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ORIGINAL RESEARCH

Genetic and Molecular Studies for Regulation of Bolting Time of Onion (*Allium cepa* L.)

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Abstract The control of bolting time in onion is an important approach for bulb and seed production, as onion plants which bolt do not produce marketable bulbs and seed yields are dependent on floral induction. However, genetic and molecular studies about bolting time in onion plants have not been examined yet to date. In order to understand the regulation of bolting time in onion plants, we conducted the genetic crosses between late bolting-type cultivar (MOS8) and very early bolting-type cultivar (Guikum). Segregation ratio of late to very early in F_2 populations indicated that this lateness trait was determined by a dominant locus. We also analyzed protein profiles in onion plants with different bolting time by a proteomics approach. Interestingly, a protein spot with significant similarities to chromodomains of mammalian chromo-ATPase/helicase-DNA-binding 1 or heterochromatin protein 1, which is involved in the histone modifications, was identified. Histone methyltransferase activity was also observed in onion plants. Taken together, these results suggest that a genetic pathway may be involved in the modulation of bolting time in onion plants, though there is no direct evidence that this protein spot obtained by proteomics is relevant to vernalization.

Keywords Onion · Bolting time · Chromodomain

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Introduction

Onion (*Allium cepa* L.), one of the oldest vegetables known to people, is the most economically significant member in the order Asparagales, which is the second valuable group of monocots (Jakse et al. 2006). Onion as a food and spice is used, usually chopped or sliced, in almost various forms including cooked vegetables and fresh salads. In addition, onion can be utilized for medicinal purposes because it contains chemical compounds believed to have anti-inflammatory, anticholesterol, anticancer, and antioxidant properties (Galmarini et al. 2001).

Onions are grown as an annual plant for commercial purpose, although they are biennial plant, which means that it takes them two seasons to go from seed to seed. Bolting (flowering) of onion is determined by two factors, the size of the plant and cold temperature. The critical size for bolting is reached when the onion forms the five-leaf stage. If onions are seeded on early fall, warm temperature will result in sufficient size for bolting. Cold temperature is also important for bolting. Earlier transplants and some onion varieties are especially susceptible to bolting during cold temperature. However, the effect of cold temperature is not a prerequisite for bolting. If onions are not at the critical size in their development, they do not accept cold as a signal to initiate bolting. Thus, sowing and transplanting at the correct time for onion plants is the most important factor to avoid premature bolting.

Genetic and molecular studies of *Arabidopsis* have revealed a complicate network of signaling pathways involved in flowering time (Boss et al. 2004; Macknight et al. 2002; Putterill et al. 2004). Four genetic pathways, which are known as the photoperiod, autonomous, vernalization, and gibberellin (GA) pathway, have been identified based on the phenotypes of flowering time mutants (Koornneef et al. 1998). The photoperiod pathway includes genes whose mutants show a late flowering phenotype under long day (LD) conditions that is not responsive to vernalization treatments. This pathway contains gene-encoding photoreceptors such as *PHYTOCHROME* (*PHY*), components of the circadian clock, clock-associated genes such as *GIGANTEA* (*GI*; Fowler et al. 1999; Park et al. 1999), and the transcriptional regulator *CONSTANS* (*CO*; Putterill et al. 1995). *FLOWERING LOCUS T* (*FT*) (Kardailsky et al. 1999; Kobayashi et al. 1999) and *SUPPRESSOR OF OVEREXPRESSION OF CO 1* (*SOC1*; Lee et al. 2000) are targets of *CO* (Samach et al. 2000). The autonomous pathway includes genes whose mutants show a late flowering independently of day length that can be rescued by vernalization. Genes included in this pathway are *FCA*, *FY*, *FVE*, *FLOWERING LOCUS D*, *FPA*, *FLOWERING LOCUS K*, and *LUMINIDEPENDENS* (Ausin et al. 2004; He et al. 2003; Kim et al. 2004; Lee et al. 1994; Lim et al. 2004; Macknight et al. 1997; Schomburg et al. 2001; Simpson et al. 2003). They regulate *FLOWERING LOCUS C* (*FLC*; Michaels and Amasino 1999), a floral repressor, expression through several different mechanisms such as histone modification and RNA binding (Simpson 2004). Some genes of this pathway are also involved in ambient temperature signaling (Blazquez et al. 2003; Lee et al. 2007). The vernalization pathway includes genes whose mutations inhibit the promotion of flowering by vernalization. Genes included in this pathway are *VERNALIZATION INSENSITIVE3*, *VERNALIZATION1*, and *VERNALIZATION2* (Gendall et al. 2001; Levy et al. 2002; Sung and Amasino 2004). The GA pathway includes genes whose mutations show a late flowering, especially under short day conditions. This pathway has GA biosynthesis genes, *FLOWERING PROMOTIVE FACTOR1*, and genes involved in GA signal transduction (Huang et al. 1998; Kania et al. 1997). GAs have been known to positively regulate the expression of floral integrator genes such as *SOC1* and *LEAFY* (Blazquez et al. 1998; Moon et al. 2003).

