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Review
Functional Plant Biology, 2010, **37**, 479–487

Goldacre Paper

The central role of the *VERNALIZATION1* gene in the vernalization response of cereals

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This paper originates from the Peter Goldacre Award 2009 of the Australian Society of Plant Scientists that was received by the author.

Abstract. Many varieties of wheat (*Triticum* spp.) and barley (*Hordeum vulgare* L.) require prolonged exposure to cold during winter in order to flower (vernalization). In these cereals, vernalization-induced flowering is controlled by the *VERNALIZATION1* (*VRN1*) gene. *VRN1* is a promoter of flowering that is activated by low temperatures. *VRN1* transcript levels increase gradually during vernalization, with longer cold treatments inducing higher expression levels. Elevated *VRN1* expression is maintained in the shoot apex and leaves after vernalization, and the level of *VRN1* expression in these organs determines how rapidly vernalized plants flower. Some alleles of *VRN1* are expressed without vernalization due to deletions or insertions within the promoter or first intron of the *VRN1* gene. Varieties of wheat and barley with these alleles flower without vernalization and are grown where vernalization does not occur. The first intron of the *VRN1* locus has histone modifications typically associated with the maintenance of an inactive chromatin state, suggesting this region is targeted by epigenetic mechanisms that contribute to repression of *VRN1* before winter. Other mechanisms are likely to act elsewhere in the *VRN1* gene to mediate low-temperature induction. This review examines how understanding the mechanisms that regulate *VRN1* provides insights into the biology of vernalization-induced flowering in cereals and how this will contribute to future cereal breeding strategies.

Additional keywords: barley, flowering, wheat.

Vernalization-induced flowering

Plants growing in temperate regions time flowering to coincide with favourable seasonal conditions. Winter frost can damage cold-sensitive floral organs, whereas heat and water stress during summer can reduce fertility, so flowering often occurs in spring when conditions are optimal (see King and Heide 2009). One cue that promotes spring flowering is prolonged exposure to cold during winter, or vernalization. Vernalization occurs in many plants (see Chouard 1960; Amasino 2004; King and Heide 2009) but the focus of this review is the molecular mechanisms that control vernalization-induced flowering in economically important cereal crops such as wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.).

The vernalization response in cereals

In temperate regions, wheat and barley are sown in autumn then over-winter before flowering in spring. When sown in spring, these same cereal varieties typically show delayed flowering or fail to flower altogether. Several researchers recognised that the cold of winter is a critical factor required to trigger flowering of these plants and that this is lacking when plants are sown in spring (see McKinney 1940; Chouard 1960). For example, Gassner showed that germinating wheat or rye (*Secale cereale* M. Bieb.) seeds at normal growth temperatures can cause a strong delay of flowering, whereas germination at low temperatures can stimulate flowering (Gassner 1918). He concluded that many cereals have a requirement for cold, or "*Kaltbedürfnis*", which must be satisfied to allow flowering (Gassner 1918). This phenomenon later came to be referred to as vernalization (*vernalis* meaning "pertaining to spring"; see Chouard 1960).

Vernalization promotes the transition to reproductive development at the shoot apex (Flood and Halloran 1984). Thus, after vernalization, the production of leaf primordia ceases and floral primordial appear at the shoot apex (Bonnet 1935, 1936; Zadoks *et al*. 1974). These develop into inflorescence branches that bear the florets (flowers) (Bonnet 1935, 1936; Zadoks *et al*. 1974). Vernalization is sufficient to trigger the transition to reproductive development, but long days are then required for rapid inflorescence development and stem elongation (Purvis 1934; Gott *et al*. 1955) (Fig. 1). Critically, vernalization is a prerequisite for the acceleration of flowering by long days, so long days cause rapid flowering only after plants have been vernalized (Purvis 1934). This combination of vernalization requirement and daylength sensitivity ensures that flowering is

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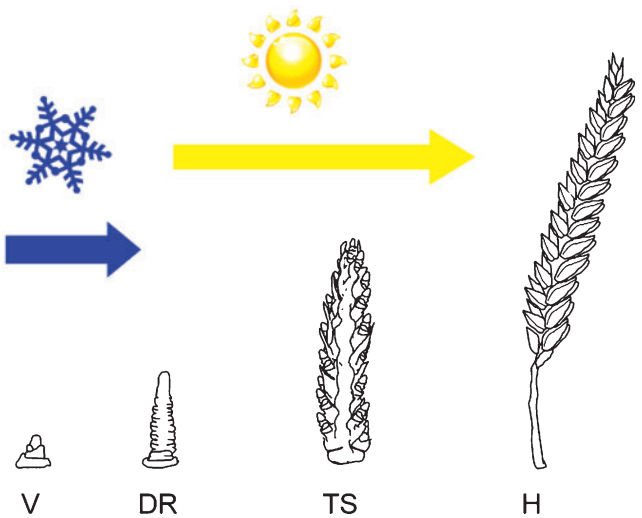

