nature plants

REVIEW ARTICLE

https://doi.org/10.1038/s41477-018-0301-z

Remembering winter through vernalisation

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Vernalisation is the programmed physiological process in which prolonged cold-exposure provides competency to flower in plants; widely found in winter and biennial species, such as *Arabidopsis*, fruit trees, vegetables and wheat. This phenomenon is regulated by diverse genetic networks, and memory of vernalisation in a life cycle mainly depends on epigenetic mechanisms. However, less is known about how to count winter-dosage for flowering in plants. Here, we compare the vernalisation genetic framework between the dicots *Arabidopsis*, temperate grasses, wheat, barley and *Brachypodium*. We discuss vernalisation mechanisms involving crosstalk between phosphorylation and *O*-GlcNAcylation modification of key proteins, and epigenetic modifications of the key gene *VRN1* in wheat. We also highlight the potential evolutionary origins of vernalisation in various species. Current progress toward understanding the regulation of vernalisation requirements provides insight that will inform the design of molecular breeding strategies for winter crops.

As sessile organisms, plants are constantly exposed to various external cues, and adjust their cell processes, metabolism and development according to the many environmental stresses they encounter. Thus, how plants respond to these environmental changes with season switches in their life cycle is a crucial issue.

Sensing seasons in plant development

Adaptation to environmental changes of the seasons allows sessile plants to survive and grow around the world. One major factor of plant adaptation to their ambient environment is the seasonal changes that trigger different developmental stages that protect plants from extreme weather stress.

Plants adjust their growth and development to the ambient environment. Low temperature is a critical factor that influences the growth, development and geographic distribution of crops[1–3]. The different capacities of crops to adapt to temperature extremes determine their geographical regions and planting times. For example, rice cultivation is limited to warm regions because lower yearly temperatures severely restrict the growth and grain-yield of rice plants[4,5]. Conversely, wheat can be cultivated in a broad swath of temperate regions because it can tolerate frosts and cold winter temperatures[6,7]. For winter wheat, prolonged exposure to cold temperatures is required to initiate the transition from vegetative growth to flowering (Fig. 1)[8–10]. How plants monitor the changing environment and adjust their growth to seasonal cues, such as cold stress and the transition from vegetative growth to flowering, is a fundamental question in determining how plants flower at the proper time and avoid frost damage to improve crop yield.

Cold-acclimation and vernalisation. In temperate regions, winter cereals are planted in autumn and flower in spring. One of the determining factors of flowering for winter cereals is the duration of exposure to the winter cold[11]. Sufficient exposure to low temperatures (0–10°C for about one month or more) enables flowering in winter cereals soon after the return of warm temperatures in spring—this process is known as vernalization[11]. Accordingly, perception of the chilling signal to trigger the defence response is a primary event during vernalisation. Perception of low temperature by plants likely invokes two sequential processes: sensing the chilling to induce tolerance responses that enable cold acclimation; and counting the dosage of vernalisation for future developmental transitions. It is not yet clear how chilling is sensed in wheat, but the COLD1–RGA1 complex is critical for cold-sensing in rice[12]. COLD1, a regulator of G-protein signalling (RGS), interacts with rice G-protein α subunit 1 (RGA1) to activate Ca^{2+} influx into the cytoplasm and triggering downstream responses, such as expression of genes encoding the transcription factors INDUCER OF CBF EXPRESSION 1 (ICE1) (also known as OsbHLH002) and C-REPEAT-BINDING FACTORS (CBFs) for chilling tolerance[12]. These downstream signalling pathways, such as the CBF-dependent signalling pathway and the mitogen-activated protein kinase 3 (MPK3)–OsbHLH002–trehalose-6-phosphate phosphatase 1 (OsTPP1) pathway, alter the expression of key cold-response genes and cause the accumulation of metabolites that enhance cold tolerance[1,13].

Wheat is planted in autumn to allow plants to acclimate to lower temperatures and subsequently tolerate prolonged cold during winter[14–16]. In the conserved cold-acclimation pathway, cold-activated ICE1 increases the expression of *CBF* genes to initiate expression of *COLD REGULATED* (*COR*) genes, which allow plants to acquire freezing tolerance[17]. The earlier and more up-regulated the *CBF* and *COR* genes are a result of cold and confer faster cold-acclimation to wheat[15,18]. Whereas increased levels of vernalisation gene, *VRN1*, mRNA after vernalisation can reduce the transcription of *CBF* and *COR* genes to cold[8,15]. Therefore, low basal levels of *VRN1* in autumn facilitate the up-regulation of *CBF* and *COR* genes in cold weather, which initiates the cold-acclimation process and improves frost-tolerance in temperate cereals[15]. In *Brachypodium distachyon* accessions with a vernalisation requirement, a similar cold-acclimation response was shown by gradually accumulating sugars, proline and *COR* gene transcripts[19]. Alternative genes, such as *COLD-INDUCED SMALL PROTEIN 1* (*CISP1*), *DEHYDRATION-RESPONSIVE ELEMENT-BINDING PROTEINS 2/3* (*DREB2/3*) and *LATE EMBRYOGENESIS ABUNDANT* (*LEA*) that were also involved in the regulation of cold acclimation in temperate grasses[7,20,21]. Cold-acclimation of cereal crops in autumn lays the foundation for their survival in the following vernalisation process during the winter. In addition to cereal crops, many herbaceous plants (known as 'biennials' or 'winter annuals') also require vernalisation. Although flowering is regulated by the interaction of multiple genetic pathways (such as the autonomous

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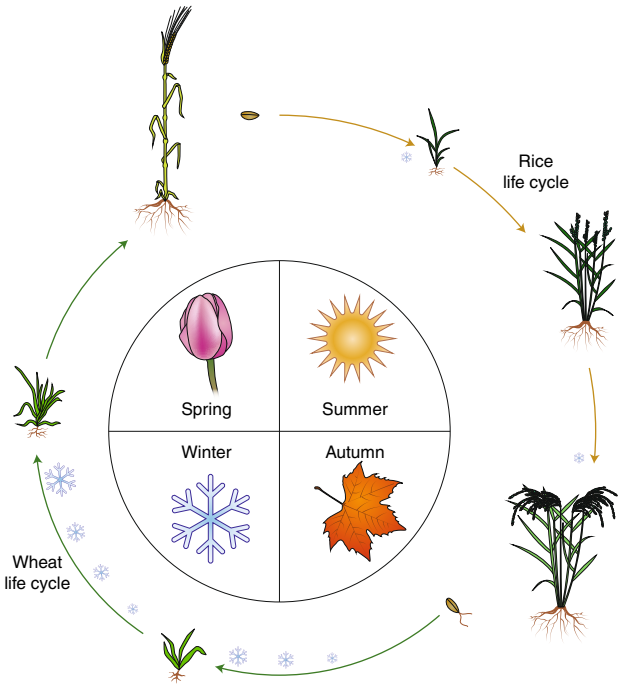

Fig. 1 | Life-cycle transitions of winter wheat and rice in response to seasonal changes. Winter wheat and rice grow in different seasons due to environmental constraints. Winter wheat can be planted in autumn to acquire frost tolerance. The prolonged low temperatures of winter delay flowering and, in spring, vernalisation and photoperiod determine when the wheat flowers. Rice shows hypersensitivity to cold stress at the seedling and reproductive stages, thus restricting its life cycle to warm conditions (for example, in northern China, rice is sown in the early summer and harvested in the autumn). The life-cycles of wheat and rice are shown by the green and yellow arrows, respectively. The snowflakes represent stages that may suffer cold stress.

and photoperiod pathways) with other variables, such as age and hormone levels (for example, gibberellin (GA) levels), vernalisation is a critical factor in promoting the floral transition in *Arabidopsis*[22]. The timing of this transition to flowering is strongly influenced by ambient environmental conditions, such as day-length and winter temperatures[23,24]. These intricate genetic networks evolved to monitor endogenous and environmental signals to precisely regulate flowering time and ensure reproductive success[25,26].

The molecular genetic network of vernalisation

Vernalisation genes with the same name but representing different genes in *Arabidopsis* and temperate grasses (wheat, barley and *B. distachyon*), such as *VRN1* and *VRN2*, are involved in the genetic network for vernalisation (Table 1)[26–28] and there is distinct diversity between the genetic pathways of vernalisation-mediated flowering in these plants. For instance, the flowering repressor gene *FLC* is suppressed by vernalisation to initiate the floral transition in spring in *Arabidopsis*[29]. In contrast, the return of warmth induces the flowering promoting gene *VRN1* to maintain higher transcript levels to ensure spring flowering in wheat and barley (Fig. 2)[30,31].

***Arabidopsis* genetic network.** There are a series of key genes involved in vernalisation in *Arabidopsis*, including *FRIGIDA* (FRI)[32] and *FLOWERING LOCUS C* (*FLC*, a MADS-box transcription factor)[33,34]. *FRI* encodes a scaffold protein that increases transcription of *FLC* by forming a large transcription activator complex composed of FRI, FRI-LIKE 1 (FRL1), FRI ESSENTIAL 1 (FES1), SUPPRESSOR OF FRI 4 (SUF4) and FLC EXPRESSOR (FLX)[35]. This complex recruits transcription factors and chromatin modifiers to activate *FLC* transcription[35]. *FLC* delays flowering in a dose-dependent manner[29,36]. High levels of *FLC* expression repress transcription of the two floral regulatory genes *FLOWERING LOCUS T* (*FT*) and *SUPPRESSOR OF OVEREXPRESSION OF CO 1* (*SOC1*), resulting in delayed flowering (Fig. 2)[37,38].

Repression of *FLC* by prolonged cold requires many genes, such as *Arabidopsis thaliana VERNALIZATION 1* (At*VRN1*), *VERNALIZATION 2* (At*VRN2*), and *VERNALIZATION INSENSITIVE 3* (*VIN3*), which are involved in histone modifications at *FLC* (refs [39–42]). At*VRN1* encodes a protein containing a B3 DNA-binding domain and a PEST region that regulates the acetylation and methylation of histone 3 at the *FLC* locus, thereby repressing its expression[41]. At*VRN2* encodes a C2H2-type zinc finger nucleoprotein, similar to the FIS2 and EMF2 polycomb group (PcG) proteins in plants and the Su(z) protein in animals[40]. At*VRN2* is one component of POLYCOMB-GROUP REPRESSIVE COMPLEX 2 (PRC2), which is required to stably maintain the repression of *FLC* on return to warmth but does not function in its initial down-regulation during vernalization[40]. Another polycomb group gene, *CURLY LEAF* (*CLF*), an orthologue of the enhancer of zeste (E(z)), encodes an H3K27me3 methyltransferase[43]. CLF is required for the repression of *FLC* by vernalisation and plays an important role in spreading H3K27me3 across the *FLC* locus after vernalization[43–45]. *VIN3*, encoding a chromatin-remodelling plant homeodomain (PHD) finger protein, is transcriptionally induced by vernalisation and returns to basal levels under warm temperatures[46]. During vernalisation, VIN3 represses *FLC* transcription by recruiting PRC2, which catalyses tri-methylation of H3K27 at the nucleation region of *FLC* (refs [39,44,47]). VIL1 (also known as VRN5) is a paralogue of VIN3 but is not induced by vernalization[48]. During vernalisation, VIN3 interacts with VRN5/VIL1 to form a heterodimer that maintains the vernalisation-inducing histone modifications required for silencing *FLC*, such as deacetylation and H3K27me3 (ref. [48]). TERMINAL FLOWER 2 (TFL2; also known as LIKE HETEROCHROMATIN PROTEIN 1, LHP1) recognizes the H3K27me3 and maintains the stable repression of *FLC* in *Arabidopsis*[49–51]. All of these genes likely cooperate to silence the *FLC* gene and maintain its repressed state by changing chromatin structure at the *FLC* locus during or after vernalisation.

Genetic network in temperate grasses. Winter cereal varieties that require vernalisation express various regulatory genes in response to this process to adjust floral initiation, such as *VRN1*, *VRN2* and *VRN3* (refs [23,26]). *VRN1*, encoding an APETALA1 (AP1)-like MADS-box transcription factor, is induced by vernalization and promotes the transition from vegetative to reproductive development[28]. *VRN2*, encoding a zinc-finger protein, is a floral repressor that delays flowering until plants are vernalized[52]. Expression of *VRN3*, which encodes a polyethanolamine-binding protein, is mediated by the photoperiod and vernalisation, and accelerates flowering[53]. During the vegetative stage of winter cereals in autumn, *VRN1* expression is low and VRN2 activity represses expression of *VRN3* (Fig. 2)[54].

