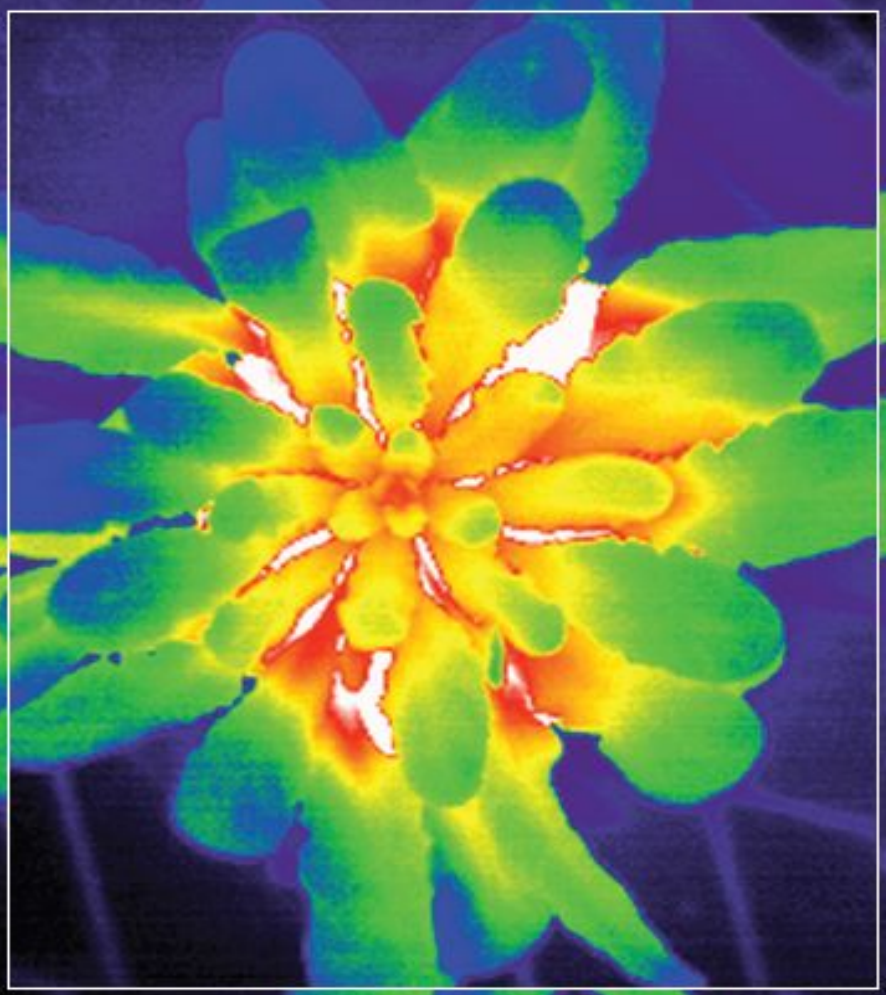
Temperature and Plant Development

EDITED BY

Keara A. Franklin and Philip A. Wigge



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Preface

Temperature is a key environmental signal regulating plant growth and development. Small changes in ambient temperature can affect a wide range of processes throughout the plant lifecycle, from seed germination and plant architecture through to flowering and reproductive development. Prolonged low-temperature treatment can act as a seasonal cue, signaling the onset of winter to prime flowering and seedling development the following spring. In addition to providing important environmental information, exposure to temperature extremes can adversely affect plant survival. The evolution of developmental adaptations to withstand prolonged cold or heat has enabled some species to exploit ecological niches in adverse habitats. In more temperate regions, many plants have evolved acclimation responses to minimize cellular damage associated with freezing and heat stress.

Plants can detect temperature changes as small as 1°C. Despite the importance of temperature in controlling plant growth and survival, our current understanding of how temperature signals are perceived is rudimentary. Suggested thermosensory mechanisms include changes in membrane fluidity, activation of membrane transport channels, altered protein activity, and the direct regulation of gene expression through altered DNA accessibility. Molecular dissection of plant temperature responses has revealed significant crosstalk with light and circadian signaling pathways. The integration of temperature and photoperiod signals provides plants with accurate seasonal information, priming adaptive responses to adverse conditions while preventing the wasteful allocation of resources in milder climates. In natural environments, plants

are subject to multiple environmental signals simultaneously, resulting in trade-offs between different stress responses. It is perhaps, therefore, not surprising that temperature has significant effects on plant immunity and defense signaling.

