Transcriptional regulation of key gene of vernalization, *FLOWERING LOCUS C*

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Summary

To adjust the timing of flowering, plants can sense the temperature and day length. Vernalization is a phenomenon where flowering is induced by a long period of low temperature, and has an important role in controlling flowering time. In *Arabidopsis thaliana, FLOWERING LOCUS C (FLC)* acts as a floral repressor, and transcription of *FLC* is repressed through the increase of tri-methylation of histone H3 Lysine 27 (H3K27me3) by vernalization. This transcriptional repression is caused by POLYCOMB REPRESSIVE COMPLEX 2 (PRC2) that functions to modify the H3K27me3. Two types of long non-coding RNAs (lncRNA) from the intragenic region of *FLC*, *COOLAIR* and *COLDAIR*, are expressed by vernalization. Notably *COLDAIR* leads to an increase of H3K27me3 with PRC2 interaction. A detailed understanding of the regulatory mechanism of the *FLC* will provide a good model of other epigenetically regulated genes.

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Introduction

Plant life cycles are influenced by seasonal environmental cues such as photoperiod or temperature. Flowering plants have evolved mechanisms for proper timing of flowering to maximize their reproductive success. One of the well-known mechanisms controlled by temperature is vernalization. Vernalization is defined as "the acquisition or acceleration of the ability to flower by a chilling treatment" (Chouard, 1960). As biennial, winter-annual or perennial plants require vernalization to prohibit the floral transition before winter and promote the floral transition in spring.

The winter annual accessions of model plant *Arabidopsis thaliana* require a vernalizing cold treatment to flower at the proper timing. Studies on vernalization using *A. thaliana* have made progress, and one key determinant involved in vernalization, *FLOWERING LOCUS C (FLC)*, has been characterized. FLC encodes a MADS box DNA-binding protein and serves to directly repress floral integrators, *FLOWERING LOCUS T (FT)* and *SUPPRESSOR OF OVEREXPRESSION OF CONSTANCE1 (SOC1)*, thus FLC acts as a floral repressor (Michaels and Amasino, 1999; Sheldon *et al*., 1999; Helliwell *et al*., 2006; Searle *et al*., 2006). *FLC* is expressed prior to cold exposure and its expression is repressed by vernalization. The expression level correlates with the chromatin state of *FLC*. Before prolonged cold exposure, *FLC* chromatin has active histone mark such as tri-methylation of histone H3 Lysine 4 (H3K4me3) (Ko *et al*., 2010). This active epigenetic state shifts to epigenetically repressed state by vernalization and reduces *FLC* expression. Vernalization results in an increase of H3K27me3, a repressive histone mark, at the *FLC* chromatin (Wood *et al*., 2006; Schmitz *et al*., 2007; De Lucia *et al*., 2008). Recently, it has been reported that long non-coding RNAs (lncRNAs) may be involved in the epigenetic repression of *FLC* chromatin (Swiezewski *et al*., 2009; Heo and Sung, 2011).

Flowering is a very important agronomic trait, because premature flowering can reduce yield and quality of crops. Therefore, understanding the mechanism of flowering time is important in the breeding strategy of crops. Some *Brassica* species promote flowering by vernalization, and there are two types of vernalization. The seed vernalization plants such as *Brassica rapa* including Chinese cabbage and turnip can perceive the low temperature at the seed germination stage as well as *A. thaliana*, and the green plant vernalization plants such as *Brassica oleracea* including cabbage and broccoli can perceive the low temperature at a certain developmental stage. The molecular mechanism of different types of vernalization has not been elucidated yet. We introduce recent studies on the regulation of *FLC* expression by lncRNAs in *A. thaliana*, and also discuss how knowledge from *A. thaliana* can help understanding the molecular mechanism of flowering time in the genus *Brassica*.