We report here the genetic and molecular evidences for regulation of bolting time in onion plants using late bolting-type cultivar (MOS8) and very early bolting-type cultivar (Guikum). We screened the proteins extracted from onion plants with different bolting time by a proteomic approach and identified a protein spot with significant similarities to chromodomains of mammalian chromo-ATPase/helicase-DNA-binding 1 (CHD1) or heterochromatin protein 1 (HP1). Furthermore, we examined in vitro histone methyltransferase (HMTase) activity assays using purified protein spots isolated from onion plants. Our results suggest that a floral genetic pathway in controlling bolting time may be involved in onion plant.

Materials and Methods

Plant Material and Growth Conditions

Two onion cultivars, MOS8 male sterile line (inbred line developed by Dong Yun Hyun) with late flowering phenotype and Guikum (inbred line provided by Kaneko seed Co., Japan) with very early flowering phenotype, were used in this study. F_1 plants produced from crosses between MOS8 and Guikum were self-pollinated to produce F_2 populations. The seeds of two onion cultivars and F_2 plants were sowed in the greenhouse for 2 months (from early September 2002 to early November 2002), and seedlings were transplanted to the field under natural environmental conditions (from early November 2002 to mid-March 2003). Onion bulbs called mother bulbs were harvested in early June 2003 and stored for 4 months in natural condition. Mother bulbs of F_2 plants and parental lines were transplanted to the field in early November 2003.

Based on segregation ratio of bolting, inheritances of F_2 generations were evaluated. Bolting was checked from transplantation into the field to the first open flower.

Two-Dimensional Polyacrylamide Gel Electrophoresis

The inner basal tissues (200 mg) isolated from onion bulbs were homogenized with lysis buffer containing 8 M urea, 2% NP-40, 5% β-mercaptoethanol, and 5% polyvinyl pyrrolidene for two-dimensional polyacrylamide gel electrophoresis (2-DE; Yang et al. 2005). Extracted protein samples (100 µg) were separated in the first dimension by isoelectric focusing (IEF) tube gel and in the second dimension by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Electrophoresis was carried out at 500 V for 30 min, followed by 1,000 V for 30 min, and 5,000 V for 1 h and 40 min. The focusing strips were immediately used for SDS-PAGE or were stored at −80°C. After electrophoresis of the first dimension, the focusing strips were incubated for 15 min in equilibration buffer I (6 M urea, 2% SDS, 50 mM Tris–HCl, pH 8.8, 30% glycerol, 1% DTT, and 0.002% bromophenol blue) and equilibration buffer II (6 M urea, 2% SDS, 50 mM Tris–HCl, pH 8.8, 30% glycerol, 2.5% iodoacetamide, and bromophenol blue). Equilibrated strips were used for SDS-PAGE as the second dimension. The gels were stained with Silver Stain Plus, and the image analysis

was performed with a FluorS MAX Multimager (Bio-Rad, Hercules, CA, USA).

N-Terminal Sequence Analysis

The proteins were electroblotted onto a polyvinylidene difluoride (PVDF) membrane (Pall, Port Washington, NY, USA) using a semidry transfer blotter (Nippon Eido) and visualized by Coomassie brilliant blue (CBB) staining. The stained protein spots were excised from the PVDF membrane and applied to the reaction chamber in a protein sequencer, Procise (Applied Biosystems, Foster city, CA). Edman degradation was performed in accordance with the standard program supplied by Applied Biosystems. The amino acid sequences were compared to known proteins deposited in the several database.