Climate change presents major challenges for global agriculture and the preservation of ecosystems and biodiversity. Current climate models predict future increases in global temperature, with potentially devastating effects on crop production. Relatively moderate increases in ambient temperature (<6°C) can invoke dramatic changes in plant development, reducing harvest yield. Increased temperature additionally accelerates flowering, promoting floral development out of synchronization with pollinator lifecycles. Indirect impacts of elevated temperature on plant development include increased pathogen susceptibility and enhanced water use. The latter may promote leaf cooling in well-watered environments but would have severe consequences in drought conditions. Conversely, exposure to sudden frost can lead to catastrophic crop losses in nonacclimated species. Understanding how plants perceive, integrate, and respond to temperature signals may provide novel molecular targets for the production of crops resilient to climate change and inform predictions as to the impact of global warming on plant ecology and biodiversity. Enhanced knowledge of plant temperature responses could additionally lead to more energy-efficient horticultural production. The stature and flowering time of glasshouse crops are commonly controlled through manipulations of light and temperature. Understanding how plants perceive and respond to small temperature changes at different times of day in diverse light environments will greatly facilitate the design of optimal growth management regimes.

This volume is designed to provide a comprehensive and up-to-date account of the role of temperature in plant development. It is aimed at all students and teachers of modern plant biology, academics with an interest in the environmental regulation of development, and policy makers working in the area of climate change, ecology, and global food security.

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1

Temperature sensing in plants

Steven Penfield and Dana MacGregor

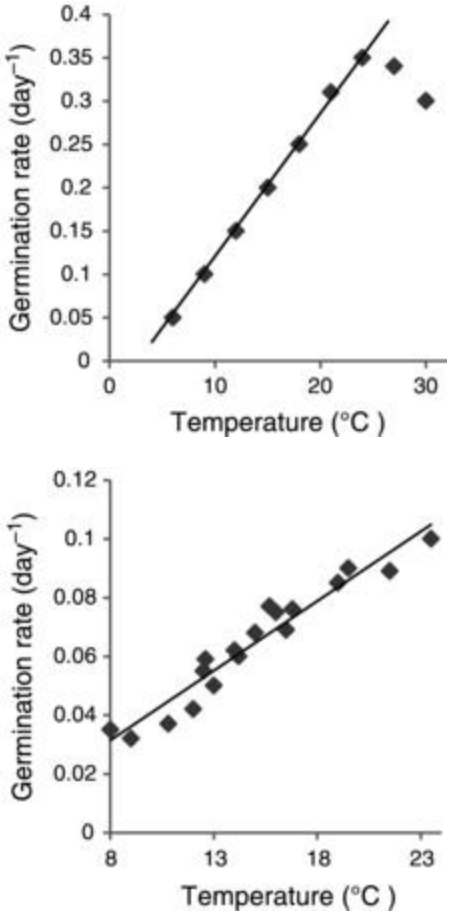
1.1 Introduction

Plants are subjected to considerable variations in temperature, both daily and annually, and are surprisingly temperature-sensitive organisms: it has been shown that levels of cytosolic calcium in plant cells can respond to as little as a 1°C temperature shift, and a 4°C diurnal temperature cycle is sufficient to entrain the circadian clock (Knight and Knight 2000; McClung et al. 2002). Developmental processes such as seed germination can be completely inhibited by 1–2°C temperature rises (Argyris et al. 2011). Despite the experimentally described responsiveness of plant physiology and development to temperature, no thermosensory molecule has yet been unequivocally identified.