Epigenetic regulation of *FLC* expression

In eukaryotes, the genome is compacted into chromatin,

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and the chromatin structure plays an important role in gene expression: Gene expression can be controlled by changes in the structure of the chromatin without changing the DNA sequence, and this phenomenon is termed "epigenetic" control. Nucleosomes are formed by a histone octamer containing two of each of the core histones H2A, H2B, H3, and H4, and 147bp of DNA is wrapped around this core. The N-terminal regions of histone proteins are subject to various chemical modifications such as methylation or acetylation, and these histone modifications are associated with gene transcription. In plants, histone deacetylation and histone methylation in H3K9 (9th lysine of H3) and H3K27 are associated with gene repression, and histone acetylation and histone methylation in H3K4 and H3K36 are associated with gene activation (He *et al*., 2004; Bastow *et al*., 2004). Histone lysine residues are able to be mono-, di-, or tri-methylated and each methylation state is associated with different functions. *FLC* expression is regulated by chromatin modification (Dennis and Peacock, 2007). Before prolonged cold exposure, *FLC* is expressed and H3K4me3 is linked with activation of *FLC* expression (Ko *et al*., 2010) (Fig. 1). The H3K4me3 is mediated by the yeast RNA polymerase II (Pol II) Associated Factor 1 (PAF1) complex, by histone H3K4 methyltransferase such as *ARABIDOPSIS TRITHORAX LIKE 1 (ATX1), ATX2,* and *ARABIDOPSIS TRITHORAX-RELATED 7 (ATXR7)*, and by complex protein associated with *Set 1 (COMPASS)*-like complex containing, *WDR5 HOMOLOG A (WDR5a), EARLY FLOWERING IN SHORT DAYS (EFS),* and *ARABIDOPSIS Ash2 RELATIVE (ASH2R)* (Zhang *et al*., 2003; Krogan *et al*., 2003; He *et al*., 2004; Oh *et al*., 2004). A prolonged cold exposure induces a plant homeo domain (PHD) finger-containing protein, *VERNALIZATION INSENSITIVE 3 (VIN3)*. *FLC* repression by vernalization is linked to the enrichment of H3K27me3, which is mediated by PLANT HOMEODOMAIN-POLYCOMB REPRESSIVE COMPLEX 2 (PHD-PRC2) mechanism (De Lucia *et al*., 2008).

Long non-coding RNA induced by cold treatment

Advanced technologies such as tiling array or RNA-sequencing using high-throughput sequencer enable us to discover long non-coding transcripts. It has been shown that some long non-coding RNAs (lncRNAs) are involved in the regulation of gene expression. For examples in mammals, lncRNAs, *Tsix or HOTAIR*, targets PRC2 to *XIST* or *HOX* gene, respectively, resulting in silencing of target genes (Zhao *et al*., 2008; Rinn *et al*., 2007). Using a custom array with single-nucleotide resolution of both strands covering 50kb region around *FLC*, lncRNAs termed cold induced long antisense intragenic RNAs (*COOLAIR*) have been found. *COOLAIR* encompasses the majority of *FLC*, from *FLC* 5' start to 3' polyadenylation sites, and is alternatively polyadenylated and spliced. Induction of *COOLAIR* occurs after 14 days of cold treatment in wild type and *vin3-4* mutant, and is earlier than *VIN3* induction (20 days after transfer to cold) (Sung *et al*., 2004) (Fig. 1). Suppression of unspliced sense *FLC* transcription was observed before the maximum induction of *VIN3*. *COOLAIR* promoter-driven antisense transcription of a reporter gene could lead to transient cold-induced repression, suggesting that *COOLAIR* contributes to early repression of sense *FLC* transcription transiently before stable repression mediated by PHD-PRC2 complex (Swiezewski *et al*., 2009). However plants having T-DNA insertions in the region covering *COOLAIR* where *COOLAIR* expression or upregulation of *COOLAIR* is not observed during cold treatment, showed normal repression of sense *FLC* by vernalization. This suggests that the production of *COOLAIR* transcripts is not an essential component of vernalization-induced repression of *FLC* (Helliwell *et al*., 2011). Further experiments will be required to clarify the possible function of *COOLAIR* during vernalization.

