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ROLE OF EPIGENETIC MECHANISMS IN PLANT RESPONSE TO LOW TEMPERATURE

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Plants are continuously exposed to various environmental stresses and they respond to them in different ways. Ambient temperature is among the most important environmental cues that directly influence plant growth and yield. Research in recent years has revealed that epigenetic mechanisms play a key role in plants' response to temperature stress. Changes in gene expression evoked by stress signals follow post-translational histone modifications, DNA methylation, histone variant incorporation, and the action of chromatin remodeling factors and Polycomb group proteins. The majority of epigenetic modifications induced by temperature stress are reversible in nature; thus, chromatin returns to its previous state after the stress has passed. Some modifications seem stable, however, due presumably to so-called stress memory. Epigenetic modifications can be inherited through mitosis and meiosis. By dint of epigenetic memory, plants can more efficiently respond to future stressful conditions, thereby increasing their potential for environmental adaptation. Recognition of the epigenetic mechanisms that take part in plants' response to changes of ambient temperature will increase our understanding of adaptations to stress conditions.

Key words: Epigenetics, vernalization, cold stress.

INTRODUCTION

In the temperate climatic zone, low temperature is a key regulatory factor influencing a wide range of transitional phases including germination, bud dormancy and flowering. Plants show high thermal acclimation potential. They have the ability to adapt and survive under cold stress. Before winter, plants stop flowering in order to protect their sensitive apical meristems from frost damage. It has been shown that cold acclimation is associated with transient and tightly controlled changes in gene expression. Among others, *COR* (Cold Regulated) and *CBF* (CRT- Binding Factor) genes become active during winter, boosting frost tolerance (Diallo et al., 2010). Another aspect of the adaptation of plants to low temperatures is a process called vernalization, which prevents precocious flowering during autumn or winter. Vernalization is a transition from vegetative to reproductive development during a period of low temperature (Kim et al., 2009). Many plants require prolonged exposure to cold during winter in order to acquire flowering competence. It is crucial for flowering not to be induced by transient exposure to cold followed by warm conditions in autumn; thus the requirement for prolonged cold. Not all plants have a vernalization requirement, and the degree of vernalization required can vary within a species. As an example, temperate-climate cereals occur in two types – spring and winter. The spring type does not require vernalization, while the winter type needs quantitative vernalization, which induces flowering, allowing it to complete the plant life cycle.

In recent years it has become increasingly clear that epigenetic mechanisms play a significant role in the plant response to environmental stress. Stress signals – hormones, metabolites, free radicals – affect genes encoding various epigenetic regulators such as histone variants, small nuclear RNA, chromatin remodeling factors, transcription factors or DNA and histones modifying enzymes. All of these factors trigger epigenetic changes in gene expression; plants with identical genomes may have different epigenomes (Amasino, 2004; Molinier et al., 2006; Chinnusamy and Zhu, 2009; Hauben et al., 2009, Rogalska et al. 2010). Epigenetics has been

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defined as "a branch of biology studying interactions between genes and their products as causes of phenotype development." At present the term is used to refer to heritable changes in genome functioning without changes in the nucleotide sequences of DNA (Allison, 2007).

PLANT CHROMATIN "SENSES" AMBIENT TEMPERATURE

The soil and air temperature are the major environmental cues regulating different developmental stages of plants. However, the mechanisms by which plants sense the temperature alterations have remained elusive. Exposure to high temperature stresses plants, leading to initiation of synthesis of heat shock proteins (HSPs). HSPs protect other proteins from denaturation, allowing the vital functions of the cell to be maintained. Compact architecture and delayed flowering are characteristics of plants growing in cooler temperatures. A moderate increase of ambient temperature induces elongation of plant axes and a transition from vegetative to generative phase in winter-type *Arabidopsis thaliana*. The floral integrator *FT* (*Flowering Time*) gene plays an undeniable role in this process (Franklin, 2010; Wigge, 2011), as the level of *FT* expression determines the thermosensitive course of the flowering pathway. However, *FT* expression is regulated mainly by the *CO* (*CONSTANS*) gene, which is not essential for perceiving temperature.

