels by vernalization also was confirmed in *Triticum monococcum* L. winter accession G3116 (data not shown).

HvFT transcript levels were very low under SDs and were up-regulated when the plants were transferred to LDs (SI Fig. 11), suggesting a strong effect of photoperiod on *HvFT* transcript levels. A similar observation was reported before in barley (29).

HvFT transcription profiles were paralleled closely by those of *VRN-H1* (Fig. 2 *C* and *D*), confirming the known interactions between these genes (9). In an opposite trend to that observed for *HvFT* and *VRN-H1*, the *VRN-H2* transcript levels in winter plants were reduced by vernalization (Fig. 2*F*). In the spring plants, *VRN-H2* levels were low even in the absence of vernalization (Fig. 2*E*), likely as a result of the higher transcript levels of *VRN1*. Previous studies suggest that *VRN1* directly or indirectly down-regulates *VRN2* (17, 23).

Transformation of Winter Wheat Plants with the Hope *TaFT* Allele. The complete genetic linkage between *FT* and *VRN3* in all mapping populations, together with the correspondence between polymorphisms in *FT* regulatory regions, *FT* transcript levels, and flowering time suggest that *FT* is in fact *VRN3*. To confirm the identity between *FT* and *VRN3*, we transformed winter wheat variety Jagger with the dominant *TaFT* allele from Hope, which carries the retrotransposon insertion (*SI Appendix, section VI*). A schematic representation of the Hope *TaFT* region cloned in the construct used in the transformation experiment is presented in Fig. 1*F*.

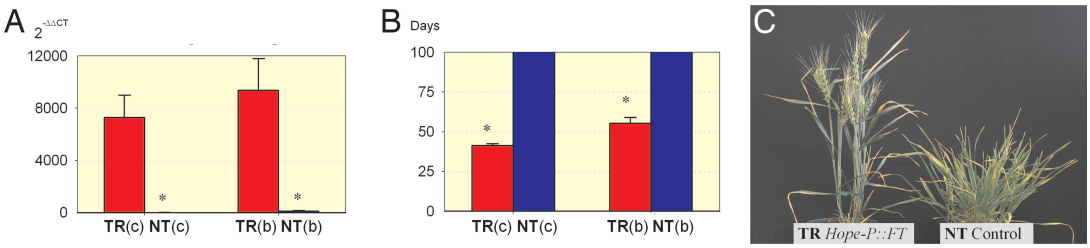

Fig. 3. Transgenic plants (red bars) and null segregants (blue bars) of winter variety Jagger transformed with the Hope promoter-*FT* construct (*Hope-P:FT*). (*A*) Transcript levels of *TaFT* in leaves from transgenic and null segregant plants at the five-leaf stage. (*B*) Flowering time of transgenic and null segregant control plants. Values are averages of six to nine plants (±SEM), and asterisks indicate significant differences ($P < 0.05$). TR, transgenic plants; NT, nontransgenic controls. Letters within parentheses indicate independent transformation events ''b'' and ''c.'' (*C*) *Hope-P:FT* transgenic plant and a nontransgenic Jagger control (86 days after sowing). All plants were grown under LD conditions without vernalization.

We analyzed two independent transgenic events (Fig. 3), including six or seven T_1 transgenic plants and eight or nine nontransgenic controls per transformation event. Leaf RNA samples were extracted from five-leaf old unvernalized plants grown under LD. Jagger plants transformed with the Hope allele showed *TaFT* transcript levels >80-fold higher than the nontransgenic controls (Fig. 3*A*). Average flowering time for the transgenic lines was typical of spring lines (40 ± 1 and 51 ± 2 days after sowing), whereas the nontransgenic plants remained in vegetative stage until the experiment was terminated 110 days after sowing (Fig. 3 *B* and *C*). The conversion of a winter wheat variety into a spring one supports the identity between *FT* and *VRN-3*.

Interactions Between *FT* and Vernalization. In both wheat and barley, factorial ANOVAs for flowering time by using vernalization treatment and *FT* allelic classes as factors yielded highly significant interactions ($P < 0.0001$) (SI Table 6). Vernalized wheat and barley plants showed smaller differences in flowering time between the two *VRN3* allelic classes than unvernalized plants (SI Fig. 12). Based on these results and the observed up-regulation of *FT* transcript levels by vernalization (Fig. 2 *A* and *B*), we conclude that, in the temperate cereals, *FT* interacts with the vernalization pathway.