Another lncRNA, *COLD ASSISTED INTRONIC NONCODING RNA (COLDAIR)*, has been identified in the first intron of the sense *FLC* transcript in the sense direction. *COLDAIR* contains a 5' cap structure but is not polyadenylated. *COLDAIR* is induced during cold exposure but expression level returned to the pre-vernalized level after more than 30 days of cold (Fig. 1). Its induction is earlier than *VIN3* and later than *COOLAIR*. The transcription start site of *COLDAIR* is within the vernalization response element (VRE), which is important for stable *FLC* repression by vernalization (Sung *et al*., 2006). *COLDAIR* interacts with one of the components of the PRC2 complex, *CURLY LEAF (CLF)*, specifically during cold exposure. Knockdown lines of *COLDAIR* by RNAi showed that *FLC* repression was not maintained when plants were returned to a warm growth condition after vernalization. Increased expression of *CLF* and enrichment of H3K27me3 by vernalization were not observed in knockdown lines of *COLDAIR*, indicating that *COLDAIR* plays a role in establishment of stable maintenance of repressed *FLC* during vernalization by recruitment of PHD-PRC2 complex to *FLC* chromatin (Heo and Sung, 2011) (Fig. 1).

Characterization of *FLC* in the genus *Brassica*

In the study of vernalization requirement and flowering time of *B. rapa*, genetic mapping and Quantitative Trait Loci (QTL) analysis have been performed. QTL analysis using vernalized populations revealed four candidate regions named *VFR2*, *FR1*, *FR2*, and *FR3* (Osborn *et al*., 1997). *VFR2* was expected to have a large effect on flowering time through the vernalization, and this region was suggested to have synteny with the region covering *FLC* in *A. thaliana* (Kole *et al*., 2001). Another four *B. rapa FLC* homologues (*BrFLC1*, *BrFLC2*, *BrFLC3*, *BrFLC5*) have been identified, and *BrFLC1*, *BrFLC2*, and *BrFLC5* corresponded to the regions of *VFR2*, *FR1*, and *FR2*, respectively (Schranz *et al*., 2002). Expression analysis indicated that *BrFLC1* and *BrFLC2* contribute to vernalization requirement of late flowering plants (Kim *et al*., 2007), and subsequent study suggested that *BrFLC2* plays a central role in flowering time and vernalization response (Zhao *et al*., 2010). Accession with a naturally occurring

