Tansley review

Temperature perception and signal transduction in plants

Author for correspondence:
Steven Penfield
Tel: +44 1904 328755
Fax: +44 1904 328762
Email: sdp5@york.ac.uk

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Steven Penfield

Centre for Novel Agricultural Products, Department of Biology, University of York, PO BOX 373, York YO190 5YW, UK

Contents

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Summary

Plants can show remarkable responses to small changes in temperature, yet one of the great unknowns in plant science is how that temperature signal is perceived. The identity of the early components of the temperature signal transduction pathway also remains a mystery. To understand the consequences of anthropogenic environmental change we will have to learn much more about the basic biology of how plants sense temperature. Recent advances show that many known plant-temperature responses share common signalling components, and suggest ways in which these might be linked to form a plant temperature signalling network.

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I. Introduction

Plants exhibit a range of responses to the temperature of their environment. Some of these responses are fast, while some involve accumulating data on temperature for several days or weeks, such as the breaking of bud dormancy or the floral promotion pathway of vernalization. Plants have been shown to measure temperature very accurately. For example, seed germination in lettuce seeds is strongly inhibited by the transition from 32°C to 33°C, a mere 1°C rise (Argyris *et al.*, 2005). Some thermogenic plants are capable of temperature homeostasis, and these have the ability to maintain a near constant inflorescence temperature, despite wide variation in the temperature of their environment (Seymour, 2001). In the twenty-first century global environmental change will alter the conditions in which we grow our crops, and in which our ecosystems thrive. It is estimated that temperature effects alone will account for the extinction of up to one-third of all European plant species (Thuiller *et al.*, 2005).

Plants depend on the perception of both high and low temperatures, both for their survival and for the regulation of key developmental events. Although environmental change is expected to increase average temperatures, this will also have important consequences for the way in which plants perceive low temperature. This is because evolution has fine-tuned plant sensitivity to low temperature according to the temperature regime of their local environment. For example, *Arabidopsis* accessions from northern Scandinavia require a much longer duration or cold for vernalization than those from warmer parts of Europe (Shindo *et al.*, 2006). In this review I will examine current state of the art knowledge on how plants measure temperature, and how this information is used to inform key developmental events, such as those shown in Fig. 1. First, plant responses to temperature will be introduced in isolation. This will begin with a discussion of extreme temperature responses and move on to introduce responses that occur over wider temperature ranges. Subsequently, I will discuss overlaps in the different signalling pathways and describe how our current knowledge points towards an integrated network mediating plant temperature signalling.

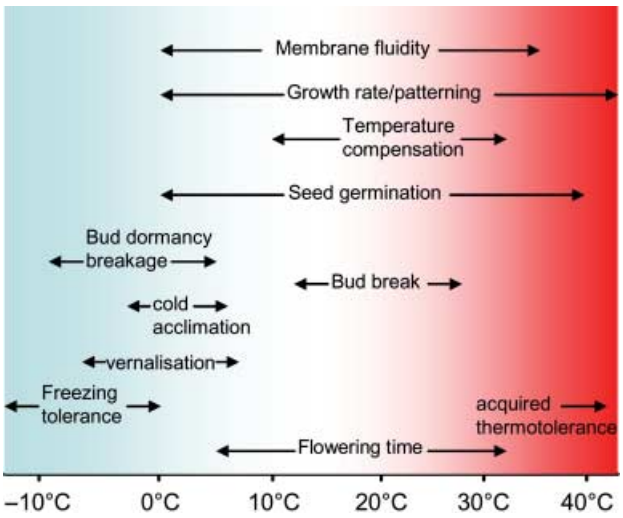

Fig. 1 Schematic representation of the temperature range of the major characterized plant responses to ambient temperature. Temperature compensation refers to the circadian clock.

II. Plant acclimatization to cold

Cold acclimatization is the process whereby plants gain a tolerance to freezing temperatures after an earlier period of (nonfreezing) chilling. This process has been extensively characterized genetically in *Arabidopsis* and will be treated only briefly here. For a more in-depth review readers are referred to the recent treatment by Chinnusamy *et al.* (2007). During the late 1990s it was shown that the *C-REPEAT BINDING FACTORS* (*CBF*s) function centrally in cold acclimatization (Stockinger *et al.*, 1997). These are members of the APETALA2 family of transcription factors and are induced transcriptionally *c.* 3–6 h after the experience of cold. When overexpressed in *Arabidopsis* or tomato they can activate a subset of *COLD-RESPONSIVE* (*COR*) gene expression (known as the CBF regulon), even in plants grown at warm temperatures and can confer freezing tolerance in the absence of cold acclimatization (Jaglo-Ottosen *et al.*, 1998; Hsieh *et al.*, 2002). The regulation of primary metabolism is an important target of the CBFs (Cook *et al.*, 2004). Aside from the CBF-mediated cold acclimatization response, the deactivation of reactive oxygen species (ROS) is also essential: mutants that accumulate ROS are hypersensitive to chilling, such as *vitamin E deficient 2* (*vte2*; Maeda *et al.*, 2006) and *frostbite1* (*fro1*; Lee *et al.*, 2002).

