Vernalization-induced flowering in cereals is associated with changes in histone methylation at the *VERNALIZATION1* gene

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Prolonged exposure to low temperatures (vernalization) accelerates the transition to reproductive growth in many plant species, including the model plant *Arabidopsis thaliana* and the economically important cereal crops, wheat and barley. Vernalization-induced flowering is an epigenetic phenomenon. In Arabidopsis, stable down-regulation of *FLOWERING LOCUS C* (*FLC*) by vernalization is associated with changes in histone modifications at *FLC* chromatin. In cereals, the vernalization response is mediated by stable induction of the floral promoter *VERNALIZATION1* (*VRN1*), which initiates reproductive development at the shoot apex. We show that in barley (*Hordeum vulgare*), repression of *HvVRN1* before vernalization is associated with high levels of histone 3 lysine 27 trimethylation (H3K27me3) at *HvVRN1* chromatin. Vernalization caused increased levels of histone 3 lysine 4 trimethylation (H3K4me3) and a loss of H3K27me3 at *HvVRN1*, suggesting that vernalization promotes an active chromatin state at *VRN1*. Levels of these histone modifications at 2 other flowering-time genes, *VERNALIZATION2* and *FLOWERING LOCUS T*, were not altered by vernalization. Our study suggests that maintenance of an active chromatin state at *VRN1* is likely to be the basis for epigenetic memory of vernalization in cereals. Thus, regulation of chromatin state is a feature of epigenetic memory of vernalization in Arabidopsis and the cereals; however, whereas vernalization-induced flowering in Arabidopsis is mediated by epigenetic regulation of the floral repressor *FLC*, this phenomenon in cereals is mediated by epigenetic regulation of the floral activator, *VRN1*.

epigenetic | MADS | intron | barley | chromatin

Plants respond to seasonal cues, such as temperature and day-length, to ensure that flowering coincides with favorable conditions. Prolonged exposure to low winter temperatures (vernalization) accelerates the progression from vegetative to reproductive growth in many plant species, including the temperate cereals (such as wheat and barley) and dicot species (such as Arabidopsis) (1–3). In both these lineages, plants retain a "memory" of the prolonged cold of winter, which stimulates flowering when days lengthen during spring (1–3). The memory of cold is then reset in the next sexual generation to ensure progeny are competent to respond to vernalization (1–3).

In Arabidopsis, the vernalization response is mediated by epigenetic regulation of the floral repressor, *FLOWERING LOCUS C* (*FLC*), which encodes a MADS-box transcription factor that represses genes involved in floral initiation, including *SUPPRESSOR OF CONSTANS 1* and *FLOWERING LOCUS T* (*FT*) (1, 4–6). *FLC* is expressed before vernalization and delays flowering, but its expression is repressed by vernalization (1, 4). *FLC* remains repressed when plants are subsequently exposed to warm temperatures, allowing activation of *FT*, which promotes flowering (1, 4). The stable down-regulation of *FLC* by vernalization is associated with an increase in the levels of repressive histone modifications at *FLC* chromatin, such as histone H3 lysine 27 di- and trimethylation (H3K27me2, H3K27me3), histone H3 lysine 9 dimethylation, and histone H4 arginine 3 symmetrical dimethylation, as well as the loss of histone modifications associated with active transcription, such as histone H3 acetylation and histone H3 lysine 4 di- and trimethylation (H3K4me2, H3K4me3) (7–13). Repression of *FLC* by vernalization involves the vernalization-dependent association of Polycomb-Group (PcG) complexes to *FLC* chromatin, which are required for addition and maintenance of H3K27me3 at *FLC* (14, 15). Taken together, these studies indicate that vernalization induces an alteration of *FLC* chromatin state from actively transcribed to stably repressed (7–15). The cellular memory of transcriptional repression of *FLC* is maintained during successive cell divisions by mitotic inheritance of repressive histone modifications at the gene (11), but active *FLC* transcription is restored in progeny, ensuring that the next generation is competent to respond to vernalization (1, 4, 16).

In temperate cereals, the vernalization response is mediated by the stable induction of a floral promoter, *VERNALIZATION1* (*VRN1*) (3, 17–19). *VRN1* encodes a FRUITFULL-like MADS-box transcription factor required for the initiation of reproductive development at the shoot apex (20–22). In vernalization-requiring cereal plants, *VRN1* is expressed at low levels and is induced by vernalization, with the level of expression being dependent on the length of cold exposure (17–19, 23–25). *VRN1* expression remains high when plants are exposed to warm temperatures following vernalization, and promotes the transition to reproductive development (17–19, 23–25). *VRN1* down-regulates the floral repressor *VERNALIZATION2* (*VRN2*), and allows long-day induction of the floral activator *FT* to accelerate subsequent stages of floral development (3, 24–26).

The vernalization response of *VRN1* shows characteristics of epigenetic regulation, in that *VRN1* is induced by vernalization, expression is maintained following vernalization, and the pre-vernalization level of *VRN1* expression is reset in the next generation (17–19, 23–25). In this article we analyze the effect of vernalization on the levels of histone modifications at the barley (*Hordeum vulgare*) *VRN1* gene (*HvVRN1*). Our study indicates that vernalization-induced flowering in cereals is mediated by epigenetic regulation of *VRN1* chromatin state. Our results suggest that regulation of the histone methylation status of *VRN1* chromatin is important for repression of *VRN1* before vernalization, for activation of *VRN1* by vernalization, and for maintaining a memory of vernalization following cold exposure.

