**FLC: A Hidden Polycomb Response Element Shows Up in Silence**

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A sizeable fraction of eukaryotic genomes is regulated by Polycomb group (PcG) and trithorax group (trxG) proteins, which play key roles in epigenetic repression and activation, respectively. In *Drosophila melanogaster*, homeotic genes are well-documented PcG targets; they are known to contain cis-acting elements termed Polycomb response elements (PREs), which bind PcG proteins and satisfy three defined criteria, and also often contain binding sites for the trithorax (trx) protein. However, the presence of PREs, or an alternative mode for PcG/trxG interaction with the genome, has not been well documented outside *Drosophila*. In *Arabidopsis thaliana*, PcG/trxG regulation has been studied extensively for the flowering repressor gene *FLOWERING LOCUS C* (FLC). Here we evaluate how PRE-like activities that reside within the FLC locus may satisfy the defined *Drosophila* criteria, by analyzing four FLC transcription states. When the FLC locus is not transcribed, the intrinsic PcG recruitment ability of the coding region can be attributed to two redundant cis-acting elements (Modules IIA and IIB). When FLC is highly expressed, trxG recruitment is to a region overlapping the transcription start site (Module I). Exposure to prolonged cold converts the active FLC state into a repressed state that is maintained after the cold period finishes. These two additional transcriptional states also rely on the same three modules for PcG/trxG regulation. We conclude that each of Modules I, IIA and IIB partially fulfills the PRE function criteria, and that together they represent the functional FLC PRE, which differs structurally from canonical PREs in *Drosophila*.

**Keywords:** cis-element • Epigenetic regulation • *FLOWERING LOCUS C* • Polycomb group • Polycomb response element • Trithorax group.

**Abbreviations:** ATX, ARABIDOPSIS TRITHORAX; ATXR7, ATX-related7; CLF, CURLY LEAF; COMPASS, complex proteins associated with Set1; EFS, EARLY FLOWERING IN SHORT DAYS; FIE, FERTILIZATION-INDEPENDENT ENDOSPERM; FLC, FLOWERING LOCUS C; FRI-C, FRIGIDA-containing complex; GUS, β-glucuronidase; HMTase, histone methyltransferase; ncRNA, non-coding RNA; PaF1-C, RNA polymerase II-associated factor1 complex; PcG, Polycomb group; PHD, plant homeodomain; Pol, polymerase; PRC, Polycomb repressive complex; PRE, Polycomb response element; trx, trithorax; trxG, trithorax group; T-DNA, transfer DNA; TSS, transcription start site; VIN3, VERNALIZATION INSENSITIVE3; VRE, vernalization response element.

**Introduction**

Polycomb group (PcG) and trithorax group (trxG) proteins play key roles in the maintenance of transcription states of numerous genes in a wide range of eukaryotes (reviewed in Simon and Tamkun 2002, Pien and Grossniklaus 2007, Schuettengruber et al. 2007, Schwartz and Pirrotta 2008). PcG proteins repress their target genes, whereas trxG proteins activate the same genes in cells that require their expression. One of the PcG complexes, Polycomb repressive complex 2 (PRC2), contains four core subunits known in *Drosophila* as E(z), Esc, Su(z)12 and Nurf55. Together, these four proteins act as a histone methyltransferase (HMTase) (trimethylation of histone H3 at lysine 27: H3K27me3), and each protein has a distinct role: E(z) is the catalytic SET domain, Nurf55 and Su(z)12 are required for nucleosome association, and Esc is able to boost the catalytic activity of E(z); none of these proteins has DNA binding ability (Cao et al. 2002, Kuzmichev et al. 2002, Muller et al. 2002). Esc can also bind to H3K27me3 to stimulate the HMTase activity of the complex, probably in relation to the self-propagation of this mark through successive
cell divisions, thus establishing a mitotically stable state (Hansen et al. 2008, Margueron et al. 2009). The second, less conserved PcG complex is PRC1, which can bind to H3K27me3 to lead to transcriptional repression (Zhou et al. 2008).