Kumar and Wigge (2010) assigned the key role in sensing the changes of ambient temperature to chromatin. In their study they investigated *arp6* mutants of *Arabidopsis thaliana*. The *ARP6* gene encodes a protein which is a subunit of the SWR1 chromatin remodelling complex. Plants with nonfunctional *ARP6* executed a constitutive developmental program typical for high temperature. *Arp6-10* mutants flowered remarkably earlier than wild-type plants at 21°C under long-day conditions and at 22°C under short-day conditions. The reaction of plants at 27°C under short-day conditions was also studied, because thermal induction of flowering is more conspicuous under short days. In these conditions, *arp6-10* mutants flowered with about five leaves. Moreover, there were effects of high temperature on hypocotyl growth, petiole elongation, HSP70 expression and flowering time. The *arp6-10* mutants grown at 17°C, 22°C and 27°C exhibited greater hypocotyl and petiole elongation than wild-type plants. Mutants grown at 17°C had even longer hypocotyls and petioles than wild-type plants grown at 22°C, with an equivalent difference between 22°C and 27°C (Raisner et al., 2005; Creyghton et al., 2008).

ARP6 is required for inserting the alternative histone H2A.Z into nucleosomes in place of H2A (Li and Liu, 2010). It is believed that the presence of the histone H2A.Z variant in nucleosomes maintains the promoters in the repressed state until an appropriate activation signal is received (Li et al., 2005). This means that the promoters of quiescent genes are kept on standby, ready for transcription. Analysis of chromatin revealed that temperature modified the nucleosome composition and accessibility to promoter sequences. To determine whether H2A.Z dynamics are indeed altered in response to temperature, Kumar and Wigge (2010) analyzed H2A.Z occupancy at the *HSP70* promoter in response to different ambient temperature. They demonstrated that nucleosomes containing the histone H2A.Z variant clearly respond to temperature changes and provide thermosensitive information to control the transcriptome, which is coordinated by ambient temperature. At lower temperature, H2A.Z nucleosomes had a high level of occupancy, and at higher temperature H2A.Z nucleosome occupancy declined, leading to increased expression of *HSP70*. These results supported work by Zilberman et al. (2008), whose study of chromatin status at the *FT* locus revealed that when plants were grown at cooler temperatures the nucleosomes at the promoter region were enriched in the H2A.Z histone variant; in plants grown at 27C the H2A.Z histones were depleted from +1 nucleosomes. Kumar and Wigge (2010) showed that H2A.Z-containing nucleosomes wrap DNA more tightly than H2A nucleosomes do. Thus, higher temperatures improve the accessibility of pol II RNA to the transcription start site. As heat/cold upregulates some genes and downregulates others (Lee et al., 2005), in the case of genes whose expression is decreased at higher temperature it might be expected that the loss of H2A.Z allows access to a repressor or appropriate DNA methyltransferases.

REMEMBERING THE COLD IN *ARABIDOPSIS THALIANA* – VERNALIZATION PROCESS

Activation of the *FT* gene by long day triggers flowering in some ecotypes of *Arabidopsis thaliana*. *FT*, which is active in leaves, encodes the protein named "mobile florigene". This protein migrates from leaves to the shoot apex, where it reacts with FD protein and activates genes that promote flower development, for instance *APETALA 1* (*AP1*) containing the MADS box (Corbesier et al., 2007). *FT* is activated by the product of *CO* (*CONSTANS*), which is subject to a daily rhythm of expression. The mechanism by which *CO* activity is controlled by day length involves both transcriptional and post-translational regulation. *CO* expression reaches maximum around 12 h after dawn and stays high until the fol-