Fig. 1. The influence of vernalization and daylength on development of the shoot apex of wheat. Vernalization-responsive varieties of wheat or barley are sown in autumn and require vernalization (blue arrow) during winter to promote the transition to reproductive development at the shoot apex. The transition to reproductive development is marked by the appearance of lateral inflorescence primordia at the shoot apex, in addition to the leaf primordia, which gives rise to distinctive double ridges (DR). After plants have been vernalized, increasing daylength (yellow arrow) can accelerate inflorescence development and stem elongation, so development proceeds rapidly to the terminal spikelet stage (TS) and eventual head emergence (H) in spring. Wheats or barleys with reduced photoperiod sensitivity or vernalization requirement will show other flowering behaviours.

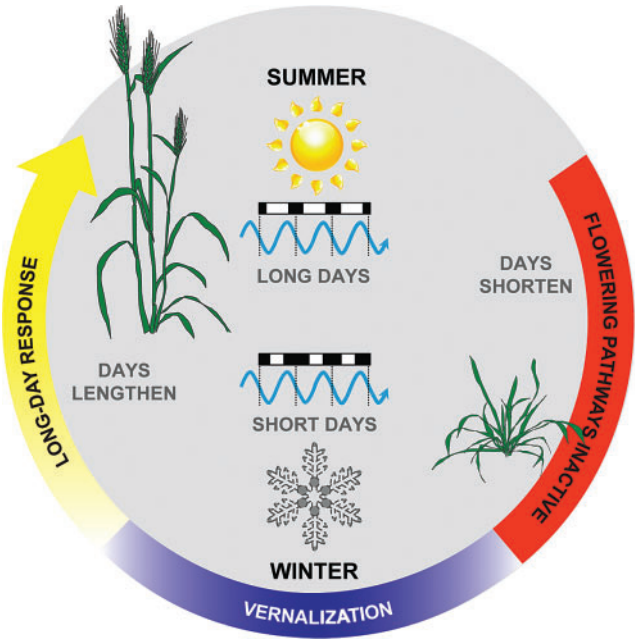

Fig. 2. Seasonal flowering responses of temperate cereals. Flowering of autumn-sown (vernalization-responsive) cereals is delayed before winter because neither daylength nor vernalization response pathways are active. During winter, plants are vernalized, promoting the transition to reproductive development and making plants competent to respond to long days. Subsequently, plants flower rapidly as days lengthen during spring.

delayed until after winter to avoid frost damage but then occurs rapidly as daylength increases during spring, thereby avoiding heat and water stress during summer (Fig. 2). A similar combination of vernalization requirement and daylength sensitivity is found in many plants from temperate zones (see Thomas and Vince-Prue 1997; King and Heide 2009).

The effect of vernalization is cumulative, so increasing durations of cold accelerate flowering to greater extents until a point when the vernalization response is saturated (Gott *et al.* 1955). Acceleration of flowering by vernalization is also temperature dependent and, typically, there is an optimal temperature for vernalization between 0 and 10°C that will saturate the vernalization response more rapidly than warmer or colder temperatures (Gassner 1918; Chouard 1960). Thus, vernalization triggers a quantitative flowering response in cereals, and the effectiveness of vernalization is both time and temperature dependent.

Vernalization is remembered

When cells from vernalized wheat are used to regenerate plants through tissue culture, the resulting plants do not require vernalization to flower (Marcińska *et al.* 1995). Similarly, wheat cells can be vernalized during tissue culture to give rise to plants that flower rapidly with no further requirement for vernalization (Whelan and Schaalje 1992). Furthermore, maturing seeds on the spike (inflorescence) can be exposed to prolonged cold and then allowed to undergo the normal process of seed drying and germination to give rise to seedlings that flower without vernalization (Gregory and Purvis 1936). These observations suggest that cereals retain a cellular memory of vernalization. The idea that plants retain a memory of vernalization is consistent with the way vernalization promotes flowering: when germinating seeds are exposed to prolonged cold, there are no visible signs of floral development at the end of vernalization, but plants undergo rapid floral development when placed at normal growth temperatures after vernalization (Purvis 1934; Flood and Halloran 1984; Sasani *et al.* 2009). So exposure to cold is remembered and this exerts an after-effect during subsequent development (Chouard 1960). After plants flower, the memory of vernalization is presumably reset in seeds to allow the vernalization response to reoccur in the next generation.