In contrast, trxG proteins counteract PcG repression and maintain active transcription states (Klymenko and Muller 2004, Papp and Muller 2006). One of the enzymatic activities of trxG proteins is trimethylation of H3K4 (H3K4me3), localized around the transcription start sites (TSSs), via the SET-domain proteins trx and Ash1 (Beisel et al. 2002, Milne et al. 2002, Nakamura et al. 2002, Smith et al. 2004). trx is the HMTase in COMPASS (complex proteins associated with Set1)-like complexes (Miller et al. 2001, Krogar et al. 2002, Mohan et al. 2011; reviewed in Shilatifard 2008). H3K4me3 can recruit multiple complexes including NURF (reviewed in Ruthenburg et al. 2007) that relaxes chromatin to enhance the accessibility of transcription factors and to activate transcription (Wysocka et al. 2006). Ash1 also catalyzes H3K36 methylation in gene coding regions (Tanaka et al. 2007, Yuan et al. 2011). In Saccharomyces cerevisiae, H3K36me2/me3 ensure transcription elongation by recruiting the histone deacetylase complex to repress cryptic internal transcription from the gene body (Carrozza et al. 2005). Both H3K4me3 and H3K36me2/me3 associate with active genes in a wide range of eukaryotes to enhance transcription (Li et al. 2007, Oh et al. 2008, Roudier et al. 2009). Like H3K27me3, these active marks may also be inherited through successive cell divisions (Cavalli and Paro 1998, Muramoto et al. 2010).

Genes regulated by both PcG and trxG proteins still require the appropriate promoter/enhancer-associated factors for transcriptional activation per se. The final transcriptional output will depend on the balance between the actions of PcG and trxG proteins, which may be established in different ways (Mendenhall and Bernstein 2008, Schwartz and Pirrotta 2008, Schuettengruber and Cavalli 2009). For example, the presence of H3K4me3 and/or H3K36me2/me3 inhibits the HMTase activity of PRC2 (Schmitges et al. 2011). In addition, certain COMPASS-like complexes also associate with the H3K27 demethylase Ultrathorax (Lee et al. 2007b) and certain PcG complexes contain an H3K4 or H3K36 demethylase (Lee et al. 2007a, Lagarou et al. 2008, Pasini et al. 2008). Moreover, PcG and trxG activities are not always mutually exclusive. Both H3K4me3 and H3K27me3 reside in many developmental genes in mammalian embryonic stem cells (Bernstein et al. 2006) as well as in FLOWERING LOCUS C (FLC) in Arabidopsis thaliana (Jiang et al. 2008), and both PcG and trxG proteins associate with some genes and regulate their expression in a balanced manner in Drosophila cells (Schwartz et al. 2010).

The fact that the core PcG complex lacks DNA binding ability raises the question of how specific genes can be targeted. Although recruitment mechanisms may be poorly conserved between organisms, a set of minimal criteria derived from the paradigmatic Drosophila homeotic genes may apply to other organisms. Schwartz and Pirrotta (2008) proposed three defining criteria for cis-elements for PcG recruitment, PREs: (1) they should generate a new binding site for PcG proteins when they are inserted at new sites in the genome; (2) they should create a domain of H3K27 trimethylation; (3) they should induce the repression or down-regulation of a reporter gene associated with them. In addition, most PREs also contain a binding site for trx (Schwartz et al. 2010). In Arabidopsis, PRC2, some PRC1 components and many trxG proteins are conserved; however, PRE function, or how PcG/trxG proteins interact with the genome, has not been well tested. Although numerous reports in animals have shown that several DNA-binding proteins and/or non-coding RNAs (ncRNAs) can function in PcG recruitment (Schwartz and Pirrotta 2008, Margueron and Reinberg 2011), only one PcG target in Arabidopsis, FLC, has been reported to be associated with a PRC2-recruiting ncRNA (Heo and Sung 2011b; reviewed in Heo and Sung 2011a).


PcG/trxG regulation has also been extensively studied for FLC, a gene encoding a MADS-box protein that serves as a potent repressor of flowering. FLC has long been recognized as a rheostat-like gene (Michaels and Amasino 1999, Sheldon et al. 1999), whose transcription level needs to be tightly adjusted according to developmental and external cues, resulting in a range of transcriptional states (active, quantitatively repressed, stable repression). Here we chose to evaluate the Drosophila PRE criteria for the case of FLC as a model PcG gene in plants, in an effort also to integrate recent information on cis-acting FLC regions (Buzas et al. 2011) and ncRNA (Heo and Sung 2011b) for PcG recruitment with four FLC transcription states.