lowing dawn (Suarez-Lopez et al., 2001). Transcription of the *CO* protein gene is regulated by products of genes encoding factors involved in day length perception or factors controlling the daily cycle (circadian clock). An example of such a gene is *GIGANTEA* (*GI*), whose product, along with other factors, binds to the *CO* gene promoter and promotes its transcription in late afternoon (Sawa et al., 2007; Greenup et al., 2009). At the post-translational level, CO protein is stable when plants are exposed to light, whereas in darkness CO is rapidly degraded through ubiquitination and the activity of the proteasome. These mechanisms combine to ensure that the peak of CO protein abundance occurs under long-day conditions when plants are exposed to light between 10 and 16 h after dawn, whereas under short-day conditions, when plants are exposed to darkness during this interval, CO protein does not accumulate and therefore *FT* is not transcribed and flowering is not promoted (Valverde et al., 2004; Jang et al., 2008). CO protein contains a zinc finger domain, and the CCT domain which structurally resembles the HAP2 (Heme Activator Protein 2) protein domain in yeast. HAP2 interacts with HAP3 and HAP4 proteins, forming a specific complex which binds to the CCAAT regulatory DNA sequence. In *Arabidopsis*, CO protein interacts with AtHAP3 and AtHAP5 proteins through the CCT domain, and this complex binds to the CAAT motif of the *FT* gene and thereby leads to its transcriptional activation (Wenkel et al., 2006).

Certain *Arabidopsis thaliana* ecotypes require prolonged cold (vernalization) to promote rapid flowering. Regulation of the vernalization response is mainly controlled by the following genes: *FLC*, *VRN1*, *VRN2* and *FT* (*VIN3*) (Sung and Amasino, 2004a; 2006). The key gene of the vernalization pathway is *FLC* – *FLOWERING LOCUS C*, which encodes a MADS-box transcription factor that represses genes involved in floral initiation, including SOC1 (*SUPPRESSOR OF CONSTANS 1*) and *FT* (*FLOWERING LOCUS T*). Before vernalization, *FLC* produces abundant mRNA; in this way the flowering reaction is suppressed, whereas its expression is repressed by vernalization. *FLC* remains repressed when plants are subsequently exposed to warm temperatures, allowing activation of *FT* and SOC1, which promote flowering (Michaels et al., 1999; Sheldon et al., 2000). Stable downregulation of *FLC* is associated with alteration of the chromatin state from actively transcribed to stably repressed. Inhibition of *FLC* expression is correlated with an increase in the levels of repressive histone modifications at *FLC* chromatin such as histone H3 lysine 27 and lysine 9 methylation, as well as the loss of histone modifications associated with active transcription, such as histone H3 acetylation and histone H3 lysine 4 methylation (Bastow et al., 2004; Finnegan et al., 2005; Finnegan and Dennis, 2007; Schmitz et al., 2008). Sung and Amasino (2004b) demonstrated that *VIN3* (Vernalization Insensitive 3), a plant homeodomain finger-containing (PHD) protein, is involved in this modification of chromatin structure. The *VIN3* gene is expressed only during cold exposure, and induction kinetics are related with the duration of cold and the strength of the vernalization response. The product of *VIN3* is a component of the chromatin-remodeling complex and takes part in diverse biochemical reactions: for instance, in cooperation between proteins, in nucleosome binding, or in phospholipid binding (Sung and Amasino, 2004a; Finnegan et al., 2005; 2007). This gene does not undergo epigenetic changes. During vernalization the protein encoded by *VIN3* binds to chromatin of the *FLC* gene, cooperates with Polycomb group proteins (PcG) and catalyzes trimethylation of lysine 9 and 27 of H3 histone. Methylation level increases at the transcription start site of the *FLC* gene, and spreads in both directions along the gene. Heo and Sung (2011) showed that long non-coding RNA plays a role in this vernalization-mediated repression of *FLC*. They identified COLD ASSISTED INTRONIC NONCODING RNA (COLDAIR), which is required for recruitment of Polycomb Repressive Complex 2 (PRC2) to the *FLC* locus. COLDAIR physically associates with a component of PRC2 and targets PRC2 to *FLC*. Sense FLC RNA (COLDAIR) transcription is activated about the 20th day of cold exposure. It is presumed that COLDAIR co-operates with the *FLC* gene as long as PcG proteins complexes are bound, which permanently repress *FLC* gene expression. Earlier, before epigenetic modification, the cold induces antisense transcription originating from the 3' end of the *FLC* gene. However, the role of antisense FLC RNA (COOLAIR – COLD INDUCED LONG ANTISENSE INTRAGENIC RNA) in the vernalization process has yet to be demonstrated, as COLDAIR but not COOLAIR interacts with PRC2.