Results

Activation of *HvVRN1* by Vernalization Is Associated with a Gain of H3K4me3 and a Loss of H3K27me3 at *HvVRN1* Chromatin. The effect of vernalization on the levels of 2 histone modifications at

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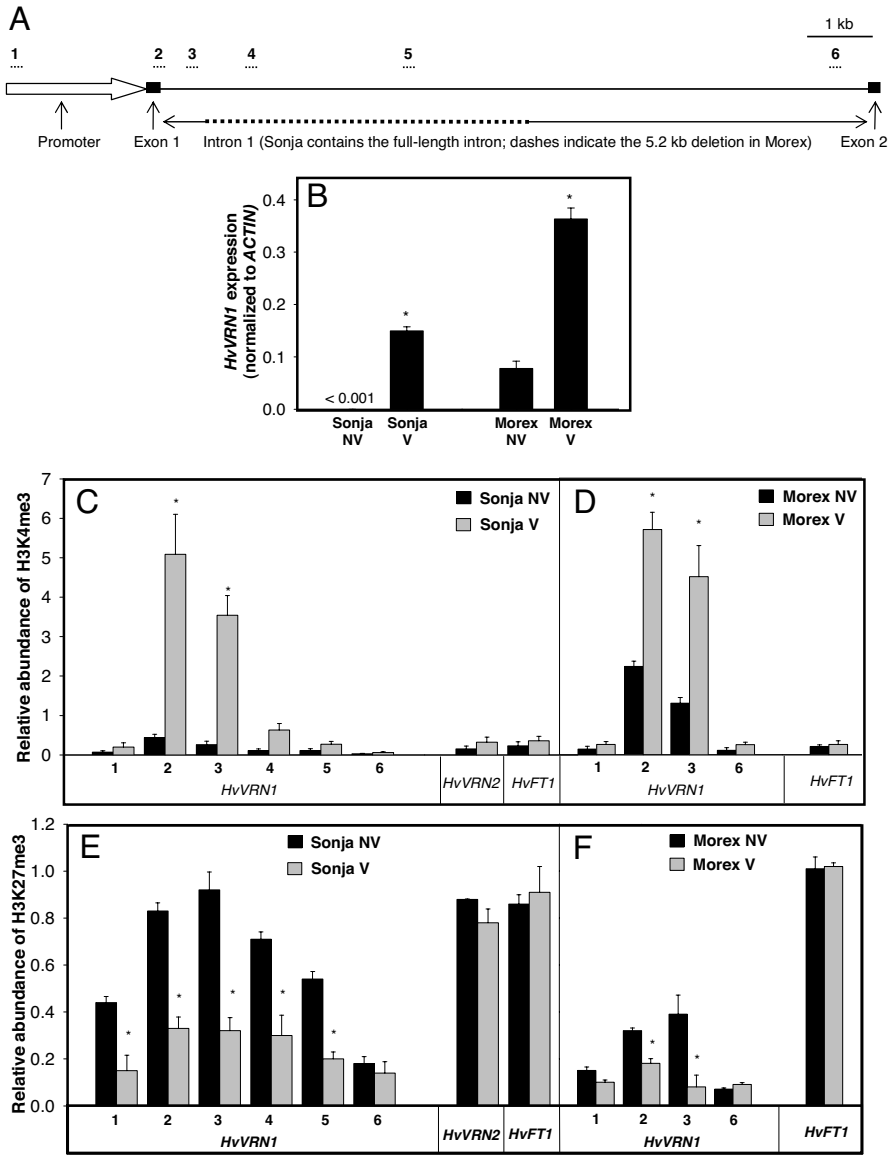

Fig. 1. The effect of vernalization on histone modifications at *HvVRN1*, *HvVRN2*, and *HvFT1* in barley seedlings. (*A*) Diagram of the 5′ end of *HvVRN1* showing the regions (1–6, *short dashed lines*) analyzed by ChIP, followed by quantitative real-time PCR. (*B*) *HvVRN1* expression in nonvernalized (NV) and vernalized (V) seedlings harvested immediately at the end of vernalization. Data represents the mean ± SEM from 6 biological replicates. (*C–F*) Relative abundance of H3K4me3 (*C* and *D*) and H3K27me3 (*E* and *F*) at *HvVRN1*, *HvVRN2*, and *HvFT1* in nonvernalized (NV) and vernalized (V) seedlings from the varieties Sonja (*C* and *E*) and Morex (*D* and *F*). Note that Morex contains a 5.2-kb deletion in the first intron of *HvVRN1* (regions 4 and 5 are deleted) and lacks *HvVRN2*. Data represents the mean ± SEM from at least 3 biological replicate experiments. An asterisk (*) indicates significantly different to NV ($P < 0.05$).