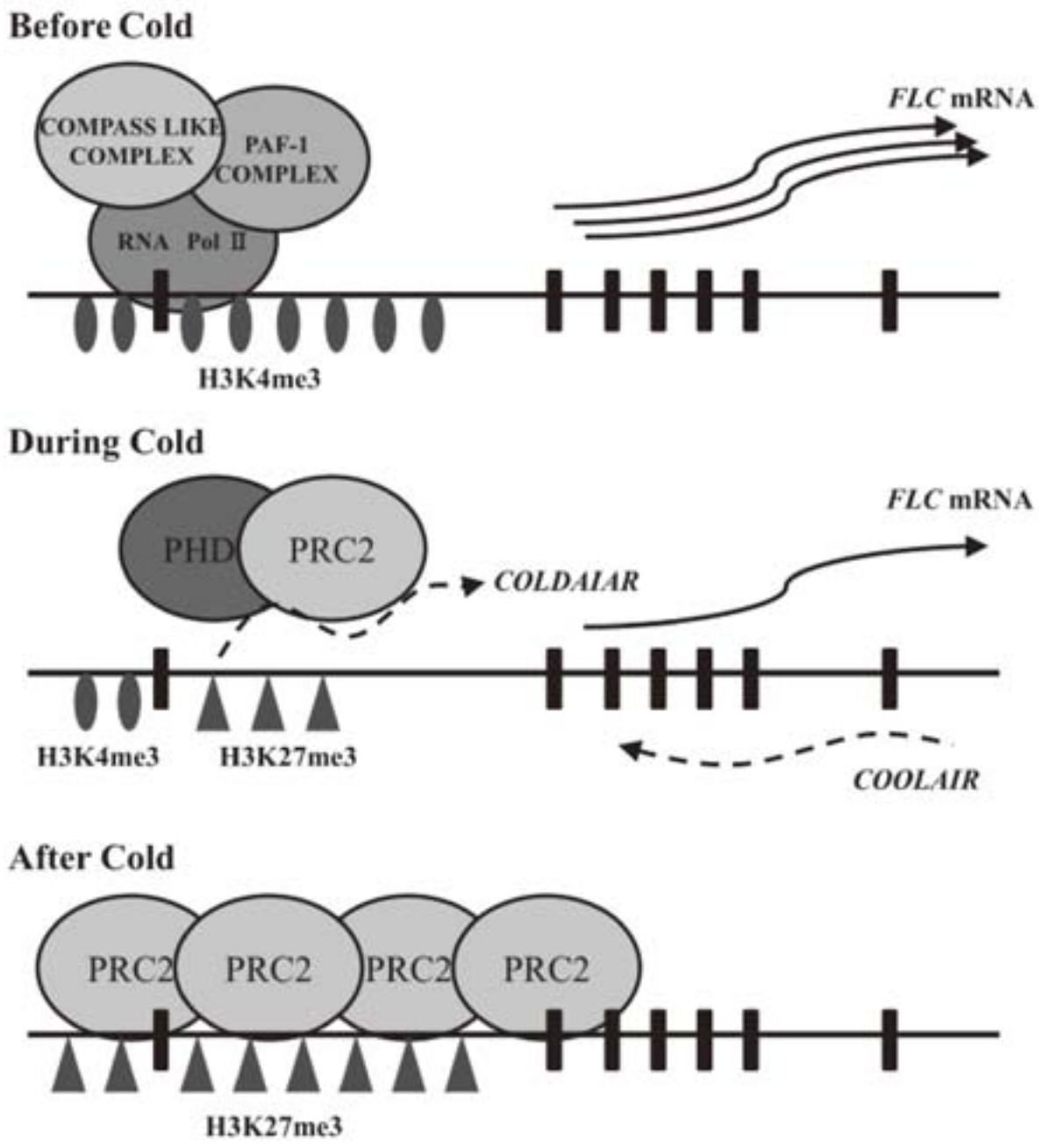

Fig 1. Epigenetic regulation of *FLC* locus by vernalization. Black boxes show the exon region. (Before Cold): Interacting of COMPASS and PAF1 at *FLC* locus results in high level of H3K4me3 therefore *FLC* is active state. (During Cold): *COOLAIR* and *COLDAIR* are transcribed at the beginning of vernalization, and then association of *COLDAIR* and PHD-PRC2 mediates H3K27me3 to establish the *FLC* repression. Repression of *FLC* is incomplete at this time point. (After Cold): As a result of enrichment of PHD-PRC2, H3K27me3 is spread in extensive *FLC* locus, and finally stable repression of *FLC* occurs.

deletion mutation in *BrFLC2* has shown early flowering (Wu *et al*., 2012). The analysis of sequence variation of *BrFLC1* indicated that a naturally occurring splicing mutation is associated with flowering time variation (Yuan *et al*., 2009).

In *B. oleracea*, *BoFLC1*, *BoFLC3*, *BoFLC4-1*, and *BoFLC5* were isolated (Schranz *et al*., 2002; Lin *et al*., 2005). Decrease in *BoFLC4-1* transcript level by vernalization correlated with an increase in *FT* transcript level, furthermore *BoFLC4-1* was down-regulated only in green plant vernalization and does not occur in the seed

vernalization (Lin *et al*., 2005). Okazaki *et al*., (2007) have identified *BoFLC2*, which has a high homology (98%) to *BoFLC4-1*, and reported that *BoFLC2* is a candidate gene for the largest QTL controlling flowering time and plays important roles in flowering through vernalization.