**trxG contribution to FLC activation**

FLC is expressed throughout vegetative development until silenced by prolonged cold in winter. How does FLC activity commence? Presumably, this involves both the removal of the repressive state from the previous cycle (termed ‘resetting’ or ‘reactivation’) and transcription factor-mediated activation during gametogenesis and/or embryogenesis (Sheldon et al. 2008, Choi et al. 2009, Yun et al. 2011). However, among the
A Polycomb response element at FLOWERING LOCUS C.

Fig. 1 Module structure of the FLC Polycomb response element. FLC exons and untranslated regions are represented by black bars, introns by white bars; upstream and downstream gray bars represent promoter and 3' FLC regions, respectively. (A) Distribution and amount of active and repressive marks in different FLC transcription states. Red, H3K4me3; orange, H3K36me2/me3; blue, H3K27me3. The thickness of each bar indicates the level of modification. The transcription level is indicated on the right by the position of the black arrowhead in the On–Off interval. 1. Lack of transcription. The thickness of each bar indicates the level of modification. The transcription level is indicated on the right by the position of the black arrowhead in the On–Off interval. 1. Lack of transcription. 2. Active transcription. The position of the black arrowhead indicates the position of the T-DNA insertion in the wild type (wt). The gray arrowhead indicates the position of the T-DNA insertion mutant fcl-20 and wild type (wt). The gray arrowhead indicates the position of the T-DNA insertion mutant fcl-20 and wild type (wt). (B) Representation of the GUS promoter construct structure (left) and GUS activity (right) during transcription states 2, 3 and 4 (defined in A). The drawing is from data in Buzas et al. (2011). The green bar represents the VRE (Sung et al. 2006a). (C) Representation of the FLC gene structure and model for FLC PRE modules. The positions of the two known ncRNAs at FLC, COLDAIR and COOLAIR are depicted. Shaded bars represent the positions of three proposed modules that form the FLC PRE. Module I is colored blue and orange because it is involved in both trxG and PcG function. Modules IIA and IIB are blue and are involved in PcG function. The size of these modules is not precisely determined experimentally. Module I is located within the region approximately +500 to +400 bp from the ATG; Modules IIA and IIB are within the regions +419 to +1,616 and +1,800 to +3,100, respectively, from the ATG. All regions in A–C are drawn to scale; scale bar, 1 kb.
both H3K4 and H3K36 (Kim et al. 2005, Zhao et al. 2005, Xu et al. 2008, Ko et al. 2010). In addition, EFS contributes to the recruitment of FRI-C to the FLC locus (Ko et al. 2010).

The trxG-responsive region of FLC

What part of FLC chromatin is important for FLC activation? Most of the trxG-like proteins described above, as well as FRI-C, associate with the region around the TSS (from the proximal promoter region to the start of the first intron) (Pien et al. 2008, Tamada et al. 2009, Ko et al. 2010, Choi et al. 2011, Jiang et al. 2011). cis-element analyses also indicate the necessity of this region for the substantial expression of FLC transgenics (Fig. 1Bii) (Sheldon et al. 2002, He et al. 2004). These data suggest that the region around the TSS possesses PRE-like trxG-recruiting activity for FLC activation and H3K4 methylation (we designate this region Module I, Fig. 1C). The presence of H3K36me2/me3 in the gene body raises the possibility that other trxG-recruiting elements may reside downstream from the TSS. EFS, like its S. cerevisiae ortholog Set2, may bind directly to RNA Pol II and add H3K36me2/me3 concomitant with the migration of RNA Pol II along the FLC gene body. Alternatively, the gene body region may be in contact with Module I via a chromatin loop, whose formation could be mediated by PRE-like elements in the gene body (Modules IIA and IIB in Fig. 1C; see below). While chromatin looping is a characteristic of certain Drosophila PREs (Bantignies and Cavalli 2011), it has never been demonstrated in plants.