In *Arabidopsis*, no association between natural variation in vernalization requirement and *FT* has been described so far. However, overexpression of *FT* (or *TSF*) strongly suppresses the *FLC*-mediated late-flowering phenotype of winter annual *Arabidopsis* accessions without affecting *FLC* mRNA levels (34). This suggests that activation of *FT* and/or *TSF* can bypass the block to flowering created by *FLC*, confirming that *FT* acts downstream of *FLC* (34). A similar result is reported here for wheat, where the increased expression of *TaFT* in transgenic winter variety Jagger bypassed the *VRN2* repression, resulting in a spring growth habit.

To test whether the interaction between vernalization and *FT* in wheat was related to the presence of the vernalization gene *VRN2*, which is unique to temperate cereals, we studied the transcript levels of *FT* in isogenic lines of *T. monococcum* differing in their *VRN2* alleles (SI Fig. 13). Spring accession Dv92 has a recessive *vrn2* allele generated by a point mutation in the CCT domain, whereas the winter accession G3116 has a functional *Vrn-2* allele (11). No significant differences in *VRN2* transcript levels ($P > 0.05$) were detected at the five-leaf stage between the isogenic lines. In contrast, at the same developmental stage, *FT* transcript levels were 170-fold higher ($P < 0.05$) in the isogenic lines carrying the mutant *vrn2* allele from Dv92 than in those carrying the dominant *Vrn2* allele at the same developmental stage (SI Fig. 13).

This result suggests that *VRN2* modulates the quantitative levels of *FT* (directly or indirectly), providing a link between the vernalization pathway and *FT* in the temperate cereals. This genetic interaction is not completely unexpected, because the CCT domain in VRN2 is related to the one present in CO, which was shown to be involved in the regulation of *FT* transcript levels (35). It is tempting to speculate that the allelic differences in the *FT* regulatory regions described in this study may be responsible for the disruption of the interactions between *FT* and *VRN2* (or a *VRN2*-regulated gene) and for their differential responses to the vernalization treatment. A tentative model summarizing the interactions between *VRN1*, *VRN2*, and *VRN3* is presented in Fig. 4.

According to this model, VRN2 is a repressor of flowering down-regulated by vernalization and SD (11, 16, 17), which negatively regulates *VRN3* and *VRN1* (directly or indirectly). *VRN3* is a promoter of flowering up-regulated by LD, which positively regulates *VRN1*, the meristem identity gene. A secondary effect of the increase in *VRN1* transcripts is the down-regulation of *VRN2*, as part of a feedback regulatory loop described in refs. 16 and 17. Unvernalized winter plants grown under LD exhibit high levels of *VRN2* transcripts and low levels of *VRN1* and *VRN3* (Fig. 2 *A*, *C*, and *E*). Vernalization under LD results in the down-regulation of *VRN2* and the up-regulation of *VRN3* and *VRN1*. Under SD, all three genes show low transcript levels, but a rapid up-regulation of *VRN1* and *VRN3* is observed when plants are transferred from SD to LD (SI Fig. 11). This model also attempts to explain the strong epistatic interactions observed among these three genes. The recessive *vrn2* allele eliminates the effect of *VRN1* and *VRN3* allelic differences on flowering time (18, 19, 36). We suggest that in the absence of a functional VRN2 repressor, the different mutations in the *VRN1* (20, 21) or *VRN3* (Fig. 1*F*) regulatory regions have no effect on flowering. It is also known that the dominant *Vrn1* and *Vrn3* alleles reduce or eliminate the effect of *VRN2* allelic differences on flowering time. We propose that a mutation in a regulatory region of *VRN1* or *VRN3* is sufficient to preclude its recognition by the *VRN2*-mediated repression and to initiate the flowering cascade.