1.2 Passive and active temperature responses in plants

Since the nineteenth century, it has been suggested that temperature affects the rates of biological reactions according to the thermodynamic principles that govern chemical reactions more generally. In this scenario, the rate of reactions whose activation energy is significantly greater than a given temperature will increase proportionally to the exponent of the temperature rise. As a guiding principle, the free energy change normally dictates that for biological reactions in biologically-relevant temperature ranges, rates will increase roughly twofold to threefold with a 10°C rise in temperature. This figure, known as the temperature coefficient or Q_{10}, became popular during the twentieth century following its popularization after the work of Van't Hoft (1896) and Arrhenius (1889). Thus, specific biological processes, from the growth of bacteria to the respiration of plants, were shown to have a Q_{10} of approximately 2–3 within a range often described as 'room temperature' (Běhrádek 1930). This law has even been applied to developmental biology and plant growth, a good example of which is the study of seed germination by Hegarty (1973). Here it was shown that the speed of germination of many common vegetable seeds showed a dependency on temperature consistent with that expected by the passive effects of temperature in biochemical reactions (Figure 1.1). In a complex biological event such as seed germination, requiring respiration, cell division, and cell elongation, it was postulated that temperature affects the rate of a single, vital rate-limiting reaction in the process. Where Q_{10}s were found to differ dramatically from 2 to 3, it was suggested that this might be due to a complex effect of temperature on many reactions with unpredictable consequences (Běhrádek 1930).

Figure 1.1 Speed of germination in seeds often shows a relationship indicative of a passive temperature response. In the lab (left) and in the field (right), carrot seeds germinate at a rate with a linear relationship with temperature, with a *Q*10 of around 2. Redrawn from Hegarty (1973).



Many types of biomolecule are expected to be subject to these types of 'passive' temperature effects including the fluidity of lipid bilayer membranes, the conformations of proteins, and the behavior of nucleic acids. Clearly, organisms need to be able to control their physiology to maintain performance of vital functions over a range of possible biochemical reaction rates. However, among this sea of events that must continue to function equivalently at multiple temperatures, one or more are used as temperature sensors by biological organisms. Here we will advance a broad definition of a temperature sensor as a

passive temperature-controlled change in configuration of a molecule or assembly of molecules that is integrated with downstream signal transduction in order to create an active signal regulating a process of adaptive significance. Such signaling pathways are referred to as ‘active’ temperature responses because during signal transduction, the effects can be amplified or buffered such that the temperature coefficient may differ significantly from 2 to 3. Plants use temperature information to allow them to synchronize their life -history with the seasons or to adapt their physiology to different temperature environments.

1.3 Temperature sensing during transcriptional regulation

Steady-state (SS) transcript levels of many plant genes are highly sensitive to temperature, and there are several documented cases where these changes are essential for known adaptive responses. In seeds, a 10°C temperature variation causes changes in approximately 10-fold more expressed genes than in seedlings (Kendall et al. 2011), showing that different plant tissues have different innate sensitivities of transcription rates to temperature. In prokaryotes, thermodynamic effects of temperature affect gene expression through the control of chromosomal supercoiling and by conformational changes in topoisomerases that control the supercoiling process. In thermophilic bacteria, the enzyme reverse gyrase acts to induce tight supercoiling even at extreme temperatures (>80°C), and the action of this enzyme appears to keep the DNA context in a configuration that permits a reasonable speed of transcription (Forterre et al. 1996). Therefore, key

to understanding the temperature-control of transcription in eukaryotes will be an analysis of how DNA interacts with its environment to control transcription rate and how this varies over temperature.

A well-studied example of the importance of temperature signaling in development is vernalization, the requirement for a prolonged cold period before plants are able to respond to floral-inductive signals. In *Arabidopsis*, the vernalization pathway acts through downregulation of the floral repressor *FLOWERING LOCUS C* (*FLC*) (reviewed in Amasino, 2010, and discussed further in Chapter 4, Sheldon et al. 1999; Michaels and Amasino 1999). In vernalization-requiring accessions, stable repression of *FLC* is achieved after several weeks of cold exposure. Here we will focus on the primary steps in responding to temperature at the *FLC* locus, of which there are two key points. The first of these is that after 2 weeks of cold, the accumulation of the repressive mark trimethylated histone 3 lysine residue 7 (H3K27) begins at the *FLC* locus. This begins to appear around the transcription start site and its presence correlates with the downregulation of *FLC* expression (Bastow et al. 2004). A second repressive mark, dimethylation of H3K9, also appears upstream of the transcription start site, and both marks are necessary for the maintenance of *FLC* repression upon transfer to cold. H3K27me3 modifications require the activity of the Polycomb Repression Complex 2 (PRC2), and mutations of several subunits of these have been identified in forward genetic screens for *Arabidopsis* mutants unable to induce or maintain the vernalized state. These mutants share a common phenotype in that the repression of *FLC* is initiated in the cold, but is not maintained upon transfer to the warm (Gendall et al. 2001; Sung and Amasino 2004; Greb et al. 2007). One of these, *VERNALIZATION INSENSITIVE3* (*VIN3*), is itself upregulated at the transcript level by prolonged low-

temperature exposure, suggesting that the temperatureregulation of *VIN3* expression is a primary event during vernalization. However, although VIN3 is necessary for the stable repression of *FLC* after vernalization, expression of *VIN3* alone cannot confer a vernalization-like response on unvernalized plants (Sung and Amasino 2004), showing that control of VIN3 and by extension PRC2 complex abundance is unlikely to be the primary temperature signal for vernalization. How temperature controls *VIN3* expression is not known.