Conclusion

Repression of *FLC* by vernalization serves as a good example of epigenetic gene silencing in plants. We introduced the repression of *FLC* mediated by *COOLAIR* and *COLDAIR*, which appears to have a key role in initial events of vernalization. By interacting with *COLDAIR*, PHD-PRC2 complex mediated a modification on the H3K27me3. Thus, it appears that *COLDAIR* can guide the PHD-PRC2 complex to H3K27me3 target site at *FLC* locus. However, it is not known how the plant responds to cold, and it is interesting to understand how *COOLAIR* and *COLDAIR* are induced by low temperatures.

In *B. rapa* and *B. oleracea*, results of QTL or expression analysis suggested that *FLC*s may be involved in flowering time. However, detailed regulatory mechanism of *FLC* homologues in *Brassica*, such as histone modification or transcription of lncRNAs, has not yet been determined. Of particular interest is the difference of the *FLC* control mechanisms in green plant vernalization and seed vernalization. Recently, it has been reported that microRNAs are involved in an age-dependent response to vernalization in *Alabis alpine* and *Cardamine flexuosa* (Bergonzi *et al*., 2013; Zhou *et al*., 2013). In the green plant vernalization of *B. oleracea*, a similar mechanism may be observed. In the future, the findings in the model plant species will allow more detailed research of vernalization and flowering time in crop species.

Reference

Bastow, R., J. S. Mylne, C. Lister, Z. Lippman, R. A. Martienssen and C. Dean. 2004. Vernalization requires epigenetic silencing of *FLC* by histone methylation. *Nature*, **427**: 164-167.

Chouard, P. 1960. Vernalization and its relations to dormancy. *Annu. Rev. Plant Physiol*., **11**: 191-238.

De Lucia, F., P. Crevillen, A. M. Jones, T. Greb and C. Dean. 2008. A PHD-polycomb repressive complex 2 triggers the epigenetic silencing of *FLC* during vernalization. *Proc. Natl. Acad. Sci. USA*., **105**: 16831-16836.

Dennis, E. S. and W. J. Peacock. 2007. Epigenetic regulation of flowering. *Curr. Opin. Plant Biol*., **10**: 520-527.

He, Y., M. R. Doyle and R. M. Amasino. 2004. PAF1-complex-mediated histone methylation of *FLOWERING LOCUS C* chromatin is required for the vernalization-responsive, winter-annual habit in *Arabidopsis*. *Genes Dev*., **18**: 2774-2784.

Helliwell, C. A., M. Robertson, E. J. Finnegan, D. M. Buzas and E. S. Dennis. 2011. Vernalization-repression of *Arabidopsis FLC* requires promoter sequences but not antisense transcripts. *PLoS ONE*, **6**: e21513.

Helliwell, C. A., C. C. Wood, M. Robertson, W. J. Peacock and E. S. Dennis. 2006. The *Arabidopsis* FLC protein interacts directly *in vivo* with *SOC1* and *FT* chromatin and is part of a high-molecular-weight protein complex. *Plant J*., **46**: 183-192.

Heo, J. B. and S. Sung. (2011). Vernalization-mediated epigenetic silencing by a long intronic noncoding RNA. *Science*, **331**: 76-79.

Ietswaart, R., Z. Wu and C. Dean. 2012. Flowering time control: another window to the connection between antisense RNA and chromatin. *Trends Genet*., **28**: 445-453.

Kim, S., B. S. Park, S. J. Kwon, J. Kim, M. H. Lim, Y. D. Park, D. Y. Kim, S. C. Suh, Y. M. Jin, J. H. Ahn and Y. H. Lee. 2007. Delayed flowering time in *Arabidopsis* and *Brassica rapa* by the overexpression of *FLOWERING LOCUS C (FLC)* homologs isolated from Chinese cabbage (*Brassica rapa* L. ssp. *pekinensis*). *Plant Cell Rep*., **26**: 327-336.

Ko, J. H., I. Mitina, Y. Tamada, Y. Hyun, Y. Choi, R. M. Amasino, B. Noh and Y. S. Noh. 2010. Growth habit determination by the balance of histone methylation activities in *Arabidopsis*. *EMBO J*., **29**: 3208-3215.