VRN1 is associated with inflorescence meristem identity, floral transition and cold tolerance in wheat[28,55,56]. This gene is orthologous to the meristem identity genes *AP1*, *CAULIFLOWER* (*CAL*) and *FRUITFULL* in *Arabidopsis*[28,57]. Hexaploid wheat carries three homologous copies of the *VRN1* gene in the long arm of chromosomes 5A, 5B and 5D, which are designated *VRN-A1*, *VRN-B1* and *VRN-D1*, respectively[30,31,58]. *VRN1* transcription is initiated after cold-exposure and maintains high levels of expression after vernalisation in both apices and leaves of winter wheat. These transcriptional dynamics may be controlled by histone modifications[31]. Both the promoter and the critical region in the first intron of *VRN1* are required for the vernalisation response[59,60]. Deletions and

Table 1 | Summary of genes involved in vernalisation in *Arabidopsis*, wheat and barley

Gene name		Description	Function	References
Arabidopsis				
FLOWERING LOCUS C (*FLC*)		A MADS-box transcription factor	Represses flowering	33,34
FRIGIDA (*FRI*)		A protein with two coiled-coil motifs as a scaffold	Promotes expression of *FLC*	32
VERNALIZATION 1 (At*VRN1*)		A protein containing a B3 DNA-binding domain and PEST regions	Represses *FLC* by epigenetic modification	41
VERNALIZATION INSENSITIVE 3 (*VIN3*)		A chromatin remodelling plant homeodomain (PHD) finger protein	Represses *FLC* by epigenetic modification	39,44,47
VERNALIZATION 5 (*VRN5*)/*VIN3-LIKE 1* (*VIL1*)		A homologue of VIN3	Represses FLC by epigenetic modification	48
FLOWERING LOCUS T (*FT*)		A small globular protein similar to the RAF kinase inhibitors	A major integrator of inductive flowering pathways	165,166
FLOWERING LOCUS D (*FD*)		A bZIP transcription factor	Interacts with FT to induce floral meristem identity genes	77
Polycomb repressive complex 2 (*PRC2*)	*FERTILIZATION INDEPENDENT ENDOSPER* (*FIE*)	A homologue of extra sex combs (ESC)	Represses *FLC* by H3K27me3 epigenetic modification	167,168
	MULTI-SUBUNIT SUPPRESSOR OF IRA 1 (*MSI1*)	Homologues of nucleosome-remodelling factor 55 kDa subunit (NURF55)	Represses *FLC* by H3K27me3 epigenetic modification	169,170
	FERTILIZATION INDE-PENDENT SEED 2 (*FIS2*)/ *EMBRYONIC FLOWER 2* (*EMF2*)/ *VERNALIZATION 2* (*AtVRN2*)	Three homologues of suppressor of zeste 12 (Su(z)12)	Represses *FLC* by H3K27me3 epigenetic modification	40,171–173
	CURLY LEAF (*CLF*)/ *SWINGER* (*SWN*)/ *MEDEA* (*MED*)	The H3K27 methyltransferases as homologues of Drosophila enhancer of zeste (E(z))	Represses *FLC* by H3K27me3 epigenetic modification	43,174,175
VIVIPAROUS 1/ABI3-LIKE 1/2 (*VAL1/2*)		A trans-acting epigenome reader and transcriptional repressor	Recognises the RY-motifs and H3K27me3 of *FLC* to recruit PRC2	119,120
LEAFY COTYLEDON 1 (*LEC1*)		A NF-Y seed-specific transcription factor	Engages EFS and SWR1c to establish an active chromatin state at *FLC*	124
EARLY FLOWERING 6 (*ELF6*)		A H3K27me3 demethylase	Partially resettes vernalized state of *FLC*	176
Wheat and barley				
VERNALIZATION 1 (*VRN1*)		An APETALA1-like MADS box transcription factor	Promotes flowering	28
VERNALIZATION 2 (*VRN2*)		A zinc-finger-CCT domain transcription factor	Represses flowering	52
VERNALIZATION 3 (*VRN3*)		A RAF kinase inhibitor–like protein, similar to *Arabidopsis* FT	Promotes flowering	53,81
VERNALIZATION-D 4 (*VRN-D4*)		Sharing with the regions *VRN-A1* as an extra copy of *VRN1*	Promotes flowering	68,69
GLYCINE-RICH RNA-BINDING PROTEIN 2 (GRP2)		A glycine-rich RNA-binding protein	Inhibites the splicing of *VRN1* pre-mRNA	67
VERNALIZATION RELATED 2 (*VER2*)		A jacalin-like lectin	Switches the sublocalization of GRP2 to increase *VRN1* expression	65–67
FD-like 2 (*FDL2*)		A homologue of Arabidopsis FD	Promotes transcription of *VRN1* by interacting with FT	78
B. distachyon				
VERNALIZATION 1 (*VRN1*)		An APETALA1-like MADS box transcription factor	Promotes flowering	89
VERNALIZATION 3 (*VRN3*)		A RAF kinase inhibitor-like protein, similar to *Arabidopsis* FT	Promotes flowering	89
REPRESSOR OF VERNALIZATION 1 (*RVR1*)		A protein containing a bromo-adjacent homology and transcriptional elongation factor S-II domain	Represses *VRN1* before cold	70
ENHANCER OF ZESTE-LIKE 1 (*EZL1*)		A homologue of *Arabidopsis* CLF	Represses *VRN1* by H3K27me3 modification before cold	132

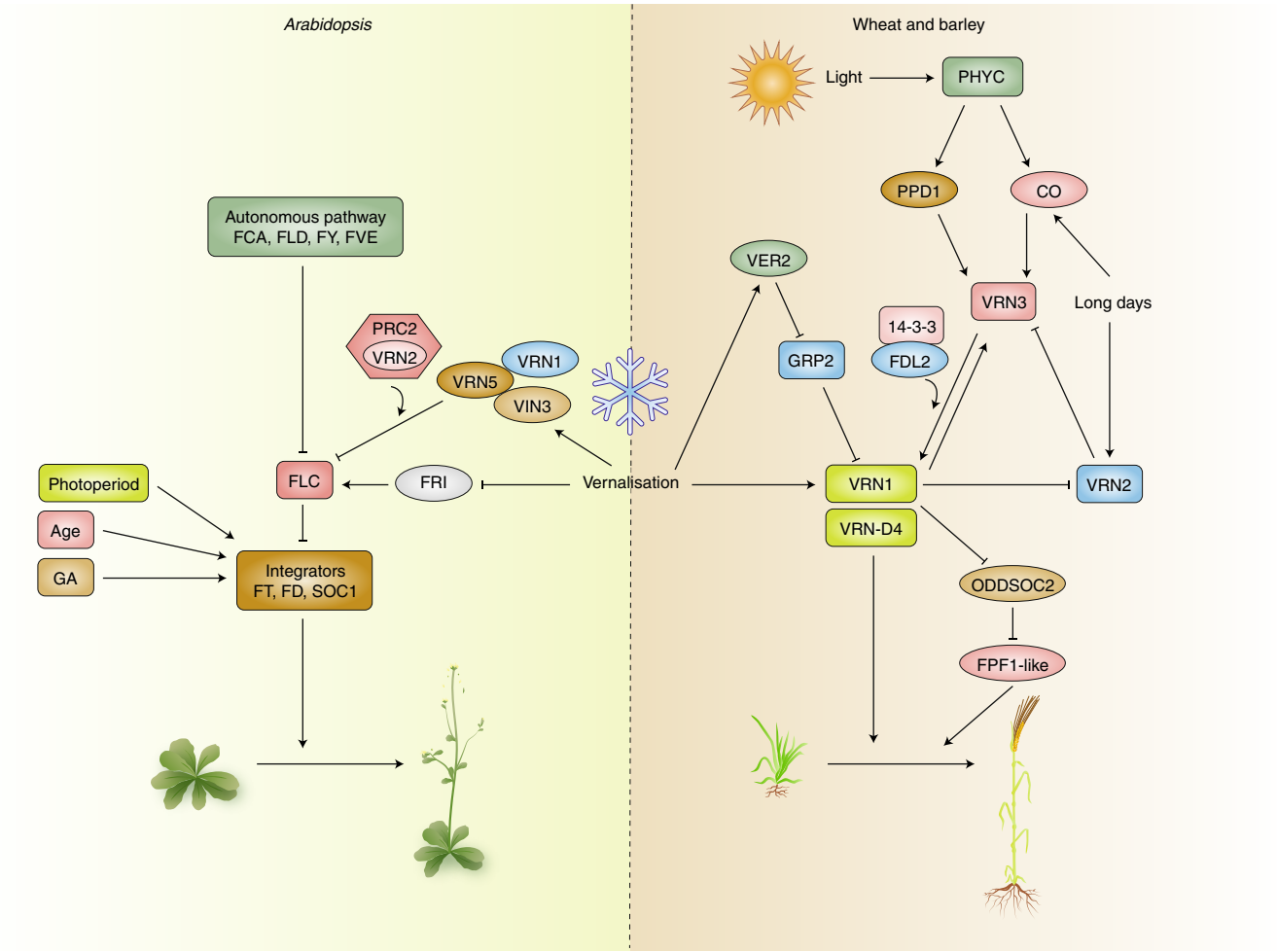

Fig. 2 | Comparison of the vernalisation gene-regulatory networks of *Arabidopsis* and wheat. There are distinct differences in the gene-regulatory networks involved in vernalisation between dicots (*Arabidopsis*) and monocots (wheat and barley). In *Arabidopsis* (left), *FLC* is a key flowering repressor that is regulated by the autonomous and vernalisation pathways. Vernalisation inhibits expression of *FLC* by PRC2- and lncRNA-mediated epigenetic regulation. AtVRN1 and VRN5 also regulate the expression of *FLC* through epigenetic modifications. Silencing of *FLC* promotes flowering by up-regulating the expression of flowering integrator genes, such as *FT* and *SOC1*. In wheat and barley, the long days of autumn induce high levels of *VRN2*, which represses the expression of *VRN3* to inhibit flowering. Cold temperatures induce high expression levels of *VRN1* through epigenetic regulation and VER2–GRP2-mediated RNA splicing. VRN-D4, a copy of VRN-A1, is also induced by vernalisation and modulates flowering. VRN1 directly represses *VRN2* expression by binding its promoter in leaves. The resulting low *VRN2* transcript levels facilitate the up-regulation of *VRN3* by long days in the spring, which is mediated by PHYC, PPD1 and CO. VRN1 can also increase the transcription of *VRN3* by binding its promoter. VRN3 further induces *VRN1* by interacting with FDL2 and 14-3-3 in the leaves, forming a positive feedback loop. VRN3 can be transported from leaves to the shoot apex to promote *VRN1* expression, initiating the switch from vegetative growth to flowering. VRN1 can also promote the expression of *FPF1*-like genes by inhibiting *ODDSOC2* transcription, thereby accelerating flowering.

insertions in the promoter of *VRN1*, and large deletions in its first intron, have been correlated with dominant alleles for spring growth habits in wheat, suggesting that these regions are extremely important for repression of *VRN1* expression before vernalization[59–61]. These mutations likely break the higher structures in chromatin or modulate epigenetic regulation of the *VRN1* locus—a question worth further investigation in the future. Allelic variation at the *VRN1* locus is one of the main sources of genetic variation in vernalisation requirements in wheat. One dominant *VRN1* allele at any of the three homoloci is sufficient to confer a spring growth habit[56,58,61,62]. Vernalisation induces higher transcript levels of *VRN-A1* with promoter mutation compared to *VRN-B1* and *VRN-D1* with first-intron deletions, so the different strengths of these alleles may be due to various underlying mutations[30,63,64]. Additional cold-induced genes mediate *VRN1* expression to 'regulate' floral initiation in wheat. *VER2*, induced by vernalization, encodes a jacalin-like lectin with high affinity for galactose and *N*-acetylglucosamine (GlcNAc)[65,66]. VER2 interacts with the glycine-rich RNA-binding protein GRP2 (an orthologue of the *Arabidopsis* GLYCINE-RICH RNA-BINDING PROTEIN 7), moving it from the nucleus to the cytoplasm. This translocation of GRP2 represses its specific RNA-binding to RIP3 in the first intron of *VRN1*, resulting in high levels of *VRN1* transcripts following prolonged cold exposure[67]. *VRN-D4*, an extra copy of *VRN-A1*, is located on chromosome 5DS and is crucial for development of the spring growth habit in wheat[68]. Three adjacent single nucleotide polymorphisms (SNPs) in the RIP3 motif of *VRN-D4* disrupt the binding of GRP2. In the RIP3 motif of *VRN-A1*, SNPs are correlated with winter and spring growth habits in wheat, which provides a novel idea for the adaptation of wheat varieties to different environments (Fig. 2)[69]. In wheat, there are likely negative regulators of *VRN1* that minimize its transcription prior to winter. Recently, in *Brachypodium*, a gene called *REPRESSOR*

OF VERNALIZATION1 (*RVR1*) has been shown to be a repressor of *VRN1* before vernalisation and to be involved in establishing a vernalisation requirement[70]. Even though VRN1 plays an important role in regulating wheat vernalisation-mediated flowering, its loss does not abolish flowering[71]. There are likely redundant flowering genes, such as *FUL2* and *FUL3* (paralogues *VRN1*), that can induce wheat flowering in the absence of functional VRN1 (ref. [71]).