HvVRN1 chromatin was measured in the vernalization-responsive barley variety Sonja. Levels of H3K4me3, a modification associated with epigenetic inheritance of active gene transcription (27–30), and H3K27me3, a modification associated with long-term gene repression (28, 30, 31), were analyzed. The *HvVRN1* gene has a large (10.8 kb) first intron containing regions associated with regulation of *HvVRN1* expression (23, 32–34). We analyzed 6 regions across the 5′ end of the *HvVRN1* gene: the promoter (2 kb upstream of the translational start, region 1), exon 1 (region 2), and 4 sites along the length of the first intron (regions 3–6) (Fig. 1*A*). To assess both the immediate and long-term effects of vernalization on histone modifications at *HvVRN1*, we examined 2 developmental stages following seed-vernalization treatment: seedlings harvested immediately at the end of vernalization (see Fig. 1), and plants that had been transferred to normal glasshouse conditions at the end of vernalization treatment and allowed to develop to the third-leaf stage (approximately 2 weeks from the end of vernalization treatment) (Fig. 2).

In Sonja seedlings, vernalization caused an increase in the levels of H3K4me3 at regions 2 and 3 of *HvVRN1* (corresponding to exon 1 and the 5′ end of intron 1), but not regions 1, 4, 5, and 6 (see Fig. 1*C*); these results parallel the induction of *HvVRN1* expression by vernalization (see Fig. 1*B*), and are consistent with previous findings that H3K4me3 occurs around the start of transcription in actively transcribing genes (28, 30). Levels of H3K27me3 were reduced by vernalization in regions 1 to 5, but not in region 6 (see Fig. 1*E*), indicating a decrease in H3K27me3 across the promoter, first exon, and first intron of *HvVRN1* (average 63% reduction). The increase in H3K4me3 and loss of H3K27me3 at *HvVRN1* suggest that vernalization promotes an active state of *HvVRN1* chromatin.

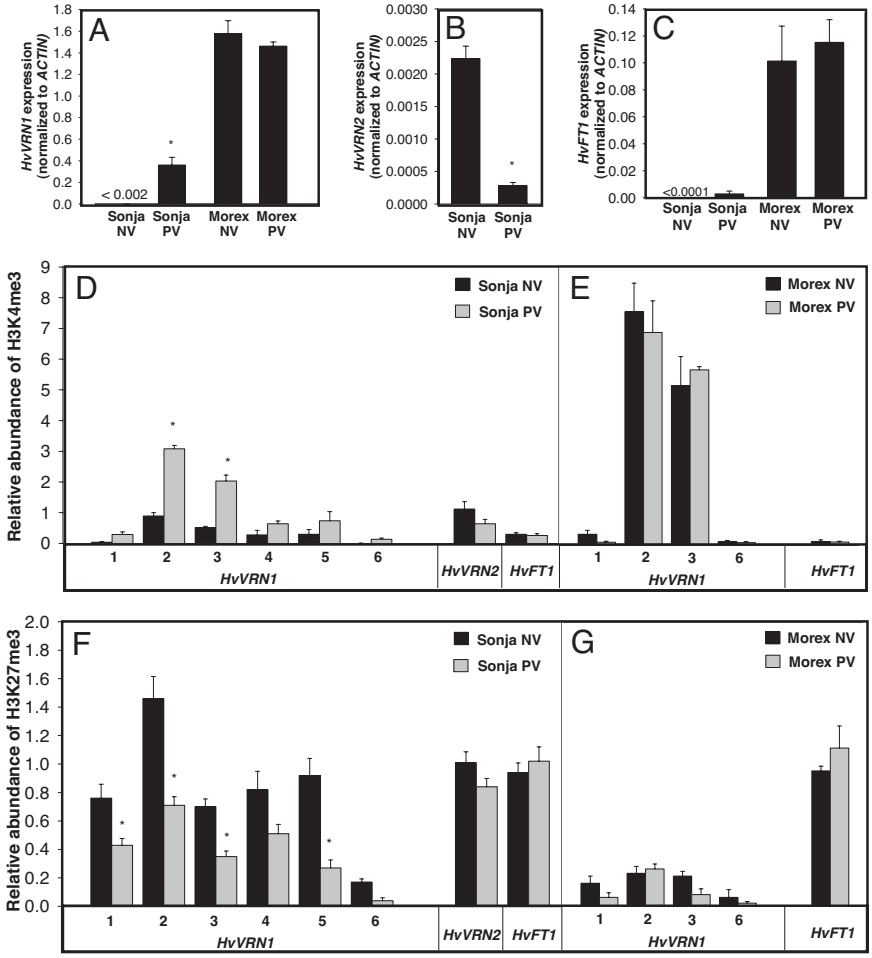

Fig. 2. The effect of seed vernalization on histone modifications at *HvVRN1*, *HvVRN2*, and *HvFT1* in leaves postvernalization. (*A–C*) *HvVRN1* (*A*), *HvVRN2* (*B*), and *HvFT1* (*C*) expression in nonvernalized (NV) and postvernalized (PV) leaves from the barley varieties Sonja and Morex. PV leaves were taken from plants at the third leaf stage grown in long days from vernalized seed. Data represents the mean ± SEM from 6 biological replicates. (*D–G*) Relative abundance of H3K4me3 (*D* and *E*) and H3K27me3 (*F* and *G*) at *HvVRN1*, *HvVRN2*, and *HvFT1* in nonvernalized (NV) and postvernalized (PV) leaves from Sonja (*D* and *F*) and Morex (*E* and *G*). *HvVRN1* regions analyzed are shown in Fig. 1*A*. Note that Morex contains a 5.2-kb deletion in the first intron of *HvVRN1* (regions 4 and 5 are deleted) and lacks *HvVRN2*. Data represents the mean ± SEM from at least 3 biological replicate experiments. An asterisk (*) indicates significantly different to NV ($P < 0.05$).

