Review

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Research on floral timing by ambient temperature comes into blossom

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The floral transition is an essential process in the life cycle of flower-bearing plants, because their reproductive success depends on it. To determine the right moment of flowering, plants respond to many environmental signals, including day length, light quality, and temperature. Small changes in ambient temperature also affect the flowering process, although our knowledge of the genetic and molecular mechanisms underlying this flowering pathway is limited. However, recent advances in *Arabidopsis* (*Arabidopsis thaliana*) have uncovered multiple molecular mechanisms controlling ambient temperature regulation of flowering, which modulate both repressing and activating factors of flowering time. At a time when temperatures are rising worldwide, understanding how plants integrate ambient temperature signals can be crucial for crop production.

A short history of flowering time research

Plants are sessile organisms that cannot migrate to more optimal locations when the environmental conditions are not favorable. Therefore, they need strategies to adapt and cope with these conditions, enabling them to increase their chance of survival and, ultimately, reproduction. It has long been noted that plants integrate environmental cues in their developmental programs to achieve this adaptation. The moment of flowering, which is heavily influenced by environmental conditions, is a key step in the life cycle of flowering plants and successful production of progeny depends on this process. When flowering starts under unfavorable conditions (e.g., just before a period of frost), seed production cannot be guaranteed.

The first descriptions of molecular mechanisms that integrate environmental cues to control flowering time date back to the early 1990s [1]. Over the succeeding years, it has become clear that different environmental signals that influence flowering time, such as day length (photoperiod), light quality, and vernalization (see Glossary), are perceived through different molecular pathways in the plant. These pathways converge at the so-called 'floral integrators', a small set of genes where all flowering time pathways come together. *FLOWERING LOCUS T* (*FT*), *SUPPRESSOR OF OVEREXPRESSION OF CO 1* (*SOC1*) and *LEAFY* (*LFY*) are regarded to be floral integrator genes [2]. Besides knowledge on the floral integrators, a plethora of genes involved in different pathways has been revealed following the first report on molecular mechanisms controlling flowering time, including *FLOWERING LOCUS C* (*FLC*) in the vernalization pathway [3], and *CONSTANS* (*CO*) [4], phytochromes, and cryptochromes [5] in both the photoperiod and light-quality pathways. By contrast, the first reports on the influence of ambient temperature on flowering time appeared much later [6,7], despite the fact that small fluctuations in temperature have dramatic effects on flowering time. However, growing signs of a rapidly changing climate with temperatures rising worldwide puts a spotlight on the importance to better understand the processes whereby fluctuations in ambient temperature influence flowering time.

The first analyses of flowering-time responses of *Arabidopsis* mutants and ecotypes to different temperatures highlighted several candidate genes as potential key regulators of the ambient temperature pathway. Most *Arabidopsis* ecotypes flower earlier at elevated temperatures, whereas some mutants and ecotypes are less affected by a

Glossary

Alternative acceptor site: in alternative splicing, usage of an alternative 3′ splice junction.
Alternative splicing: the process at which particular exons or introns are excluded or included from the precursor of mRNA to form multiple mature mRNAs.
Chromatin immunoprecipitation (ChIP): a technique used to study binding of a protein of interest to DNA *in vivo*.
Co-immunoprecipitation: technique used to study the proteins bound to a protein of interest by precipitating the intact protein complex along with the known protein.
Cryptochromes: photoreceptors sensitive to blue light.
Electrophoretic mobility shift assay (EMSA): technique used to study binding of a protein of interest to a DNA probe of interest *in vitro*.
Epistatic: when expression of one gene depends on the presence of another, nonallelic, gene.
Floral integrator: gene that integrates signals from different flowering-time pathways that can initiate flowering when sufficiently activated.
Hypocotyl: the stem of a seedling below the cotyledons.
Juvenile phase: early phase of vegetative development at which the plant is not yet competent to flower.
Mutually exclusive exon: in alternative splicing, one of two exons is retained in a splicing event, but not both.
Orthologous genes: genes from different species that share a common ancestral gene.
Paralogous genes: genes derived from a duplication within a genome.
Petiole: the stalk of a leaf that attaches the leaf blade to the stem.
Phytochromes: photoreceptors sensitive to red and far-red light.
Proteasome: protein complex that degrades unneeded or damaged proteins.
RNA polymerase II (Pol II): an enzyme responsible for the synthesis of mRNA by transcription of DNA.
Vernalization: a prolonged period of cold, winter-like temperatures by which the plant acquires the competence to flower.

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temperature switch. Among these are mutants for the MADS-box genes *SHORT VEGETATIVE PHASE* (*SVP*) and an ecotype with a deletion of *FLOWERING LOCUS M* (*FLM*), which have lost their ability to respond to fluctuation in ambient temperatures [8,9]. Until recently, the exact underlying molecular mechanism was unknown. However, recent studies [10–15] show the presence of multiple mechanisms, such as alternative splicing and stability of encoding proteins, involved in ambient temperature-regulated floral timing for genes previously placed in different flowering pathways [16–19]. Here, we review newly identified genetic and molecular mechanisms involved in ambient temperature-regulated flowering time and explore the question of how these different mechanisms and pathways relate to each other.

Open sesame: does warmth evict H2A.Z to open the gates for transcriptional activation?