The molecular basis of vernalization-induced flowering in cereals

The *VERNALIZATION1* gene (*VRN1*) controls vernalization-induced flowering in cereals (see Trevaskis *et al.* 2007*a*; Distelfeld *et al.* 2009). *VRN1* encodes a MADS box transcription factor (a class of transcription factor named after the archetypal genes *MCM1*, *AGAMOUS*, *DEFICIENS*, *SRF1*) related to genes that promote flowering in other plant species (Danyluk *et al.* 2003; Trevaskis *et al.* 2003; Yan *et al.* 2003). *VRN1* transcripts are present at low basal levels but increase during prolonged cold treatment (Danyluk *et al.* 2003; Trevaskis *et al.* 2003; Yan *et al.* 2003). This response is quantitative, with longer cold treatments inducing higher transcript levels (Danyluk *et al.* 2003; Yan *et al.* 2003; von Zitzewitz *et al.* 2005; Sasani *et al.* 2009). This parallels the degree to which

flowering is accelerated by increasing lengths of cold treatment (Sasani *et al*. 2009). Thus, it seems likely that *VRN1* is a cold-activated promoter of flowering that mediates vernalization-induced flowering in cereals. The activity of *VRN1* might be essential for flowering in cereals, since a mutant of Einkorn wheat (*Triticum monococcum*) that lacks *VRN1* is unable to flower (Shitsukawa *et al*. 2007). However, a recent study shows that the non-flowering phenotype of this mutant might also be partly due to deletion of genes flanking *VRN1* (Distelfeld and Dubcovsky 2010).

VRN1 acts at the shoot apex and in leaves to promote flowering

Vernalization activates expression of *VRN1* in the leaf and shoot apex, and elevated expression of *VRN1* is maintained in these tissues following vernalization (Yan *et al*. 2003; Sasani *et al*. 2009). *VRN1* is likely to have distinct functions in each of these organs. At the shoot apex, expression of *VRN1* is likely to promote the transition to reproductive development, so the production of leaves ceases and inflorescence development begins (Gocal *et al*. 2001; Loukoianov *et al*. 2005; Hemming *et al*. 2008; Preston and Kellogg 2008; Sasani *et al*. 2009) (Fig. 3). *VRN1*-like genes play similar roles in promoting phase transition in other plants,

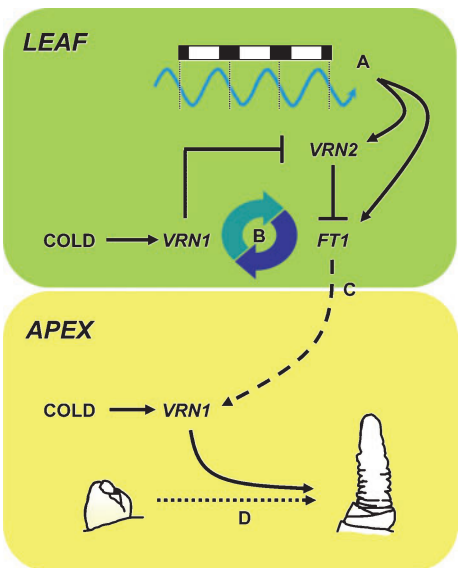

Fig. 3. Specific roles for *VRN1* in different organs of vernalized plants. Prior to winter, *VRN1* is expressed at low levels in the leaf and shoot apex, while *VRN2* is expressed in leaves during longer days, inhibiting long-day induction of *FT1* (*A*). After vernalization, *VRN1* is expressed in the shoot apex and leaves. In the leaves, *VRN1* downregulates *VRN2*, allowing long-day induction of *FT1*. *VRN1* might also activate *FT1* through *VRN2* independent mechanisms. Since *FT1* can further activate expression of *VRN1* in leaves, this results in a positive feedback loop (*B*). This might strengthen the long-day flowering response in spring. *VRN1* is also expressed at the shoot apex of vernalized plants, where it promotes the transition to reproductive development irrespective of daylength (*C*). Since *FT1* is likely to encode a mobile floral promoter, the FT1 protein might be transported to the shoot apex (*D*) where it could further upregulate *VRN1* when plants experience long days after vernalization.

including *Arabidopsis thaliana* (L.) Heynh (Ferrándiz *et al*. 2000). In the leaves, expression of *VRN1* unlocks the long-day flowering response, allowing increasing daylength to accelerate flowering in spring (Trevaskis *et al*. 2007*a*; Hemming *et al*. 2008; Preston and Kellogg 2008) (Fig. 3).