Matrix-Assisted Laser Desorption/Ionization–Time-of-Flight Mass Spectrometry

Excised protein spots were destained, dehydrated, reduced with DTT, alkylated with iodoacetamide, and digested with trypsin in accordance with the recommended procedure. Samples were then analyzed by matrix-assisted laser desorption/ionization–time-of-flight mass spectrometry (MALDI–TOF MS) on a Voyager-DE STR machine (Applied Biosystems, Framingham MA). Peptide mass fingerprints were matched to the NCBI database entries using the MS-Fit program (http://prospector.ucsf.edu/prospector/4.0.7/html/msfit.htm). The following criteria were used for database search: mass tolerance of 100 ppm, a minimum of four matched peptides, and >15% sequence coverage.

In Vitro HMTase Activity Assays

HMTase assays were carried out at 30°C for 1 h in 20-µl volumes containing 50 mM Tris–HCl, pH 8.5, 20 mM KCl, 10 mM $MgCl_2$, 10 mM β-mercaptoethanol, 250 mM sucrose, 8 µg/µl histone from calf thymus (Roche, USA), 220 nCi of *S*-adenosyl-L-[methyl-^{3}H]-methionine ([^{14}C]SAM), and 200 µg of purified protein spots prepared from onion plants. Reactions were stopped by adding SDS loading buffer, and proteins were separated in 16% SDS-PAGE. After Coomassie staining and destaining, the signals were detected by PhosphoImager (Fuji BAS, Japan).

Results and Discussion

Genetic Inheritance of Bolting in Onion Plants

In order to understand the genetic control of bolting in onion plants, we crossed late bolting-type cultivar (MOS8, days to bolting=165–170 days) with very early bolting-type cultivar (Guikum, days to bolting=130–135 days). The bolting phenotypes of F_1 generations were similar to those of late bolting-type cultivars (data not shown). This suggests that genetic loci affecting bolting may be present in onion plants. Subsequent analysis of the inheritance distribution in F_2 generations was shown in Fig. 1. The distribution pattern and segregation ratio (late bolting/early bolting = about 3:1) indicated that bolting time depended on the segregation of any gene where the dominant allele confers lateness. Furthermore, bolting phenotypes of onion cultivars were reduced by long exposure to cold (E.T. Lee, personal communication). Given the crosses between late and very early bolting onion varieties and effects of low temperature in onion plants, it appears likely that the genetic basis involved in the regulation of bolting time in onion is similar to that of vernalization requirement in plant species (Sung and Amasino 2005). Genetic and molecular studies in various winter-annual and summer-annual acces-