Is there a role for chromatin remodeling in the regulation of floral timing by ambient temperature? The DNA of eukaryotes is organized into chromatin, which comprises repeating units called nucleosomes. Each nucleosome presents a core comprising eight proteins (histones) with DNA wrapped around it. Similar to most eukaryotes, plant nucleosomes contain two copies of each of the canonical histones H2A, H2B, H3, and H4. The basic structural function of nucleosomes is that they tightly pack the DNA to fit into the nucleus. However, they also have an important function in the control of gene expression. This can be achieved through post-translational modifications of histone tails, such as methylation, acetylation, SUMOylation, and phosphorylation, and replacement of the canonical histones with several histone variants [20]. One of the important histone variants is H2A.Z, which can be incorporated into chromatin by histone replacement, whereby the canonical histone H2A is replaced by H2A.Z.

Plants with a mutation in *ACTIN-RELATED PROTEIN 6* (*ARP6*), which is part of the SWR1 complex that is responsible for H2A.Z deposition [21], show serrated leaves and a bushy phenotype, and occasionally have flowers with more than four petals [22]. Moreover, these mutant plants display early flowering and petiole and hypocotyl elongation, resembling phenotypes of warm-grown wild type plants [11]. The transcriptome of the *arp6* mutant grown at 12°C was compared with wild type plants transferred to a higher temperature and revealed a substantial overlap between mis-expressed genes in the mutant and the differentially expressed genes from wild type plants shifted to higher temperatures. This indicated that the *arp6* mutant, which is impaired in H2A.Z incorporation, was also affected in terms of its temperature response. To investigate the putative role of H2A.Z in temperature responses, chromatin immunoprecipitation (ChIP) analysis was used to study H2A.Z occupancy at the transcriptional start site (TSS) of several loci. When plants were shifted to a higher temperature, all studied loci showed a significant decrease in H2A.Z occupancy, independent of their transcriptional response. The nucleosomes positioned directly upstream (−1) and downstream (+1) of the TSS in the locus encoding the temperature-responsive protein HEAT SHOCK PROTEIN 70 (HSP70) were used to investigate the nucleosomal dynamics in more detail. At the +1 nucleosome, which contains H2A.Z at relatively low temperatures, shifting to a higher temperature decreased H2A.Z occupancy and to a lesser extent occupancy of the control histone H3. At the −1 nucleosome, which is devoid of H2A.Z, the same temperature shift did not reduce histone H3 occupancy, indicating that nucleosome occupancy in general is not affected by a temperature shift. This led the authors to suggest that the presence of H2A.Z in the nucleosome contributes to the dynamic responses to temperature. However, whether the authors used the appropriate position to study H2A.Z dynamics is under debate, because more recent research has shown that it is H2A.Z enrichment in the gene body rather than at the TSS that correlates with gene responsiveness [23]. Nevertheless, *arp6* mutants showed a constitutive open conformation of the +1 nucleosome of *HSP70*, and phenocopy warm-grown plants, which is in line with the hypothesis that H2A.Z is involved in temperature responses. Regarding the biological function of the suggested H2A.Z mechanism, it is important to realize that *arp6* mutants are still temperature responsive, including earlier flowering upon higher temperature. This suggests that, in a wild type situation, H2A.Z depletion functions as an enabler, rather than an activator of the higher temperature response. In such a scenario, transcription factors can be differentially regulated upon shift to a higher temperature and can only access their targets when H2A.Z depletion has occurred. With respect to flowering control, H2A.Z functions as an additional control post and a gatekeeper that circumvents transcription of flowering genes at lower temperatures, despite the presence of inductive signals (Figure 1A).

A role for chromatin remodeling and H2A.Z is not an unknown phenomenon in flowering time regulation, because it is well established that the floral repressor *FLC* is epigenetically silenced upon prolonged cold, winter-like temperatures (vernalization) [24,25]. When expressed, FLC interacts with SVP, and these two proteins may function together in repressing *FT* and *SOC1* [17] (Figure 2). Under nonvernalizing conditions, the FRIGIDA (FRI) protein recruits chromatin modifiers at the TSS of the *FLC* locus, among which is the RNA polymerase II (Pol II)-associated factor 1 complex (PAF1c). This leads to modification of nucleosomes and dynamic exchange of H2A by H2A.Z, which facilitates transcription of *FLC* by Pol II. At least part of this system seems to be conserved for paralogs of *FLC*, named *FLOWERING LOCUS M* (*FLM*) and *MADS AFFECTING FLOWERING* (*MAF*)*2-5*, because mutants deficient in components of PAF1c showed a modest (in case of *MAF2*) to marked silencing of *FLM* and *MAF3-5*, just like *FLC* [26]. Moreover, H2A.Z enrichment has been reported at the *MAF4* and *MAF5* loci [21]. As discussed below (see 'Alternative splicing and protein stability of repressive MADS-domain transcription factors'), FLM, and to a lesser extend MAF2–MAF4, have an important function in ambient temperature-regulated floral timing. It remains unclear whether putative transcriptional regulation of *FLM* and the *MAF*s at the chromatin level in an *FLC*-like manner is of biological relevance, because the *MAF*s, and especially *FLM*, seem to be regulated mainly by alternative splicing rather than by alternative expression.