Additional variation in vernalisation requirements in wheat is determined by *VRN2* (ref. [52]). Analyses of genetic epistatic interactions indicate that *VRN1* and *VRN2* share a genetic pathway[72]. *VRN2*, as a repressor of flowering, encodes a zinc-finger-CCT domain transcription factor and is down-regulated by vernalisation and short days[52,72,73]. Therefore, this CCT domain contributes the vernalisation requirement in winter wheat and barley. Mutants or deletions in the CCT domain eliminate this vernalisation requirement[72,74,75].

VRN3, a promoter of flowering, encodes an RAF kinase inhibitor-like protein and is induced in leaves by long days. This protein in wheat is functionally similar to FLOWERING LOCUS T in *Arabidopsis*[53,76]. VRN3 promotes *VRN1* expression by interacting with the bZIP transcription factor FDL2, which is an orthologue of *Arabidopsis* FD that directly binds ACGT elements in the promoter region of *VRN1* (refs [77,78]). The VRN3–FDL2 interaction requires the 14-3-3C protein to form a florigen activation complex (FAC), which can regulate other flowering promoters in wheat and barley[79]. VRN3 can integrate the vernalisation and photoperiod signals to accelerate flowering. In the long days of autumn, the low expression level of *VRN1* leads to high levels of *VRN2*, which represses the expression of *VRN3* to inhibit flowering[71,80]. In winter, short days and prolonged exposure to low temperatures up-regulates *VRN1* (refs [28,31]). VRN1 can directly bind the promoter of *VRN2* to decrease its transcription, and can increase the transcription of *VRN3* by binding to its promoter in barley[8]. Thus, the increased expression of *VRN1* after winter represses the transcription of *VRN2*, which enables the transcription of *VRN3* under long-day conditions in spring[80–82]. *VRN3* expression is also mediated by the photoperiod genes *PPD1* and *CO*, as well as the competition to interact with nuclear factor-Y (NF-Y) transcription factors between CO and VRN2 (ref. [83]). Light-dependent PHYTOCHROME C (PHYC) mediates the transcriptional activation of *PPD1* and *CO* to up-regulate *VRN3* in wheat[84,85]. VRN3 further induces *VRN1* in the leaves to form a positive-feedback regulatory loop. In addition, VRN3 is transported to the shoot apex from leaves, where it up-regulates *VRN1* expression to a threshold level that initiates the switch from vegetative to reproductive growth (Fig. 2)[86]. In barley, the grass-specific MADS box gene *ODDSOC2*, an orthologue of *FLC* in monocots, was found to function in the vernalisation process[87]. VRN1 can down-regulate the transcription of *ODDSOC2* by directly binding to its promoter, reducing expression of the *FPF1*-like gene, an orthologue of *Arabidopsis FLOWERING PROMOTING FACTOR 1* (*FPF1*), to inhibit flowering[8,87,88]. The VRN1–VRN2–VRN3 regulatory modules can integrate vernalisation and the photoperiod signal to precisely mediate the flowering time of cereals in the spring.

The temperate grass model *B. distachyon* shares a conserved mechanism of vernalisation with wheat and barley on temperate grass orthologues of *VRN1* and *VRN3* (ref. [89]). In *Brachypodium*, the expression pattern of *VRN1* in its flowering response depends on vernalisation[90,91]; where increased expression levels of *VRN1* and *VRN3* promote the transition to flowering[90,92]. Despite *VRN2* acting as a floral inhibitor in *Brachypodium*, it is not repressed by vernalisation through *VRN1* but is induced by cold, contrary to the *VRN2* gene in wheat and barley[92]. The functional diversity of *VRN2* in the vernalisation of various grass species is still a 'black box'.

Interestingly, cold acclimation is decreased in vernalized plants, which may indicate a trade-off between development and defence. The vernalisation gene *VRN1* can down-regulate the expression of *CBF* by binding to its promoter, facilitating the transition from vegetative to reproductive development after vernalization[8]. A similar regulatory mechanism is found in *Brachypodium*, in which the expression of *VRN1* reduces freezing tolerance by decreasing the expression of cold-responsive genes in vernalized and spring growth habit plants[93]. Thus, the expression of *VRN1* can coordinate cold-acclimation and vernalisation-mediated flowering in temperate grass. Similar patterns have also been shown in rice cold-tolerance, where OsMADS57 can coordinate cold-stress responses and developmental transitions through switching its binding targets, although rice does not require vernalisation for flowering[94].

The perception of vernalisation and memory of winter

Vernalisation requires that plants both sense their exposure to cold temperatures and form a 'memory' of that exposure to ensure they flower in response to warmer temperatures in spring[95]. Indeed, sensing the vernalization dosage is required for the transition from vegetative growth to flowering. Previous studies that used localized chilling and grafting methods suggested that shoot apices (including young leaf primordia and the shoot apical meristem) are responsible for the perception of cold-temperature signals for flowering[96,97]. However, these technical approaches failed to distinguish between cold-sensing at the meristem and in young leaves. Further evidence indicates that the vernalisation signal is perceived in leaves. In sugar beet, young leaves were induced to produce the floral stimulus by vernalisation[98]. Flowering plants were regenerated from vernalised leaves of *Luannari biennis* and *Thlaspi arvense* but not from non-vernalised leaves[99–101]. More precise molecular analyses, such as in situ RNA hybridization of the vernalisation-induced gene *VER2*, also indicate that immature leaves are the key tissues for sensing cold temperatures during vernalisation[66]. These data suggest that young leaves are the main tissue to perceive the vernalisation signal for flowering.

After the sensing step, plants must maintain the effect of vernalisation through mitotic divisions to successfully transition to flowering in spring[102]. This cold-induced mitotic transmission is an effect of vernalisation memory (or winter memory)[103], which was first described in the henbane plant in the 1940s[104]. Vernalised henbane plants can sustain vegetative growth throughout non-inductive photoperiods, and can then flower after shifting to inductive photoperiods[105]. Thus the stable effect of vernalisation can be remembered through cell divisions under warm conditions[103]. This vernalisation memory has also been verified in temperate grasses. For example, *VRN1* is stably induced after vernalisation and thus likely contributes to the memory of vernalisation in *Brachypodium* and cereals, such as wheat and barley[91,106]. The effects of insufficient vernalisation can be reversed by exposure to high temperatures (~35°C) in a process known as devernalisation[66,104,107]. Further studies have demonstrated that the mechanism of vernalisation memory is related to the epigenetic regulation of key vernalisation genes.

Counting winter dosage with epigenetic marks

A requirement for vernalisation (and therefore winter) ensures that plants grow vegetatively over winter and flower the next spring. Counting the winter dosage is mainly regulated by the histone epigenetic alteration of *FLC* in *Arabidopsis and VRN1* in cereal crops.

Activation of FLC by epigenetic histone modifications. Before vernalisation, *FLC* is expressed at a high levels due to the presence of histone modifications in its promoter region that promote transcription, including trimethylation of histone 3 at lysine 4 (H3K4me3) and at lysine 36 (H3K36me3). FRI acts as a scaffold with its paralogue FRL1, DNA-binding protein SUF4, transactivating protein FLX4 and the zinc finger-containing protein FES1, forming a transcriptional activating complex[35]. This FRI complex increases *FLC* expression by increasing the levels of H3K4 and

H3K36 trimethylation, H3 and H4 acetylation and H2A.Z deposition at the *FLC* by recruiting the COMPASS-like complex, SET DOMAIN GROUP 25 (SDG25), the H3K36me3 methyltransferase EARLY FLOWERING IN SHORT DAYS (EFS), and the chromatin-remodelling SWR1 complex[5,108–110]. This group of transcriptional activators ensures high levels of *FLC* expression to prevent flowering before spring. Cold-activated stress protein WRKY34 promotes the expression of *CULLIN 3A* (*CUL3A*) resulting in the proteasome-dependent degradation of the FRI complex by a CUL3A-based ubiquitin E3 ligase to slightly repress transcription of *FLC* (Fig. 3)[111]; in an example of protein degradation for coordinating stress and flowering development.

PcG-mediated silencing of *FLC* during vernalisation. The polycomb repressive complex 2 (PRC2), which silences gene expression by inducing trimethylation of histone H3 at lysine 27 (H3K27me3) at target gene loci, is a popular working model to explain vernalisation at the molecular level[112,113]. The epigenetic silencing of *FLC* during vernalisation may be divided into two phases in *Arabidopsis*: the initial silencing of expression, and the subsequent spreading and maintenance of the silenced state. During prolonged cold exposure during winter, *VIN3* and the long non-coding (lnc)RNAs *COLD-INDUCED LONG ANTISENSE INTRAGENIC RNA* (*COOLAIR*) and *COLD ASSISTED INTRONIC NONCODING RNA* (*COLDAIR*), down-regulate *FLC* (refs [47,114,115]). The antisense lncRNA *COOLAIR* starts downstream of the *FLC* poly(A) site and terminates within the *FLC* promoter. *COOLAIR* expression is induced soon after exposure to low temperatures and peaks after 10 days of cold[116]. During vernalisation, *COOLAIR* accelerates the transcriptional repression of *FLC* by reducing H3K36me3 at the *FLC* locus[116]. *COLDAIR* maintains this *COOLAIR*-initiated repression of *FLC*. *COLDAIR* is derived from the first intron of *FLC* and is induced slowly by cold temperatures, reaching its maximum expression level after 20 days of cold. The sense lncRNA *COLDAIR* interacts with CLF, a component of the PRC2 complex, to recruit PRC2 to the *FLC* locus to repress *FLC* transcription[117]. An additional sense lncRNA *COLDWRAP* (*COLD OF WINDTER-INDUCED NONCODING RNA FROM THE PROMOTER*), derived from the *FLC* promoter and highly expressed after winter cold, associates with *COLDAIR* to retain the PRC2 complex at *FLC* to establish the stable repressed state of *FLC* (ref. [118]). Before vernalisation, VIVIPAROUS 1/ABI3-LIKE factors VAL1 and VAL2 recruit LHP1, the PRC2 complex, components of the apoptosis- and splicing-associated protein (ASAP) complex, and HDA19 to the nucleation region of *FLC*. This recruitment of regulatory factors is achieved by VAL1 and VAL2 recognition of two RY motifs and H3K27me3 markers in the nucleation region of *FLC* (refs [119,120]). To initiate *FLC* repression, VIN3 associates with PRC2 to form the active PHD–PRC2 complex, resulting in a peak of the repressive chromatin marker H3K27me3 at the nucleation region (Fig. 3)[121]. Both *COLDAIR* and VAL1/2 can recruit PRC2 complexes at the *FLC* locus and initiate the repression of *FLC*, but how they cooperate to regulate this repression is still unknown. It is likely that they function in the relay to maintain the repressed state of the *FLC* nucleation region during prolonged cold exposure by targeting the same site or adjacent sites to the *FLC* locus[44,122]. It is also possible that they work together to strongly initiate the repression of *FLC* by increasing H3K27 trimethylation at the nucleation region. This will be an interesting problem to resolve in the future.