The cellular memory of transcriptional repression of *FLC* is maintained during successive cell divisions by mitotic inheritance of repressive histone modifications at the gene (Sung et al., 2006). The key players in keeping the cellular memory are Polycomb group (PcG) proteins (de Lucia et al., 2008). Long-term *FLC* repression requires the activity of the *FRIGIDA* (*FRI*) gene; its activity is required to maintain the vernalization response (Amasino and Michaelis, 2010). In other words, FRI is a determinant of the vernalization response in different *Arabidopsis thaliana* ecotypes. Other regulatory genes that form a part of the so-called autonomous pathway act via transcriptional regulation of the floral repressor *FLC*. Some of them encode histone deacetylases, which reduce *FLC* gene expression levels (Greenup et al., 2009).

EPIGENETIC CONTROL OF VERNALIZATION IN WINTER CEREALS

The vernalization response has evolved independently in winter cereals and *Arabidopsis thaliana* (Fig. 1). In cereals cultivated in the temperate climate zone (e.g., wheat, barley, oat, rye) there are three main genes involved in the vernalization pathway: *VRN1*(*AP1*), *VRN2* and *VRN3* (Yan et al., 2006; Distelfeld et al., 2009). Genetic variation of these genes is used in cereal breeding programs to develop varieties suitable for different climatic zones.

The vernalization response is mediated by stable induction of *VRN1* gene promoter. This gene encodes a MADS box transcription factor required for the initiation of reproductive development at the shoot apex. Before vernalization *VRN1* is expressed at low levels. *VRN1* transcript levels increase gradually during vernalization, with longer cold treatments inducing higher expression levels. *VRN1* expression remains high when plants are exposed to warm temperatures following vernalization, and promotes the transition to reproductive development (Trevaskis et al., 2007). *VRN1* downregulates the floral repressor *VRN2*, and allows induction of the floral activator *FT* (VNR3) to accelerate subsequent stages of floral development (Greenup et al., 2009). *VRN2* encodes a protein, with zinc finger and CCT domains, which is a transcription factor predominant in the winter type (Li and Liu, 2010). The activity of this gene is blocked by vernalization. *VRN2* represses flowering until plants become vernalized and the level of *VRN1* expression increases. Mutation of the CCT domain or deletion of the entire *VRN2* gene corresponds with recessive alleles for spring growth habit which eliminate the vernalization requirement (Dubcovsky et al., 2007; Li and Liu, 2010). Summing up, *VRN1* is a key gene of vernalization activated by prolonged cold, enabling the transition from vegetative to generative development in the shoot apex. This gene also triggers a long-day response in leaves. There are *VRN1* alleles in some wheat and barley varieties, which carry mutations in the promoter or first intron. These mutations make the gene active without vernalization and reduce the vernalization response (Finnegan and Dennis, 2007; Oliver et al., 2009).

HOW PLANTS REMEMBER VERNALIZATION

Plants have the ability to measure a period of cold during winter and to "remember" this during cold exposure in the spring (Sung and Amasino, 2004a). As prolonged cold causes changes in the chromatin structure at specific loci, the vernalization signal is transmitted stably through mitotic divisions (epigenetic effects are often heritable, in the sense that they are passed on from one cell generation to the next). As described above, vernalization promoted flowering through epigenetic repression of the flowering repressor *FLC* in *Arabidopsis*. Subsequent studies have revealed that the mechanism of *FLC* repression involves a series of modifications of FLC chromatin which ultimately result in a stable repressed state. Generally, epigenetic variations can also be transmitted from parents to progeny (Jablonka and Raz 2009). However, the vernalization signal is not transmitted through meiosis; the signal is simply "erased" (Putteril et al., 2004). Active *FLC* transcription is restored in progeny, ensuring that the next generation is competent to respond to vernalization.