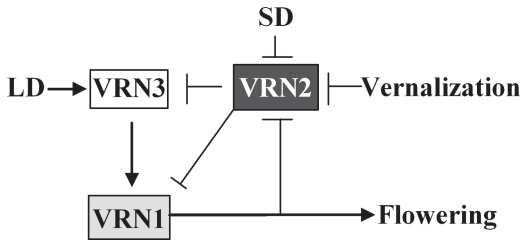

Fig. 4. Hypothetical model summarizing our current understanding of the genetic interactions among the three cloned Triticeae vernalization genes (see *Interactions Between* FT *and Vernalization* for explanation).

In summary, this study provides strong evidence supporting the identity between *FT* and *VRN3* in wheat and barley. It also shows that allelic variation in *FT* is associated with large differences in flowering time and that there are significant interactions between *FT* allelic variation and vernalization requirements in these species. This allelic variation provides an additional source of adaptive diversity to these economically important crops.

Materials and Methods

Genetic and Physical Maps. *SI Appendix*, section I, describes the accessions and markers used in the wheat- (SI Table 1) and barley- (SI Table 3) mapping populations. The complete list of the barley BACs used to construct the physical contigs and the sequencing coverage for each sequenced BAC is available in *SI Appendix*, section II. The phylogenetic analysis is described in *SI Appendix*, section III

Allelic Variation. The description of the materials used for the characterization of the *FT* allelic differences is included in *SI Appendix*, section IV. This includes a list of the wheat accessions tested for the presence of the retrotransposon insertion on the *TaFT* promoter (SI Table 4). The map comparisons used to determine the location of the QTLs for flowering time discovered in the cross Fredrickson × Stander (37) on barley chromosome arm 7HS also is included in this section.

Transcription Profiles and Transgenic Plants. The materials and methods used in the expression experiments are presented in *SI Appendix*, section V. This information includes the environmental conditions and the primers used in the quantitative PCR experiments (SI Table 5). The constructs and procedures used in the transgenic experiments are detailed in *SI Appendix*, section VI, whereas the statistical analyses for the interactions between *FT* and vernalization are presented in *SI Appendix*, section VII.

We thank the Okayama University (Kurashiki, Japan), the John Innes Centre (Norwich, U.K.), and the National Small Grain Collection (Aberdeen, ID) for the germplasm used in this study; L. Valarikova, Xiaoqin Zhang, Lydia Li, and Quyen Lam for excellent technical assistance; and Dr. P. San Miguel (Purdue University, West Lafayette, IN) for the BAC sequencing. This work was supported by U.S. Department of Agriculture National Research Initiative Grant 2005-02356. G.T. thanks the Argentinean Fulbright Commission for an Exchange Program grant.