Memory and resetting of vernalisation. In *Arabidopsis*, H3K27me3 induced by the PRC2 complex (silencing the *FLC* gene) and H3K4me3 induced by the COMPASS complex (activating the expression of *FLC*) are involved in the memory of the repressed and activated states, respectively, of chromatin through cell division[35,45,121,123,124]. Active histone modifications, such as H3K4me3, promote high levels of *FLC* expression before winter, and the active state of *FLC* is maintained until winter. During vernalisation, the PcG-mediated nucleation of *FLC* in intron 1 represses its expression, and this silencing is remembered during subsequent growth and development under warmer temperatures[125,126]. Vernalisation memory requires the spread of H3K27me3 across the entire region of *FLC* through cell divisions following return to warmth, in which DNA polymerase α, DNA polymerase ε, ESD7, CLF, EMF2, MSI1 and LHP1 play important roles[123]. Maintenance of *FLC* repression allows *Arabidopsis* to flower in spring[45,123,127], but how the silenced state of *FLC* is erased in the next generation to enable high levels of *FLC* expression before winter is still unclear. Recently, the seed-specific transcription factor *LEC1* was found to bind the promoter of *FLC* and promote a shift from the vernalized chromatin state, characterized by repressive H3K27me3, to the active chromatin state, characterized by permissive H3K4me3 and H3K36me3 (ref. [124]). This epigenetic change deletes the vernalisation memory and reactivates expression of *FLC* in the pro-embryo. This active state of *FLC* is also passed on to the following seedling stage by mitosis, preventing flowering before or during winter. The jumonji-domain-containing protein EARLY FLOWERING 6 (ELF6), which possesses H3K27me3 demethylase activity, also functions in deleting vernalisation memory and reactivating the expression of *FLC* in the pro-embryo[128]. However, how the *LEC1* and ELF6 together regulate the deletion of vernalisation memory, is still unknown.

Epigenetic regulation of vernalisation in temperate grasses. In temperate grasses, the epigenetic regulation of vernalisation is distinctly different from that of *Arabidopsis*. In wheat and barley, the target gene regulated by epigenetic modification is mainly the promoting gene *VRN1* instead of a repressor of flowering[10,106]. *VRN1* expression is induced by vernalisation and is maintained at high levels on return to warm conditions. *VRN1* possesses a large first intron of about 10 kb that contains regulatory regions for vernalisation. Epigenetic modifications in both the first intron and the promoter of *VRN1* are involved in the epigenetic memory of vernalisation in wheat and barley[59,106,129]. Before vernalisation, the repressive mark H3K27me3 is deposited in *VRN1* locus to inhibit *VRN1* transcription in barley and wheat[106]. During vernalisation, the active histone modification marks H3K4me3 and H3K36me3 are gradually increased at *VRN1*, while H3K27me3 levels decrease[59,67,106,129]. The epigenetic activation of *VRN1* can be maintained on returned to warm conditions. The polycomb complex and Trithorax-group complex are responsible for the maintenance of H3K27me3 and H3K4me3 levels[130,131]. It was also shown that the CURLY LEAF orthologue ENHANCER OF ZESTE-LIKE 1 (EZL1) in *Brachypodium* is involved in depositing repressive H3K27me3 chromatin marks around *VRN1* before cold[132]. In *Brachypodium*, there is a decrease in H3K27me3, as well as an increase in H3K4me3, around *VRN1* during cold that associated with the stably induced expression of *VRN1* after the cold[70,87,91]. The integrated data demonstrate that *VRN1* likely plays a role in memory of the vernalized state through epigenetic regulation. There may also be alternative candidates for genes that are related to the memory of winter in grasses. A number of genes in *Brachypodium* were identified, for which the expression patterns and epigenetic modifications (H3K27me3 and H3K4me3) changed during cold, that are maintained after 7 days post-vernalization[133,134]. In *Brachypodium*, *VRN3*, similar to *VRN1*, also showed coordinated changes in H3K4me3 and H3K27me3 that were epigenetically regulated during vernalization[134].

This vernalisation-memory-associated switch from H3K27me3 to H3K4me3 at the key vernalisation gene locus may be due H3K27me3 demethylase activity, H3K4 methyltransferase activity and binding elements in *VRN1*. Intricate complexes (such as epigenetic modules) are likely needed to sense prolonged cold temperature and regulate the maintenance of the active state of *VRN1*

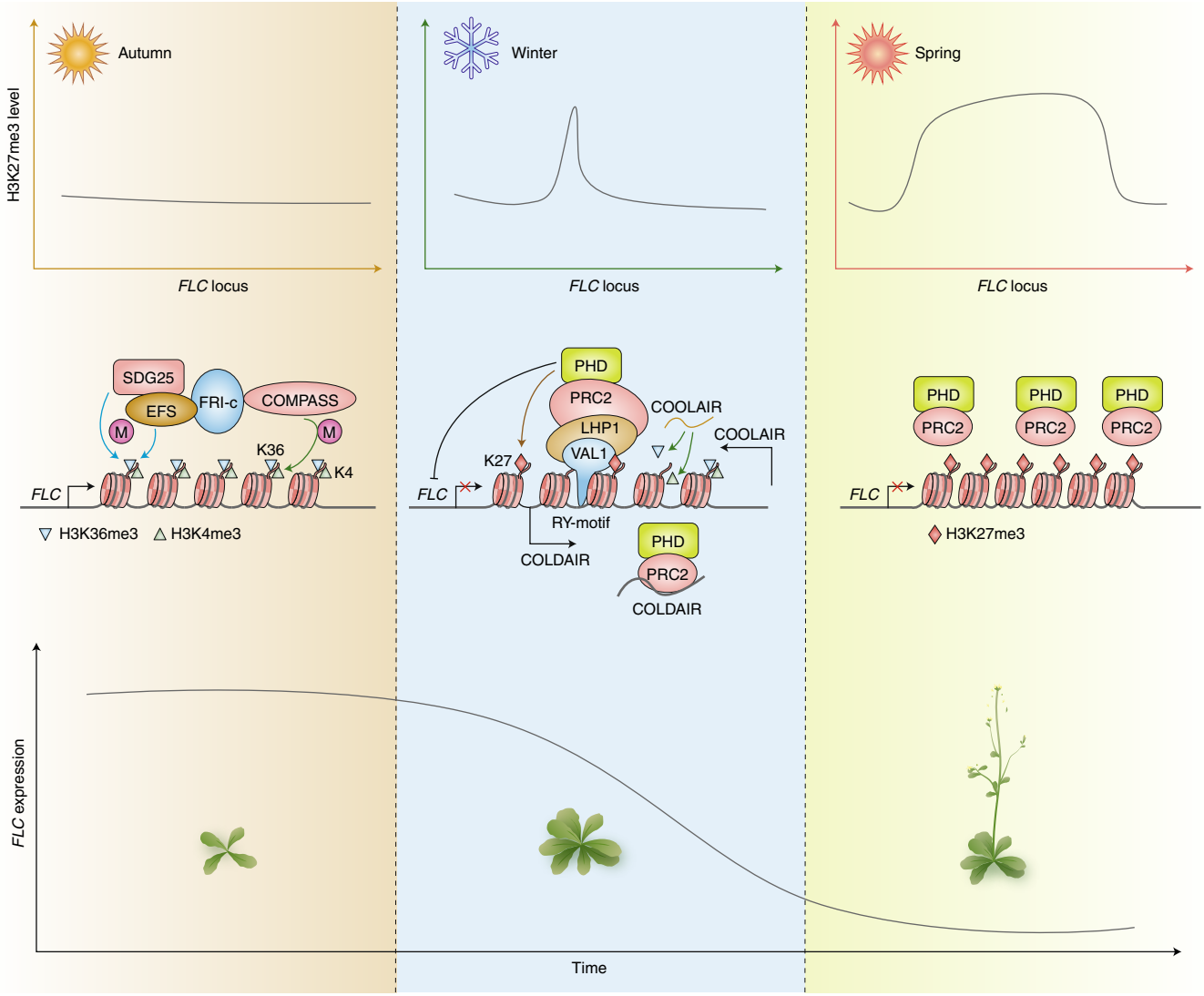

Fig. 3 | Epigenetic silencing of *FLC* to count the duration of vernalisation. A profile of *FLC* regulation for counting vernalisation with epigenetic marks in *Arabidopsis*. Before vernalisation (in the autumn), FRI increases expression of *FLC* by recruiting proteins to form the FRI-complex (FRI-c), which increases the level of the active histone marks H3K4me3 and H3K36me3 at the *FLC* locus by recruiting COMPASS-like H3K4 methyltransferase complex, SDG25 and EFS. The high expression level of *FLC* inhibits *Arabidopsis* flowering before or during winter. During vernalisation (in the winter), *FLC* is gradually silenced by high levels of H3K27me3 at the nucleation region of *FLC*, which is jointly regulated by the PHD–PRC2 complex and decreasing levels of H3K4me3 and H3K36me3. Prolonged low temperatures in winter induce the expression of non-coding RNAs *COOLAIR* and *COLDAIR*, and the expression of PHD finger protein VIN3 at different stages of vernalisation. VAL1 recognizes the RY motifs and H3K27me3 markers in the *FLC* nucleation region to recruit LHP1 and the PHD–PRC2 complex, resulting in the peak of H3K27me3 at this region. *COLDAIR* interacts with CURLY LEAF (CLF, a component of the PRC2 complex), to recruit PRC2 and enhance repression of *FLC*. On return to warmth (in spring), the silenced state of *FLC* is maintained by the spread of H3K27me3 across the entire region of *FLC* through cell divisions, allowing *Arabidopsis* to flower. Here, the H3K27me3 level at the *FLC* locus (top) is consistent with the chromatin structure of *FLC* (middle).

or other candidate genes after vernalisation. This key fundamental question should be addressed with new data in the future.

O-GlcNAc signalling in the vernalisation response

O-GlcNAc signal was reported to be involved in the etiology of human diseases, such as cancer and diabetes, through affecting protein phosphorylation status, stability, localization and/or interaction with other proteins. In plants, previous studies have also shown that *O*-GlcNAc signalling, including metabolism, has a crucial role in regulating the vernalisation response for flowering.

Metabolic characters during vernalisation. During prolonged cold exposure, several metabolic changes occur before morphological changes at the shoot apex. There are three sequential stages in vernalisation: the inducing period, the accelerating period and the steadying period[66]. During the different stages of vernalisation, several programmed metabolic changes occur sequentially, including oxidative phosphorylation to produce energy during the inducing period (a dehydrogenase-enhanced stage), and nucleic acid and protein metabolism in the accelerating period[66]. Monosaccharide levels are significantly higher in cold-treated plants[16] and the addition of glucose can reduce the number of required vernalized-days of winter wheat before flowering[135,136]. In fact, approximately 2–5% of intracellular glucose enters the hexosamine biosynthesis pathway (HBP), which eventually produces uridine diphosphate-*N*-acetylglucosamine (UDP-GlcNAc)[137].

***O*-GlcNAc signal and its regulation in vernalisation responsiveness.** UDP-GlcNAc is the direct donor of the nutrient sensor *O*-GlcNAc[138]. Two conserved enzymes, *O*-GlcNAc transferase and *O*-GlcNAcase, mediate the reversible addition and removal of *O*-GlcNAc to and from the serine or threonine of target proteins, similar to protein phosphorylation[139]. Indeed, the same sites are often targets for both *O*-GlcNAcylation and phosphorylation, resulting in the 'Yin–Yang' model of antagonism between these two modifications at both the global proteome level and at specific amino acids on particular proteins[140–142]. In *Arabidopsis*, the OGTs SECRET AGENT (SEC) and SPINDLY (SPY) have been functionally characterized in plant development and growth, and double-mutant seedlings have been shown to be inviable[143–145]. *O*-GlcNAcylated proteins are also identified as mediators of the circadian clock, flowering time and floral organ development through effects on transcription, chromatin remodelling and epigenetic gene regulation in *Arabidopsis*[146]. Recently, ARABIDOPSIS HOMOLOGUE OF TRITHORAX 1 (ATX1) was shown to be modified by *O*-GlcNAcylation, and *O*-GlcNAc signalling can regulate flowering by changing the activity of ATX1 to affect the epigenetic methylation modifications at *FLC* locus in *Arabidopsis*[147]. In wheat, *O*-GlcNAcylation of GRP2 and phosphorylation of VER2 together mediate the vernalisation process[65,67]. A jacalin-like lectin, VER2, shows binding specificity to *N*-acetylglucosamine and galactose[65]. Knockdown of *VER2* causes a delay in flowering, and overexpression of *VER2* can accelerate flowering in winter wheat during vernalisation, suggesting its overexpression can partially replace vernalisation treatment to induce flowering[67]. During vernalisation, VER2 binds to *O*-GlcNAcylated proteins[65,66], such as *O*-GlcNAcylated GRP2 (ref. [67]). In fact, prolonged exposure to cold temperatures simultaneously increases *O*-GlcNAcylation of the flowering repressor GRP2 in the cytoplasm and phosphorylation of the flowering activator VER2 in the nucleus. This crosstalk between phosphorylation and *O*-GlcNAcylation, regulates the interaction of VER2 and GRP2, as well as modulating the transcription of key vernalisation response gene *VRN1* (which contains the specific RNA-binding site RIP3 that affect flowering in winter wheat) (Fig. 4). As VER2 binds diverse range of *O*-GlcNAcylated proteins, there may also be other *O*-GlcNAcylated proteins involved in regulation of vernalisation response[65].