Kole, C., P. Quijada, S. D. Michaels, R. M. Amasino and T. C. Osborn. 2001. Evidence for homology of flowering-time genes *VFR2* from *Brassica rapa* and *FLC* from *Arabidopsis thaliana*. *Theor. Appl. Genet*., **102**: 425-430.

Krogan, N. J., J. Dover, A. Wood, J. Schneider, J. Heidt, M. A. Boateng, K. Dean, O. W. Ryan, A. Golshani, M. Johnston, J. F. Greenblatt and A. Shilatifard. 2003. The Paf1 complex is required for histone H3 methylation by COMPASS and Dot1p: linking transcriptional elongation to histone methylation. *Mol. Cell*, **11**: 721-729.

Lin, S. I., J. G. Wang, S. Y. Poon, C. L. Su, S. S. Wang and T. J. Chiou. 2005. Differential regulation of *FLOWERING LOCUS C* expression by vernalization in cabbage and *Arabidopsis*. *Plant Physiol*., **137**: 1037-1048.

Liu, F., V. Quesada, P. Crevillén, I. Bäurle, S. Swiezewski and C. Dean 2007. The *Arabidopsis* RNA-binding protein *FCA* requires a lysine- specific demethylase 1 homolog to downregulate *FLC*. *Mol. Cell*, **28**: 398-407.

Michaels, S. D. and R. M. Amasino. 1999. *FLOWERING LOCUS C* encodes a novel MADS domain protein that acts as a repressor of flowering. *Plant Cell*, **11**: 949-956.

Michaels, S. D. and R. M. Amasino. 2001. Loss of *FLOWERING LOCUS C* activity eliminates the late-flowering phenotype of *FRIGIDA* and autonomous pathway mutations but not responsiveness to vernalization. *Plant Cell*, **13**: 935-941.

Oh, S., H. Zhang, P. Ludwig and S. van Nocker. 2004. A mechanism related to the yeast transcriptional regulator Paf1c is required for expression of the *Arabidopsis FLC/MAF* MADS box gene family. *Plant Cell*, **16**: 2940-2953.

Okazaki, K., K. Sakamoto, R. Kikuchi, A. Saito, E. Togashi, Y. Kuginuki, S. Matsumoto and M. Hirai. 2007. Mapping and characterization of *FLC* homologs and QTL analysis of flowering time in *Brassica oleracea*. *Theor. Appl. Genet*.,

114: 595-608.

Osborn, T. C., C. Kole, I. A. Parkin, A. G. Sharpe, M. Kuiper, D. J. Lydiate and M. Trick. 1997. Comparison of flowering time genes in *Brassica rapa*, *B. napus* and *Arabidopsis thaliana*. *Genetics*, **146**: 1123-1129.

Rinn, J. L., M. Kertesz, J. K. Wang, S. L. Squazzo, X. Xu, S. A. Brugmann, L. H. Goodnough, J. A. Helms, P. J. Farnham, E. Segal and H. Y. Chang. 2007. Functional demarcation of active and silent chromatin domains in human *HOX* loci by noncoding RNAs. *Cell*, **129**: 1311-1323.

Schmitz, R. J. and R. M. Amasino. 2007. Vernalization: A model for investigating epigenetics and eukaryotic gene regulation in plants. *Biochim. Biophys. Acta.*, **1769**: 269-275.

Schranz, M. E., P. Quijada, S. B. Sung, L. Lukens, R. Amasino and T. C. Osborn. 2002. Characterization and effects of the replicated flowering time gene *FLC* in *Brassica rapa*. *Genetics*, **162**: 1457-1468.

Searle, I., Y. He, F. Turck, C. Vincent, F. Fornara, Kröber, R. A. Amasino and G. Coupland. 2006. The transcription factor FLC confers a flowering response to vernalization by repressing meristem competence and systemic signaling in *Arabidopsis*. *Genes Dev.*, **20**: 898-912.

Sheldon, C. C., J. E. Burn, P. P. Perez, J. Metzger, J. A. Edwards, W. J. Peacock and E. S. Dennis. 1999. The *FLF* MADS box gene: A repressor of flowering in *Arabidopsis* regulated by vernalization and methylation. *Plant Cell*, **11**: 445-458.