Important in the upstream regulation of *CBF* expression is the basic helix–loop–helix (bHLH) transcription factor INDUCER OF CBF EXPRESSION1 (ICE1; Chinnusamy *et al.*, 2003). *ICE1* is a constitutively expressed promoter of *CBF* gene expression. The key to ICE1 activity is that the protein is stabilized in the nucleus during cold treatments (Chinnusamy *et al.*, 2003). It is regulated post translationally by at least two proteins HIGH EXPRESSION OF OSMOTICALLY RESPONSIVE GENES1 (HOS1) and SIZ1 (SAP and MIZ1). HOS1 is a ring finger protein that ubiquitinates ICE1, marking it for destruction by the proteasome, and *hos1* mutants display increased freezing tolerance and high ICE1 protein levels (Ishitani *et al.*, 1998; Dong *et al.*, 2006). SIZ1 sumoylates ICE1 and this either activates or stabilizes the protein (Miura *et al.*, 2007). Interestingly, the HOS1 protein also shows cold-induced translocation from the cytoplasm to the nucleus.

There is also a significant component of the cold acclimatization response that is regulated independently of the CBFs. One of the most important additional regulators is *ZAT12*. *ZAT12* has a complex role in cold acclimatization, activating the expression of some cold-responsive genes, but inhibiting the activation of the *CBF*s (Vogel *et al.*, 2005).

III. The acquisition of thermotolerance

Plants exhibit a complex response to extreme high temperatures. The term basal thermotolerance describes the plant response to high temperature in the absence of any period of acclimatization. Basal thermotolerance is highly dependent on salicylic acid (SA) action (Clarke *et al.*, 2004). After as little as 15 min exposure to high temperatures plants begin to acclimatize, and the maximum tolerated temperature increases (Kaplan *et al.*, 2004). Acclimatization requires the expression of heat-shock proteins (HSPs), which appear to protect plants against oxidative stress. One of most important of these, HSP101, is encoded by the *HOT1* locus (Hong & Vierling 2000; Queitsch *et al.*, 2000). Loss of *HOT1* reduces acquired thermotolerance, but does not affect basal thermotolerance. In *Arabidopsis* a HSP transcription factor cascade has been described in which some HSPs further induce the expression of others. Two HSPs, HSP1 and HSP3, have been reported to be redundantly required for *HSP101* transcriptional induction by heat shock, but the *hsp1 hsp3* double mutant has only a mild deficit in acquired thermotolerance (Lohmann *et al.*, 2004). In tomato, HSF1A is believed to have the important role at the head of the transcriptional cascade inducing acquired thermotolerance (Mishra *et al.*, 2002).

A key role for ROS has been proposed in acquired thermotolerance. Larkindale *et al.* (2005) found a role for cytoplasmic NADPH oxidases, ROS generating enzymes previously found to be required for abscisic acid (ABA) signalling in seeds and guard cells. It has been proposed that ROS production either by stress or by NADPH oxidases directly activates the transcription of HSPs, and that HSPs may be ROS sensors (Larkindale & Huang, 2004; Volkov *et al.*, 2006). Alternatively, ROS may be required for ABA signalling during the acquisition of thermotolerance. The involvement of ABA (see below) and tolerance to ROS suggest strong parallels with other stress responses such as drought and cold. Indeed, a metabolomic study of thermotolerance showed wide overlap of heat- and cold-induced changes in plant metabolism (Kaplan *et al.*, 2004).