Recently it has been suggested that the observed quantitative repression of *FLC* by increasing durations of cold reflects a bistable switch in *FLC* repression occurring in ever greater numbers of cells during vernalization (Angel et al. 2011). In support of this, non-saturating vernalization exposures result in a cell autonomous response in which some cells are silenced for *FLC:GUS* expression, while many cells continue to express at high levels. After 2 weeks of cold, H3K27me3 levels begin to increase around the transcription start site of *FLC* full-length transcript, suggesting this is a primary response to cold in the vernalization pathway (Angel et al. 2011). The dynamics of this increase correlate well with the timing of the increase in *VIN3* expression, suggesting that the VIN3 protein might play a role in the targeting of the PRC2 complex to the H3K27me3 nucleation site. However, the lack of any predicted or known sequence specificity of VIN3 for any DNA sequence and the inability of *VIN3* overexpression to induce a vernalization response suggest that H3K27me3 modification in response to VIN3 cannot alone explain the temperature responsiveness of *FLC* transcript SS levels.

The *FLC* locus also produces at least two noncoding RNAs that appear to have a role in the vernalization process. The first, designated *COOLAIR*, is a long antisense transcript that covers the entire *FLC* locus and has a promoter that can

independently confer cold responsiveness to a reporter gene independently of gene context (Swiezewski et al. 2009). This latter observation appears to tie temperature responsiveness to transcriptional control, rather than RNA stability. Importantly, *COOLAIR* expression occurs in *vin3* mutants and was also shown to confer downregulation of the sense *FLC* transcript, suggesting that coldresponsiveness is VIN3 independent. However, *COOLAIR* is unlikely to be solely responsible for the downregulation of sense *FLC* expression in *Arabidopsis*, since T-DNA insertion mutants lacking the *COOLAIR* transcript but expressing a functional FLC protein continue to show a robust vernalization response (Helliwell et al. 2011). More recently, a second noncoding but this time sense transcript-designated *COLDAIR* has been identified with a role in the vernalization response (Heo and Sung 2011). The *COLDAIR* transcript is transcribed from the first intron, a region of *FLC* long known to have a role in the control of vernalization, and has as part of its promoter an approximately 300 bp sequence known as the vernalization response element (VRE; Sung et al. 2006). *COLDAIR* knockdown lines show reduced repression of *FLC* by vernalization. It is therefore suggested that the *COLDAIR* transcript controls H3K27me3 nucleation at the *FLC* coding transcription start site (Heo and Sung 2011). All this activity occurs long before *VIN3* expression increases, suggesting that activity surrounding the transcriptional promotion of *COLDAIR* is a key primary step in responding to temperature during vernalization. A key question for future vernalization response remains understanding the mechanism of how temperature regulates *FLC* expression.

In wheat, a key player in the vernalization response is the MADS-box transcription factor VERNALIZATION1 (VRN1, see Trevaskis et al. 2007). Instead of cold conferring transcriptional repression, expression of VRN1 is activated