Sung, S and R. M. Amasino. 2004. Vernalization in *Arabidopsis thaliana* is mediated by the PHD finger protein VIN3. *Nature*, **427**: 159-164.

Sung, S., Y. He, T. W. Eshoo, Y. Tamada, L. Johnson, K. Nakahigashi, K. Goto, S. E. Jacobsen and R. M. Amasino. 2006. Epigenetic maintenance of the vernalized state in *Arabidopsis thaliana* requires LIKE HETEROCHROMATIN PROTEIN 1. *Nat. Genet.*, **38**: 706-710.

Swiezewski, S., F. Liu, A. Magusin and C. Dean. 2009. Cold-induced silencing by long antisense transcripts of an *Arabidopsis* Polycomb target. *Nature*, **462**: 799-802.

Wood, C. C., M. Robertson, G. Tanner, W. J. Peacock, E. S. Dennis and C. A. Helliwell. 2006. The *Arabidopsis thaliana* vernalization response requires a polycomb-like protein complex that also includes VERNALIZATION INSENSITIVE 3. *Proc. Natl. Acad. Sci. USA.*, **103**: 14631-14636.

Wu, J., K. Wei, F. Cheng, S. Li, Q. Wang, J. Zhao, G. Bonnema and X. Wang. 2012. A naturally occurring InDel variation in *BraA.FLC.b (BrFLC2)* associated with flowering time variation in *Brassica rapa*. *BMC Plant Biol.*, **12**: 151

Yuan, Y. X., J. Wu, R. F. Sun, X. W. Zhang, D. H. Xu, G. Bonnema and X. W. Wang. 2009. A naturally occurring splicing site mutation in the *Brassica rapa FLC1* gene is associated with variation in flowering time. *J. Exp. Bot.*, **60**: 1299-1308.

Zhang, H., C. Ransom, P. Ludwig and S. van Nocker. 2003. Genetic analysis of early flowering mutants in *Arabidopsis* defines a class of pleiotropic developmental regulator required for expression of the flowering-time switch *FLOWERING LOCUS C*. *Genetics*, **164**: 347-358.

Zhao, J., B. K. Sun, J. A. Erwin, J. J. Song and J. T. Lee. 2008. Polycomb proteins targeted by a short repeat RNA to the mouse X chromosome. *Science*, **322**: 750-756.

Zhao, J., V. Kulkarni, N. N. Liu, D. P. Del Carpio, J. Bucher and G. Bonnema. 2010. *BrFLC2 (FLOWERING LOCUS C)* as a candidate gene for a vernalization response QTL in *Brassica rapa*. *J. Exp. Bot.*, **61**:1817-1825.

春化の鍵遺伝子である *FLOWERING LOCUS C* の転写制御について

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要　約

植物は温度や日長を認識することで開花するタイミングを調節している。春化は、植物が長期間の低温に遭遇することにより花成が誘導される現象であり、開花期を制御する重要な役割を担っている。シロイヌナズナ（*Arabidopsis thaliana*）の開花抑制遺伝子である *FLOWEING LOCUS C(FLC)* では、春化によって、転写抑制にかかわるヒストンマークであるヒストンH3の27番目のリジン残基のトリメチル化（H3K27me3）を介した転写抑制が生じる。この遺伝子サイレンシングにはH3K27me3をターゲットとするPOLYCOMB RPRESSIVE COMPLEX 2（PRC2）複合体が関与する。これは動植物で保存された転写制御メカニズムである。*FLC* 遺伝子座内から転写される長鎖非翻訳RNA（long non-coding RNA: lncRNA）である *COOLAIR* と *COLDAIR* は、春化によって誘導される。特に、*COLDAIR* はPRC2と結合することで、*FLC* 遺伝子座内のH3K27me3レベルの増加をもたらす。*FLC* の制御機構が詳細に理解されれば、他のエピジェネティックな制御を受ける遺伝子のモデルになるだろう。

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