IV. Abscisic acid and extreme temperature responses

Abscisic acid biosynthesis is an absolute requirement for cold acclimatization and for acquired thermotolerance (Gilmour & Thomashow, 1991; Larkindale & Vierling, 2005). Both ABA-deficient and -insensitive mutants display a lack of freezing tolerance after cold acclimatization (Gilmour & Thomashow, 1991). In addition, overexpression of *ABSCISIC ACID INSENSITIVE 3* (*ABI3*) confers increased freezing tolerance on *Arabidopsis* (Tamminen *et al.*, 2001). ABA levels do increase transiently during cold exposure, but this increase is markedly less than that observed during drought (Lang *et al.*, 1994). It has also been observed that ABA levels increase upon cold exposure even in chilling sensitive species, so the exact function of this increase in ABA levels is open to debate. The ABA levels in cold-acclimatizing *Arabidopsis* may be regulated by the MYB transcription factor HOS10 (Zhu *et al.*, 2005). In *hos10* mutants freezing tolerance after cold acclimatization is reduced, and the expression of *NCED3* (a 9-*cis*-epoxycarotenoid dioxygenase required for ABA synthesis) is repressed. ABA action occurs partly through regulation of the *CBF*s, and partly through a CBF-independent pathway.

At high temperatures ABA synthesis is also required for acquired thermotolerance, but not basal thermotolerance (Larkindale & Vierling, 2005). Acquired thermotolerance was also disrupted in the ABA signalling mutants *abi1-1* and *abi2-1*, but not *abi3*. Abscisic acid also has a key role in the inhibition of germination at high temperatures (Tamura *et al.*, 2006; Toh *et al.*, 2008). In contrast to wild-type seeds, ABA biosynthetic mutants show strong germination at higher temperatures, and temperature induces the expression of several ABA biosynthetic genes including *ABA1* and *NCED9*. By contrast, low temperatures induce ABA catabolism in seeds (Finch-Savage *et al.*, 2007).

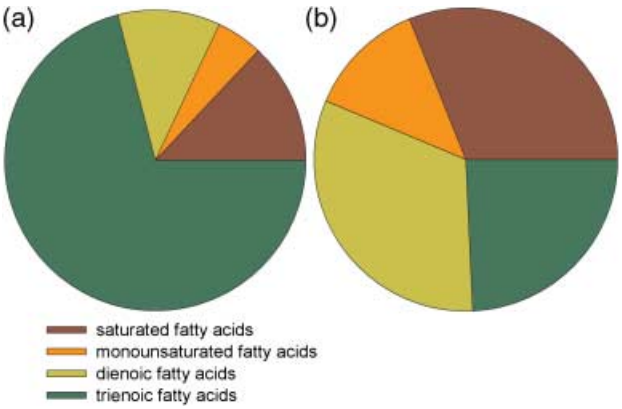

Fig. 2 The fatty acid composition of *Arabidopsis* leaves growing at 15°C (a) and 35°C (b). Data extracted from Falcone *et al*. (2004). Note the high level of polyunsaturated fatty acids in plants grown at the lower temperature.

V. Altering plant fatty acid composition affects plant tolerance to temperature extremes

Plants respond to higher temperatures by increasing the content of saturated and monounsaturated fatty acids, while at lower temperatures increased synthesis of polyunsaturated (predominantly trienoic) fatty acids are evident (Wallis & Browse, 2002; Falcone *et al.*, 2004; Fig. 2). In this way, plants and other organisms maintain constant membrane fluidity, despite environmental temperature variation. Pre-empting temperature changes by overexpression or knock-down of individual fatty acid desaturase isoforms appears to increase

plant tolerance to temperature extremes. For example, tobacco plants with lower polyunsaturated fatty acids owing to silencing of a plastidial omega-3 desaturase displayed increased tolerance to temperatures above 33°C (Murakami *et al.*, 2000). By contrast, transgenic tobacco plants overexpressing the *Arabidopsis* plastidial omega-3 *FATTY ACID DESATURASE 7* (*FAD7*) contained increased trienoic fatty acids and showed increased tolerance to chilling when transferred from 25°C to 4°C (Kodama *et al.*, 1995). This work suggests that modification of trienoic fatty acid content is important in plant adaptation to extreme temperatures. Zhang *et al.* (2005) provide good evidence that at least in terms of high-temperature tolerance, the modification of plastidial trienoic fatty acids is most important. Overexpression of the *Arabidopsis FAD8* gene (encoding a plastid-localized omega-3 desaturase) in tobacco cell cultures predominantly increased the amount of plastid trienoic fatty acids, while overexpression of *Arabidopsis FAD3* affected total trienoic fatty acid levels. Yet, the *FAD8* transgenic lines were more compromised in their tolerance to heat, suggesting that the plastid has a greater requirement for the correct maintenance of membrane fluidity at higher temperatures than other parts of the cell.