Studies in *A. thaliana* have shown that *FLOWERING LOCUS T* (*FT*) mediates the long-day flowering response (Kardailsky *et al*. 1999; Kobayashi *et al*. 1999). Expression of *FT* is controlled by interactions between the circadian clock and light receptors, such that the FT protein is produced in leaves when daylight extends into the late afternoon and exceeds a critical length (Suárez-López *et al*. 2001; An *et al*. 2004; Valverde *et al*. 2004). The FT protein is then transported to the shoot apex where it promotes floral development (Corbesier *et al*. 2007; Tamaki *et al*. 2007). Thus, FT is proposed to be the main component of the 'florigen', the leaf-derived signal that triggers flowering at the shoot apex (Zeevaart 2008).

In temperate cereals, the *FT-like 1* (*FT1*) gene (also known as *VRN3*) seems to play a role similar to that of *FT* in *A. thaliana*, and is induced by long days to accelerate flowering (Turner *et al*. 2005; Yan *et al*. 2006; Faure *et al*. 2007). In vernalization-responsive cereals, *FT1* is expressed only after plants are vernalized (Yan *et al*. 2006; Hemming *et al*. 2008). It seems that expression of *VRN1* is required for long-day induction of *FT1* (Hemming *et al*. 2008). *VRN1* might promote expression of *FT1* via regulatory interactions with *VRN2* (Trevaskis *et al*. 2007*a*; Hemming *et al*. 2008). *VRN2* is a repressor of flowering that is expressed in leaves in long days (Yan *et al*. 2004*a*; Dubcovsky *et al*. 2006; Trevaskis *et al*. 2006) and it seems likely that *VRN2* delays flowering by suppressing long-day induction of *FT1* (Hemming *et al*. 2008). *VRN1* downregulates *VRN2* (Loukoianov *et al*. 2005; Trevaskis *et al*. 2006), so downregulation of *VRN2* by *VRN1* provides a mechanism to allow long-day induction of *FT1* in the leaves of vernalized plants (Hemming *et al*. 2008; Sasani *et al*. 2009) (Fig. 3). It is not known whether the VRN1 protein interacts directly with the *VRN2* gene or whether other genes mediate this regulatory interaction.

Active alleles of *VRN1* reduce vernalization requirement and adapt crops to different climates

Wheat and barley crops are often grown in regions with mild winter conditions where vernalization does not occur. In other regions, crops are sown in spring to avoid harsh winter conditions and so do not experience vernalization. To adapt crops to these regions, breeders have produced varieties that flower without vernalization (see Chouard 1960). Varieties of wheat and barley that flower without vernalization are referred to as spring types, whereas those that require vernalization to flower are termed winter types.

Genetic analyses have shown that *VRN1* is a major determinant of the vernalization requirement in wheat and barley (Pugsley 1971; Takahashi and Yasuda 1971). Some alleles of *VRN1* are expressed without prior cold treatment and these allow flowering without vernalization (Danyluk *et al*. 2003; Trevaskis *et al*. 2003; Yan *et al*. 2003). Increased expression of *VRN1* from these 'active alleles' is associated either with insertions and deletions in the promoter (wheat) or with deletions in the first intron (wheat and barley) of the *VRN1* gene

(Yan *et al*. 2004*b*; Fu *et al*. 2005) (Fig. 4). Presumably, these regions contain sequences required to maintain low levels of *VRN1* expression until plants are vernalized.

Comparison of the promoter regions from wild-type versus active alleles of *VRN1* has identified a critical region near the transcriptional start site that is altered in some active alleles of *VRN1* (Pidal *et al*. 2009). This region might contain a binding site for a repressor protein that is required to maintain *VRN1* in an inactive state until plants are vernalized. Alternatively, the conformation of DNA in this region might limit the rate of transcriptional initiation, and mutations might remove this limit.

Studies in barley have shown that the first intron of *VRN1* contains a broad region that is required for repression of *VRN1* before winter. Around a dozen different alleles of *VRN1* have been identified in barley (Fu *et al*. 2005; von Zitzewitz *et al*. 2005; Cockram *et al*. 2007; Szucs *et al*. 2007; Hemming *et al*. 2009). Some lack large sections of the ~11 kb first intron, while others lack smaller sections (Fu *et al*. 2005; von Zitzewitz *et al*. 2005; Cockram *et al*. 2007; Szucs *et al*. 2007; Hemming *et al*. 2009). Typically, alleles lacking larger sections are more active and are associated with earlier flowering without vernalization, whereas alleles lacking smaller segments are associated with only a moderate increase in *VRN1* activity and weaker promotion of flowering (Szucs *et al*. 2007; Hemming *et al*. 2009). Comparison of the regions deleted in these different active alleles has identified a core region within the first intron that seems to be important for repression of *VRN1* (Hemming *et al*. 2009). In addition to this core region, sections at the 5′ end of the intron (towards the promoter) also seem to play an important role in maintaining repression of *VRN1*, and alleles with large deletions that remove both the core region and the 5′ end of the intron typically have the highest activity (Szucs *et al*. 2007; Hemming *et al*. 2009). An insertion of a mobile genetic element in the 5′ end of the first intron is also associated with active expression of *VRN1* without vernalization (Stockinger *et al*. 2007; Hemming *et al*. 2009).

