1 Temperature sensing in plants

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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 Hoff (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).

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 temperature-regulation 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 cold responsiveness 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 Hoff 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

![Diagram](image.png)

**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 temperature controlled 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.
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.

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 fulfill 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 trans- 
porters in the TRP family of potassium channels are necessary for sens-
ing 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 depol -
arization 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 acclima-
tion (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 tem-
perature 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 mech-
anism 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-
duced 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 \([\text{Ca}^{2+}]_{\text{cyt}}\) 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 \([\text{Ca}^{2+}]_{\text{cyt}}\) sensitivity. This analysis was extended by Whalley et al. (2011) who developed an elegant system in which \([\text{Ca}^{2+}]_{\text{cyt}}\) 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 \([\text{Ca}^{2+}]_{\text{cyt}}\), 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 \([\text{Ca}^{2+}]_{\text{cyt}}\) 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 \([\text{Ca}^{2+}]_{\text{cyt}}\) oscillations, suggesting that \([\text{Ca}^{2+}]_{\text{cyt}}\) 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.
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 over-expression 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 $[\text{Ca}^{2+}]_{\text{cyt}}$. 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 one member, CIPK3, is induced transcriptionally by cold and is required for
cold-responsive gene expression (Kim et al. 2003). CBLs and CIPKs may therefore be part of a positive feedback pathway that amplifies cold signals in plants.

1.5 A role for membrane fluidity as an upstream temperature sensor?

The control of membrane fluidity is evident across organisms from mammals to microorganisms. In plants, manipulation of fatty acid content can impair ability to survive temperature extremes, including chilling and heat stress (Miquel et al. 1993; Kodama et al. 1995; Murakami et al. 2000). In keeping with the hypothesis that homeostasis of membrane fluidity is required for normal cellular functions and stress resistance, depleting unsaturated fatty acids, for instance, through \textit{fad2} mutation, exposes plants to chilling injury, whereas overexpression of \textit{FAD7} improves chilling tolerance (Miquel et al. 1993; Kodama et al. 1995). Homeostasis of membrane fluidity is achieved by the regulation of the activity of fatty acid desaturases, at either the protein or the transcriptional level (Gibson et al. 1994; Matsuda et al. 2005). Interest in the possibility that membrane fluidity could act as a temperature sensor in plants comes primarily from the study of prokaryotic systems, in which a signal transduction cascade is initiated by changes in fluidity that result in small changes in membrane thickness (see Penfield et al. 2012). In this system, a protein with a dual role as phosphatase and kinase switches from a phosphatase to a kinase as membrane thickness increases with decreasing temperature (Cybulski et al. 2010). This in turn activates a phosphorelay that increases transcription of a fatty acid desaturase, whose products act to restore membrane fluidity to its original level.

Homologues of these proteins are not conserved in plants, and the plant fluidity sensor remains to be discovered. However, in homeostasis lays the major problem with this system as a general temperature sensor: if fluidity is constant across multiple temperatures, how can fluidity act as an absolute temperature sensor? Conclusive evidence that changes in membrane fluidity regulate processes other than membrane composition are currently lacking in any experimental system, from prokaryotes to eukaryotes. In plants, the main evidence comes from the study of the application of chemicals which artificially alter membrane fluidity such as dimethylsulfoxide (DMSO, a rigidifier) and benzyl alcohol, a liquefier. Application of these compounds leads to alteration in temperature-controlled transcription (Orvar et al. 2000; Sangwan et al. 2001). However, genetic manipulation of membrane fluidity does not result in similar changes, leading to speculation that the effects of DMSO and BA were not mediated by their effect on fluidity. Measuring fluidity is not straightforward, but recently an elegant assay using photoquenching of membrane-bound GFP allowed direct
measurements of fluidity in plants for the first time (Martiniere et al. 2011). This study showed that membrane fluidity is not perfectly homeostatic in plants, but subject to diurnal variation. This variation is only incompletely counterbalanced by daily changes in composition, probably due to the slow turnover rates of membrane lipids. This work too could not find a convincing link between fluidity changes and the control of adaptive temperature responsiveness, suggesting that the major role of temperature sensing by membrane fluidity changes may be to control the downstream activity of fatty acid desaturases and maintain membrane fluidity homeostasis.