The availability of mutants has allowed us to see the importance of regulated fatty acid metabolism for plant adaptation to different temperatures. For example, *Arabidopsis* plants without a functional *FAD2* gene appear normal at 22°C, but when grown at 12°C they fail elongate their stems (Miquel *et al.*, 1993). If *fad2* mutant plants are incubated for a prolonged period at 6°C they eventually succumb, even though wild-type plants are capable of withstanding chilling temperatures indefinitely (Miquel *et al.*, 1993). Another mutant, *fab1*, suffers chilling damage during prolonged incubation at 2°C, but like *fad2*, *fab1* can withstand a period of a few days at chilling temperatures largely unscathed (Wu *et al.*, 1997). Further, *Arabidopsis fad* mutants *fad5* and *fad6* also show an increased sensitivity to chilling (Kunst *et al.*, 1989). It still remains unclear why these mutant plants have the phenotypes they do, and why certain fatty acid profiles are deleterious at specific temperatures. One suppressor of the *fab1* chilling phenotype is an allele of *fad5*, itself in isolation predisposing plants to chilling injury (Barkan *et al.*, 2006). One possibility is that the shape of the lipid molecules found in the *fab1 fad5* double mutants can somehow increase the fluidity of the thylakoid membrane compared with that of *fab1* (Barkan *et al.*, 2006). Another possibility is that changes in the abundance of lipids with signalling roles are important in the manifestation of some of these phenotypes. For example, the *fab2* mutant has an extreme dwarf phenotype as a consequence of massively increased ROS and SA signalling. This is cause by alterations in the level of oleic acid (18:1) which has a key signalling role in *Arabidopsis* (Kachroo *et al.*, 2004). Both SA and ROS are regulators of the response to extreme temperatures (Larkindale *et al.*, 2005), so it may be that changes in the abundance of lipids with signalling roles underlie the failure of some *fad* mutants to survive certain temperature treatments. Hence it may be premature to link all temperature-specific phenotypes of *fad* mutants to alterations in membrane fluidity.

VI. Temperature regulates the protein stability of fatty acid desaturases

The temperature input to the regulation of fatty acid metabolism appears to be in the form of the regulation of the transcription and protein stability of the various FAD isoforms. *Arabidopsis FAD8* is induced at the level of transcription at lower temperatures, whereas no change in *FAD7* expression was found (Gibson *et al.*, 1994). However, most evidence points to regulated protein turnover as the key mechanism for the temperature regulation of fatty acid synthesis. In wheat root tips the abundance of the endoplasmic reticulum-localized FAD3 protein was found to be higher at 10°C than at 30°C, but this was not reflected in changes of the level of the mRNA transcript (Horiguchi *et al.*, 2000). Interestingly, oilseed rape FAD3 has increased stability at low temperatures even when expressed in yeast cells, indicating that the mechanism for the temperature regulation of fatty acid metabolism might be conserved between plants and fungi (Dyer *et al.*, 2001). In *Arabidopsis* there is evidence that the FAD8 protein is less stable at higher temperatures (27°C) than at low temperatures. This high temperature instability was lost if a *C*-terminal 44 amino acid FAD8 protein subdomain was deleted (Matsuda *et al.*, 2005). In domain-swapping experiments replacement of the *C*-terminus of FAD7 with that of FAD8 could confer high-temperature instability on the FAD7 protein (Matsuda *et al.*, 2005).

VII. Membrane fluidity and temperature sensing

In both yeast and cyanobacteria it has been hypothesized that temperature-mediated alteration of membrane fluidity may itself be the primary temperature sensing event, and there has been much speculation that the same might be true in higher plants (Murata & Los, 1997). *Synechocystis* cells deficient in fatty acid desaturases, and therefore with altered membrane fluidity, have defects in temperature-regulated gene expression (Inaba *et al.*, 2002). In plants, pharmacological studies using agents that modify the fluidity of cell membranes have shown that it is possible to modify temperature-regulated gene expression. Commonly, benzyl alcohol is used to promote membrane fluidity, while dimethylsulphoxide (DMSO) is used to artificially rigidify membranes. For example, the low-temperature induced expression of the alfalfa gene *CAS30* was shown to be blocked by benzyl alcohol in cell cultures, and freezing tolerance of the cells was reduced (Orvar *et al.*, 2000). Furthermore, it has been shown that in oilseed rape cell cultures that benzyl alcohol blocked the cold inducibility of another gene, known as *BN115*, whereas

DMSO could induce *BN115* in the absence of a cold treatment (Sangwan *et al.*, 2001). However, concerns about nonspecific effects of these membrane-altering agents, coupled with a lack of any evidence for defects in temperature-regulated gene expression in *Arabidopsis* desaturase mutants (that have altered membrane lipid composition and therefore presumably altered fluidity), have lead to scepticism that this could be a possible temperature sensing system in plants (Somerville & Browse, 1996).

