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Minireview

Vernalization in cereals

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Abstract

How vernalization - exposure to a period of cold - induces flowering in *Arabidopsis* has been intensively investigated at the genetic and moleular levels. Recent papers, including one in *BMC Plant Biology*, shed light on changes in gene regulation that occur on vernalization in cereals.

Flowering is a critical stage in the life history of a plant. The time to flower must coincide with favorable conditions so that viable seeds can be produced, ensuring the continued survival of the species in subsequent generations. Vernalization, a prolonged period of low temperature, is one environmental stimulus that ensures that flowering occurs in the appropriate season of the year - spring - and many plant species, including both broadleaf plants (the dicots) and grass-like plants (the monocots), require vernalization to stimulate flowering [1]. Those plants that need vernalization often require an additional environmental cue, long daylength, to ensure that flowering occurs in spring. The environmental cues of vernalization and long days act sequentially and in concert to promote spring flowering.

As the location of a plant is fixed, its life cycle needs to fit the annual cycle of the regional climate. In the temperate regions of the planet, where there are distinct seasonal variations in both temperature and daylength, plant species have evolved responses that ensure that their life cycle, particularly the shift from vegetative to reproductive growth, fits the annual climate cycle such that flowering and seed formation occur at the most propitious time.

Vernalization has several unique properties. One is that the initial perception and response to the period of cold needs to occur in dividing cells, such as in germinating seedlings, and can often be separated from the time of flowering by weeks and even months. A molecular memory of vernalization is maintained during the subsequent vegetative growth of the plant, until at some point in its development long days trigger the actual flowering response. A second feature is that in all species, both monocot and dicot, with a vernalization requirement to stimulate flowering, there is a process of resetting to the default state before the germination of the seed of the next generation, such that plants of that generation will not flower unless they too have been exposed to a vernalization period. These properties have provided a longstanding physiological and developmental puzzle, unable to be understood until molecular analyses were available. Writing in *BMC Plant Biology*, Winfield *et al.* [2] put another piece of the puzzle in place for cereals by a genome-wide transcriptome analysis that identifies upregulation of the genes for the biosynthesis of the growth hormone gibberellin (GA) in plants grown under conditions mimicking the British winter.

Molecular aspects of vernalization

The key characteristics of vernalization apply both to the cereals and to dicots such as *Arabidopsis*, but there are differences in the response pathways in these two groups of plants. Some of the genes involved are common to the two groups, but other genes differ not only in their identity but in their mode of action. However, in both groups the vernalization response is due to the regulation of key genes by epigenetic modification - that is, modifications to chromatin that do not alter the DNA sequence itself.

The response pathway was first worked out in *Arabidopsis* [1]. The key epigenetic changes accompanying vernalization in this species operate on *FLOWERING LOCUS C* (*FLC*). *FLC* codes for a repressor of flowering, ensuring that vegetative growth continues through the harsher weather of winter. Vernalization results in histone modifications that repress *FLC* [3]. Repression of the locus is accompanied by increased levels of trimethylation of lysine 27 (K27me3) in histone H3, an epigenetic mark associated with the repressed state. The H3K27me3 modification is added by Polycomb group proteins, chromatin-remodeling proteins that have homologs in *Drosophila*, mammals, worms and other plants. Polycomb repressive complex 2 (PRC2) acts as a histone methylase and is responsible for the epigenetic downregulation of *FLC*. The absence of the repressor protein FLC following vernalization then permits two other genes, *FLOWERING LOCUS T* (*FT*) and *SUPPRESSOR OF OVER EXPRESSION OF CONSTANS* (*SOC1*), to be expressed, and the activity of their gene products triggers the genes that control flower development (Figure 1). *FT* encodes a protein that acts as a mobile flowering signal, or 'florigen', traveling via the phloem from the leaf to the apex to cause flower formation [3,4].

Vernalization in cereals

As the molecular mechanisms behind the vernalization response in *Arabidopsis* became clearer, there was increasing opportunity to ask about the molecular basis of the vernalization response in cereals. These plants - barley, wheat, rye and oats - are some of the most important food crops in the world. Plant breeders have known for many decades that some crops require vernalization. Examples of these are the winter wheats and winter barleys, whereas other lines of the same species - spring wheats and spring barleys - do not require vernalization in order to flower. Until recently, there was no knowledge of the mechanism of the vernalization response in cereals except for the genetic definition of some key genes.

The expectation was that the *Arabidopsis* mechanism would be operating in cereals because the vernalization responses had similar properties - the need for dividing cells, the mitotic memory of the winter treatment, discontinuity between the time of the response initiation and the actual flowering time, and the resetting to the default situation for the next generation.

	Arabidopsis	Winter cereals
Without vernalization	*FLC* active and represses *SOC1* and *FT* Flowering inhibited	*VRN1* not active *VRN2* active, represses *FT* Flowering inhibited
With vernalization	*FLC* repressed *SOC1* and *FT* active *FT* induced by long days Flowering promoted	*VRN1* active *VRN2* repressed *FT* active, induced by long days Flowering promoted

Figure 1
Diagram showing the key genes controlling vernalization in *Arabidopsis* and cereals.