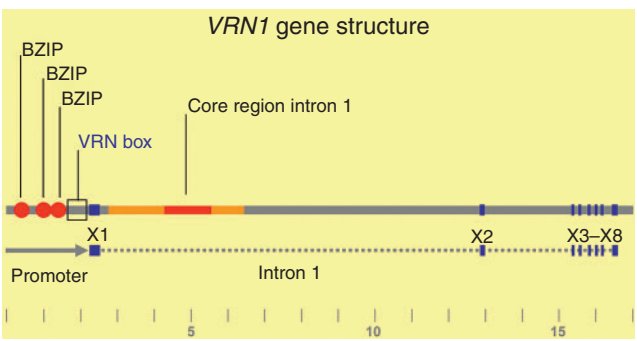

Fig. 4. Structure of the *VRN1* gene. Schematic representation of the *VRN1* locus, showing putative regulatory elements, promoter, and intron and exon positions. Binding sites for FD-like proteins are indicated by basic leucine zipper binding sites. The promoter region that contributes to repression of *VRN1* before winter is boxed (the so-called VRN box). The regions within the first intron that are required for repression of *VRN1* are highlighted. Red shows the core region, which is absent from alleles of *VRN1* that are expressed without vernalization; orange shows parts of the intron that are conserved in wheat and barley *VRN1* genes. Exon regions are indicated by blue columns labelled X1 (Exon 1) to X8.

Repressive histone modifications occur within the first intron of *VRN1*

The state of chromatin (the packaging of DNA and associated histones into higher order structures) appears to be an important determinant of *VRN1* activity (Oliver *et al*. 2009). Analysis of histone modifications at the *VRN1* locus in barley plants that have not been vernalized has shown that the chromatin at *VRN1* contains a specific histone modification typically associated with an inactive chromatin state and long-term transcriptional repression: histone-3-lysine-27-trimethylation (H3K27Me3) (Oliver *et al*. 2009). This modification is found within the first intron of *VRN1* and also at the promoter (Oliver *et al*. 2009). Deposition of H3K27Me3 at these sites might contribute to repression of *VRN1* before winter. In other organisms, H3K27Me3 is deposited by the polycomb repressor complex 2 (PRC2) (Cao *et al*. 2002). The intron of *VRN1* might contain sequences targeted by such a complex, explaining why this region is required to maintain repression of *VRN1*; however, the sequence motifs targeted by plant PRC2 complexes are not known.

When plants are vernalized, the level of H3K27Me3 at the *VRN1* locus decreases and histone modifications typically associated with an active chromatin state appear (H3K4Me3), suggesting that vernalization induces a change in the state of chromatin at the *VRN1* locus (Oliver *et al*. 2009). Chromatin modifications can be inherited through cell divisions, so these epigenetic modifications, and the associated changes in chromatin state, might allow *VRN1* to remain active after vernalization. This could contribute to a cellular memory of vernalization in cereals (Fig. 5).

Mechanisms that activate expression of *VRN1* in response to low temperatures

Prolonged exposure to cold activates expression of *VRN1*, but how this occurs is not known. The regulatory regions in the first intron are not required for cold induction of *VRN1*, since exposure to low temperatures can further activate expression of *VRN1* alleles that lack most of the first intron (Trevaskis *et al*. 2007*b*; Hemming *et al*. 2009). So while the first intron is important to maintain low levels of *VRN1* transcripts before winter, cold activation of *VRN1* is probably mediated by other parts of the gene. Transient transformation experiments using tobacco leaves (*Nicotiana benthamiana* L.) suggest that fusion of a 1 kb region of the *VRN1* promoter to a reporter gene (Green Fluorescent Protein) can mediate cold induction of reporter gene activity (Kane *et al*. 2007). This suggests that cold activation of *VRN1* is controlled by regulatory elements in the promoter. Presumably these elements are targeted by a temperature response mechanism that is conserved in cereals (monocots: *Poaceae*) and tobacco (dicot: *Solanaceae*). Unfortunately the study of Kane *et al*. (2007) did not examine whether the increase in reporter gene activity seen after cold treatment resulted from increased transcription or simply from increased stability of the reporter protein, so the results of this experiment are inconclusive.