Vernalisation evolution

Grasses (Poaceae) originated about 70 million years ago, likely in the understory of tropical forests. The colonization of temperate zones by Pooideae, a subfamily of grasses, was likely enabled by the evolution of specific inducible genetic modules from deeply conserved stress-response pathways[148,149]. In temperate latitudes, some plant species have evolved the ability to use prolonged cold winters as a regulatory factor, which confers them with competence for spring flowering to maximize their reproductive success[150]. The evolution of the vernalisation response resulted in the wide spread of the Pooideae planting area and facilitated the radiation of Pooideae into temperate areas[150,151]. Other Poaceae taxa, such as Bambusoideae and Ehrhartoideae, do not require vernalisation, likely due to their restricted distribution in tropical or subtropical regions. This is true even in highland rice varieties, which can tolerate chilling but display no vernalization response[152]. Although the rice gene *GHD7* (*GRAIN NUMBER, PLANT HEIGHT AND HEADING DATE 7*) is an orthologue of the wheat vernalisation gene *VRN2*, it only has the ancestral function of repressing flowering under long days[92,153].

Despite evidence for an early origin of vernalisation responsiveness in Pooideae, the VRN1–VRN2–VRN3 regulatory modules may have been established during core Pooideae evolution (such as in wheat and barley) to precisely mediate flowering[92,150]. In fact, *VRN2* is not down-regulated by vernalisation through VRN1 in *Brachypodium*[90,92]. However, the up-regulation of *VRN1* by prolonged cold was likely a key early event in Pooideae evolution[92,150,152,154], while the FUL-like MADS box genes (VRN1)–FT regulatory feedback loop probably developed prior to vernalisation in cereals, as it is highly conserved in the flowering control pathway[53,82,90,155]. Thus, the VRN1–VRN2–VRN3 regulatory module in vernalisation is likely to have occurred after the divergence of Brachypodieae from the core Pooideae. Mutations in the promoter region and first intron of *VRN1* may have increased variations in growth habit (ranging from winter to spring growth) and therefore increased evolutionary potential. Most of the wild Triticeae species possess a winter growth habit with the ancestral recessive *VRN1* allele, however domestication and artificial selection favoured various dominant *VRN1* alleles that conferred the spring growth habit. This evolution of the spring type from the winter type resulted in adaptation of crops to temperate regions[156]. Phylogenetic analyses indicate that *VRN2*-like genes underwent a duplication event before the diversification of grasses, resulting in two grass clades—the *CO9* clade and the *VRN2*/*GHD7* clade[92]. On the other hand, the *Brachypodium VRN2* gene, unlike *VRN2* in wheat and barley, is induced by long days in vernalised plants[90,92]. It seems that the function of this gene in vernalisation is evolving rapidly in grasses. Nevertheless, the function of VRN2/GHD7 in repressing flowering is conserved under long days. The rice *GHD7* gene is induced by long days and represses the transcription of *HEADING DATE 3A* (*HD3A*, a homologue of *FT*) to inhibit flowering[157]. Within the grass lineage, *GHD7* may function to induce flowering in response to photoperiod, but may have nothing to do with the response to vernalisation, probably due to the lack of low temperatures required for vernalisation in tropical or subtropical regions[92].

The vernalisation-regulated flowering pathway is involved in both monocots and eudicots. In monocots, such as wheat and barley, the genes controlling the vernalisation response function mainly in the VRN1–VRN2–VRN3 regulatory loop. However, this is different from the FRI–FLC module in *Arabidopsis*[31,121]. The mechanism of counting the duration of vernalisation also differs between monocots and eudicots. The polycomb complex mediates the stable epigenetic silencing of *FLC* by H3K27me3 in *Arabidopsis*, whereas the epigenetic activation and maintenance of *VRN1* expression is regulated by increased H3K4me3 in wheat and barley[67,106,123]. These distinct vernalisation systems are probably the result of convergent evolution between monocots and eudicots living in similar environments. Orthologues of *FLC*, *ODDSOC2* in *Brachypodium* and barley, or *AGL33* in wheat, were identified to act similarly as flowering repressors[158,159]. Even in accessions of *Brachypodium*, such as Bd21 and BdTR3C, down-regulation of *ODDSOC2* by prolonged cold exposure is associated with increased H3K27me3 at *ODDSOC2* locus and is maintained when the plants are returned to warm temperatures[158]. This provides a fresh perspective on the differences in the evolution of vernalisation between monocots and eudicots.

The vernalisation-regulated flowering pathway is also divergent in different dicotyledonous species. In sugar beet, as a new mode of life-cycle-control in dicotyledonous species, a different regulatory module BOLTING TIME CONTROL 1 (BvBTC1)–BvFT1–BvFT2 has evolved in response to vernalisation, compared with that in *Arabidopsis*[76,160]. During vernalisation, the pseudo-response regulator (PRR) gene Bv*BTC1* is upregulated, suppressing expression of the flowering repressor gene Bv*FT1* and promoting expression of the flowering activator gene Bv*FT2* (ref. [76]). Despite the various vernalisation regulatory loops in *Arabidopsis* and beet, an FLC orthologue (FLC-LIKE 1; BvFL1) has been identified in beet. Complementation analyses of Bv*FL1* in *Arabidopsis*, and down-regulation of Bv*FL1* by vernalization in beet, suggest its conserved floral repressor function[161]. However, instead of being epigenetically maintained in a transcriptionally silent state after vernalisation, expression of Bv*FL1* returns to pre-vernalisation levels, which is very different from that in *Arabidopsis*[160–162]. In galegoid legumes (another dicotyledonous

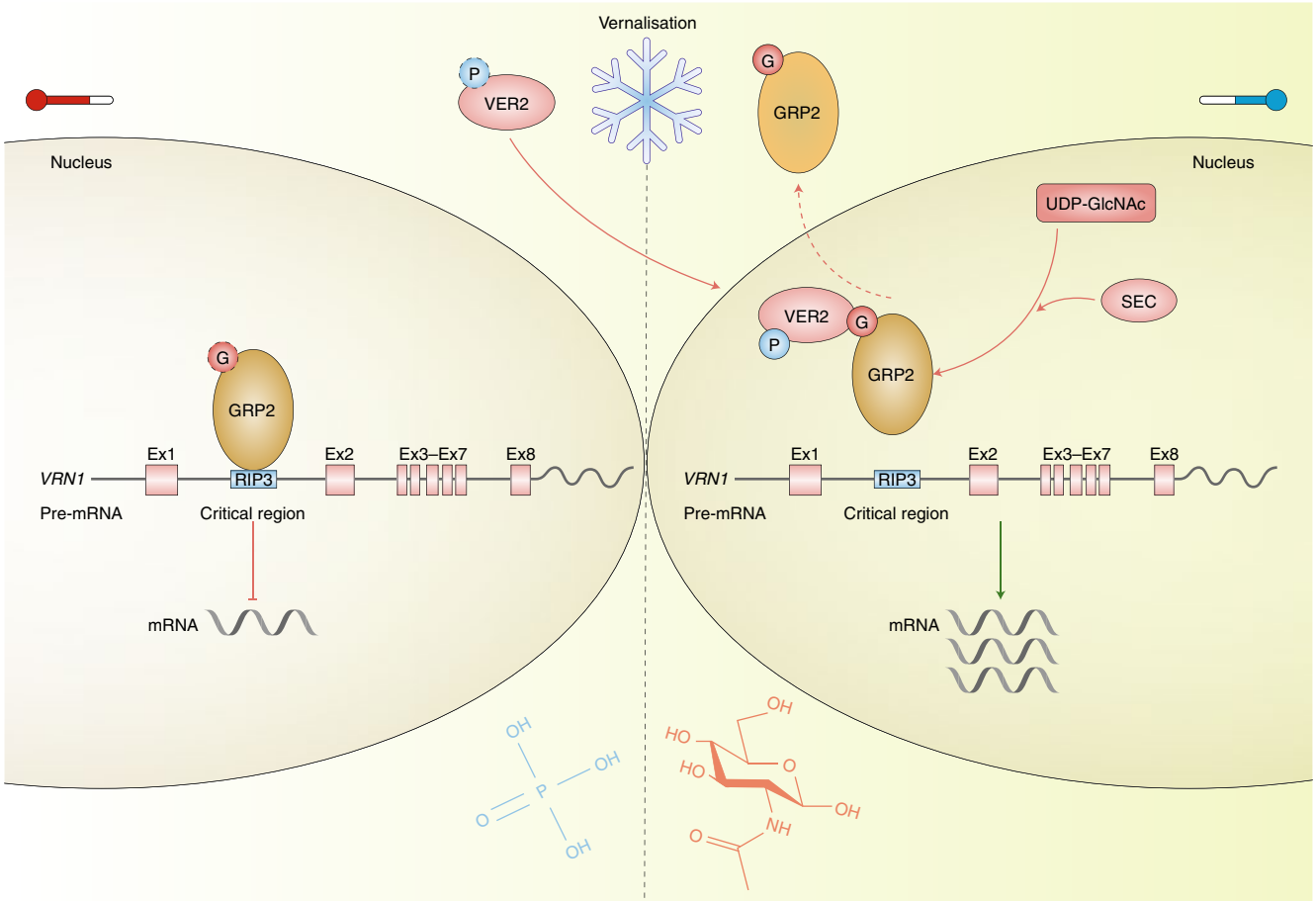

Fig. 4 | The regulatory role of the *O*-GlcNAc signal in vernalisation. Before vernalisation, GRP2 (an RNA-binding protein) binds to the RIP3 motif in the first intron of *VRN1* pre-mRNA, inhibiting its splicing to keep the level of *VRN1* mRNA low (left). Prolonged cold temperature exposure gradually increases the level of phosphorylated-VER2 in the nucleus. At the same time, phosphorylated-VER2 can interact with *O*-GlcNAcylated GRP2, induced by vernalisation, which leads to release of *VRN1* pre-mRNA and accumulation of *VRN1*. This accumulation promotes flowering of plants in the spring (right). The crosstalk between phosphorylation of VER2 and *O*-GlcNAcylation of GRP2 mediates vernalisation by regulating the expression of *VRN1* in wheat. The blue molecular structure represents phosphoric acid; the orange molecular structure shows acetylglucosamine (GlcNAc). SEC, SECRET AGENT.

species), *Arabidopsis FLC*-like genes appear to be absent, and a vernalisation-independent mechanism exists. Interestingly, the number of *FT* orthologues are higher in legumes (which evolved into three subclades *FTa*, *FTb* and *FTc*, with different functions in the legume species)[163]. The *FTa1* gene from *Medicago truncatula* is necessary for the response to vernalisation, whereas vernalisation responsiveness is mediated by *FTc1* gene in *Lupinus angustifolius*[158,164]. Both conservation and divergence were likely to have occurred during the evolution of vernalisation in dicotyledons.

Perspectives

The major fundamental questions of vernalisation are how plants sense the vernalisation signal and count the dosage of prolonged cold exposure, along with how these processes can be applied to crop production.