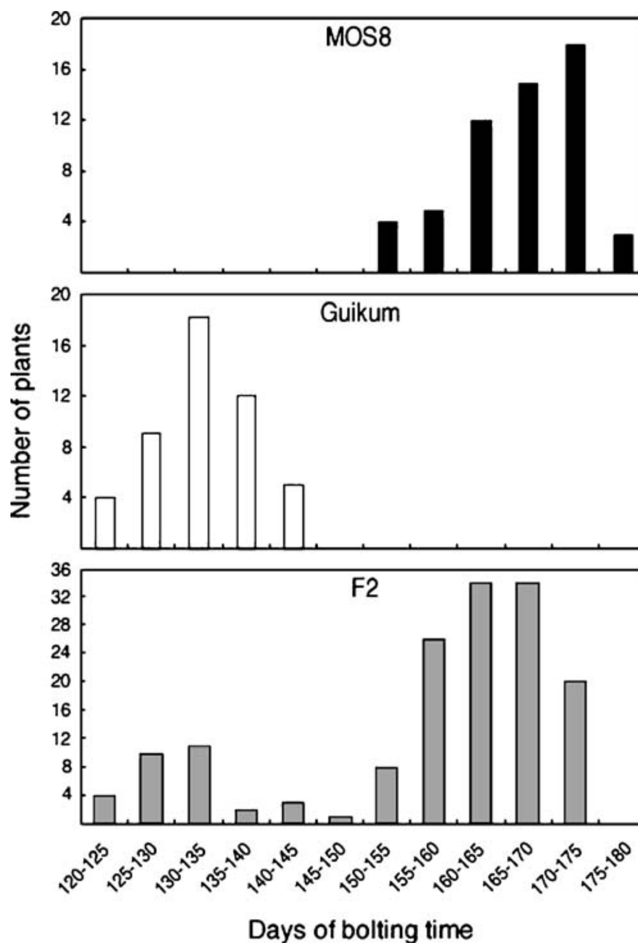

Fig. 1 Distribution pattern of bolting time in F_2 populations derived from crosses between MOS8 (late bolting type) and Guikum (very early bolting type) onion cultivars. These onion\cultivars used in this study were inbred lines. The days of bolting time were calculated when the onion plants were bolted up to 80% in total population

sions of *Arabidopsis* as a model plant have shown that *FRIGIDA* and *FLC* have important functions in distinguishing winter-annual and summer-annual habits in *Arabidopsis* accessions (Clarke and Dean 1994; Gazzani et al. 2003; Shindo et al. 2005). Another plant species are also determined by a relatively small number of loci, either dominant or recessive locus. With *Hyoscyamus niger* (henbane), the biennial habit is governed by a single dominant locus, whereas this habit is governed by a single recessive locus in *Beta vulagris* (sugar beet; Abegg 1936; Lang 1986).

2-DE Analysis in Onion Plants

In order to examine the components involved in the control of bolting time in onion, we checked protein profiles by a proteomics approach using MOS8 and Guikum. The inner basal tissues of each onion bulbs grown for 96 days after transplanting were used for proteomics experiment because bolting is initiated in this region after cold treatment (Fig. 2a). Initial 2-DE analysis of soluble proteins from onion plants was performed using an IEF range of pH 3 to 6 (data not shown). Because use of appropriate pH gradient is an effective way to reduce overlapping spots, additional 2-DE with pH 4 to 6 immobilized pH gradient (IPG) strips was performed (Fig. 2b). After CBB staining, several differences in protein accumulation profiles were detected in onion plants with different bolting time. Although many spots were differentially accumulated in onion plants, we failed to obtain sufficient amounts from many different spots for protein sequencing. Thus, we chose seven protein spots significantly changed in accordance with the degree of bolting time. The amino acid sequences of the differentially regulated proteins were analyzed by protein sequencing. Homology searches were performed using the FASTA search tool. N-terminal sequences were successfully obtained for only one protein (spot 7). The remaining proteins were analyzed by MALDI–TOF MS. Among the other six proteins, three proteins (spots 1, 5, and 6) were not identified, whereas three proteins (spots 2, 3, and 4) were identified as actin, tubulin, and keratin. The result was summarized in Table 1.

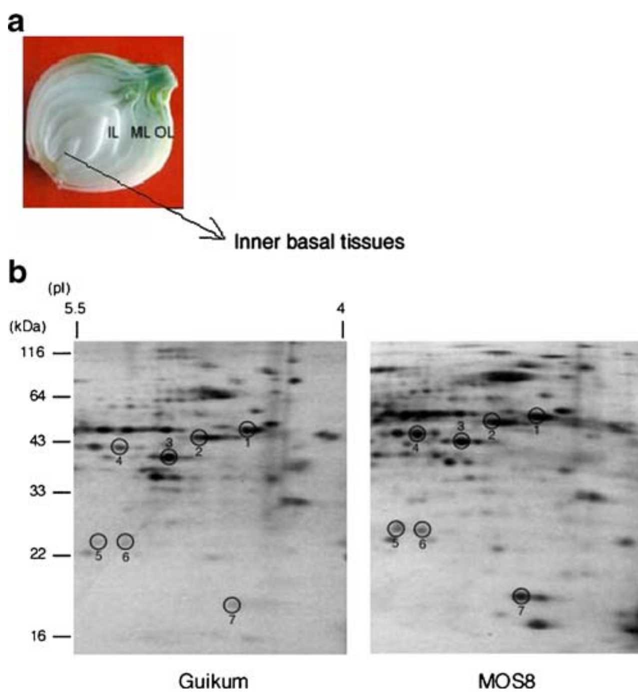

Fig. 2 Two-dimensional gel electrophoresis of proteins isolated from onion plants (MOS8 and Guikum). **a** The inner basal tissues of onion bulb used for proteomic analysis. *IL* inner layer, *ML* middle layer, *OL* outer layer. **b** Protein analysis was performed using medium-range IPG strips with pH range from 4 to 6. The protein spots were identified by protein sequencing and MALDI–TOF MS analysis. Molecular masses (kilodalton) are shown on the left and pI ranges at the top corners of each figure