VIII. The role of gibberellin in temperature-regulated growth

Temperature has strong connection with growth rate: some plants cease growth completely at low temperatures. A growing body of evidence suggests that plants use endogenous hormones to couple their growth rate to temperature, rather than be subject to a passive temperature inhibition of growth (such as through a temperature affect on metabolic rate). Wheat Reduced height3 (Rht3) dwarf varieties, defective in gibberellin (GA) signalling, show normal growth at lower temperatures (10°C). However, unlike wild type they cannot increase their growth rate when placed at 20°C (Tonkinson *et al.*, 1997). Therefore it seems that in some ways plants with a deficit in either GA content or signalling may resemble plants growing at lower temperatures. Adding exogenous GA also promotes wild type wheat growth at 10°C, suggesting that temperature may act by regulating the level of active GAs in the plant.

More good evidence for a central role for GAs in temperature responses come from studies of thermoperiodism. A thermoperiodic growth response is defined as growth promoted by increasing the differential between the daytime maximum and the night-time minimum temperature, rather than simply correlating with the average temperature (Went, 1944). In many species high thermoperiodic differentials increase stem elongation, and it has been shown that this is accompanied by changes in GA metabolism. Exogenous GA application can blind fuchsia, lilies, campanulas and peas to thermoperiodic affects, presumably by saturating the system (Tangerås, 1979; Moe, 1990; Grindal *et al.*, 1998). On the molecular level it has been shown that increased thermoperiodic growth in pea stems is mediated by the transcriptional repression of the GA-catabolizing enzyme *GA 2-oxidase* (Grindal *et al.*, 1998; Stavang *et al.*, 2005), and is accompanied by increases in GA biosynthetic gene expression. Increasing temperature has also been shown to increase *GA 20-oxidase* transcript levels in citrus, and this was accompanied by an increase in bioactive GA content (Vidal *et al.*, 2003). Cold treatment can also affect the expression of genes involved in GA metabolism. In *Arabidopsis*, 4°C treatment upregulates *GA 2-oxidase* and represses the transcript levels of at least two *GA 20-oxidase* isoforms (Lee *et al.*, 2005). Hence, there is a large body of evidence that implicates the regulation of GA metabolism as a key output of temperature signalling pathways regulating growth.

IX. Temperature-regulated GA synthesis controls seed germination

Seed germination has long been a paradigm for abiotic signalling research, and experiments with this system led to the discovery of key plant signalling pathways, such as that of phytochrome (Borthwick *et al.*, 1952). Seed germination also presents an attractive system to study temperature signalling, because experiments can be quick, easy to quantify, and because a wide variety of behaviours are available. Seeds of some species require very specific temperatures for germination; some require a single temperature shift or an alternating temperature regime (Bewley & Black, 1994).

Seed germination control is but an extreme case of growth regulation, and in *Arabidopsis* is well known to require the regulation of hormone metabolism. In particular, the action of gibberellin in temperature responses is well established. Temperature regulates the levels of GA-metabolizing genes in both lettuce, and *Arabidopsis*. Increased levels of bioactive GAs are produced after a germination-promoting low-temperature treatment of *Arabidopsis* seeds, and this is mediated by upregulation of *GA 3-OXIDASE* (GA3OX) gene expression, and concomitant downregulation of GA 2-oxidase, the GA-catabolic enzyme (Yamauchi *et al.*, 2004). This regulation occurs at the level of transcription. A specific isoform of GA 3-oxidase, *GA3OX1*, is required for cold-induced germination and is regulated by the SPATULA (SPT) transcription factor (Yamauchi *et al.*, 2004; Penfield *et al.*, 2005). SPT is a member of the PHYTOCHROME INTERACTING FACTOR 3 clade of bHLH transcription factors, and functions in the regulation of seed germination, seedling and flower development (Penfield *et al.*, 2005). The semidominant *spt-2* mutation blocks seed germination in response to low temperature treatments, but permits germination after other stimuli such as light, nitrate or after-ripening. The fact that *spt-2* blocks temperature- but not light-responsive germination, suggests that PIF transcription factors can act directly on the temperature signalling, without a parallel impact on light responses. Fascinatingly, low temperature promotes GA accumulation in *Arabidopsis* seeds, whereas in vegetative tissues the opposite is true, with low temperature promoting a decrease in GA levels. At some stage in early seedling development a switch from the seed state to the vegetative state must occur, yet little is known of how this might be achieved.