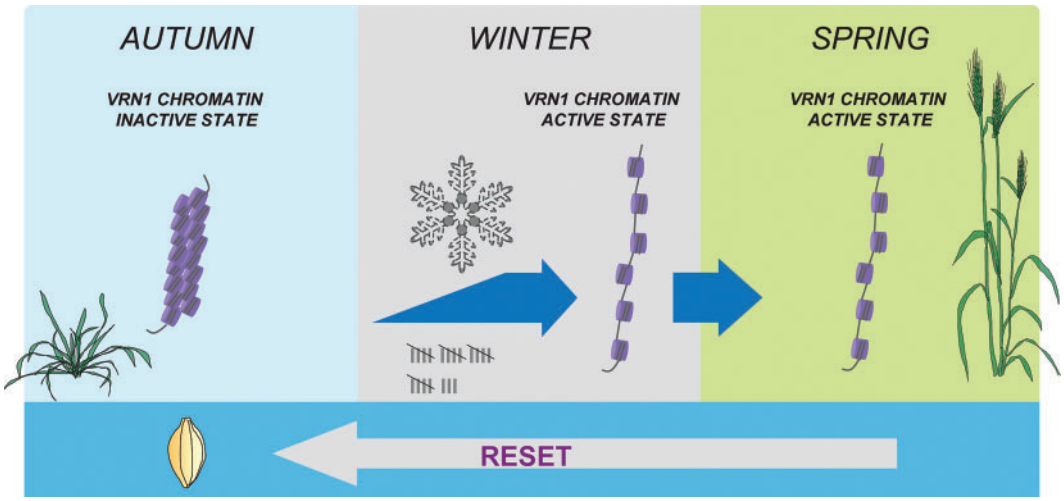

Fig. 5. Chromatin state and the vernalization response in cereals. The *VRN1* locus is maintained in an inactive chromatin state before winter, preventing expression of *VRN1* and delaying flowering. When plants are vernalized, *VRN1* chromatin shifts to an active state and *VRN1* is expressed. *VRN1* remains active in spring to provide a memory of vernalization but is restored to an inactive state in the next generation.

Genes that control the daylength flowering response can influence the vernalization requirement

Some wheats and barleys carry alleles of *FT1* (*VRN3*) that are expressed at high levels and cause rapid flowering without vernalization (Takahashi and Yasuda 1971; Yan *et al*. 2006). Other wheats and barleys lack a functional *VRN2* gene and so flower rapidly in long day conditions, without any requirement for vernalization (Takahashi and Yasuda 1971; Dubcovsky *et al*. 1998; Yan *et al*. 2004*a*). In these genotypes, activation of the long-day flowering response without prior cold treatment overcomes the normal requirement for vernalization.

VRN2 and *FT1* (*VRN3*) have different effects on flowering behaviour. Active alleles of *FT1* cause rapid inflorescence initiation and development, irrespective of daylength or vernalization (Yan *et al*. 2006). Loss of *VRN2* also causes rapid inflorescence initiation and development without vernalization, but only in long-day conditions (Karsai *et al*. 2005; Hemming *et al*. 2008). By comparison, active alleles of *VRN1* mimic the effects of vernalization by promoting inflorescence initiation and allowing long days to accelerate subsequent stages of inflorescence development. The way each gene influences overall flowering behaviour determines how these genes are utilised to produce wheats and barleys that flower without vernalization. For example, active alleles of *FT1* seem to be useful in regions with extreme winters and short summer growing seasons, where rapid flowering is beneficial regardless of growing conditions (Takahashi and Yasuda 1971).

The daylength–flowering response pathway influences *VRN1* expression

VRN1 is expressed without prior vernalization in varieties that carry active alleles of *FT1* (Yan *et al*. 2006). In *A. thaliana*, FT interacts with a 'basic leucine zipper' transcription factor, FD, to activate target genes that include MADS box transcription factors that are related to *VRN1*; *FRUITFUL* and *APETALLA1* (Abe *et al*. 2005; Teper-Bamnolker and Samach 2005; Wigge *et al*. 2005; Corbesier *et al*. 2007). The wheat FT1 protein can interact with the FD-like protein 2 (TaFDL2), which can, in turn, bind to the promoter of *VRN1* (Li and Dubcovsky 2008). Thus, in cereals, FT1 might activate expression of *VRN1* in leaves and the shoot apex through interactions with FD-like proteins (Li and Dubcovsky 2008). Activation of *VRN1* by *FT1* might also occur in varieties that lack *VRN2*, where rapid induction of both *FT1* and *VRN1* occurs when plants are exposed to long days (Yan *et al*. 2006; Trevaskis *et al*. 2007*b*).