Transcription activity counteracts PcG activity at FLC

During vegetative development, in addition to the trxG-supported active transcription, FLC is also regulated by PcG, as evidenced mainly by its de-repression in all PcG mutants tested (Wood et al. 2006, Greb et al. 2007, Jiang et al. 2008, Doyle and Amasino 2009, Sheldon et al. 2009). The PcG regulation of FLC during vegetative development, as measured by the presence of H3K27me3 at the FLC locus, is especially clear when FLC transcription is perturbed, for example in fri or other FLC activator mutants, or by deletion of the DNA sequence containing the TSS (flc-2, see Fig. 1A1) (Zhang et al. 2007, De Leiva et al. 2008, Jiang et al. 2008, Oh et al. 2008, Doyle and Amasino 2009, Tamada et al. 2009, Buzas et al. 2011). [The only known exception is atx1, where both FLC expression and H3K27me3 levels are reduced (Pien et al. 2008)]. In contrast, in accessions with active FLC transcription, the levels of PRC2 binding and H3K27me3 are lower than those in accessions with basal FLC transcription (Doyle and Amasino 2009, Tamada et al. 2009, Buzas et al. 2011), suggesting that the active FLC accessions possess a mechanism to counteract PcG binding and/or H3K27 trimethylation. Indeed, maximum levels of H3K27me3 can be detected when a transgenic region that retains the full PcG-recruiting properties (tFLC transgene, Fig. 1A1) is not transcribed. Moreover, the H3K27me3 levels are drastically reduced after transcription is induced (Buzas et al. 2011).

PRE-like elements at FLC

Where within the 12 kb region of the FLC locus might specific PREs be located? Recently, the sense ncRNA COLDAIR was found to initiate within the proximal half of the first intron (we designate this region Module IIA, Fig. 1C) (Heo and Sung 2011b). Its promoter seems to coincide or overlap with the previously described vernalization response element (VRE, green bar in Fig. 1B and C; see also later) (Sung et al. 2006a). Importantly, COLDAIR associates with CURLY LEAF (CLF), an H3K27 HMTase, and recruit PRC2 to FLC, facilitating H3K27 trimethylation during the vegetative phase as well as in response to cold (Heo and Sung 2011b). However, the proximal half of the first intron is not the only putative PRE-containing sequence within FLC. The flc-2 deletion mutant, which lacks all of the promoter upstream region, including the proximal half of the first intron/VRE (Fig. 1A1) (Michaels and Amasino 1999), is still able to recruit maximum levels of H3K27me3 (Buzas et al. 2011), indicating the presence of a second putative PRE, possibly in the distal half of the first FLC intron or further downstream. From β-glucuronidase (GUS) reporter constructs, it is clear that repressive elements are located redundantly in the proximal and distal halves of the first intron (Fig. 1Bii, iii, iv) (Sheldon et al. 2002). Therefore, it is unlikely that the second PRE is located outside the first intron (we designate the distal half of the first intron Module IIB, Fig. 1C). Modules I, IIA and IIB together contribute to PcG/TrxG recruitment during normal vegetative growth as well as when plants sense the prolonged cold (vernalization) (see below).

Conversion from the active to the repressed state during cold treatment

During vegetative growth, flowering is repressed by FLC expression, which is supported by the FLC activators (i.e. trxG proteins and FRI-C) (Crevillen and Dean 2011). However, during winter cold, this active FLC state can be quantitatively converted into a repressed state: the reduction in FLC expression is proportional to the duration of cold (Sung and Amasino 2004, Sheldon et al. 2006, Finnegan and Dennis 2007, Greb et al. 2007, Angel et al. 2011). This process involves many factors. Among them, the plant homeodomain (PHD)-finger protein VIN3 is the only factor whose expression is induced specifically during cold (Sung and Amasino 2004, Wood et al. 2006, Kim et al. 2010, Finnegan et al. 2011). The gradual increase in VIN3 expression is tightly correlated with the gradual decrease in FLC expression (Sung and Amasino 2004, Sung et al. 2006b, Greb et al. 2007). VIN3 thus appears to play a central role in FLC repression during cold. VIN3 associates with PRC2 during cold (Wood et al. 2006, De Lucia et al. 2008). Given the ability of the PHD-finger motif to bind H3K4me3
(Ruthenburg et al. 2007), it is likely that VIN3 binds to Module I (containing H3K4me3) to recruit PRC2 to FLC (Sung et al. 2006b, Wood et al. 2006, Greb et al. 2007, De Lucia et al. 2008). Indeed, the FLC region responsive to the initial cold exposure is Module I: both H3K4me3 and H3K27me3 levels in this region undergo quantitative changes (a decrease and an increase, respectively; Fig. 1A3) (Finnegan and Dennis 2007, Angel et al. 2011).