Repression of *HvVRN1* Expression Before Vernalization Is Associated with High Levels of H3K27me3. In nonvernalized Sonja seedlings, high levels of H3K27me3 were detected at the *HvVRN1* gene (see Fig. 1*E*), suggesting that the presence of H3K27me3 at *HvVRN1* chromatin is associated with repression of the gene before vernalization. In some varieties of wheat and barley, deletions within the first intron of *VRN1* are associated with activation of *VRN1* expression without vernalization treatment (23, 32–34). The barley variety Morex contains a large (5.2 kb) deletion in the first intron of *HvVRN1* (i.e., regions 4 and 5 are absent) (see Fig. 1*A*) (32), and Morex exhibits significantly higher *HvVRN1* expression in nonvernalized seedlings compared to Sonja ($P < 0.05$) (see Fig. 1*B*). In nonvernalized Morex seedlings, levels of H3K4me3 at regions 2 and 3 of *HvVRN1* were significantly higher than in Sonja ($P < 0.05$, compare NV in Fig. 1 *C* and *D*), while levels of H3K27me3 at regions 1, 2, and 3 of *HvVRN1* were significantly lower in Morex than Sonja ($P < 0.05$, compare NV in Fig. 1 *E* and *F*). These data show that the activation of *HvVRN1* in nonvernalized Morex seedlings is associated with low levels of H3K27me3 at *HvVRN1*. Taken together, these results indicate that repression of *HvVRN1* before vernalization is associated with high levels of H3K27me3 at the gene, and suggest that regions of the first intron could be important for determining levels of H3K27me3 at *HvVRN1* before vernalization.

Similar to Sonja, vernalization caused an increase in *HvVRN1* expression in Morex seedlings (see Fig. 1*B*), along with an increase in H3K4me3 and a reduction of H3K27me3 at regions 2 and 3 of *HvVRN1* (see Fig. 1 *D* and *F*). These results show that in a variety with high basal levels of *HvVRN1*, vernalization further activates *HvVRN1* chromatin state in seedlings.

Vernalization Does not Affect Levels of H3K4me3 and H3K27me3 at *HvVRN2* and *HvFT1* in Seedlings. In addition to *HvVRN1*, the effect of vernalization on histone modifications at *HvVRN2* and *HvFT1*, which regulate the long-day flowering response, was examined (3, 24–26, 35). *HvVRN2* and *HvFT1* do not contain a large first intron, and our analysis was restricted to 1 region within exon 1 for these 2 genes. Additionally, Morex lacks the *HvVRN2* locus (36, 37), so *HvVRN2* was only analyzed in Sonja.

HvVRN2 and *HvFT1* were expressed at low levels in seedlings (*HvVRN2*, undetectable; *HvFT1*, mean expression relative to *ACTIN* = less than 0.0002). In both nonvernalized and vernalized seedlings, *HvVRN2* and *HvFT1* contained low levels of H3K4me3 but were enriched for H3K27me3 (see Fig. 1 *C–F*). In contrast to *HvVRN1*, vernalization did not affect the levels of either histone modification at *HvVRN2* or *HvFT1* (see Fig. 1

C–F). These results agree with previous data showing that the seed-vernalization response in cereals involves cold-activation of *VRN1* independently of day-length response pathways (3, 24).

Histone Modifications at *HvVRN1* Are Maintained Postvernalization. A key feature of the vernalization response in cereals is the maintenance of high *VRN1* expression levels when plants are exposed to warm growth temperatures following vernalization (see Fig. 2*A*) (17–19, 23–25). In vernalization-requiring varieties, such as Sonja, this is associated with a rapid transition of the shoot apex from vegetative to reproductive development [supporting information (SI) Fig. S1]. Without vernalization, *VRN1* expression remains low (see Fig. 2*A*) and the shoot apex remains vegetative (see Fig. S1). We examined the long-term effect of seed vernalization on the levels of H3K4me3 and H3K27me3 at *HvVRN1* chromatin by measuring these histone modifications in plants grown from nonvernalized or vernalized seedlings. In Sonja, this revealed a similar pattern of histone modifications at *HvVRN1* to that observed in vernalized seedlings; in postvernalized Sonja leaves, H3K4me3 levels were higher in regions 2 and 3 of *HvVRN1* (see Fig. 2*D*), while H3K27me3 levels were lower across the gene (see Fig. 2*F*) compared to nonvernalized leaves. These data indicate that vernalization-induced changes in histone methylation at *HvVRN1* are maintained in leaves following seed vernalization.

Developmental Induction of *HvVRN1* Is Associated with High Levels of H3K4me3 and Low Levels of H3K27me3. In contrast to Sonja, Morex plants rapidly initiate flowering and *HvVRN1* is expressed in leaves without vernalization treatment (see Fig. 2A and Fig. S1) (23, 24). Morex plants derived from nonvernalized and vernalized seed had similar levels of *HvVRN1* expression (see Fig. 2*A*), which suggests that the high level of *HvVRN1* expression in these plants is because of developmental induction of *HvVRN1* independent of vernalization treatment. Consistent with the high level of *HvVRN1* expression in Morex, levels of H3K4me3 were high at regions 2 and 3 of *HvVRN1*, and levels of H3K27me3 were low across the gene in both nonvernalized and postvernalized leaves, and vernalization did not affect the levels of these modifications (see Fig. 2 *E* and *G*). These data show that the developmental induction of *HvVRN1* in a variety that flowers without vernalization is associated with high levels of H3K4me3 and low levels of H3K27me3 at the gene.