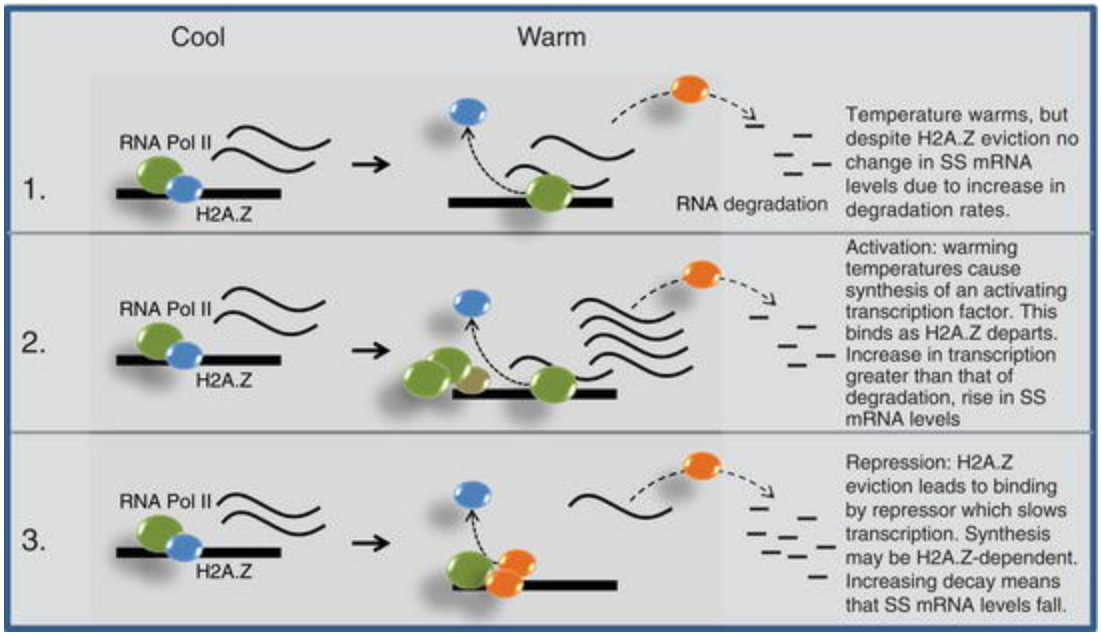
during vernalization to promote flowering. Repression of *VRN1* expression also requires the first intron (Fu et al. 2005; Cockram et al. 2007), but cold activation appears to be primarily driven by elements in the *VRN1* promoter. So, in wheat, the first intron is again required for repression, but not for cold activation. The similarities and differences between *Arabidopsis FLC* and wheat *VRN1* regulation are striking and highlight how much of our understanding of these processes reflects the transcriptional regulatory processes downstream of the temperature-sensing pathways and much less is known of how temperature signals are sensed. In addition, it is still completely unknown how vernalization integrates temperature signaling with time in order to measure the duration of the cold signal during winter.

The complex kinetics of the control of *FLC* expression has led to the search for alternative models for studying the control of transcription by temperature. Several plant genes seem to have quantitative responses of SS mRNA levels to environmental temperature over a wide temperature range. Good examples of these in *Arabidopsis* are *HEAT SHOCK PROTEIN 70* (*HSP70*) and *COLD-REGULATED 15a* (*COR15a*), which increase transcript abundance in response to increasing and decreasing temperatures, respectively (Penfield 2008; Kumar and Wigge 2010). A genetic screen for *HSP70* mis-regulation has been used to identify genes necessary for correct temperature responses in the ambient temperature range (between about 12°C and 27°C) (Kumar and Wigge 2010). The first mutants characterized were novel alleles of *ACTIN-RELATED PROTEIN6* (*ARP6*), encoding a component of the plant SWR1 complex required for the deposition of the histone 2A variant H2A.Z into chromatin (Mizuguchi et al. 2004). ARP6 is necessary for coordinating the temperature transcriptome, since the warm-temperature transcriptome is constitutively expressed at

lower temperatures in *arp6* alleles, lacking H2A.Z incorporation. Consistent with this observation, H2A.Z nucleosomes are evicted from chromatin at higher temperatures, enabling RNA Pol II to transcript genes such as *HSP70* that are induced at higher temperatures (Kumar and Wigge 2010).

An interesting feature of H2A.Z biology is that the effect of H2A.Z nucleosomes on transcription appears to be locus specific, since, for example, in the case of *FLC*, H2A.Z deposition is correlated with transcription of the gene (Deal et al. 2005), while in the case of *HSP70*, and genes involved in the phosphate starvation response, H2A.Z loss results in upregulation of expression (Smith et al. 2010). When H2A.Z occupancy is analyzed by chromatin immunoprecipitation (ChIP), higher temperatures result in a decrease in occupancy (Kumar and Wigge 2010), and this effect appears to be independent of the transcriptional status of the gene, suggesting that eviction of H2A.Z occurs in response to warmer temperature, independent of effects on transcription (Franklin 2010; Kumar and Wigge 2010). For SS mRNA levels to rise with increasing temperature, transcription rates must increase at a rate exceeding that of mRNA degradation rates. If it is assumed that mRNA degradation rates follow a simple Arrhenius–Van't Hoft relationship with temperature, additional mechanisms in addition to changes in nucleosome occupancy may be required to cause a gain in SS transcript levels. An increase in SS levels may require a positive feedback loop, either induction of an activator of transcription (Figure 1.2) or a factor which stabilizes mRNA. Larger effects of increasing temperature on decay rates may act to lower SS RNA levels even in the presence of increasing transcription. It seems likely that H2A.Z nucleosome eviction is likely tied to other processes in order to drive temperature-regulated changes in SS mRNA levels.