PRC2 recruitment is also markedly enhanced by COLDAIR, originating from Module IIA (Fig. 1C) (Heo and Sung 2011b). COLDAIR expression is detected during plant growth without cold, and is further increased during cold. Importantly, in vin3, in all PcG single mutants tested and in COLDAIR-suppressed lines, FLC expression is still moderately reduced in the cold (Gendall et al. 2001, Sheldon et al. 2006, Wood et al. 2006, Greb et al. 2007, Sheldon et al. 2009, Heo and Sung 2011b). It is possible that an as yet unknown cold-specific factor is redundantly responsible for the FLC repression during cold. Alternatively, redundancy between VIN3 and other reported VIN3-like proteins (Sung et al. 2006b, Greb et al. 2007, Kim and Sung 2010), between VIN3 and COLDAIR, or both, may account for the initial repression. COOLAIR, a class of antisense ncRNAs that are transcribed from the 3’ region of the FLC gene (Fig. 1C), represents another cold-induced (Swiezewski et al. 2009, Hellwell et al. 2011, Heo and Sung 2011b) but redundant factor (Sheldon et al. 2002, Sung et al. 2006a, Hellwell et al. 2011) in FLC repression during vernalization. Nevertheless, the initial FLC repression during cold is accompanied by a reduction in H3K4me3 levels in wild-type as well as in COLDAIR-suppressed lines (Heo and Sung 2011b). It is still unknown which factor(s) account for the reduced H3K4me3 levels.

Later in the cold, after 6 weeks, the FLC gene body region also exhibits moderately increased H3K27me3 levels (Fig. 1A3) (Finnegan and Dennis 2007, Angel et al. 2011), most probably originating from Modules IIA and/or IIB. COLDAIR may be involved in this process, although the mechanism underlying this methylation is still largely elusive. We hypothesize that the lack of transcription (i.e. RNA Pol II migration) at this late cold stage may lead to a reduction in H3K36me2/me3 levels, which could release the allosteric inhibition of PRC2 HMTase activity by K36me2/me3 and allow full PcG activity of Modules IIA and/or IIB (Fig. 1A3). In support of this hypothesis, a similar experimental case is the flc-20 mutant (Fig. 1A2), where H3K36me3 is reduced and H3K27me3 is enhanced in the region of no transcription (Buzas et al. 2011).

Stable FLC repression following cold

The stable FLC repression following cold bears a prominent epigenetic hallmark, namely the ‘memory of winter’, recognized very early by Wellensiek (1962, 1964). Once the cold period (winter) is finished, Arabidopsis plants experience warm temperatures (spring) and flower early by being able to ‘remember’ the FLC transcription state from the cold period, such that the level of repression is proportional to the duration of cold (reviewed in Schmitz and Amasino 2007, Kim et al. 2009).

Which part of the FLC chromatin is responsible for maintenance of FLC repression after cold? Extensive data support a redundant contribution from Modules IIA and IIB in stably maintaining FLC repression after cold. First, GUS reporter assays indicate that the proximal and distal halves of the first long intron (corresponding to Modules IIA and IIB, respectively) can confer stable repression on the FLC-GUS transgene (Fig. 1Bii, iii, iv) (Sheldon et al. 2002). Secondly, within Module IIA, a narrower DNA region of approximately 300 bp termed the VRE (green bar in Fig. 1B, C) was also shown to contribute to maintenance of FLC repression (Sung et al. 2006a). In addition, COLDAIR is required for maintenance of FLC repression and is transcribed from a promoter presumed to reside within Module IIA (Fig. 1C), with an overlapping (by at least 100 bp) minimal promoter responsible for increased COLDAIR transcription in the cold (Heo and Sung 2011b). Thirdly, the FLC gene body (including Modules IIA and IIB) becomes coated with H3K27me3 after plants are returned to warm temperatures (Finnegan and Dennis 2007, Angel et al. 2011). Importantly, H3K27me3 marking cannot take place in all cells: mitotically quiescent cells do not undergo the FLC maintenance phase (Finnegan and Dennis 2007). This is consistent with early reports describing cell division as a requirement for the acceleration of flowering time by cold (Wellensiek 1962, Wellensiek 1964). It is likely that DNA replication (more specifically, histone H3 variant replacement) needs to take place prior to the H3K27me3 increase (Finnegan and Dennis 2007). Indeed, there are two situations where a delay in H3K27me3 increase following the reduction in FLC transcription has been observed. First, H3K27me3 levels in the gene body stay low within 1 d after cold treatment and only start to increase subsequently (Finnegan and Dennis 2007, Angel et al. 2011). Secondly, there is a delay of a few days before H3K27me3 increases after cessation of transcription of a cold-independent inducible transgene (Buzas et al. 2011). These two observations also further support the idea that Modules IIA and IIB have the same function before cold and after cold in recruiting PcG.