***HvVRN2* and *HvFT1* Remain Enriched for H3K27me3 Postvernalization.** Expression of *HvVRN2* and *HvFT1* is influenced by exposure to long days following vernalization treatment (3, 24–26, 35). Seed vernalization treatment caused a reduction in *HvVRN2* expression in Sonja leaves (see Fig. 2*B*), while *HvFT1* expression was induced but remained low (see Fig. 2*C*). *HvFT1* expression was significantly higher in Morex compared to Sonja, regardless of vernalization treatment ($P < 0.05$) (see Fig. 2*C*), probably because of either the absence of *HvVRN2*, a repressor of *HvFT1*, in Morex (36, 37), or the particular *HvFT1* allele present in Morex (35). Vernalization did not affect the levels of H3K4me3 or H3K27me3 at *HvVRN2* or *HvFT1* in leaves, and both genes were enriched for H3K27me3 (see Fig. 2 *D–G*). These data indicate that, unlike *VRN1*, regulation of expression of *VRN2* and *FT* in response to vernalization is not associated with changes in the levels of H3K4me3 and H3K27me3.

Discussion

In this article, we have analyzed the effect of vernalization on histone modifications at the *HvVRN1* gene. Our study demonstrates that induction of *HvVRN1* transcription by vernalization involves changes in histone methylation at *HvVRN1*. Before vernalization, the occurrence of H3K27me3 at *HvVRN1* is associated with repression of the gene. Vernalization causes increased levels of H3K4me3 and decreased levels of H3K27me3 at *HvVRN1* chromatin, and these changes are retained postvernalization, suggesting that vernalization promotes an active state of *HvVRN1* chromatin that is maintained following vernalization treatment. Our study suggests that the memory of vernalization in cereals involves epigenetic inheritance of histone modifications associated with active transcription at *VRN1*.

In plants and other organisms, maintenance of H3K4me3 and H3K27me3 levels is achieved through the action of Trithorax-group and PcG protein complexes, respectively (38–43). Putative core Trithorax-group and PcG complex components and potential interacting proteins have been identified in cereals (44, 45), and it is likely that these complexes are involved in the addition and maintenance of H3K4me3 and H3K27me3 at *VRN1*. Repression of the floral activation genes *AGL19* and *FT* in Arabidopsis involves PcG-mediated maintenance of H3K27me3 at these genes (40, 46). The occurrence of H3K27me3 at *VRN1* and repression of the gene before vernalization may be mediated by PcG complexes, which may require PcG-binding elements within the gene, although such elements have not yet been defined in plants (42). In Arabidopsis, regions of the first intron of *FLC* are required for the maintenance of *FLC* repression following vernalization (47), and the vernalization-dependent association of a PcG complex to *FLC* occurs specifically within the first intron (15). Deletions within the first intron of *VRN1* that are associated with high basal *VRN1* expression may lack PcG-binding elements, resulting in lower H3K27me3 levels. The vernalization-induced loss of H3K27me3 at *VRN1* may result from histone demethylase activity, but H3K27me3 demethylases have not yet been identified in plants.

VRN2 and *FT* are involved in determining flowering-time as part of the day-length response pathway in cereals (3, 24–26, 35). Both *VRN2* and *FT* are repressed in germinating seedlings and are marked by H3K27me3, suggesting that they are targets of PcG-mediated repression in this tissue. In Arabidopsis, PcG-mediated H3K27me3 at *FT* chromatin limits expression of *FT*, and mutations of PcG-complex components cause *FT* induction and precocious flowering (46). Here, we have shown that H3K27me3 enrichment at *FT* chromatin occurs in cereals, suggesting that PcG complexes play a conserved role in the repression of *FT* in both Arabidopsis and the cereals. In contrast to *VRN1*, vernalization did not affect the levels of H3K4me3 or H3K27me3 at *VRN2* and *FT*, suggesting that vernalization directs changes in the chromatin state at *VRN1* but not *VRN2* or *FT*.

In conclusion, our study has shown that, like Arabidopsis, vernalization in barley results in an alteration of chromatin state at a key floral regulatory gene. However, in contrast to the PcG-mediated repression of *FLC* in Arabidopsis, vernalization promotes an active chromatin state of *VRN1* in barley. A key question to be addressed in the future is how the plant's perception of cold directs the changes in the chromatin state of these genes.