Figure 1.2 Control of SS transcript abundance by the temperature-dependent association of H2A.Z-containing nucleosomes with transcription start sites. (1) A passive affect alone does not produce an increase in SS levels, because mRNA degradation rates too are affected. (2) The eviction of H2A.Z allows an activator to bind. The abundance of this may also be temperaturecontrolled or a positive feedback through autoactivation. (3) Eviction allows a repressor to bind, allowing nucleosome eviction to depress transcription rates. After Kumar and Wigge (2010) and Franklin (2010). RNA Pol II– RNA polymerase II. For color detail, please see color plate section.



While H2A.Z nucleosomes are required for the normal behavior of the ambient temperature transcriptome, it has not been demonstrated directly that they are themselves thermosensors. To fulfil our definition of a temperature sensor, we must also show that our sensor is linked by a signal transduction pathway to processes of adaptive significance. Have plants exploited the temperature responsiveness of the association of DNA and H2A.Z nucleosomes to confer thermosensitivity to important developmental or physiological processes? As we are

primarily concerned with temperature regulation of transcription, the imperative is to discover temperature- and *arp6*-dependent changes in gene expression that underlie plant adaptation to variable environments. *arp6* mutants do indeed show phenotypes that suggest they are compromised in their ability to regulate important temperature-controlled plant processes, such as the timing of flowering and hypocotyl elongation (Deal et al. 2005; Kumar and Wigge 2010). In *arp6*, flowering is earlier than wild type in long days and short days. If H2A.Z nucleosomes are rate limiting for the expression of key flowering regulators, such as *FT*, then higher temperatures could cause flowering by triggering H2A.Z eviction. Supporting this hypothesis, the transcription factor PIF4 directly activates *FT* in a temperature-dependent manner, and the ability of PIF4 to bind to the *FT* promoter is temperature dependent (Kumar et al. 2012). However, in short days shifting *arp6* plants from 22°C to 27°C results in a large decrease in flowering time, demonstrating that a substantial portion of the thermosensory response is ARP6 independent. This suggests that other components alongside SWR1 and H2A.Z have an important role in thermosensation controlling flowering time. Taken together, these results suggest that chromatin accessibility appears to be temperature regulated, but whether this is a direct response to temperature or controlled by a temperature-regulated chromatin modifying pathway remains to be seen.

Warm temperatures increase *Arabidopsis* hypocotyl elongation in an auxin-dependent manner (Gray et al. 1998). This process requires the activity of the PIF4 transcription factor (Koini et al. 2009), and the binding of PIF4 to auxin biosynthesis genes has been shown to be temperature regulated, supporting a role for temperature-mediated changes in chromatin accessibility in controlling this process (Franklin et al. 2011). Consistent with this, *arp6*

have elongated hypocotyls (Kumar and Wigge 2010). In a close parallel to the role of ARP6 in the control of flowering time, *arp6* hypocotyls do elongate in response to temperature but show a reduced response. This appears to reinforce the idea that this pathway must function redundantly with others to control ambient temperature responses in plants.