As to the similarity to *Arabidopsis*, the answer was both yes and no. One of the genetically defined genes, *VRN1*, was shown to be the key response gene. But in this case the cold treatment induces gene activity, rather than repressing it as in *FLC* in *Arabidopsis*. *VRN1* is a promoter of the transition from the vegetative to reproductive state of the growing shoot apex. Induction of *VRN1* is accompanied by the repression of another genetically defined gene, *VERNALISATION 2* (*VRN2*), which, when active, prevents transcriptional activity of the *FT* gene and production of the mobile flowering signal [4] (Figure 1).

A recent paper by Oliver *et al.* [5] has shown that the induction of *VRN1* in barley is epigenetic, and involves histone modifications of the same type as occur in *FLC* in *Arabidopsis*. However, these change in opposite directions to those in *FLC*. In *VRN1* there is a decrease in H3K27me3, the mark of a transcriptionally inactive gene, and an increase in trimethylation of lysine 4 of H3 (H3K4me3), a mark of an active gene.

Integration of the vernalization and daylength pathways

One of the similarities between the dicot and monocot systems is the way in which the vernalization response is integrated with the other environmental cue of increasing daylength. In both types of plants, the *FT* gene is induced into transcriptional activity by the lengthening days of spring. The mechanisms enabling the *FT* gene to respond are different, but both relieve the repression of *FT*. In *Arabidopsis* the absence of *FLC* activity enables *FT* to respond to long days, and in the cereals the absence of *VRN2* activity