Gibberellin action has been shown to be mediated by the GA-dependent destabilisation of the DELLA proteins in *Arabidopsis* and rice (Silverstone *et al.*, 2001). Consistent with this work, DELLA repression of growth is required to confer a low-temperature requirement on *Arabidopsis* seed germination, and DELLA mutants show abnormal temperature responses in seeds (Penfield *et al.*, 2006).

X. Temperature regulates auxin levels to control plant growth rhythms

Evidence from many studies including those in *Arabidopsis* suggests that certain times of the day are important for plant growth. Dusk is the time of maximum growth rate of *Arabidopsis* hypocotyls, at least when entrained in 12 h light : 12 h dark cycles (Dowson-Day & Millar, 1999). The exact time of maximum growth may vary with photoperiod, but essentially involves some part of the dark period (Nozue *et al.*, 2007). High growth rate correlates with increases in some species of GA, but also with an increased indoleacetic acid (IAA) content of plant tissues (Thingnaes *et al.*, 2003). In *Arabidopsis* hypocotyls, high temperature (here 29°C) causes an increase in free IAA, and this mediates a marked cell elongation response (Gray *et al.*, 1998). This elevated temperature response was shown to be independent of ABA or ethylene, but was compromised in the brassinosteroid-deficient mutant *de-etiolated 2* (*det2*), or in well-known auxin signalling mutants. Lee *et al.*, (2005) showed that decreasing temperature caused a decrease in the expression of the auxin-responsive reporter DR5:GUS, consistent with the idea that free auxin levels are temperature responsive, at least in some tissues. Therefore temperature signals may act to modify auxin synthesis or distribution in the plant, and this could represent a growth-regulating mechanism. Auxin-responsive gene expression is increased during the growth period of *Arabidopsis* hypocotyls, suggesting a role for auxin signalling in rhythmic growth (Nozue *et al.*, 2007). Notably, auxin signal strength has also recently been shown to be gated by the clock (Covington & Harmer, 2007). This points to a mechanism whereby auxin and GA act together to provide a rhythmic growth-promoting output that is strongly dependent on the ambient temperature. Indeed a recent microarray experiment using aspen vascular bundles showed that GA-regulated transcription was but a subset of that regulated by auxin (Björkland *et al.*, 2007).

XI. The circadian clock: temperature entrainment and compensation

Rhythmic growth requires the action of the plant circadian clock. This is composed of a coupled set of transcriptional feedback loops. These are capable of circadian oscillations in constant environments, but in a real-world situation are entrained by environmental inputs to synchronize them with the 24-h cycle of the earth's rotation. Plant circadian clocks can be entrained solely by a rhythm of two alternating temperatures, demonstrating that a temperature signal is transduced to the circadian oscillator. A difference of just 4°C between subjective night and day temperatures is sufficient to entrain the *Arabidopsis* clock (McClung *et al.*, 2002).

The current model for the *Arabidopsis* clock consists of at least three interlocking transcriptional feedback loops (Locke *et al.*, 2006; Fig. 3). In loop one the expression of the MYB transcription factors *CIRCADIAN CLOCK ASSOCIATED 1* (*CCA1*) and *LATE ELONGATED HYPOCOTYL* (*LHY*) peak in the morning and act together to repress the transcription of the pseudo-response regulator (PRR) protein *TIMING OF CAB EXPRESSION 1* (*TOC1*; also known as *PRR1*). By evening *CCA1* and *LHY* expression has declined and *TOC1* expression increases. TOC1, via an unknown component acts to reactivate *LHY* and *CCA1* expression. A second loop involves CCA1, LHY, PRR7 and PRR9, and a third loop requires TOC1 and GIGANTEA (GI). It has been shown that the rhythmic expression of the core clock components *LHY*, *CCA1* and *TOC1* can be entrained by temperature cycles alone (Salome and McClung, 2005). Analysis of mutants has led to the conclusion that closely related proteins PRR7 and PRR9 are critical for temperature entrainment. The *ppr7 ppr9* double mutant shows an abnormal period of *TOC1*, *LHY* and *CCA1* expression when entrained using temperature cycles alone, but not when entrained by light cycles (Salome & McClung, 2005).