1. Putterill J, Robson F, Lee K, Simon R, Coupland G (1995) *Cell* 80: 847–857.
2. Hayama R, Yokoi S, Tamaki S, Yano M, Shimamoto K (2003) *Nature* 422:719–722.
3. Kobayashi Y, Kaya H, Goto K, Iwabuchi M, Araki T (1999) *Science* 286:1960–1962.
4. Kardailsky I, Shukla VK, Ahn JH, Dagenais N, Christensen SK, Nguyen JT, Chory J, Harrison MJ, Weigel D (1999) *Science* 286:1962–1965.
5. Lifschitz E, Eviatar T, Rozman A, Shalit A, Goldshmidt A, Amsellem Z, Alvarez JP, Eshed Y (2006) *Proc Natl Acad Sci USA* 103:6398–6403.
6. Kojima S, Takahashi Y, Kobayashi Y, Monna L, Sasaki T, Araki T, Yano M (2002) *Plant Cell Physiol* 43:1096–1105.
7. Böhlenius H, Huang T, Charbonnel-Campaa L, Brunner AM, Jansson S, Strauss SH, Nilsson O (2006) *Science* 312:1040–1043.
8. Huang T, Bohlenius H, Eriksson S, Parcy F, Nilsson O (2005) *Science* 309:1694–1696.
9. Wigge PA, Kim MC, Jaeger KE, Busch W, Schmid M, Lohmann JU, Weigel D (2005) *Science* 309:1056–1059.
10. Abe M, Kobayashi Y, Yamamoto S, Daimon Y, Yamaguchi A, Ikeda Y, Ichinoki H, Notaguchi M, Goto K, Araki T (2005) *Science* 309:1052–1056.
11. Yan L, Loukoianov A, Blechl A, Tranquilli G, Ramakrishna W, SanMiguel P, Bennetzen JL, Echenique V, Dubcovsky J (2004) *Science* 303:1640–1644.
12. Michaels SD, Amasino RM (1999) *Plant Cell* 11:949–956.
13. Sheldon CC, Burn JE, Perez PP, Metzger J, Edwards JA, Peacock WJ, Dennis ES (1999) *Plant Cell* 11:445–458.
14. Searle I, He YH, Turck F, Vincent C, Fornara F, Krober S, Amasino RA, Coupland G (2006) *Gene Dev* 20:898–912.
15. Bastow R, Mylne JS, Lister C, Lippman Z, Martienssen RA, Dean C (2004) *Nature* 427:164–167.
16. Dubcovsky J, Loukoianov A, Fu D, Valarik M, Sanchez A, Yan L (2006) *Plant Mol Biol* 60:469–480.
17. Trevaskis B, Hemming MN, Peacock WJ, Dennis ES (2006) *Plant Physiol* 140:1397–1405.
18. Dubcovsky J, Chen C, Yan L (2005) *Mol Breed* 15:395–407.
19. Tranquilli GE, Dubcovsky J (2000) *J Hered* 91:304–306.
20. Yan L, Helguera M, Kato K, Fukuyama S, Sherman J, Dubcovsky J (2004) *Theor Appl Genet* 109:1677–1686.
21. Fu D, Szucs P, Yan L, Helguera M, Skinner J, Hayes P, Dubcovsky J (2005) *Mol Gen Genomics* 273:54–65.
22. Yan L, Loukoianov A, Tranquilli G, Helguera M, Fahima T, Dubcovsky J (2003) *Proc Natl Acad Sci USA* 100:6263–6268.
23. Loukoianov A, Yan L, Blechl A, Sanchez A, Dubcovsky J (2005) *Plant Physiol* 138:2364–2373.
24. Yasuda S (1969) *Barley Newsl* 12:57–58.
25. Law CN (1966) *Genetics* 53:487–498.
26. Law CN, Wolfe MS (1966) *Can J Genet Cytol* 8:462–470.
27. Law CN, Worland AJ (1997) *New Phytol* 137:19–28.
28. Chao S, Sharp PJ, Worland AJ, Warham EJ, Koebner RMD, Gale MD (1989) *Theor Appl Genet* 78:495–504.
29. Turner A, Beales J, Faure S, Dunford RP, Laurie DA (2005) *Science* 310:1031–1034.
30. Yan L, von Zitzewitz J, Skinner J, Hayes PM, Dubcovsky J (2005) *Genome* 48:905–912.
31. Dubcovsky J, Luo, MC, Zhong, GY, Bransteiter R, Desai A, Kilian A, Kleinhofs A, Dvorak J (1996) *Genetics* 143:983–999.
32. Yu Y, Tomkins JP, Waugh R, Frisch DA, Kudrna D, Kleinhofs A, Brueggeman RS, Muehlbauer GJ, Wise RP, Wing RA (2000) *Theor Appl Genet* 101:1093–1099.
33. Takahashi R (1983) *Catalogue of the Barley Germplasm Preserved at the Okayama University* (Institute of Agricultural and Biological Sciences, Okoyama University, Kurashiki, Japan).
34. Michaels SD, Himelblau E, Kim SY, Schomburg FM, Amasino RM (2005) *Plant Physiol* 137:149–156.
35. Suarez-Lopez P, Wheatley K, Robson F, Onouchi H, Valverde F, Coupland G (2001) *Nature* 410:1116–1120.
36. Takahashi R, Yasuda S (1971) in *Barley Genetics II (Proceedings of the Second International Barley Genetics Symposium*, ed Nilan RA (Washington State Univ Press, Pullman, WA), pp 388–408.
37. Mesfin A, Smith KP, Dill-Macky R, Evans CK, Waugh R, Gustus CD, Muehlbauer GJ (2003) *Crop Sci* 43:307–318.
38. Livak KJ, Schmittgen TD (2001) *Methods* 25:402–408.