Counting the dosage of vernalisation with epigenetic marks. The genetic and epigenetic regulatory networks of vernalisation differ considerably between temperate grasses and *Arabidopsis*. In *Arabidopsis*, flowering repressor *FLC* is the key gene that responds to vernalization[34]. The PcG-mediated epigenetic silencing of *FLC* tracks the duration of vernalisation and initiates the floral transition[45]. The vernalisation-mediated recruitment of H3K27me3 to the nucleation site of *FLC* initiates gene repression and, on return to warm conditions, the repressed state of *FLC* can be maintained and mitotically inherited by spreading H3K27me3 around the promoter and coding region of the *FLC* locus. This ensures flowering of *Arabidopsis* in the spring[121]. In barley, VRN1 binds to the promoter of *VRN2* to repress its expression. Thus repression of *VRN2* may not be involved in counting the vernalisation process[8,106]. Interestingly, in temperate grasses (such as wheat, barley and *Brachypodium*), the flowering promoter gene *VRN1* tracks the duration of prolonged cold with increased H3K4me3, rather than H3K27me3, and maintains a high level of *VRN1* expression even after vernalisation[67,106,132]. A number of genetic analyses hint that the promoter and first intron of *VRN1* play a vital role in its epigenetic regulation[59,67,129].

The potential mechanism of sensing vernalisation signal. The perception of the vernalisation signal is a process that balances the cold defence response with plant development following prolonged exposure cold temperatures. A potential theory for the quantitative activation of flowering by vernalisation in cereals is that cold-temperature-induced transcription leads to a gradual change in *VRN1* expression by erasing repressive chromatin marks[106]. Another alternative hypothesis is that the duration of vernalisation could be recorded by the modifications of key proteins, such as phosphorylation of VER2 and *O*-GlcNAcylation on GRP2 (ref. [67]). In wheat, crosstalk between gradually phosphorylated VER2 and *O*-GlcNAcylated

GRP2 during vernalisation can regulate the expression of *VRN1* in response to vernalisation, which implies that the Yin-Yang relation of phosphorylation and *O*-GlcNAcylation may involve sensing the vernalisation signal and counting the duration of prolonged cold (Fig. 4). Further analysis of this relationship may provide new avenues of research on the perception of vernalisation dosage.

Up to now, the genetic and epigenetic regulatory mechanisms of vernalisation have only been well studied in *Arabidopsis*. The reference genome sequence of hexaploid wheat may shed new light on key fundamental questions about complex crop genomes, such as how plants perceive vernalisation and measure its dosage, as well as how vernalisation memories are formed and erased. How epigenetic marks and Yin-Yang modifications coordinate dosage counting is a particularly interesting question to be addressed in the future.

Received: 2 January 2018; Accepted: 12 October 2018;
Published online: 26 November 2018

References

1. Zhu, J. K. Abiotic stress signaling and responses in plants. *Cell* **167**, 313–324 (2016).
2. Guo, X. Y., Liu, D. F. & Chong, K. Cold signaling in plants: insights into mechanisms and regulation. *J. Integr. Plant Biol.* **60**, 745–756 (2018).
3. Liu, J. Y., Shi, Y. T. & Yang, S. H. Insights into the regulation of C-repeat binding factors in plant cold signaling. *J. Integr. Plant Biol.* **60**, 780–795 (2018).
4. Bin Rahman, A. & Zhang, J. Preferential geographic distribution pattern of abiotic stress tolerant rice. *Rice* **11**, 10 (2018).
5. Kim, H. Y., Ko, J., Kang, S. & Tenhunen, J. Impacts of climate change on paddy rice yield in a temperate climate. *Glob. Change Biol.* **19**, 548–562 (2013).
6. Laudencia-Chingcuanco, D. et al. Genome-wide gene expression analysis supports a developmental model of low temperature tolerance gene regulation in wheat (*Triticum aestivum* L.). *BMC Genomics* **12**, 299 (2011).
7. Morran, S. et al. Improvement of stress tolerance of wheat and barley by modulation of expression of DREB/CBF factors. *Plant Biotechnol. J.* **9**, 230–249 (2011).
8. Deng, W. et al. Direct links between the vernalization response and other key traits of cereal crops. *Nat. Commun.* **6**, 5882 (2015).
9. Fjellheim, S., Boden, S. & Trevaskis, B. The role of seasonal flowering responses in adaptation of grasses to temperate climates. *Front Plant Sci.* **5**, 432 (2014).
10. Oliver, S. N., Deng, W., Casao, M. C. & Trevaskis, B. Low temperatures induce rapid changes in chromatin state and transcript levels of the cereal VERNALIZATION1 gene. *J. Exp. Bot.* **64**, 2413–2422 (2013).
11. Chouard, P. Vernalization and its relations of dormancy. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **11**, 191–238 (1960).
12. Ma, Y. et al. COLD1 confers chilling tolerance in rice. *Cell* **160**, 1209–1221 (2015).
13. Zhang, Z. et al. OsMAPK3 phosphorylates OsbHLH002/OsICE1 and inhibits its ubiquitination to activate *OsTPP1* and enhances rice chilling tolerance. *Dev. Cell* **43**, 731–743 (2017).
14. Fowler, D. B., Chauvin, L. P., Limin, A. E. & Sarhan, F. The regulatory role of vernalization in the expression of low-temperature-induced genes in wheat and rye. *Theor. Appl. Genet.* **93**, 554–559 (1996).
15. Galiba, G. et al. Regulatory genes involved in the determination of frost tolerance in temperate cereals. *Plant Sci.* **176**, 12–19 (2009).
16. Shahryar, N. & Maali-Amiri, R. Metabolic acclimation of tetraploid and hexaploid wheats by cold stress-induced carbohydrate accumulation. *J. Plant Physiol.* **204**, 44–53 (2016).
17. Li, Q. et al. Transcriptomic insights into phenological development and cold tolerance of wheat grown in the field. *Plant Physiol.* **176**, 2376–2394 (2018).
18. Pearce, S. et al. Large deletions in the CBF gene cluster at the Fr-B2 locus are associated with reduced frost tolerance in wheat. *Theor. Appl. Genet.* **126**, 2683–2697 (2013).
19. Colton-Gagnon, K. et al. Comparative analysis of the cold acclimation and freezing tolerance capacities of seven diploid *Brachypodium distachyon* accessions. *Ann. Bot.* **113**, 681–693 (2014).
20. Sasaki, K., Christov, N. K., Tsuda, S. & Imai, R. Identification of a novel LEA protein involved in freezing tolerance in wheat. *Plant Cell Physiol.* **55**, 136–147 (2014).
21. Ying, M. & Kidou, S. Discovery of novel cold-induced CISP genes encoding small RNA-binding proteins related to cold adaptation in barley. *Plant Sci.* **260**, 129–138 (2017).
22. Shrestha, R., Gomez-Ariza, J., Brambilla, V. & Fornara, F. Molecular control of seasonal flowering in rice, arabidopsis and temperate cereals. *Ann. Bot.* **114**, 1445–1458 (2014).
23. Andres, F. & Coupland, G. The genetic basis of flowering responses to seasonal cues. *Nat. Rev. Genet.* **13**, 627–639 (2012).
24. Casal, J. J. & Questa, J. I. Light and temperature cues: multitasking receptors and transcriptional integrators. *New Phytol.* **217**, 1029–1034 (2018).
25. Franks, S. J., Sim, S. & Weis, A. E. Rapid evolution of flowering time by an annual plant in response to a climate fluctuation. *Proc. Natl Acad. Sci. USA* **104**, 1278–1282 (2007).
26. Greenup, A., Peacock, W. J., Dennis, E. S. & Trevaskis, B. The molecular biology of seasonal flowering-responses in Arabidopsis and the cereals. *Ann. Bot.* **103**, 1165–1172 (2009).
27. Distelfeld, A., Li, C. & Dubcovsky, J. Regulation of flowering in temperate cereals. *Curr. Opin. Plant Biol.* **12**, 178–184 (2009).
28. Yan, L. et al. Positional cloning of the wheat vernalization gene *VRN1*. *Proc. Natl Acad. Sci. USA* **100**, 6263–6268 (2003).
29. Bastow, R. et al. Vernalization requires epigenetic silencing of *FLC* by histone methylation. *Nature* **427**, 164–167 (2004).
30. Nowak, M. et al. Analysis of *VRN1* gene in triticale and common wheat genetic background. *Sci. Agricola* **71**, 380–386 (2014).
31. Trevaskis, B. The central role of the *VERNALIZATION1* gene in the vernalization response of cereals. *Funct. Plant Biol.* **37**, 479–487 (2010).
32. Johanson, U. et al. Molecular analysis of FRIGIDA, a major determinant of natural variation in Arabidopsis flowering time. *Science* **290**, 344–347 (2000).
33. Michaels, S. D. & Amasino, R. M. FLOWERING LOCUS C encodes a novel MADS domain protein that acts as a repressor of flowering. *Plant Cell* **11**, 949–956 (1999).
34. Sheldon, C. C. et al. The molecular basis of vernalization: the central role of FLOWERING LOCUS C (FLC). *Proc. Natl Acad. Sci. USA* **97**, 3753–3758 (2000).
35. Choi, K. et al. The FRIGIDA complex activates transcription of *FLC*, a strong flowering repressor in Arabidopsis, by recruiting chromatin modification factors. *Plant Cell* **23**, 289–303 (2011).
36. Alexandre, C. M. & Hennig, L. FLC or not FLC: the other side of vernalization. *J. Exp. Biol.* **59**, 1127–1135 (2008).
37. Michaels, S. D. et al. Integration of flowering signals in winter-annual Arabidopsis. *Plant Physiol.* **137**, 149–156 (2005).
38. Sheldon, C. C., Finnegan, E. J., Dennis, E. S. & Peacock, W. J. Quantitative effects of vernalization on FLC and SOC1 expression. *Plant J.* **45**, 871–883 (2006).
39. Bond, D. M., Dennis, E. S. & Finnegan, E. J. Hypoxia: a novel function for VIN3. *Plant Signal. Behav.* **4**, 773–776 (2009).
40. Gendall, A. R., Levy, Y. Y., Wilson, A. & Dean, C. The *VERNALIZATION 2* gene mediates the epigenetic regulation of vernalization in Arabidopsis. *Cell* **107**, 525–535 (2001).
41. Levy, Y. Y. et al. Multiple roles of Arabidopsis VRN1 in vernalization and flowering time control. *Science* **297**, 243–246 (2002).
42. Searle, I. et al. The transcription factor FLC confers a flowering response to vernalization by repressing meristem competence and systemic signaling in Arabidopsis. *Genes Dev.* **20**, 898–912 (2006).
43. Doyle, M. R. & Amasino, R. M. A single amino acid change in the enhancer of zeste ortholog CURLY LEAF results in vernalization-independent, rapid flowering in Arabidopsis. *Plant Physiol.* **151**, 1688–1697 (2009).
44. Wood, C. C. et al. 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 (2006).
45. Yang, H. et al. Distinct phases of Polycomb silencing to hold epigenetic memory of cold in Arabidopsis. *Science* **357**, 1142–1145 (2017).
46. Sung, S. & Amasino, R. M. Vernalization in *Arabidopsis thaliana* is mediated by the PHD finger protein VIN3. *Nature* **427**, 159–164 (2004).
47. Bond, D. M., Dennis, E. S., Pogson, B. J. & Finnegan, E. J. Histone acetylation, VERNALIZATION INSENSITIVE 3, FLOWERING LOCUS C, and the vernalization response. *Mol. Plant* **2**, 724–737 (2009).
48. Greb, T. et al. The PHD finger protein VRN5 functions in the epigenetic silencing of Arabidopsis FLC. *Curr. Biol.* **17**, 73–78 (2007).
49. Mylne, J. S. et al. LHP1, the Arabidopsis homologue of HETEROCHROMATIN PROTEIN1, is required for epigenetic silencing of FLC. *Proc. Natl Acad. Sci. USA* **103**, 5012–5017 (2006).
50. Turck, F. et al. Arabidopsis TFL2/LHP1 specifically associates with genes marked by trimethylation of histone H3 lysine 27. *PLoS Genet.* **3**, 855–866 (2007).
51. Sung, S. et al. Epigenetic maintenance of the vernalized state in *Arabidopsis thaliana* requires LIKE HETEROCHROMATIN PROTEIN 1. *Nat. Genet.* **38**, 706–710 (2006).
52. Yan, L. et al. The wheat VRN2 gene is a flowering repressor down-regulated by vernalization. *Science* **303**, 1640–1644 (2004).
53. Yan, L. et al. The wheat and barley vernalization gene VRN3 is an orthologue of FT. *Proc. Natl Acad. Sci. USA* **103**, 19581–19586 (2006).