Materials and Methods

Plant Material. For seed vernalization treatments, barley seeds were sown in soil, pots were covered with aluminium foil, and then incubated at 2 °C ± 1 °C for 49 days as previously described (25); at this time-point, seedlings have germinated and show no signs of etiolation. Previous experiments showed that vernalization for 35 to 63 days accelerated flowering (25), and so a vernalization treatment of 49 days was chosen for the present study. Vernalized seedlings (above-ground coleoptiles plus internal leaves, ≈3 cm in length after 49 days vernalization) were harvested directly in the cold. Nonvernalized seedlings were derived from seeds sown in aluminium foil-covered pots of soil and germinated in the dark in a glasshouse at 19 °C ± 2 °C; seedlings were harvested 5 days after sowing, at which time the coleoptiles were the same length as vernalized seedlings. At this time-point, the shoot apex is vegetative (see Fig. S1). For the analysis of postvernalized leaves, pots of vernalized seed were transferred to sunlit glasshouses, the foil was removed, and plants were

maintained under long days (16-h light/8-h dark, with supplementary lighting used when natural light levels dropped below 200 μE). Leaf tissue (the third leaf from each plant) was harvested in the middle of the light period at the third-leaf stage (Zadoks scale Z = 13) (48), which occurred approximately 2 weeks after vernalization. Nonvernalized leaf tissue was harvested from plants maintained in the same glasshouse from nonvernalized seed, also at the third-leaf stage.

RNA Isolation, cDNA Synthesis, and Quantitative PCR. Total RNA was isolated using the Qiagen RNeasy Plant Miniprep kit (Qiagen), then treated with DNase (Promega) and used for cDNA synthesis with an oligo dT primer and SuperScript III (Invitrogen) according to the manufacturer's instructions. Quantitative real-time PCR was performed to measure levels of gene expression using a Rotor-Gene 3000 Real-Time Cycler (Corbett Research) as previously described (26). DNA concentrations were determined using the comparative quantification method as previously described (9). *ACTIN* was used for normalization of gene expression as described previously (24, 26). Primer pairs for *HvVRN1*, *HvVRN2*, *HvFT1*, and *ACTIN* have previously been described (24, 26). Expression results for each gene were measured in the same PCR run in Sonja and Morex.

Chromatin Immunoprecipitation. For the ChIP analysis of leaf tissue, ChIP was performed essentially as previously described (49), with some modifications. Leaf tissue was harvested and immediately cross-linked under vacuum for 10 min in buffer containing 0.4 M sucrose, 10 mM Tris (pH 8), 1 mM EDTA, 1 mM PMSF, and 1% formaldehyde; then glycine was added to a final concentration of 0.1 M and the vacuum was reapplied for 5 min. Leaves were briefly rinsed in water, frozen in liquid nitrogen, and stored at −80 °C. For the isolation of chromatin, leaves (2 g) were ground in liquid nitrogen and added to 5-ml lysis buffer [50 mM Hepes (pH 7.5), 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.1% sodium deoxycholate, 0.1% SDS, 1 mM PMSF] containing 100 μl of Complete protease inhibitors (Roche, 1 tablet dissolved in 1-ml lysis buffer). Samples were subjected to sonication to shear the DNA into fragments of $\approx$500 bp, and then centrifuged for 1 min at 16,000 $\times$ *g* (4 °C) to pellet debris. The supernatant was removed to a fresh tube, a further 100 μl of Complete protease inhibitors was added, and precleared with Protein A agarose beads (Upstate) for 1 h at 4 °C on a rotary shaker. Following centrifugation for 2 min at 800 $\times$ *g* (4 °C), aliquots of the supernatant were taken for immunoprecipitation with the appropriate antibody. The extract and antibody was incubated overnight at 4 °C on a rotary shaker, then Protein A agarose beads were added and the incubation continued for an additional 2 h. The beads were then washed with 2$\times$ low salt buffer, 2$\times$ medium salt buffer, and 1$\times$ LNDET buffer, on a rotary shaker at 4 °C for 10 min per wash. A further 2$\times$ TE washes were performed for 5 min per wash, with a final 1$\times$ TE wash at room temperature. Immunoprecipitates were eluted from the beads with 2 $\times$ 150 μl incubations with 1% SDS, 0.1 M $NaHCO_3$ on a rotary shaker at room temperature for 15 min. Reverse cross-linking was performed as previously described (49), followed by a DNA cleanup with the Qiagen Qiaquick PCR cleanup kit (Qiagen). DNA was then used for quantitative real-time PCR as described below. For the ChIP analysis of seedlings, nuclei were extracted from 2-g seedlings as previously described (50), and the resultant chromatin was used for immunoprecipitation, as described for leaf tissue. Antibodies recognizing H3K4me3 and H3K27me3 were obtained from Upstate Biotechnology. The antibody against histone H3 was purchased from Abcam.

The amount of genomic DNA precipitated in ChIP assays was quantified by quantitative real-time PCR as described above. Primer pairs are shown in Table S1; each was verified to amplify a single product consisting of the expected target sequence. For each primer pair, the amount of DNA precipitated using anti-H3K4me3 or anti-H3K27me3 antibodies was normalized to the amount precipitated by an anti-H3 antibody from the same sample to correct for differences in ChIP input DNA. The genes for *ACTIN* (enriched for H3K4me3) (Fig. S2) or *BARLEY MADS 9* (51) (*BM9*, enriched for H3K27me3) (Fig. S3) were used for normalization to compare vernalized and nonvernalized samples (expression of these genes does not change with vernalization). No-antibody control reactions were performed in parallel with each antibody reaction to verify that the immunoprecipitated DNA was enriched for the control genes (*ACTIN* for H3K4me3, *BM9* for H3K27me3, all for H3). Take-off values for *ACTIN* in the H3K4me3 immunoprecipitations and *BM9* in the H3K27me3 immunoprecipitations were comparable (within 1 cycle) between Sonja and Morex samples, enabling a direct comparison of the normalized H3K4me3 and H3K27me3 levels between Sonja and Morex. The data presented is the relative amount of precipitated DNA normalized to either *ACTIN* (for H3K4me3) or *BM9* (for H3K27me3), and each graph represents the mean of at least 3 biological replicate experiments $\pm$ SEM.