Although activation of *FT1* can induce expression of *VRN1* in some genotypes, the relevance of this to vernalization-induced flowering is unclear. In autumn-sown (vernalization-responsive) varieties, *VRN1* is induced by cold during winter when days are short (Danyluk *et al*. 2003; von Zitzewitz *et al*. 2005; Trevaskis *et al*. 2006) and *FT1* is not expressed (Hemming *et al*. 2008). Similarly, vernalization induces expression of *VRN1* in seeds germinated in darkness where *FT1* is not expressed (Sasani *et al*. 2009). So *FT1* is unlikely to mediate induction of *VRN1* by cold. Instead, long-day induction of *VRN1* might occur in spring if plants are partially vernalized – after mild winters, for example – and this could compensate for incomplete vernalization. Similarly, long-day activation of *VRN1* might allow some varieties to flower eventually even if plants do not experience vernalization. Activation of *VRN1* by long days might also be important after the transition to reproductive development, since long days can enhance expression of *VRN1* in leaves after the initiation inflorescence (Sasani *et al*. 2009). This might occur through a positive feedback mechanism whereby *FT1* enhances expression of *VRN1*, which further activates *FT1* (Fig. 3). This might strengthen the long-day flowering response once inflorescence development begins.

Utilising genetic diversity in *VRN1* for crop improvement

Different alleles of *VRN1* can generate a wide spectrum of vernalization requirements and have been used to adapt varieties to a wide range of growing regions and sowing times (Pugsley 1971; Takahashi and Yasuda 1971). Preliminary surveys of genetic diversity at the *VRN1* locus have shown how breeders have utilised particular alleles of *VRN1* to breed cultivars adapted to specific regions. For example, an active allele of *VRN1* on the D genome is common in wheats from some growing regions and might offer an adaptive benefit relative to other alleles (Zhang *et al*. 2008). Similarly, some alleles of *VRN1* gene are prevalent in European barleys (Cockram *et al*. 2007; Hemming *et al*. 2009).

The prevalence of an allele within a breeding program might indicate an adaptive benefit of this allele in a particular region, but might also reflect breeding history, since a founding variety or recurrent parent can bias breeding material towards a particular *VRN1* genotype. Therefore, genetic surveys alone might not identify the optimal *VRN1* genotype for a particular region. To determine which *VRN1* alleles are most suitable for use in Australian wheat and barley varieties, we used DNA diagnostic techniques to identify different alleles of *VRN1* amongst diverse cultivars, breeding lines and landraces of wheat (3000 accessions) and barley (4400 accessions) (Hemming *et al*. 2009; S. Fieg and B. Trevaskis, unpubl. data). The different alleles thus identified have been introgressed into elite wheat and barley varieties, and are undergoing recurrent backcrossing to develop near-isogenic lines that vary for *VRN1* genotype but otherwise have similar genetic backgrounds. These can be tested for performance in different regions with different sowing times, and will provide valuable information to Australian cereal breeders.

An alternative approach being used to identify alleles of *VRN1* that are suited to different Australian growing regions is to identify *VRN1* alleles that are common among Australian cereal breeding programs and then analyse the association of these alleles with crop performance among diverse materials grown in different regions over multiple years. This approach makes good use of existing pedigree and phenotype data, and is a powerful way to identify favourable alleles of *VRN1* within existing breeding material. This strategy is currently being used to identify useful alleles of *VRN1* among Australian wheat breeding material (see Eagles *et al*. 2009).

The relationship between *VRN1*, vernalization requirement and frost tolerance

Autumn-sown wheats and barleys, which require vernalization to flower, can acclimatise to low temperatures and are subsequently able to survive freezing conditions during winter. This process is known as cold acclimation (reviewed in Galiba *et al*. 2009). The longer plants are exposed to low temperatures, the more frost tolerance increases. This continues until the point when further cold treatment has no additional impact on flowering time (the vernalization saturation point) then frost tolerance begins to decrease (Vasiljev 1934 cited by McKinney 1940; Roberts 1979; Mahfoozi *et al*. 2001; Prasil *et al*. 2004). Thus, it seems that cold acclimation and the vernalization response are interconnected.