54. Dubcovsky, J. Regulation of flowering time in wheat and barley. *Comp. Biochem. Phys. A* **141**, 263–264 (2005).
55. Dhillon, T. et al. Regulation of freezing tolerance and flowering in temperate cereals: the VRN-1 connection. *Plant Physiol.* **153**, 1846–1858 (2010).
56. Golovnina, K. A., Kondratenko, E. Y., Blinov, A. G. & Goncharov, N. P. Molecular characterization of vernalization loci VRN1 in wild and cultivated wheats. *BMC Plant Biol.* **10**, 168 (2010).
57. Ferrandiz, C., Gu, Q., Martienssen, R. & Yanofsky, M. F. Redundant regulation of meristem identity and plant architecture by FRUITFULL, APETALA1 and CAULIFLOWER. *Development* **127**, 725–734 (2000).
58. Konopatskaia, I. et al. VRN1 genes variability in tetraploid wheat species with a spring growth habit. *BMC Plant Biol.* **16**, 244 (2016).
59. Fu, D. L. et al. Large deletions within the first intron in VRN-1 are associated with spring growth habit in barley and wheat. *Mol. Genet. Genomics* **273**, 54–65 (2005).
60. Yan, L. et al. Allelic variation at the VRN-1 promoter region in polyploid wheat. *Theor. Appl. Genet.* **109**, 1677–1686 (2004).
61. Muterko, A., Kalendar, R. & Salina, E. Novel alleles of the VERNALIZATION1 genes in wheat are associated with modulation of DNA curvature and flexibility in the promoter region. *BMC Plant Biol.* **16**, 9 (2016).
62. Kamran, A., Randhawa, H. S., Yang, R. C. & Spaner, D. The effect of VRN1 genes on important agronomic traits in high-yielding Canadian soft white spring wheat. *Plant Breeding* **133**, 321–326 (2014).
63. Kosner, J. & Pankova, K. The detection of allelic variants at the recessive vrn loci of winter wheat. *Euphytica* **101**, 9–16 (1998).
64. Shcherban, A. B., Khlestkina, E. K., Efremova, T. T. & Salina, E. A. The effect of two differentially expressed wheat VRN-B1 alleles on the heading time is associated with structural variation in the first intron. *Genetica* **141**, 133–141 (2013).
65. Xing, L. et al. Phosphorylation modification of wheat lectin VER2 is associated with vernalization-induced *O*-GlcNAc signaling and intracellular motility. *PLoS ONE* **4**, e4854 (2009).
66. Yong, W. D. et al. Vernalization-induced flowering in wheat is mediated by a lectin-like gene VER2. *Planta* **217**, 261–270 (2003).
67. Xiao, J. et al. *O*-GlcNAc-mediated interaction between VER2 and TaGRP2 elicits *TaVRN1* mRNA accumulation during vernalization in winter wheat. *Nat. Commun.* **5**, 4572 (2014).
68. Kippes, N. et al. Fine mapping and epistatic interactions of the vernalization gene VRN-D4 in hexaploid wheat. *Mol. Genet. Genomics* **289**, 47–62 (2014).
69. Kippes, N. et al. Identification of the *VERNALIZATION 4* gene reveals the origin of spring growth habit in ancient wheats from South Asia. *Proc. Natl Acad. Sci. USA* **112**, 5401–5410 (2015).
70. Woods, D. P. et al. Establishment of a vernalization requirement in *Brachypodium distachyon* requires REPRESSOR OF VERNALIZATION1. *Proc. Natl Acad. Sci. USA* **114**, 6623–6628 (2017).
71. Chen, A. & Dubcovsky, J. Wheat TILLING mutants show that the vernalization gene VRN1 down-regulates the flowering repressor VRN2 in leaves but is not essential for flowering. *PLoS Genet.* **8**, e1003134 (2012).
72. Distelfeld, A. et al. Genetic and molecular characterization of the *VRN2* loci in tetraploid wheat. *Plant Physiol.* **149**, 245–257 (2009).
73. Dubcovsky, J. et al. Effect of photoperiod on the regulation of wheat vernalization genes *VRN1* and *VRN2*. *Plant Mol. Biol.* **60**, 469–480 (2006).
74. Dubcovsky, J., Chen, C. L. & Yan, L. L. Molecular characterization of the allelic variation at the VRN-H2 vernalization locus in barley. *Mol. Breeding* **15**, 395–407 (2005).
75. Li, Y. & Xu, M. CCT family genes in cereal crops: A current overview. *Crop J.* **5**, 449–458 (2017).
76. Pin, P. A. et al. An antagonistic pair of FT homologs mediates the control of flowering time in sugar beet. *Science* **330**, 1397–1400 (2010).
77. Abe, M. et al. FD, a bZIP protein mediating signals from the floral pathway integrator FT at the shoot apex. *Science* **309**, 1052–1056 (2005).
78. Li, C. & Dubcovsky, J. Wheat FT protein regulates *VRN1* transcription through interactions with FDL2. *Plant J.* **55**, 543–554 (2008).
79. Li, C., Lin, H. & Dubcovsky, J. Factorial combinations of protein interactions generate a multiplicity of florigen activation complexes in wheat and barley. *Plant J.* **84**, 70–82 (2015).
80. Distelfeld, A. & Dubcovsky, J. Characterization of the maintained vegetative phase deletions from diploid wheat and their effect on VRN2 and FT transcript levels. *Mol. Genet. Genomics* **283**, 223–232 (2010).
81. Hemming, M. N., Peacock, W. J., Dennis, E. S. & Trevaskis, B. Low-temperature and daylength cues are integrated to regulate FLOWERING LOCUS T in barley. *Plant Physiol.* **147**, 355–366 (2008).
82. Shimada, S. et al. A genetic network of flowering-time genes in wheat leaves, in which an APETALA1/FRUITFULL-like gene, VRN1, is upstream of FLOWERING LOCUS T. *Plant J.* **58**, 668–681 (2009).
83. Li, C., Distelfeld, A., Comis, A. & Dubcovsky, J. Wheat flowering repressor VRN2 and promoter CO2 compete for interactions with NUCLEAR FACTOR-Y complexes. *Plant J.* **67**, 763–773 (2011).
84. Chen, A. et al. PHYTOCHROME C plays a major role in the acceleration of wheat flowering under long-day photoperiod. *Proc. Natl Acad. Sci. USA* **111**, 10037–10044 (2014).
85. Pearce, S. et al. RNA-seq studies using wheat PHYTOCHROME B and PHYTOCHROME C mutants reveal shared and specific functions in the regulation of flowering and shade-avoidance pathways. *BMC Plant Biol.* **16**, 141 (2016).
86. Muterko, A. F., Balashova, I. A., Fayt, V. I. & Sivolap, Y. M. Molecular-genetic mechanisms of regulation of growth habit in wheat. *Cytol. Genet.* **49**, 58–71 (2015).
87. Greenup, A. G. et al. ODDSOC2 Is a MADS box floral repressor that is down-regulated by vernalization in temperate cereals. *Plant Physiol.* **153**, 1062–1073 (2010).
88. Sharma, N. et al. A Flowering Locus C homolog is a vernalization-regulated repressor in Brachypodium and is cold regulated in wheat. *Plant Physiol.* **173**, 1301–1315 (2017).
89. Higgins, J. A., Bailey, P. C. & Laurie, D. A. Comparative genomics of flowering time pathways using *Brachypodium distachyon* as a model for the temperate grasses. *PLoS ONE* **5**, e10065 (2010).
90. Ream, T. S. et al. Interaction of photoperiod and vernalization determines flowering time of *Brachypodium distachyon*. *Plant Physiol.* **164**, 694–709 (2014).
91. Woods, D. P., Ream, T. S. & Amasino, R. M. Memory of the vernalized state in plants including the model grass Brachypodium distachyon. *Front Plant Sci.* **5**, 99 (2014).
92. Woods, D. P. et al. Evolution of VRN2/Ghd7-like genes in vernalization-mediated repression of grass flowering. *Plant Physiol.* **170**, 2124–2135 (2016).
93. Feng, Y., Yin, Y. & Fei, S. *BdVRN1* expression confers flowering competency and is negatively correlated with freezing tolerance in *Brachypodium distachyon*. *Front Plant Sci.* **8**, 1107 (2017).
94. Chen, L. et al. OsMADS57 together with OsTB1 coordinates transcription of its target OsWRKY94 and D14 to switch its organogenesis to defense for cold adaptation in rice. *New Phytol.* **218**, 219–231 (2018).
95. Sung, S. Epigenetic memory of winter by plants. *Integr. Comp. Biol.* **54**, 203 (2014).
96. Curtis, O. & Chang, H. The relative effectiveness of the temperature of the crown as contrasted with that of the rest of the plant upon the flowering of celery plants. *Am. J. Botany*, 1047–1048 (1930).
97. Melchers, G. Versuche zur genetik und entwicklungs-physiologie der blühreife. *Biol. Zentralbl.* **56**, 567–570 (1936).
98. Crosthwaite, S. K. & Jenkins, G. I. The role of leaves in the perception of vernalizing temperatures in sugar-beet. *J. Exp. Bot.* **44**, 801–806 (1993).
99. Metzger, J. D. Localization of the site of perception of thermoinductive temperatures in *Thlaspi arvense* L. *Plant Physiol.* **88**, 424–428 (1988).
100. Wellensiek, S. J. Dividing cells as locus for vernalization. *Nature* **195**, 307 (1962).
101. Wellensiek, S. J. Dividing cells as prerequisite for vernalization. *Plant Physiol.* **39**, 832 (1964).
102. Sung, S. & Amasino, R. M. Vernalization and epigenetics: how plants remember winter. *Curr. Opin Plant Biol.* **7**, 4–10 (2004).
103. Amasino, R. Vernalization, competence, and the epigenetic memory of winter. *Plant Cell* **16**, 2553–2559 (2004).
104. Lang, A. & Melchers, G. Vernalization und devernalisation bei einer zweijahrigen pelanze. *Z. Naturforsch. B* **2**, 444–449 (1947).
105. Lang, A. in *Encyclopedia of Plant Physiology* Vol. 15 (ed. Ruhland, W.) 1380–1536 (Springer-Verlag, Berlin, 1965).
106. Oliver, S. N. et al. Vernalization-induced flowering in cereals is associated with changes in histone methylation at the *VERNALIZATION1* gene. *Proc. Natl Acad. Sci. USA* **106**, 8386–8391 (2009).
107. Yong, W. D. et al. Cloning and characterization of vernalization-related gene (ver203F) cDNA 3' end. *Chinese Science Bulletin* **44**, 1289–1294 (1999).
108. Berr, A. et al. SET DOMAIN GROUP25 encodes a histone methyltransferase and is involved in FLOWERING LOCUS C activation and repression of flowering. *Plant Physiol.* **151**, 1476–1485 (2009).
109. Deal, R. B., Topp, C. N., McKinney, E. C. & Meagher, R. B. Repression of flowering in Arabidopsis requires activation of FLOWERING LOCUS C expression by the histone variant H2A. Z. *Plant Cell* **19**, 74–83 (2007).
110. Pien, S. et al. ARABIDOPSIS TRITHORAX1 dynamically regulates FLOWERING LOCUS C activation via histone 3 lysine 4 trimethylation. *Plant Cell* **20**, 580–588 (2008).
111. Hu, X. et al. Proteasome-mediated degradation of FRIGIDA modulates flowering time in Arabidopsis during vernalization. *Plant Cell* **26**, 4763–4781 (2014).