Statistical Analysis. Data were analyzed using the Student's *t* test and deemed significant if $P < 0.05$.

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1. Sheldon CC, Rouse DT, Finnegan EJ, Peacock WJ, Dennis ES (2000) The molecular basis of vernalization: the central role of *FLOWERING LOCUS C* (*FLC*). *Proc Natl Acad Sci USA* 97:3753–3758.
2. Sung S, Amasino R (2005) Remembering winter: Toward a molecular understanding of vernalization. *Ann Rev Plant Biol* 56:491–508.
3. Trevaskis B, Hemming MN, Dennis ES, Peacock WJ (2007) The molecular basis of vernalization-induced flowering in cereals. *Trends Plants Sci* 12:352–357.
4. Michaels SD, Amasino RM (1999) *FLOWERING LOCUS C* encodes a novel MADS domain protein that acts as a repressor of flowering. *Plant Cell* 11:949–956.
5. Helliwell CA, Wood CC, Robertson M, Peacock WJ, Dennis ES (2006) The Arabidopsis FLC protein interacts directly in vivo with *SOC1* and *FT* chromatin and is part of a high molecular-weight protein complex. *Plant J* 46:83–192.
6. Searle I, et al. (2006) The transcription factor FLC confers a flowering response to vernalization by repressing meristem competence and systemic signaling in Arabidopsis. *Genes Dev* 20:898–912.
7. Bastow R, et al. (2004) Vernalization requires epigenetic silencing of *FLC* by histone methylation. *Nature* 427:164–167.
8. Sung S, Amasino R (2004) Vernalization in Arabidopsis thaliana is mediated by the PHD finger protein VIN3. *Nature* 427:159–164.
9. Finnegan EJ, et al. (2005) The downregulation of *FLOWERING LOCUS C* (*FLC*) expression in plants with low levels of DNA methylation and by vernalization occurs by distinct mechanisms. *Plant J* 44:420–432.
10. Sung S, et al. (2006) Epigenetic maintenance of the vernalized state in *Arabidopsis thaliana* requires LIKE HETEROCHROMATIN PROTEIN 1. *Nat Genet* 38:706–710.
11. Finnegan EJ, Dennis ES (2007) Vernalization-induced trimethylation of histone H3 lysine 27 at *FLC* is not maintained in mitotically quiescent cells. *Curr Biol* 17:1978–1983.
12. Greb T, et al. (2007) The PHD finger protein VRN5 functions in the epigenetic silencing of *Arabidopsis FLC. Curr Biol* 17:73–78.
13. Schmitz RJ, Sung S, Amasino RM (2008) Histone arginine methylation is required for vernalization-induced epigenetic silencing of *FLC* in winter-annual *Arabidopsis thaliana. Proc Natl Acad Sci USA* 105:411–416.
14. Wood CC, et al. (2006) The *Arabidopsis thaliana* vernalization response requires a polycomb-like protein complex that also includes VERNALIZATION INSENSITIVE 3. *Proc Natl Acad Sci USA* 103:14631–14636.
15. De Lucia F, Crevillen P, Jones AME, Greb T, Dean C (2008) A PHD-Polycomb Repressive Complex 2 triggers the epigenetic silencing of *FLC* during vernalization. *Proc Natl Acad Sci USA* 105:16831–16836.
16. Sheldon CC, et al. (2008) Resetting of *FLOWERING LOCUS C* expression after epigenetic repression by vernalization. *Proc Natl Acad Sci USA* 105:2214–2219.
17. Danyluk J, et al. (2003) TaVRT-1, a putative transcription factor associated with vegetative to reproductive transition in cereals. *Plant Physiol* 132:1849–1860.
18. Trevaskis B, Bagnall DJ, Ellis MH, Peacock WJ, Dennis ES (2003) *MADS box* genes control vernalization-induced flowering in cereals. *Proc Natl Acad Sci USA* 100:13099–13104.
19. Yan L, et al. (2003) Positional cloning of the wheat vernalization gene *VRN1*. PNAS 100:6263–6268.
20. Murai K, Miyamae M, Kato H, Takumi S, Ogihara Y (2003) WAP1, a wheat *APETALA1* homolog, plays a central role in the phase transition from vegetative to reproductive growth. *Plant Cell Physiol* 44:1255–1265.
21. Preston JC, Kellogg EA (2006) Reconstructing the evolutionary history of paralogous *APETALA1/FRUITFULL*-Like genes in grasses (*Poaceae*). *Genetics* 174:421–437.
22. Shitzukawa N, et al. (2007) The einkorn wheat (*Triticum monococcum*) mutant, *maintained vegetative phase*, is caused by a deletion in the *VRN1* gene. *Genes Genet Syst* 82:167–170.
23. von Zitzewitz J, et al. (2005) Molecular and structural characterization of barley vernalization genes. *Plant Mol Biol* 59:449–467.
24. Trevaskis B, Hemming MN, Peacock WJ, Dennis ES (2006) *HvVRN2* responds to daylength, whereas *HvVRN1* is regulated by vernalization and developmental status. *Plant Physiol* 140:1397–1405.
25. Sasani S, et al. The influence of vernalization and daylength on the expression of flowering-time genes in the leaves and shoot apex of barley (*Hordeum vulgare*). *J Exp Bot*, in press.
26. Hemming MN, Peacock WJ, Dennis ES, Trevaskis B (2008) Low-temperature and daylength cues are integrated to regulate *FLOWERING LOCUS T* in barley. *Plant Physiol* 147:355–366.
27. Kim TH, et al. (2005) A high-resolution map of active promoters in the human genome. *Nature* 436:876–880.
28. Roh TY, Cuddapah S, Cui K, Zhao K (2006) The genomic landscape of histone modifications in human T cells. *Proc Natl Acad Sci USA* 103:15782–15787.
29. Wysocka J, et al. (2006) A PHD finger of NURF couples histone H3 lysine 4 trimethylation with chromatin remodeling. *Nature* 442:86–90.
30. Barski A, et al. (2007) High-resolution profiling of histone methylations in the human genome. *Cell* 129:823–837.
31. Zhang X, et al. (2007) Whole-genome analysis of histone H3 lysine 27 trimethylation in Arabidopsis. *PLOS Biol* 5:1026–1035.