A further key feature of circadian clocks is their ability to perform robustly across a wide range of temperature regimes. This feature is known as temperature compensation and has been documented in the circadian clocks of animals, plants and fungi (Rensing & Ruoff, 2002). In *Arabidopsis* the

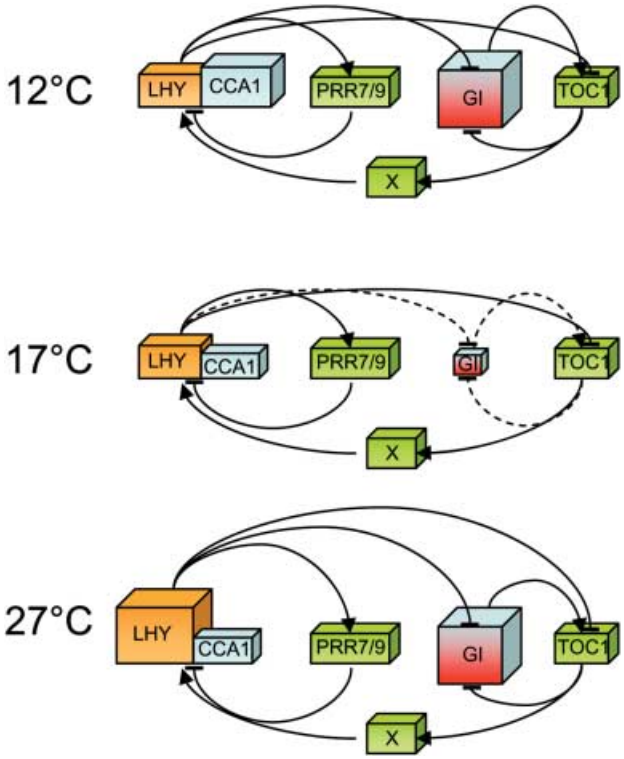

Fig. 3 Temperature compensation of the *Arabidopsis* circadian clock, after Gould *et al*. (2006). Size of boxes indicates the relative contribution of each protein at the indicated temperatures.

circadian clock components *LHY*, *CCA1* and *TOC1* show temperature-specific effects on the oscillation of their transcript abundance. For example *TOC1* amplitude is greatly increased at 27°C compared with 17°C, and the amplitudes of *CCA1* and *LHY* are correspondingly decreased at the higher temperature (Gould *et al.*, 2006). LHY is most important in maintaining CAB2:LUC period at 27°C, whereas CCA1 is more important at 12°C. Interestingly, temperature compensation is lost in *gi-11* mutants. High variation in period length of leaf movements and CAB2:LUC expression at high and low temperatures was observed in the *gi-11* null alleles that greatly exceeded that of wild type (Gould *et al.*, 2006). However, at 17°C GI appears to have little or no role in circadian rhythms. The function of GI is apparently to maintain the 24 h rhythm of the clock when the temperature deviates from 17°C.

XII. Temperature regulates flowering time independently of GA synthesis

Gibberellin is a key promoter of the floral transition in many species, and in *Arabidopsis* GA acts directly on the expression of the *LEAFY* gene to promote flowering. Yet the genetically determined temperature signalling pathways regulating flowering converge on the floral promoter FT, and there remains no evidence for a direct role for GA in temperature-promoted flowering.

Winter annual species undergo the process of vernalization, the promotive response of flowering in response to a prolonged period of cold. Those interested in the details of the current knowledge of the vernalization response are directed to recent comprehensive reviews (Sung & Amasino, 2005). Suffice to say here that the expression of the MADs box transcription factor *FLOWERING LOCUS C* (*FLC*) is central to conferring the vernalization requirement (Sheldon *et al.*, 2000). After prolonged cold, transcriptional activation of the VERNALISATION INSENSITIVE 3 (VIN3) transcription factor promotes the epigenetic silencing of *FLC* expression, leading to early flowering in the presence of an inductive photoperiod (Sung & Amasino, 2004; Bastow *et al.*, 2004).