Interestingly, 16 amino acid sequences of a spot (spot 7) showed significant similarities to several chromodomain regions of mammalian CHD1 or HP1 proteins, though we could not find confident protein homologous to this spot because of short amino acid sequences and poorly characterized onion genome (Fig. 3). The chromodomain appears to be a well-conserved motif because it can be found in wide range of organisms such as protists, plants, amphibians, and mammals (Eissenberg 2001). Furthermore, proteins with this chromodomain are known as both a positive and negative regulator of gene expression in various developmental processes (Hall and Georgel 2007). For instance, two tandem chromodomains of CHD1 protein has known to interact with methylated lysines on histones, which include H3K4me, H3K36me, and H3K79me, associated with active chromatin, thereby inducing active transcription (Flanagan et al. 2005; Sims et al. 2005). However, chromodomain of HP1 protein recognizes and binds to H3K9me for promotion of heterochromatin formation (Jacobs and Khorasanizadeh 2002; Nielsen et al. 2002). Therefore, chromatin remodeling factors with chromodomains may play an important role in regulating gene expression because there is a dramatic change in the chromatin in meristematic regions such as inner basal tissues used in this study. Although there is no evidence that this protein spot is relevant to the regulation of bolting time by vernalization, this observation raises the possibility that chromatin remodeling factors may be conserved in onion plants.

In Vitro HMTase Activity Assays in Onion Plants

In order to assess whether histone methylation correlated with bolting time of onion plants, we performed in vitro HMTase activity assays using purified protein spots with significant similarities to chromodomains of mammalian

Table 1 Identification of onion proteins whose abundance varied significantly among onion plants with different bolting time

Spot no.[a]	pI/kDa[b]	Sequences[c]	Homologous protein (%)	Accession no.
1	4.8/46	N-blocked/MS[d]	Not hit	–
2	5.0/43	N-blocked/MS	Actin 1 (96)	P53504
3	5.1/39	N-blocked/MS	Tubulin alpha 2 chain (89)	Q96460
4	5.2/40	N-blocked/MS	Keratin, type II cytoskeletal 1 (90)	P04264
5	5.4/23	N-blocked/MS	Not hit	–
6	5.2/23	N-blocked/MS	Not hit	–
7	4.9/17	N-ARTLQTARRSTGGKAP	Chromodomains of mammalian CHD1 or HP1 proteins (93)	2B2W_D 3FDT_T 1GUW_B 1KNE_P

[a] Spot numbers are shown in Fig. 2

[b] pI and molecular mass (kilodalton) are from the gel in Fig. 2

[c] N-terminal amino acid sequences are determined by Edman degradation

[d] MALDI–TOF MS

CHD1 or HP1 isolated from two onion cultivars (MOS8 and Guikum) with calf thymus histones as substrate (Fig. 4a). Amino acid sequences of the purified spots used in this assay were confirmed (data not shown). The purified protein spots were able to methylate histone proteins in examined onion plants, indicating that the spots are associated with HMTase activity. Furthermore, the differences of HMTase activity were observed in onion plants, though equal amounts of calf thymus histones were used in this assay (Fig. 4a, b). However, chromodomains of chromatin remodeling factors like mammalian CHD1 or HP1 generally acts as a binding module for methylated lysines on histones. This could be explained by SET domain-containing histone methyltransferase (Yeates 2002) being present in extracts from onion cultivars. We cannot exclude the possibility that this purified protein spot is histone methyltransferase with chromodomain like SUV39H1 protein (Brehm et al. 2004; Koonin et al. 1995).