112. Xiao, J. et al. Cis and trans determinants of epigenetic silencing by Polycomb repressive complex 2 in Arabidopsis. *Nat. Genet.* **49**, 1546–1552 (2017).
113. Zhou, Y. et al. Telobox motifs recruit CLF/SWN-PRC2 for H3K27me3 deposition via TRB factors in Arabidopsis. *Nat. Genet.* **50**, 638–644 (2018).
114. Heo, J. B., Lee, Y. S. & Sung, S. Epigenetic regulation by long noncoding RNAs in plants. *Chromosome Res.* **21**, 685–693 (2013).
115. Kim, D. H. & Sung, S. The binding specificity of the PHD-finger domain of VIN3 moderates vernalization response. *Plant Physiol.* **173**, 1258–1268 (2017).
116. Csorba, T., Questa, J. I., Sun, Q. & Dean, C. Antisense COOLAIR mediates the coordinated switching of chromatin states at FLC during vernalization. *Proc. Natl Acad. Sci. USA* **111**, 16160–16165 (2014).
117. Heo, J. B. & Sung, S. Vernalization-mediated epigenetic silencing by a long intronic noncoding RNA. *Science* **331**, 76–79 (2011).
118. Kim, D. H. & Sung, S. Vernalization-triggered intragenic chromatin loop formation by long noncoding RNAs. *Dev. Cell* **40**, 302–312 (2017).
119. Questa, J. I. et al. Arabidopsis transcriptional repressor VAL1 triggers Polycomb silencing at FLC during vernalization. *Science* **353**, 485–488 (2016).
120. Yuan, W. et al. A cis cold memory element and a trans epigenome reader mediate Polycomb silencing of FLC by vernalization in Arabidopsis. *Nat. Genet.* **48**, 1527–1534 (2016).
121. Zhu, D., Rosa, S. & Dea, nC. Nuclear organization changes and the epigenetic silencing of *FLC* during vernalization. *J. Mol. Biol.* **427**, 659–669 (2015).
122. Kim, D. H., Zografos, B. R. & Sung, S. Mechanisms underlying vernalization-mediated VIN3 induction in Arabidopsis. *Plant Signal. Behav.* **5**, 1457–1459 (2010).
123. Velanis, C. N. & Goodrich, J. Vernalization and epigenetic inheritance: a game of histones. *Curr. Biol.* **27**, 1324–1326 (2017).
124. Tao, Z. et al. Embryonic epigenetic reprogramming by a pioneer transcription factor in plants. *Nature* **551**, 124 (2017).
125. Hepworth, J. & Dean, C. Flowering Locus C's lessons: conserved chromatin switches underpinning developmental timing and adaptation. *Plant Physiol.* **168**, 1237–1245 (2015).
126. Whittaker, C. & Dean, C. The FLC Locus: a platform for discoveries in epigenetics and adaptation. *Ann Rev Cell Dev. Biol.* **33**, 555–575 (2017).
127. Berry, S. et al. Local chromatin environment of a Polycomb target gene instructs its own epigenetic inheritance. *eLife* **4**, e07205 (2015).
128. Crevillen, P. et al. Epigenetic reprogramming that prevents transgenerational inheritance of the vernalized state. *Nature* **515**, 587 (2014).
129. Diallo, A. O. et al. Expression of vernalization responsive genes in wheat is associated with histone H3 trimethylation. *Mol. Genet. Genomics* **287**, 575–590 (2012).
130. Chittock, E. C., Latwiel, S., Miller, T. & Mueller, C. W. Molecular architecture of polycomb repressive complexes. *Biochem. Soc. T* **45**, 193–205 (2017).
131. Simon, J. A. & Tamkun, J. W. Programming off and on states in chromatin: mechanisms of Polycomb and trithorax group complexes. *Curr. Opin. Genet. Dev.* **12**, 210–218 (2002).
132. Lomax, A. et al. An ortholog of CURLY LEAF/ENHANCER OF ZESTE like-1 is required for proper flowering in Brachypodium distachyon. *Plant J.* **93**, 871–882 (2018).
133. Huan, Q. et al. Transcriptome-wide analysis of vernalization reveals conserved and species-specific mechanisms in Brachypodium. *J. Integr. Plant Biol.* **55**, 696–709 (2013).
134. Huan, Q., Mao, Z., Chong, K. & Zhang, J. Global analysis of H3K4me3/H3K27me3 in *Brachypodium distachyon* reveals VRN3 as critical epigenetic regulation point in vernalization and provides insights into epigenetic memory. *New Phytol.* **219**, 1373–1387 (2018).
135. Bernier, G. & Perilleux, C. A physiological overview of the genetics of flowering time control. *Plant Biotechnol. J.* **3**, 3–16 (2005).
136. Trione, E. J. Metabolic changes associated with vernalization of wheat. I. carbohydrate and nitrogen patterns. *Plant Physiol.* **41**, 277–281 (1966).
137. Teo, C. F., Wollaston-Hayden, E. E. & Wells, L. Hexosamine flux, the *O*-GlcNAc modification, and the development of insulin resistance in adipocytes. *Mol. Cell Endocrinol.* **318**, 44–53 (2010).
138. Rafie, K. et al. Recognition of a glycosylation substrate by the *O*-GlcNAc transferase TPR repeats. *Open Biol.* **7**, 170078 (2017).
139. Nagel, A. K. & Ball, L. E. *O*-GlcNAc transferase and *O*-GlcNAcase: achieving target substrate specificity. *Amino Acids* **46**, 2305–2316 (2014).
140. Hu, P., Shimoji, S. & Hart, G. W. Site-specific interplay between *O*-GlcNAcylation and phosphorylation in cellular regulation. *FEBS Lett.* **584**, 2526–2538 (2010).
141. Butkinaree, C., Park, K. & Hart, G. W. O-linked beta-N-acetylglucosamine (*O*-GlcNAc): extensive crosstalk with phosphorylation to regulate signalling and transcription in response to nutrients and stress. *Biochim. Biophys. Acta* **1800**, 96–106 (2010).
142. Slawson, C. & Hart, G. W. Dynamic interplay between *O*-GlcNAc and O-phosphate: the sweet side of protein regulation. *Curr. Opin. Struct. Biol.* **13**, 631–636 (2003).
143. Olszewski, N. E., West, C. M., Sassi, S. O. & Hartweck, L. M. *O*-GlcNAc protein modification in plants: Evolution and function. *Biochim. Biophys. Acta* **1800**, 49–56 (2010).
144. Zentella, R. et al. *O*-GlcNAcylation of master growth repressor DELLA by SECRET AGENT modulates multiple signaling pathways in Arabidopsis. *Genes Dev.* **30**, 164–176 (2016).
145. Zentella, R. et al. The Arabidopsis O-fucosyltransferase SPINDLY activates nuclear growth repressor DELLA. *Nat. Chem. Biol.* **13**, 479–485 (2017).
146. Xu, S. L. et al. Proteomic analysis reveals *O*-GlcNAc modification on proteins with key regulatory functions in Arabidopsis. *Proc. Natl Acad. Sci. USA* **114**, 1536–1543 (2017).
147. Xing, L. et al. Arabidopsis *O*-GlcNAc transferase SEC targets histone methyltransferase ATX1 to regulate flowering. *EMBO J.* **37**, e98115 (2018).
148. Humphreys, A. M. & Linder, H. Evidence for recent evolution of cold tolerance in grasses suggests current distribution is not limited by (low) temperature. *New Phytol.* **198**, 1261–1273 (2013).
149. Poethig, R. S. Phase change and the regulation of developmental timing in plants. *Science* **301**, 334–336 (2003).
150. McKeown, M. et al. Evidence for an early origin of vernalization responsiveness in temperate pooideae grasses. *Plant Physiol.* **172**, 416–426 (2016).
151. Christin, P. A. et al. Molecular dating, evolutionary rates, and the age of the grasses. *Syst. Biol.* **63**, 153–165 (2014).
152. Zhong, J., Robbett, M., Poire, A. & Preston, J. C. Successive evolutionary steps drove Pooideae grasses from tropical to temperate regions. *New Phytol.* **217**, 925–938 (2018).
153. Xue, W. et al. Natural variation in Ghd7 is an important regulator of heading date and yield potential in rice. *Nat. Genet.* **40**, 761–767 (2008).
154. Woods, D. P. et al. Genetic architecture of flowering-time variation in Brachypodium distachyon. *Plant Physiol.* **173**, 269–279 (2017).
155. Tsuji, H. & Taoka, K. Florigen signaling. *Enzymes* **35**, 113–144 (2014).
156. Shcherban, A. B. & Salina, E. A. Evolution of VRN-1 homoeologous loci in allopolyploids of Triticum and their diploid precursors. *BMC Plant Biol.* **17**, 188 (2017).
157. Weng, X. et al. Grain number, plant height, and heading date7 is a central regulator of growth, development, and stress response. *Plant Physiol.* **164**, 735–747 (2014).
158. Nelson, M. N. et al. The loss of vernalization requirement in narrow-leafed lupin is associated with a deletion in the promoter and de-repressed expression of a Flowering Locus T (FT) homologue. *New Phytol.* **213**, 220–232 (2017).
159. Ruelens, P. et al. FLOWERING LOCUS C in monocots and the tandem origin of angiosperm-specific MADS-box genes. *Nat. Commun.* **4**, 2280 (2013).
160. Vogt, S. H. et al. The FLC-like gene BvFL1 is not a major regulator of vernalization response in biennial beets. *Front. Plant Sci.* **5**, 146 (2014).
161. Reeves, P. A. et al. Evolutionary conservation of the FLOWERING LOCUS C-mediated vernalization response: evidence from the sugar beet (*Beta vulgaris*). *Genetics* **176**, 295–307 (2007).
162. Pfeiffer, N. et al. Genetic analysis of bolting after winter in sugar beet (*Beta vulgaris* L.). *Theor. Appl. Genet.* **127**, 2479–2489 (2014).
163. Hecht, V. et al. The pea GIGAS gene is a FLOWERING LOCUS T homolog necessary for graft-transmissible specification of flowering but not for responsiveness to photoperiod. *Plant Cell* **23**, 147–161 (2011).
164. Laurie, R. E. et al. The Medicago FLOWERING LOCUS T homolog, MtFTa1, is a key regulator of flowering time. *Plant Physiol.* **156**, 2207–2224 (2011).
165. Putterill, J. & Varkonyi-Gasic, E. FT and florigen long-distance flowering control in plants. *Curr. Opin. Plant Biol.* **33**, 77–82 (2016).
166. Turck, F., Fornara, F. & Coupland, G. Regulation and identity of florigen: FLOWERING LOCUS T moves center stage. *Annu. Rev. Plant Biol.* **59**, 573–594 (2008).
167. Oliva, M. et al. FIE, a nuclear PRC2 protein, forms cytoplasmic complexes in *Arabidopsis thaliana*. *J. Exp. Bot.* **67**, 6111–6123 (2016).
168. Spillane, C. et al. Interaction of the Arabidopsis polycomb group proteins FIE and MEA mediates their common phenotypes. *Curr. Biol.* **10**, 1535–1538 (2000).
169. Derkacheva, M. et al. Arabidopsis MSI1 connects LHP1 to PRC2 complexes. *EMBO J.* **32**, 2073–2085 (2013).
170. Steinbach, Y. & Hennig, L. Arabidopsis MSI1 functions in photoperiodic flowering time control. *Front. Plant Sci.* **5**, 77 (2014).
171. Kim, S. Y., Zhu, T. & Sung, Z. R. Epigenetic regulation of gene programs by EMF1 and EMF2 in Arabidopsis. *Plant Physiol.* **152**, 516–528 (2010).
172. Luo, M. et al. Expression and parent-of-origin effects for FIS2, MEA, and FIE in the endosperm and embryo of developing Arabidopsis seeds. *Proc. Natl Acad. Sci. USA* **97**, 10637–10642 (2000).

173. Tang, X. et al. Synergistic repression of the embryonic programme by SET DOMAIN GROUP 8 and EMBRYONIC FLOWER 2 in Arabidopsis seedlings. *J. Exp. Bot.* **63**, 1391–1404 (2012).
174. Spillane, C. et al. Positive darwinian selection at the imprinted MEDEA locus in plants. *Nature* **448**, 349–352 (2007).
175. Xu, Y. et al. Regulation of vegetative phase change by SWI2/SNF2 chromatin remodeling ATPase BRAHMA. *Plant Physiol.* **172**, 2416–2428 (2016).
176. Yu, X. et al. Modulation of brassinosteroid-regulated gene expression by Jumonji domain-containing proteins ELF6 and REF6 in Arabidopsis. *Proc. Natl Acad. Sci. USA* **105**, 7618–7623 (2008).

Acknowledgements

We gratefully acknowledge funding from NSFC for the basic science centre program (31788103) and the major state basic research program of China (973).

Author contributions

S. X. wrote a draft of this article. K. C. designed the outline of the manuscript and polished the article.

Competing interests

The authors declare no competing interests.

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