1.6 Temperature sensing by proteins

So far we have considered the roles of cation transporters and direct transcriptional responses to temperature and how these have been co-opted for the regulation of adaptive processes in plants. There is also ample evidence that transcription factors are themselves subject to a post-translational regulation by temperature, although it is not always clear where the temperature signal is perceived. A few examples are given as follows.

One of the best-characterized pathways that directly links a change in temperature to a protein level response is the control of INDUCER OF COLD EXPRESSION 1 (ICE1) protein levels, an MYC-type basic helix-loop-helix transcription factor, by HIGH EXPRESSION OF OSMOTICALLY RESPONSIVE GENES 1 (HOS1). In this process, the level of CBF transcript is determined by the concentration of the transcriptional activator ICE1, whose levels are reduced by cold. HOS1 encodes an E3 ubiquitin ligase that is involved in targeting ICE1 for degradation by the proteasome (Ishitani et al. 1998; Lee et al. 2001; Dong et al. 2006). When the plant is experiencing a warm temperature, GFP-tagged HOS1 is located in the cytoplasm and its transcript levels are constant (Lee et al. 2001). Within 10 min of a shift to cold, HOS1 transcript levels drop dramatically, allowing for a brief alleviation of ICE1 ubiquitination, and because polyubiquitination targets proteins for degradation by the proteasome, the levels of ICE1 then increase (Dong et al. 2006). The mechanism that results in the reduction in HOS1 transcript level in response to a drop in temperature is unidentified, but it is short lived. After being in the cold for 1 h, HOS1 transcript levels return to pre-cold levels, and GFP-tagged HOS1 is found preferentially in the nucleus. With HOS1 levels rising in the nucleus, it is able to interact with and ubiquitinicate ICE1, targeting it to the proteasome for degradation so that CBF3 and CBF-targeted transcripts return to low levels (Dong et al. 2006). Thus, the temperature-controlled accumulation of HOS1 in either the nucleus or cytoplasm is proposed to be critical in the way in which the ICE1 protein transiently activates CBF transcription after cold shock.
A second related process where temperature-regulated transcription factors control adaptive processes in plants is the temperature control of growth. Low temperatures inhibit plant growth, not just through a passive effect on reaction rates, but through an active process of the regulation of plant hormone levels, notably gibberellins and auxin (Penfield 2008). Increases in auxin levels that accompany warm temperatures require PHOTOTRANSFORMING FACTOR 4 (PIF4), a protein whose DNA-binding activity is altered with increasing temperatures (discussed in Chapter 3; Franklin et al. 2011; Kumar et al. 2012). It is currently not clear whether PIF4 itself is affected by temperature, for instance, through a post-translational modification or complexing with inhibitory factors, or whether PIF4 is competing with another factor, such as histone binding (Kumar and Wigge 2010). In this context, it is useful to consider two further PIFS whose abundance is directly affected by temperature, SPATULATA (SPT) and LONG HYPOCOTYL IN FAR-RED1 (HFR1). SPT controls temperature-responsive seed dormancy and growth (Penfield et al. 2005; Sidaway-Lee et al. 2010), with mutants having high growth rates even at low temperatures and overexpressors showing a dwarf phenotype only if the temperature is low. Importantly, cold elevates SPT protein levels leading to growth repression, in a manner that closely parallels the function of DELLA proteins. In contrast, HFR1 protein levels and HFR1 transcription are increased at warm temperatures (Foreman et al. 2011). It appears that temperature control of multiple PIFs and their activities underlie plant growth responses to temperature, although the mechanism by which temperature affects PIF protein levels and activity remains unknown.