Is there an FLC PRE?

We propose that there are three FLC cis-acting regulatory regions (Modules I, IIA and IIB) that together are needed to confer the FLC transcriptional states during vegetative growth and vernalization via the combined antagonistic PcG/trxG action and satisfy all three Drosophila PRE criteria proposed by Schwartz and Pirrotta (Fig. 1C).

Module I is the binding site for trxG proteins and the DNA-binding proteins associated with FRI-C activation.
Consistent with the general role of trxG proteins in maintaining active transcription through mitotic division, FLC activation driven by some transcription factor could be the initial event during early embryogenesis, followed by trxG ‘memorizing’ activity throughout vegetative growth. Module I is also the region undergoing quantitative changes in both H3K4me3 and H3K27me3 that accompany the FLC quantitative transcriptional repression during cold. Finally, Module I also functions after cold, when the H3K27me3 domain extends over the promoter. Module I has not been directly tested for an ectopic ability to recruit either PcG proteins or trxG proteins. It remains possible that Module I contains a PRE itself, or that it uses PRE-like activity from Module II via a chromatin loop (Bantignies and Cavalli 2011).

Interestingly, Modules IIA and IIB each appear to have PRE-like activity. To verify this, Buzas et al. (2011) reinserted this region as a transgene into random genomic locations (tFLC, Fig. 1A1) and confirmed that it satisfies criterion 2 for a Drosophila PRE in creating a domain of H3K27me3. Given that all H3K27me3 in Arabidopsis is FIE dependent, and that FIE associates with the gene body in the absence of transcription in the flc-20 mutant (Fig. 1A1) (Buzas et al. 2011), the first criterion for a PRE (generating a binding site for PcGs) can be clearly inferred. In addition to the recruitment in the absence of transcription, Module II is also required after cold for maintenance of the stably repressed state (Sheldon et al. 2002, Sung et al. 2006a).

Most probably, during vegetative development, recruitment of PcG to Module IIA is mediated by the ncRNA COLDAIR, an activity that is boosted during cold (Heo and Sung 2011b). It is not clear when or how COLDAIR activity commences, whether it is before or after the FLC activation, or whether it is driven by cryptic or specific COLDAIR promoter activity. One plausible scenario is that the H3K4me3 and H3K36me2/me3 active marks are deposited first (therefore, COLDAIR expression starts after FLC expression). As recently reported by Schmitges et al. (2011), PcG HMTase activity can be modulated by the presence of K4me3 and K36me2/me3 on the histone H3 tail; the quantitative repression during cold may be achieved in this way. However, it is possible that the sequence of events does not take place in all cells, especially because not all cells have the same vernalization response (Finnegan and Dennis 2007). In the case of Module IIB, it is not clear if recruitment of PcG may also be mediated by another ncRNA initiated within this region, a DNA-binding factor or an as yet unforeseen mechanism.

Finally, the third criterion for a Drosophila PRE is the repression of an associated reporter gene. An FLC region including all Modules (Fig. 1B1) was shown to be able to confer the vernalization response to an adjacent nptII selection gene (Finnegan et al. 2004, Sheldon et al. 2009), thus also satisfying this PRE criterion. We note that AGAMOUS is another Arabidopsis gene shown to satisfy this criterion (Schubert et al. 2006).

Final remarks
Key information on the exact binding sites and/or other specific features within the FLC PRE is still missing. In addition, the interpretation of results obtained from transgenic constructs may be made difficult if trans-acting features exist in the native and/or transgenic FLC sequences. Quantitative differences in histone marks, as measured by chromatin immunoprecipitation, are probably also due to some extent to distinctly marked cell populations. For these reasons, it is premature to distinguish additional PRE features that might operate in plants. Nevertheless, this discussion provides some evidence that the three minimal Drosophila criteria for PREs are also testable, and may generally apply, in plants. An additional clue is that PRE-like activity in other genes may be revealed in the absence of transcription, as it has been here for FLC.

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