Studies on vernalization in cereals have demonstrated that the pathway is different, but that basic mechanisms that sense prolonged cold could be conserved. The memory of vernalization in cereals results from alterations in histone H3 lysine methylation levels throughout the extent of the *VRN1* gene (Oliver et al., 2009). The increase of H3K9 trimethylation and decrease of H3K27 trimethylation suggest that vernalization promotes an active chromatin state within *VRN1*. The level of H3K27 methylation within this gene was high before vernalization. The influence of vernalization on H3K4 and H3K27 methylation levels in *VRN2* and *FT*1 genes were also investigated. There was a high level of H3K27me3 and low level of H3K4me3 either in vernalized seedlings or those not exposed to prolonged cold, unlike the "vernalized" *VRN1* gene. This means that vernalization did not influence the chromatin of these genes. In plants and other organisms the H3K3 and H3K27 trimethylation levels can be maintained through the action of trithorax and Polycomb proteins. However, it seems that in the *VRN1* gene present in cereals there are no sequence motifs to which the protein can potentially bind. Reduction of the H3K27 trimethylation level may result from the action of histone demethylases. Interestingly, in germinating caryopses the *VRN2* and *FT* genes are blocked by PcG proteins, which recognize methylated H3K27 sites, whereas in the shoot apex these proteins are not involved in regulation of *VRN1* (Finnegan et al., 2005; Greenup et al., 2009; Oliver et al., 2009). Some data indicate that vernalization is mediated by DNA demethylation (Li and Liu, 2010).

COLD ACCLIMATION VERSUS VERNALIZATION

In plants, germ-line cells are more susceptible than vegetative meristems to frost; hence even small differences in developmental stages can influence the

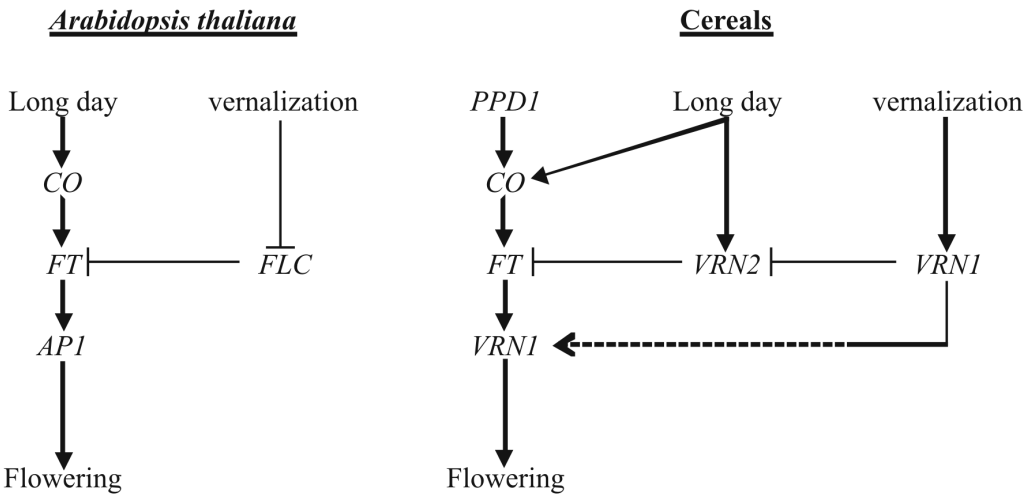

Fig. 1. Comparison of molecular pathways regulating flowering time in *Arabidopsis* and winter cereals. Vernalization and long days promote flowering in *Arabidopsis* and winter cereals. *CONSTANS* (*CO*) genes sense long days and activate *FLOWERING LOCUS* T (*FT*) expression, whereas in cereals this role is ascribed to the *PHOTOPERIOD1* (*PPD1*) gene. Before vernalization the *FT* gene is induced by long-day conditions in *Arabidopsis* or in cereals. However, the vernalization pathway develops independently in *Arabidopsis* and cereals. In *Arabidopsis*, *FLOWERING LOCUS C* (*FLC*) inhibits long-day induction of the *FT* gene, while *FLC* is blocked by vernalization. In winter cereals, before winter the *VRN2* gene represses *FT* expression, while *VRN1* is activated by vernalization, further repressing *VRN2* and activating *FT*. In *Arabidopsis* the vernalization pathway and the long-day response meet at *FT*, which is known as a floral integrator. In cereals the *VRN2* gene is a floral integrator. In both *Arabidopsis* and winter cereals, flowering induction triggers the expression of floral identity meristem genes, for instance APETALLA1 (*AP1*) in *Arabidopsis*. The *VRN1* gene acts as a flowering time gene in the vernalization pathway and as a floral identity meristem gene during reproductive development (Greenup et al., 2009).