32. Fu D, et al. (2005) Large deletions within the first intron in *VRN-1* are associated with spring growth habit in barley and wheat. *Mol Gen Genomics* 273:54–65.
33. Cockram J, et al. (2007) Haplotype analysis of vernalization loci in European barley germplasm reveals novel *VRN-H1* alleles and a predominant winter *VRN-H1/VRN-H2* multi-locus haplotype. *Theor Appl Genet* 115:993–1001.
34. Szucs P, et al. (2007) Validation of the *VRN-H2/VRN-H1* epistatic model in barley reveals that intron length variation in *VRN-H1* may account for a continuum of vernalization sensitivity. *Mol Genet Genomics* 277:249–261.
35. Yan L, et al. (2006) The wheat and barley vernalization gene *VRN3* is an orthologue of *FT*. *Proc Natl Acad Sci USA* 103:19581–19586.
36. Yan L, et al. (2004) The wheat *VRN2* gene is a flowering repressor down-regulated by vernalization. *Science* 303:1640–1644.
37. Dubcovsky J, Chen C, Yan L (2005) Molecular characterization of the allelic variation at the *VRN-H2* vernalization locus in barley. *Mol Breed* 15:395–407.
38. Hanson RD, et al. (1999) Mammalian Trithorax and Polycomb-group homologues are antagonistic regulators of homeotic development. *Proc Natl Acad Sci USA* 96:14372–14377.
39. Gendall A, Levy Y, Wilson A, Dean C (2001) The *VERNALIZATION* 2 gene mediates the epigenetic regulation of vernalization in Arabidopsis. *Cell* 107:525–535.
40. Schoenrock N, et al. (2006) Polycomb-group proteins repress the floral activator *AGL19* in the *FLC*-independent vernalization pathway. *Genes Dev* 20:1667–1678.
41. Saleh A, Al-Abdallat A, Ndamukong I, Alvarez-Venegas R, Avramova Z (2007) The *Arabidopsis* homologs of trithorax (ATX1) and enhancer of zeste (CLF) establish ''bivalent chromatin marks'' at the silent *AGAMOUS* locus. *Nucleic Acids Res* 35:6290–6296.
42. Schuettengruber B, Chourrout D, Vervoort M, Leblanc B, Cavalli G (2007) Genome regulation by Polycomb and Trithorax proteins. *Cell* 128:735–745.
43. Pien S, et al. (2008) ARABIDOPSIS TRITHORAX1 dynamically regulates *FLOWERING LOCUS C* activation via histone 3 lysine 4 trimethylation. *Plant Cell* 49:580–588.
44. Springer NM et al. (2003) Comparative analysis of SET domain proteins in maize and Arabidopsis reveals multiple duplications preceding the divergence of monocots and dicots. *Plant Physiol* 132:907–925.
45. Fu D, Dunbar M, Dubcovsky J (2007) Wheat *VIN3*-like PHD finger genes are up-regulated by vernalization. *Mol Genet Genomics* 277:301–313.
46. Jiang D, Wang U, Wang Y, He Y (2008) Repression of *FLOWERING LOCUS C* and *FLOWERING LOCUS T* by the *Arabidopsis* Polycomb Repressive Complex 2 components. *PLoS ONE* 3:e3404.
47. Sheldon CC, Conn AB, Dennis ES, Peacock WJ (2002) Different regulatory regions are required for the vernalization-induced repression of *FLOWERING LOCUS C* and for the epigenetic maintenance of repression. *Plant Cell* 14:2527–2537.
48. Zadoks JC, Chang TT, Konzak CF (1974) A decimal code for the growth stage of cereals. *Weed Res* 14:415–421.
49. Johnson LM, Cao X, Jacobsen SE (2002) Interplay between two epigenetic marks: DNA methylation and histone H3 lysine 9 methylation. *Curr Biol* 12:1360–1367.
50. Gendrel A, Lippman Z, Martienssen R, Colot V (2005) Profiling histone modification patterns in plants using genomic tiling microarrays. *Nat Meth* 3:213–218.
51. Schmitz J, et al. (2000) Cloning, mapping and expression analysis of barley *MADS-box* genes. *Plant Mol Biol* 42:899–913.