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Onion      ARTLQTARRSTGGKAP----
2B2W_D     ARTXQTARKSTGGKAPRKQY
3FDT_T     ARTKQTARXSTGGKA-----
1GUW_B     ARTXQTARXSTGGKAPGG--
1KNE_P     ARTKQTARXSTGGKAY----
           *** **** *****
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Fig. 3 Multiple alignments of amino acid sequences between onion protein spot and other homologous proteins. Identical amino acid residues are denoted by *asterisks*. *2B2W_D* chain D-tandem chromodomains of human CHD1 complexed with histone H3 tail containing trimethyllysine 4, *3FDT_T* chain T-crystal structure of the complex of human chromobox homolog 5 with H3K9(Me)3 peptide, *1GUW_B* chain B-structure of the chromodomain from mouse HP1 beta in complex with the lysine 9-methyl histone H3 N-terminal peptide, *1KNE_P* chain P-chromodomain of HP1 complexed with histone H3 tail containing trimethyllysine 9

Conclusions

Our results suggest that a genetic pathway may be involved in the control of bolting time in onion plants by genetic inheritance, though the regulation of bolting in onion plants may be more complexly governed by several loci. Although it is very difficult to identify confident proteins in onion plants with uncharacterized genome, it appears likely that chromatin remodeling factors involved in histone modification may be conserved in onion plant. Although molecular and genetic analyses of flowering time in *Arabidopsis* have identified several floral promotion and repression pathways, our knowledge of the floral pathways in other economically important crops is limited. Thus, the quantitative trait locus mapping and the use of high-throughput experiments such as genomics will provide a

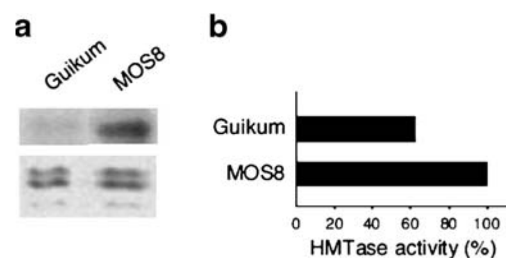

Fig. 4 In vitro HMTase activity in onion plants (MOS8 and Guikum). **a** Fluorography of ^{3}H-methyl calf thymus histone. 200 µg of purified spots containing protein homologous to human CHD1 isolated from two onion cultivars grown for 96 days after transplanting was used in this assay (upper panel). Reaction mixtures were analyzed by 16% SDS-PAGE and autoradiography. Equal amounts of each reaction were confirmed by coomassie blue stain profiles of calf thymus histones (lower panel). **b** Quantitation of HMTase assay in MOS8 and Guikum

better understanding of the regulation of bolting time in onion.

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References

Abegg FA (1936) A genetic factor for the annual habit in beets and linkage relationship. J Agric Res 53:493–511

Ausin I, Alonso-Blanco C, Jarillo JA, Ruiz-Garcia L, Martinez-Zapater JM (2004) Regulation of flowering time by FVE, a retinoblastoma-associated protein. Nat Genet 36:162–166

Blazquez MA, Green R, Nilsson O, Sussman MR, Weigel D (1998) Gibberellins promote flowering of arabidopsis by activating the LEAFY promoter. Plant Cell 10:791–800

Blazquez MA, Ahn JH, Weigel D (2003) A thermosensory pathway controlling flowering time in Arabidopsis thaliana. Nat Genet 33:168–171

Boss PK, Bastow RM, Mylne JS, Dean C (2004) Multiple pathways in the decision to flower: enabling, promoting, and resetting. Plant Cell 16(Suppl):S18–S31

Brehm A, Tufteland KR, Aasland R, Becker PB (2004) The many colours of chromodomains. Bioessays 26:133–140

Clarke JH, Dean C (1994) Mapping FRI, a locus controlling flowering time and vernalization response in Arabidopsis thaliana. Mol Gen Genet 242:81–89

Eissenberg JC (2001) Molecular biology of the chromo domain: an ancient chromatin module comes of age. Gene 275:19–29

Flanagan JF, Mi LZ, Chruszcz M, Cymborowski M, Clines KL, Kim Y, Minor W, Rastinejad F, Khorasanizadeh S (2005) Double chromodomains cooperate to recognize the methylated histone H3 tail. Nature 438:1181–1185