1.4 Sensing cold: A role for plasma membrane calcium channels in plants

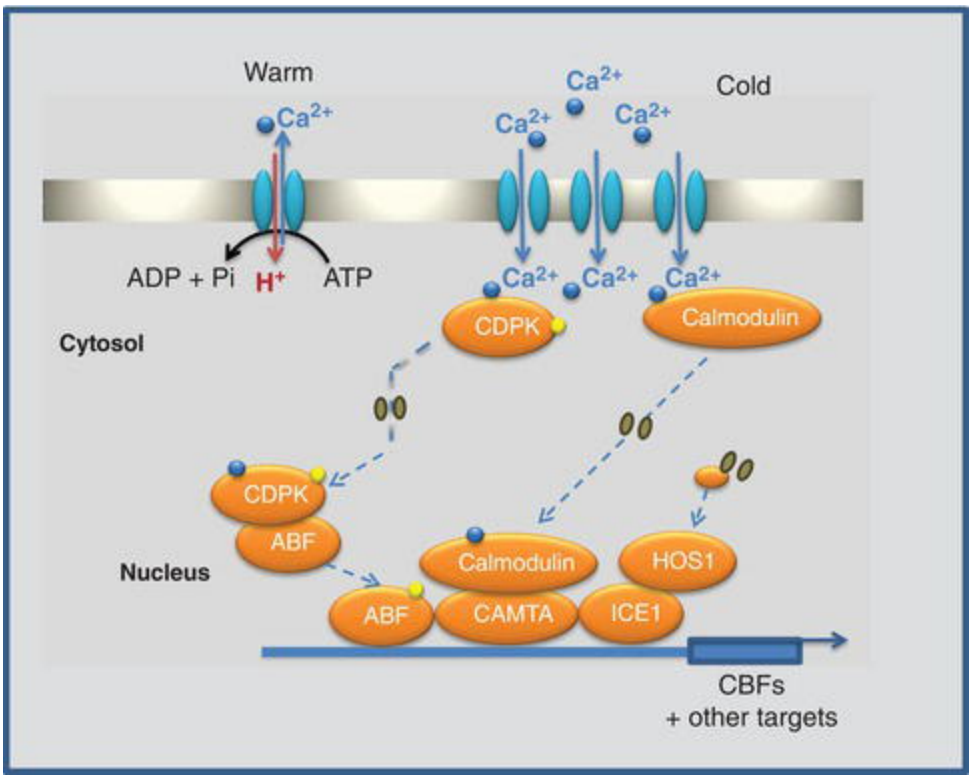
In animals, it is now well established that voltage-dependent action transporters in the TRP family of potassium channels are necessary for sensing temperature (Peier et al. 2002). Plants do not contain conserved relatives of these proteins, suggesting that the mechanism for temperature sensing is not shared. However, electrophysiological evidence suggests that plant cell membranes depolarize with a decrease in temperature and that this depolarization is accompanied by an influx of calcium in to the cytosol (Minorsky 1989; Knight et al. 1991, 1996). The degree of depolarization and increase in cytosolic calcium is dependent not only on the degree of cooling but also on the rate (Plieth et al. 1999). This type of response has parallels with the downstream transcriptional activation of the C-repeat binding factor (CBF) family, involved in plant cold acclimation (discussed in Chapter 2). For instance, when plants are shifted from a growth temperature of 20–10°C, the corresponding increase in *CBF* transcript levels is less than that resulting from the larger change in temperature from 20°C to 4°C (Zarka et al. 2003). For *Arabidopsis* grown at 20°C, a shift to 14°C or lower is sufficient to induce *CBF*

transcripts to detectable levels, and plants acclimated to growth at 4°C will induce a *CBF* response when shifted below 0°C (Zarka et al. 2003). Interestingly, daily calcium oscillations also modulate the responsiveness of cytosolic free calcium concentrations ($[Ca^{2+}]^{cyt}$) to cold, providing a potential mechanism for circadian gating (Dodd et al. 2006). Calcium influx after cold appears to cause the depolarization rather than be a response to it, because calcium channel blockers such as lanthanum also block cold-induced membrane depolarization (Lewis and Spalding 1998). This shows that extracellular calcium influx is a primary plant response to cold and cooling. Despite many efforts to identify the types of channel responding to the cold stimulus, none have been found, possibly indicating a high degree of genetic redundancy. Given that these responses occur within a minute or so of a cold pulse, it is likely that these are early signaling events in the detection of a cold stimulus. Ion influx is an attractive system for generating an active temperature signal since work has previously been done to establish the resting membrane potential, enabling a large response to be achieved by simply opening the channel, exploiting the resting potential to amplify the temperature signal. The next question to answer is whether these cold-induced oscillations in $[Ca^{2+}]^{cyt}$ led to a downstream signal transduction cascade.