Circadian clocks are also temperature sensitive and must maintain a constant 24-hour period across a broad range of temperatures (discussed in Chapter 6). This defining feature of circadian oscillators is especially interesting and requires a mechanism to buffer period against the effect of temperature on individual rates, such as transcription and mRNA decay, termed temperature compensation. For instance, transcription and mRNA decay rates, on average, increase by 3.5-fold for a 10°C temperature rise in plants (Sidaway-Lee and Penfield, unpublished data). The *Arabidopsis* circadian clock is composed of a series of interlocking negative feedback loops with dawn- and dusk-expressed components. The genes that make up the core clock components include a pair of highly homologous MYB transcription factors called CIRCADIAN CLOCK-ASSOCIATED 1 (CCA1) and LATE ELONGATED HYPOCOTYL (LHY) which function synergistically and have partially redundant functions in the repression of dusk-expressed TIMING OF CAB EXPRESSION1 (TOC1), ELF3, ELF4, AND LUX ARRYTHMO (LUX; see McWatters and Devlin, 2011, for a recent review of our understanding of clock architectures in plants). Genetic studies have identified various clock genes without which temperature compensation
fails, including LHY, PRR9, and GI (Edwards et al. 2006; Gould et al. 2006; Salome et al. 2010). However, a recent systems biology approach produced the first temperature-compensated model of the Arabidopsis circadian clock and predicted that there is one major temperature-sensitive clock protein and that is LHY (Gould et al. 2013). As temperature increases, LHY protein levels increase and this in turn increases the time after dawn at which the transcript levels of TOC1 and the other evening expressed genes, all inhibited by LHY, start to rise (Gould et al. 2013). This increase in LHY protein levels slows the clock and therefore balances faster rates in other reactions and allows the clock to maintain a 24-hour period at warmer temperatures. How this rise in LHY protein levels is mediated and whether the temperature sensitivity of LHY can be exploited for downstream signaling is currently unknown.

Several components of the LHY/CCA1 to PRR regulatory loop have additionally been tied to control of CBF transcript accumulation. Plants that are arrhythmic in constant conditions due to the loss of PSEUDO-RESPONSE REGULATOR 9 (PRR9), PSEUDO-RESPONSE REGULATOR 7 (PRR7), and PSEUDO-RESPONSE REGULATOR 5 (PRR5) have constitutively elevated levels of CBF transcripts and increased survival when challenged with freezing, suggesting that PRR9, PRR7, and PRR5 act as direct negative regulators of CBF transcripts (Nakamichi et al. 2009). Because LHY/CCA1 and these PRR proteins are locked in a translational feedback loop, it is possible that the PRR proteins are acting indirectly through LHY and CCA1. Support for this hypothesis has recently been published in a paper by Dong et al. (2011) in which the authors present ChIP experiments demonstrating direct binding of GFP-tagged CCA1 to the CBF1–CBF3 promoters. Their evidence indicates that LHY also plays a role in regulating CBF transcript levels as single lhy or cca1 mutants have nearly wild-type levels and rhythms of CBF2 and CBF3 (Dong et al. 2011). Experiments with lhy cca1 double mutants show that LHY and CCA1 are necessary for the rhythmic accumulation of CBF and CBF-target gene transcripts under constant temperature and for their induction in response to a challenge with cold (Dong et al. 2011). Therefore, it is now clear that CBF transcript levels are rhythmically regulated by the activity of LHY and CCA1 and that their induction in response to cold requires these MYB transcription factors, which are themselves temperature regulated.

1.7 Summary

The evidence presented so far suggests that there are likely to be several temperature sensors in plants, all of which have roles to play in adaptive plant responses to varying environmental temperatures. A multitude of
unknowns also suggests that as a field, we have a long way to go before we can say we understand how plants sense temperature. Further research into how protein levels are temperature regulated and how temperature-controlled transcription controls plant development will undoubtedly lead to further important insights into the way in which plants cope with living in seasonal environments and suggest mechanisms through which changes in environmental temperature might act to drive evolution.

References