Fowler S, Lee K, Onouchi H, Samach A, Richardson K, Morris B, Coupland G, Putterill J (1999) GIGANTEA: a circadian clock-controlled gene that regulates photoperiodic flowering in Arabidopsis and encodes a protein with several possible membrane-spanning domains. EMBO J 18:4679–4688

Galmarini CR, Goldman IL, Havey MJ (2001) Genetic analyses of correlated solids, flavor, and health-enhancing traits in onion (Allium cepa L.). Mol Genet Genomics 265:543–551

Gazzani S, Gendall AR, Lister C, Dean C (2003) Analysis of the molecular basis of flowering time variation in Arabidopsis accessions. Plant Physiol 132:1107–1114

Gendall AR, Levy YY, Wilson A, Dean C (2001) The *VERNALIZATION 2* gene mediates the epigenetic regulation of vernalization in *Arabidopsis*. Cell 107:525–535

Hall JA, Georgel PT (2007) CHD proteins: a diverse family with strong ties. Biochem Cell Biol 85:463–476

He Y, Michaels SD, Amasino RM (2003) Regulation of flowering time by histone acetylation in Arabidopsis. Science 302:1751–1754

Huang S, Raman AS, Ream JE, Fujiwara H, Cerny RE, Brown SM (1998) Overexpression of 20-oxidase confers a gibberellin-overproduction phenotype in Arabidopsis. Plant Physiol 118:773–781

Jacobs SA, Khorasanizadeh S (2002) Structure of HP1 chromodomain bound to a lysine 9-methylated histone H3 tail. Science 295:2080–2083

Jakse J, Telgmann A, Jung C, Khar A, Melgar S, Cheung F, Town CD, Havey MJ (2006) Comparative sequence and genetic analyses of asparagus BACs reveal no microsynteny with onion or rice. Theor Appl Genet 114:31–39

Kania T, Russenberger D, Peng S, Apel K, Melzer S (1997) FPF1 promotes flowering in Arabidopsis. Plant Cell 9:1327–1338

Kardailsky I, Shukla VK, Ahn JH, Dagenais N, Christensen SK, Nguyen JT, Chory J, Harrison MJ, Weigel D (1999) Activation tagging of the floral inducer *FT*. Science 286:1962–1965

Kim HJ, Hyun Y, Park JY, Park MJ, Park MK, Kim MD, Lee MH, Moon J, Lee I, Kim J (2004) A genetic link between cold responses and flowering time through FVE in Arabidopsis thaliana. Nat Genet 36:167–171

Kobayashi Y, Kaya H, Goto K, Iwabuchi M, Araki T (1999) A pair of related genes with antagonistic roles in mediating flowering signals. Science 286:1960–1962

Koonin EV, Zhou S, Lucchesi JC (1995) The chromo superfamily: new members, duplication of the chromo domain and possible role in delivering transcription regulators to chromatin. Nucleic Acids Res 23:4229–4233

Koornneef M, Alonso-Blanco C, Peeters AJ, Soppe W (1998) Genetic control of flowering time in Arabidopsis. Annu Rev Plant Physiol Plant Mol Biol 49:345–370

Lang A (1986) Hyoscyamus niger. CRC Press, Boca Raton

Lee I, Aukerman MJ, Gore SL, Lohman KN, Michaels SD, Weaver LM, John MC, Feldmann KA, Amasino RM (1994) Isolation of LUMINIDEPENDENS: a gene involved in the control of flowering time in Arabidopsis. Plant Cell 6:75–83

Lee H, Suh SS, Park E, Cho E, Ahn JH, Kim SG, Lee JS, Kwon YM, Lee I (2000) The AGAMOUS-LIKE 20 MADS domain protein integrates floral inductive pathways in *Arabidopsis*. Genes Dev 14:2366–2376

Lee JH, Yoo SJ, Park SH, Hwang I, Lee JS, Ahn JH (2007) Role of SVP in the control of flowering time by ambient temperature in Arabidopsis. Genes Dev 21:397–402

Levy YY, Mesnage S, Mylne JS, Gendall AR, Dean C (2002) Multiple roles of Arabidopsis VRN1 in vernalization and flowering time control. Science 297:243–246