Efforts to determine transcriptional responses to elevated $[Ca^{2+}]^{cyt}$ have helped to identify an ongoing cold signal transduction influencing gene expression (summarized in Figure 1.3). Experiments which have involved the pharmacological manipulation of $[Ca^{2+}]^{cyt}$ showed that elevations in $[Ca^{2+}]^{cyt}$ led to the activation of transcription of genes with ABA RESPONSE ELEMENTS (ABREs) in their promoters (Galon et al. 2010). If a LUCIFERASE reporter gene driven by ABREs is transformed into tobacco,

luciferase activity displays $[Ca^{2+}]^{cyt}$ sensitivity. This analysis was extended by Whalley et al. (2011) who developed an elegant system in which $[Ca^{2+}]^{cyt}$ oscillations were induced by applying voltages to seedlings floating in cuvettes. Here it was found that genes containing several types of cis-elements in their promoters were induced by $[Ca^{2+}]^{cyt}$, including ABREs, CAMTA-binding elements, C-repeats, and TCP-binding sites. Many of the induced genes have previously been shown to be cold responsive (Zarka et al. 2003). Together this work shows convincingly that changes in $[Ca^{2+}]^{cyt}$ can be transduced as a signal to promoters and affect gene expression. Carpaneto et al. (2007) showed that mutants deficient in cold signaling components were not deficient in the control of $[Ca^{2+}]^{cyt}$ oscillations, suggesting that $[Ca^{2+}]^{cyt}$ oscillations are truly upstream of all known signal transduction. This leaves us with the problem of elucidating the elements of the signal transduction pathway between cold-induced calcium oscillations and gene expression.

Figure 1.3 Schematic of our current understanding of cold perception and signaling in plants, showing possible signal transduction pathways from cytosolic free calcium increases to transcriptional control. For abbreviations see main text. For color detail, please see color plate section.



Calcium-dependent protein kinases (CDPKs) have been shown to directly bind and phosphorylate the ABRE-BINDING FACTOR (ABF) subfamily of bZIP transcription factors. Pharmacological inhibition of CDPKs impairs cold responsiveness and freezing plant tolerance (Tähtiharju et al. 1997). Several CDPKs have been shown to bind ABF1–ABF4, modulating their activity and having downstream consequences for abscisic acid (ABA) signaling (Zhu et al. 2007; Zhao et al. 2011). ABFs appear to be integrating points for multiple signalling pathways, including those for drought, salt, ABA, and cold sugar responses, perhaps explaining why there is substantial overlap between the transcriptional responses to these signals. Multiple abiotic signals phosphorylate ABFs so this is likely to be an integration point of multiple stress signals. However, to date, no ABF has been shown to be phosphorylated in response to cold, so it remains open whether this is the signal transduction pathway through which cold signals are propagated.

A second possibility is a central role for calmodulin and calmodulin- binding proteins. Inhibition of calmodulin signaling impairs freezing tolerance in *Arabidopsis* (Tähtiharju et al. 1997), whereas calmodulin overexpression induces high levels of *COR* gene expression (Townley and Knight 2002). A dissection of the *CBF2* promoter revealed a conserved binding element of the calmodulin-binding CAMTA family of transcription factors which was able to confer cold responsiveness to a reporter gene (Doherty et al. 2009). Of the six CAMTA transcription factors in *Arabidopsis*, CAMTA3 could be shown to bind the conserved *CBF2* promoter element, whereas deletion of either *CAMTA1* or *CAMTA3* both impaired the acquisition of freezing tolerance. This work clearly demonstrated a role for CAMTAs in cold signal transduction. An interesting feature of this study is that the authors report that loss of CAMTAs also impairs the ability of the *CBF2* promoter to respond to calcium signals that result from mechanical stimulation (Knight et al. 1991). This suggests that the calmodulin signal transduction pathway is not specific for cold but can potentially carry signals from multiple stresses to downstream target promoters (Doherty et al. 2009), some of which also stimulate increases in $[Ca^{2+}]^{cyt}$. Thus, it is again possible that cold signaling uses a pathway that is shared with other processes and may not itself exist as a separable entity.

A final class of calcium signal transduction proteins worthy of consideration as transducers of a cold-induced calcium influx are the calcineurin B-like proteins (CBLs; Luan et al. 2002). CBLs are small calcium-binding proteins which function through the binding of a large family of serine/threonine protein kinases known as CBL-INTERACTING PROTEIN KINASES (CIPKs). This pathway also appears to have overlapping functions in the response to multiple stresses and in ABA signal transduction. At least