survival of plants in freezing conditions. These differences may reflect the allelic action of *PPD* genes, which regulate initiation of the generative stage by the photoperiod or the activity of vernalization genes (*VRN*) influencing frost tolerance. It is worth noting that *VRN* genes are regulated by prolonged cold but not frost. Similarly, frost acclimation requires long-time exposure to cold temperatures (Turner et al., 2005; Beales et al., 2007).

The degree of freezing tolerance has been found to diminish gradually after the reproductive phase is initiated. Some experiments conducted with *Triticum monococcum* mutants with deletion of the *VRN1* gene – *mpv*/*mpv* (*Maintained Vegetative Phase*) – showed that homozygous plants (*mpv*/*mpv*) cannot flower while heterozygous plants (*Mvp*/-) carrying one functional *VRN1* copy can flower normally. A high level of *VRN1* transcription under long-day conditions was detected in heterozygous plants, whereas frost tolerance was reduced as well as the transcription level of many cold-induced genes, among others *CBF* (also known as *Dehydration Responsive Elements*) and *COR* genes (Dhillon et al., 2010). *CBF* genes encode transcription factors which interact with the conservative CCGAC sequence motif (C-repeat (CRT)/dehydration element DRE) located at promoter sites of many genes involved in early drought and cold response (Galiba et al., 2009). Hence it is suggested that the *VRN1* gene is required for initiation of the regulatory cascade that downregulates the cold acclimation pathway. The action of this gene is accompanied by additional genes regulated by long days, which are required for *COR* gene blocking. Numerous studies with near-isogenic lines and QTL mapping studies have pointed to *VRN1* as an important regulator of freezing tolerance (Francia et al., 2004; Limin and Fowler, 2006; Galiba et al., 2009). The results indicate that allelic variation in *VRN1* is sufficient to determine the differences in the degree of freezing tolerance, suggesting that quantitative trait loci for freezing tolerance previously mapped on this chromosome region are likely a pleiotropic effect of *VRN1* rather than the effect of a separate closely linked locus of *FR1* (*Frost Resistance-1*) (Limin and Fowler, 2006; Dhillon et al., 2010). Studies in *Arabidopsis thaliana* have shown that core histone (mainly H4) acetylation and deacetylation play a key role in gene activation/repression during cold acclimation and freezing tolerance. Another study in *A. thaliana* confirmed that siRNA participates in the freezing acclimation pathway (Putterill et al., 2004).

ROLE OF SMALL RNAs IN COLD STRESS RESPONSE

Small noncoding RNAs regulate various biological processes by causing either transcriptional gene silencing (TGS) or posttranscriptional gene silencing (PTGS) (Baulcombe, 2004). Regulation at the transcriptional level involves histone modification and DNA methylation (Schramke and Allshire, 2004; Khraiwesh et al., 2010), whereas at the posttranscriptional level small RNAs mediate RNA degradation (Baumberger and Baulcombe, 2005) or repress translation (Lanet et al., 2009). Two main classes of small regulatory RNAs have been distinguished by their different modes of biogenesis and function: microRNAs (miRNAs) and small interfering RNAs (siRNAs). Recent evidence indicates that both miRNAs and siRNAs play a role in abiotic stress response (Sunkar et al., 2007; Khraiwesh et al., 2012). Stress-induced miRNAs target negative regulators of stress responses or positive regulators of processes inhibited by stresses, and that several of the newly identified miRNAs exhibit tissue-specific or developmental stage-specific expression patterns. Stress conditions cause plants to over- or underexpress certain miRNAs or to synthesize new miRNAs to cope with stress. Zhou et al. (2008) found that nineteen microRNA genes of eleven microRNA families in *Arabidopsis thaliana* are upregulated by cold stress. Six of them were induced, while the remaining five showed either transient or mild regulation under cold stress. Cold stress also changed the expression of siRNAs in wheat (Yao et al., 2010) and *Populus* (Lu et al., 2008), and in both these genera some miRNAs were upregulated while others were downregulated under cold stress.