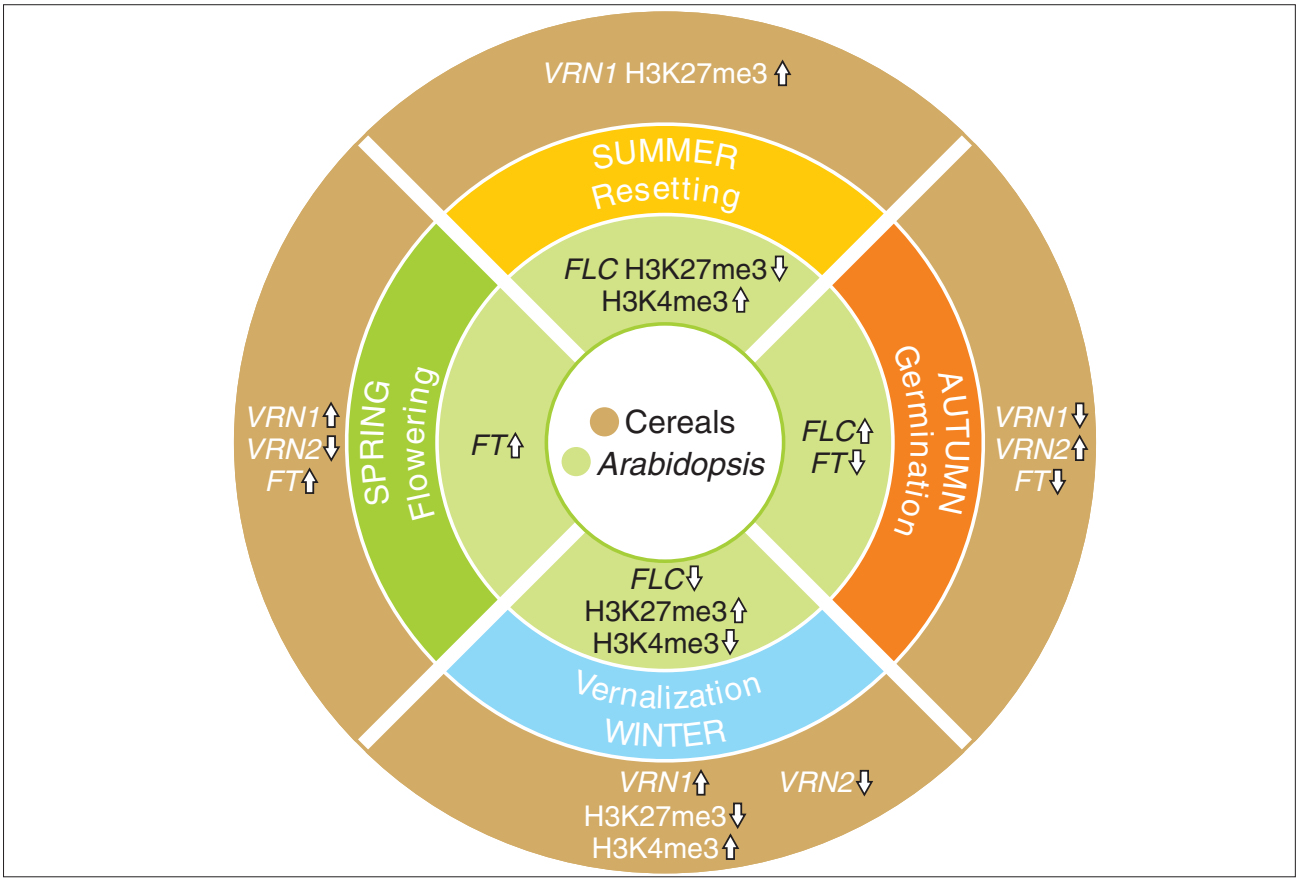

Figure 2
The integration of the vernalization and daylength pathways. In summer, seed formation and resetting occur. In autumn, seeds germinate but must not flower. In winter, vernalization occurs and readies FT for induction by the long days of spring. Flowering occurs in spring. The epigenetic regulation by histone modifications is shown.

similarly enables *FT* induction by long days. In both cereals and *Arabidopsis* the *FT* response is activated in leaf tissue and the FT protein is translocated from the leaves to the growing apex where it interacts with the genes that induce floral morphogenesis [6,7]. These similarities and differences between cereals and *Arabidopsis* are summarized in Figure 2, which illustrates the epigenetic responses at the key genes as they occur in different seasons of the year.

Mitotic memory and resetting

One of the remarkable features of vernalization-induced flowering is the mitotic memory system that operates through the cell generations in the developing plant. The histone modifications on the key regulatory genes are inherited mitotically. Equally remarkable, and this applies to both dicots and monocots, is that the system is reset to the default position in the next generation. Once again, the germinating seeds or seedlings need to be vernalized if the new generation of plants is to flower.

Resetting in *Arabidopsis* occurs early in the development of the new embryo; the male-derived copy of *FLC* delivered from the pollen becomes active in the single-celled zygote. The gene first becomes active even earlier, during the development of pollen in the pre-meiotic anthers, but then activity is lost and only becomes evident again in the zygote. Activity is not restored to the female-derived copy of *FLC* until the early globular stage, when the embryo consists of approximately 16-32 cells [8].

Resetting also occurs in cereals, but nothing is known of the timing of the activity-phase change. In both cereals and *Arabidopsis* the detail of the mechanisms involved in resetting have not been described, but the appropriate changes in the histone activity marks have been identified.

Gibberellin may play a role in short-day vernalization

Common to both *Arabidopsis* and the cereals is the fact that the vernalization response readies the *FT* gene to be induced into activity by longer daylengths. If *FT* cannot be induced, because of a deletion or some other mutation, one might expect that flowering will not occur. This is, however, not the case in either *Arabidopsis* or cereals. In the absence of *FT* activity, flowering is delayed in long-day conditions, but if short days are imposed experimentally, there is no effect on flowering time.

So does the vernalization response have other targets that can act as flowering stimulators? One possibility was suggested by reports that the biosynthetic pathway of the growth hormone GA is activated in the apex of vernalized plants. This was first described in the dicot *Thlaspi*, a relative of *Arabidopsis* [9]. In *Arabidopsis*, GA is essential for flowering in short days.

In a recent analysis of genome-wide gene transcription during vernalization in wheat, Winfield *et al.* [2] show that the activity of key GA biosynthetic genes also increases in short-day vernalization in cereals. Consistent with GA activity, the cereal shoot apex lengthens during vernalization and subsequent growth in short days, so these results suggest that GA may be a back-up mechanism to the FT pathway in short days in cereals as well.

Future questions

Can we conclude that the mystery of vernalization-induced flowering is solved? The answer is that it is only partly worked out. Several important issues still confront us. One is the mechanism of perception of the low temperature. Plants are known to respond via a number of different biochemical pathways to frost or other cold conditions [10]. Are the sensors of vernalization low temperatures the same? How is the signal pathway transduced to bring about the epigenetic changes in the key genes?

In *Arabidopsis*, one gene has been found that acts upstream from *FLC* repression and is essential for the vernalization response. *VERNALISATION INSENSITIVE 3* (*VIN3*) is induced during the period of low temperature and its protein product associates with the PRC2 complex responsible for the trimethylation of histone H3K27 residues [11].

An understanding of the mechanism of resetting in *Arabidopsis* and cereals is a particular goal of future research. Although the timing of resetting in *Arabidopsis* is known, the pathway that reactivates *FLC* is not.

Research into the molecular mechanisms of vernalization has made great advances since the discovery of the role of *FLC* in *Arabidopsis*. The nature of the epigenetic regulation of *FLC* has been determined and the proteins necessary for its downregulation and the mitotic memory during subsequent growth have been identified. The need for dividing cells as the target for vernalization has been addressed, as have the first steps in the resetting phenomenon. Vernalization in cereals has been shown to be similar yet different to that in *Arabidopsis*. All this means that the developmental process controlling the switch from vegetative to reproductive phase in the apical meristem, the most critical developmental transition in plants, is now one of the best-understood epigenetic controls in any organism.

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