Activation of *VRN1* might cause the decrease in frost tolerance that begins when plants are fully vernalized. This hypothesis is supported by genetic studies that show active alleles of *VRN1* reduce frost tolerance (Roberts 1990; Hayes *et al*. 1993; Fowler *et al*. 1996; Koemel *et al*. 2004; Limin and Fowler 2006). For example, a comparison of near-isogenic lines that differ in *VRN1* genotype has shown that varieties with active alleles of *VRN1*, which flower without vernalization, have a greatly reduced capacity to acclimate to cold compared with lines with wild-type alleles of *VRN1*, which require vernalization to flower (Koemel *et al*. 2004; Limin and Fowler 2006). At present, it is not clear whether this is a direct consequence of induction of *VRN1* or an indirect consequence, caused by the effect that *VRN1* has on other genes or on plant development *per se*. Regardless of the mechanism, this relationship between *VRN1* activity and frost tolerance is important and has implications for cereal breeders, since altering vernalization requirement can also affect winter survival.

The evolution of vernalization-induced flowering

Vernalization occurs in a wide range of plants. Genes controlling vernalization-induced flowering have now been isolated from temperate cereals (monocots: *Poaceae*) and the model plant *A. thaliana* (dicot: *Brassicaceae*). In *A. thaliana*, the vernalization response is mediated *by FLOWERING LOCUS C* (*FLC*) (Michaels and Amasino 1999; Sheldon *et al*. 1999). *FLC* encodes a MADS box floral repressor that is downregulated by vernalization (Michaels and Amasino 1999; Sheldon *et al*. 1999). This contrasts with the situation in temperate cereals, where no *FLC*-like genes occur and instead *VRN1* is activated by vernalization to promote flowering (see Trevaskis *et al*. 2007*a*; Greenup *et al*. 2009). It seems that the vernalization response has evolved independently in these distantly related angiosperms.

Although different genes control the vernalization response in cereals and *A. thaliana*, there are parallels between the pathways controlling vernalization-induced flowering in these plants. For example, vernalization is a prerequisite for long-day induction of both *FT* and *FT1* (Michaels and Amasino 1999; Sheldon *et al*. 1999; Michaels *et al*. 2005; Yan *et al*. 2006; Hemming *et al*. 2008). In cereals, *VRN2* downregulates *FT1* before winter, directly or indirectly (Yan *et al*. 2006; Hemming *et al*. 2008), and in *A. thaliana*, *FLC* interacts directly with the *FT* gene sequence to repress transcription (Helliwell *et al*. 2006). Furthermore, in both cereals and in *A. thaliana*, the state of chromatin at key MADS box transcription factors plays an important role in regulating the vernalization response. The repressive chromatin mark H3K27Me3 is required for repression of *VRN1* before winter in cereals and for repression of *FLC* after winter in *A. thaliana* (Schubert *et al*. 2006; Sung *et al*. 2006; Wood *et al*. 2006; Finnegan and Dennis 2007; De Lucia *et al*. 2008; Oliver *et al*. 2009). These parallels are probably the result of convergent evolution.

Directions for future research

One key area for further research is in understanding how plants sense cold and how increasing durations of cold cause increased expression of *VRN1*. Another key question is how *VRN1* promotes flowering. More specifically, what are the direct targets of *VRN1*? By using molecular tools such as reporter gene fusions, microarrays and targeted mutagenesis, it should

be possible to address these questions. In this regard, barley is a useful genetic model, since it is transformable and large collections of genetically diverse barley accessions exist in cereal seed banks. There are also many mapping populations, including recombinant inbred lines and doubled haploids, which can be used to identify potential regulators of *VRN1*.

Conclusions

The activity of *VRN1* underlies many of the physiological features of vernalization-induced flowering in cereals, so understanding how *VRN1* is regulated will provide further insights into the biology of the vernalization response in these plants. A better understanding of the regulation and molecular functions of *VRN1* will also have important implications for cereal breeding programs. It should eventually be possible to predict with precision how different alleles of *VRN1* will influence flowering behaviour in different genetic backgrounds or different environments, and to tailor breeding strategies accordingly. Knowledge developed in temperate cereals will also be relevant to related grasses, including a range of economically important pasture grasses. For these reasons, the vernalization response of cereals, the plants where vernalization-induced flowering was first recognised, will be an important area for ongoing research.

Acknowledgements

I thank my friends and colleagues Steve Swain, Aaron Greenup, Sarah Fieg, Sandra Oliver and Megan Hemming for reading drafts of this manuscript and providing constructive suggestions. I also gratefully acknowledge both the Commonwealth Scientific and Industrial Research Organisation, and the Grains Research and development Corporation for long-term support of this research.

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Manuscript received 12 March 2010, accepted 18 April 2010