Both miRNAs and siRNAs are loaded into AGO (ARGONAUTE) protein-containing RISC (RNA-Induced Silencing Complex) which guides target regulation at the posttranscriptional level or at the transcriptional level through a pathway termed RNA-directed DNA methylation (RdDM). The latter mechanism (RdDM) seems to be evolutionarily significant, as transgenerational effects in plants are associated with alterations in methylation of genomic DNA and as the epigenetic memory of stress may cause favorable adaptive changes in plants (Chinnusamy and Zhu, 2009). Boyko et al. (2010) found that stress-induced transgenerational responses in *Arabidopsis* depended on altered DNA methylation and small RNA-silencing pathways. One of the factors contributing to these changes involves mobile genetic elements. Different stress factors may decrease the methylation level of these sequences (Kalinka et al. 2009), leading to their activation and transposition. Cold was found to downregulate *MET1*, resulting in demethylation of mobile genetic elements in *Zea mays* (Steward et al., 2002) and *Antirrhinum majus* (Hashida et al., 2006). Ito et al. (2011) showed that some retrotransposons become active in *Arabidopsis* seedlings subjected to stress. The siRNA pathway plays a crucial role in restricting retrotransposition triggered by environmental stress. As changes in methylation at mobile genetic element insertions affect nearby genes, mobility bursts may generate novel, stress-responsive regulatory gene networks. However, it is the miRNA pathway that seems more involved in stress adaptation responses.

Although a large number of siRNAs and miRNAs have been identified, only a few dozen small RNAs have been annotated with specific functions. The spectrum of miRNAs action especially is supposed to be extremely wide. Alterations in the level of miRNAs during stress change the gene expression profiles influencing plant growth and development. Most miRNAs are involved in overlapping regulatory networks (Khraiwesh et al., 2012). For example, miR172 seems to play double roles: mRNA cleavage and translation repression (Jones-Rhoades et al., 2006). Four targets of miR172 in *Arabidopsis thaliana* encode AP2 (APETALA) transcription factors (Aukerman and Sakai, 2003). Thus, elevated expression of miR172 represses translation of AP2 targets and results in early flowering and defects in floral organ identity (Chen, 2004; Axtell and Bartel, 2005). Cold-responsive miRNA genes may be involved in many signaling pathways (Zhou et al., 2008). Some cold-inducible miRNA genes may affect auxin signaling pathways, and upregulation of these miRNAs through auxin pathways promotes lateral root development (Xie et al., 2000, 2002; Jones-Rhoades and Bartel, 2004). Another example is miR169, which may inhibit the expression of six *XTH* (xyloglucan endotransglucosylase/hydrolase) genes. Many members of the *XTH* family have been confirmed to function in cell elongation by loosening the cell wall (Rose et al., 2002; Shikata et al., 2004; Vissenberg et al., 2005).

CONCLUSION

Ambient temperature regulates multiple aspects of plant development. There are two sides to thermal acclimation. The first involves temporary and transient effects, while the second is of evolutionary significance. Changes of gene expression caused by stress depend on post-translation chemical modifications of histones and the level of DNA methylation, which evoke specific chromatin changes in the area of the key genes. The majority of the modifications induced by stress are reversible to the initial level once the stress factor disappears. However, there exists the "memory of the stress signal", which

remains in the form of chromatin modifications. Epigenetic mechanisms can stably alter transcriptional activities of genes, and those can be transmitted through mitosis and sometimes also meiosis. The presence of "stress memory" keeps plants prepared for upcoming stresses. One of the best-studied examples of plants' adaptation to low temperature is vernalization. Although vernalization responses differ between species, the basic mechanism is common. Regulation of the vernalization response is controlled by specific loci whose chromatin structure undergoes specific modification; downregulation or upregulation of these genes influence the expression of other genes. Up to now many genetic pathways and regulatory mechanisms have been elucidated, but further studies obviously are required for a full understanding of cold acclimation mechanisms in plants.

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