Lim MH, Kim J, Kim YS, Chung KS, Seo YH, Lee I, Hong CB, Kim HJ, Park CM (2004) A new Arabidopsis gene, FLK, encodes an RNA binding protein with K homology motifs and regulates flowering time via FLOWERING LOCUS C. Plant Cell 16:731–740

Macknight R, Bancroft I, Page T, Lister C, Schmidt R, Love K, Westphal L, Murphy G, Sherson S, Cobbett C, Dean C (1997) FCA, a gene controlling flowering time in Arabidopsis, encodes a protein containing RNA-binding domains. Cell 89:737–745

Macknight R, Duroux M, Laurie R, Dijkwel P, Simpson G, Dean C (2002) Functional significance of the alternative transcript processing of the Arabidopsis floral promoter FCA. Plant Cell 14:877–888

Michaels SD, Amasino RM (1999) *FLOWERING LOCUS C* encodes a novel MADS domain protein that acts as a repressor of flowering. Plant Cell 11:949–956

Moon J, Suh SS, Lee H, Choi KR, Hong CB, Paek NC, Kim SG, Lee I (2003) The *SOC1* MADS-box gene integrates vernalization and gibberellin signals for flowering in *Arabidopsis*. Plant J 35:613–623

Nielsen PR, Nietlispach D, Mott HR, Callaghan J, Bannister A, Kouzarides T, Murzin AG, Murzina NV, Laue ED (2002) Structure of the HP1 chromodomain bound to histone H3 methylated at lysine 9. Nature 416:103–107

Park DH, Somers DE, Kim YS, Choy YH, Lim HK, Soh MS, Kim HJ, Kay SA, Nam HG (1999) Control of circadian rhythms and photoperiodic flowering by the Arabidopsis GIGANTEA gene. Science 285:1579–1582

Putterill J, Robson F, Lee K, Simon R, Coupland G (1995) The CONSTANS gene of Arabidopsis promotes flowering and encodes a protein showing similarities to zinc finger transcription factors. Cell 80:847–857

Putterill J, Laurie R, Macknight R (2004) It's time to flower: the genetic control of flowering time. Bioessays 26:363–373

Samach A, Onouchi H, Gold SE, Ditta GS, Schwarz-Sommer Z, Yanofsky MF, Coupland G (2000) Distinct roles of CONSTANS target genes in reproductive development of *Arabidopsis*. Science 288:1613–1616

Schomburg FM, Patton DA, Meinke DW, Amasino RM (2001) FPA, a gene involved in floral induction in Arabidopsis, encodes a protein containing RNA-recognition motifs. Plant Cell 13:1427–1436

Shindo C, Aranzana MJ, Lister C, Baxter C, Nicholls C, Nordborg M, Dean C (2005) Role of FRIGIDA and FLOWERING LOCUS C in determining variation in flowering time of Arabidopsis. Plant Physiol 138:1163–1173

Simpson GG (2004) The autonomous pathway: epigenetic and post-transcriptional gene regulation in the control of Arabidopsis flowering time. Curr Opin Plant Biol 7:570–574

Simpson GG, Dijkwel PP, Quesada V, Henderson I, Dean C (2003) FY is an RNA 3′ end-processing factor that interacts with FCA to control the Arabidopsis floral transition. Cell 113:777–787

Sims RJ 3rd, Chen CF, Santos-Rosa H, Kouzarides T, Patel SS, Reinberg D (2005) Human but not yeast CHD1 binds directly and selectively to histone H3 methylated at lysine 4 via its tandem chromodomains. J Biol Chem 280:41789–41792

Sung S, Amasino RM (2004) Vernalization in Arabidopsis thaliana is mediated by the PHD finger protein VIN3. Nature 427:159–164

Sung S, Amasino RM (2005) Remembering winter: toward a molecular understanding of vernalization. Annu Rev Plant Biol 56:491–508

Yang G, Inoue A, Takasaki H, Kaku H, Akao S, Komatsu S (2005) A proteomic approach to analyze auxin- and zinc-responsive protein in rice. J Proteome Res 4:456–463

Yeates TO (2002) Structures of SET domain proteins: protein lysine methyltransferases make their mark. Cell 111:5–7