Aside from vernalization, variations in the growth temperature can have dramatic effects on flowering time. In general, increasing temperature promotes flowering with a shorter vegetative phase, and by increasing growth rate. Some evidence suggests that this temperature dependence of flowering time is diurnally regulated. In *Arabidopsis* high temperature at night is a more potent floral inducer than high temperature during the day (Thingnaes *et al.*, 2003). In some plants, including orchids, this relationship is reversed, and high daytime temperatures are better floral promoters (Blanchard & Runkle, 2006). *Arabidopsis* mutants in the so-called autonomous pathway such as *fca*, *fve* and *fy* are insensitive to the promotion of flowering by increasing temperature (Blazquez *et al.*, 2003). Since their primary function in flowering regulation is the maintenance of low *FLC* expression, this work again points to *FLC* as a determinant of temperature-regulated flowering. Further support for this hypothesis comes from studies of natural variation in temperature responsive flowering in *Arabidopsis*, which have shown that FLC and its close homologue FLOWERING LOCUS M (FLM) are key regulators of temperature-responsive flowering (Balasubramanian *et al.*, 2006). This temperature promotion even occurs in vernalized plants, or plants with constitutively low *FLC* expression. It seems that despite strong epigenetic repression of *FLC*, temperature-dependent variation in FLC activity is still important in these plants. In the case of FLM there is evidence that temperature-regulated alternative splicing may be important (Balasubramanian *et al.*, 2006). Like vernalization, ambient growth temperature affects *FLC* transcript levels (Lee *et al.*, 2007), suggesting that *FLC* transcription could be the target of the temperature signal. Yet *flc-3* loss-of-function mutants retain temperature responsive flowering (Blazquez *et al.*, 2003). Therefore it is likely that FLC functions redundantly in the temperature regulation of flowering, perhaps with FLM and SHORT VEGETATIVE PHASE (SVP), both of which also control *FT* transcription and have a role in the temperature response (Lee *et al.*, 2007).

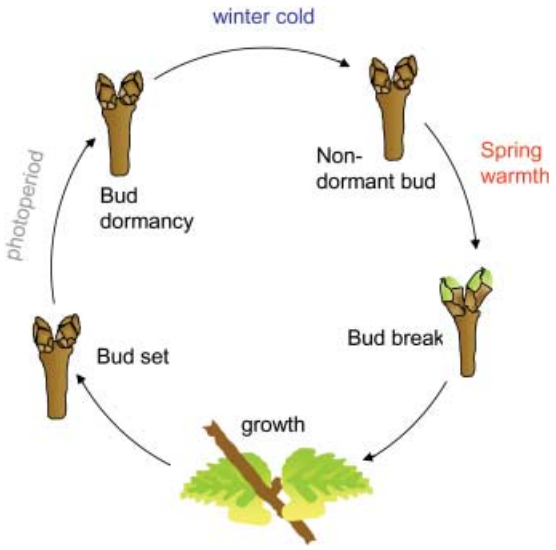

Fig. 4 The annual cycle of bud dormancy in perennial plants growing in temperate regions, and the environmental signals driving the cycle.

XIII. Temperature-responsive periods control annual growth in trees

Perennial plants growing in temperate regions follow an annual cycle of growth (Fig. 4). Bud burst in spring is followed by summer flowering and the appearance of new buds in the autumn. It is proposed that climate warming caused by human activity is affecting the timing of bud burst in spring. Recent media focus on the timing of bud break

makes this one of the most widely appreciated temperature-controlled traits in plants (Prentice *et al.*, 1992), yet one of the least well studied at the molecular level. In the autumn the newly formed buds enter a period of dormancy, which is controlled by decreasing day length in most species. However, in some species of the Rosaceae, including apple and pears, sensing of low temperature is the prime inducer of bud dormancy (Heide & Prestrud, 2005).

Bud dormancy is terminated by a prolonged period of chilling during winter. In some cases the chilling period needs to be as long as 2 months, and this process at least superficially resembles the effect of vernalization on the flowering of winter annual species. In the spring, chilled buds are competent to receive the signal from increasing ambient temperatures and bud break then occurs. There are strong parallels between the processes involved in bud dormancy and seed dormancy, and these are the two most pervasive temperature-regulated processes in higher plants.

Knowledge of the regulation of the bud dormancy cycle at the molecular level is limited, primarily because of the lack of available model species. However, it has recently been shown that the short day promotion of bud dormancy in poplar induction requires the CO/FT module found to regulate photoperiodic flowering (Böhlenius *et al.*, 2006). A recent report also links a Norway spruce FT homologue PaFT4 to the temperature regulation of bud burst, although these experiments were preliminary and this relationship requires further testing (Gyllenstrand *et al.*, 2007). Interestingly, there is evidence that increasing the temperature of the roots can nonlocally promote bud break. This suggests a role for a temperature-dependent mobile signal from root tissues in the breaking of bud dormancy (Greer *et al.*, 2006). Root to shoot signalling in the control of bud dormancy has been described extensively in annual plants (Foo *et al.*, 2001), although evidence for temperature regulation is lacking.

XIV. Temperature response pathways share common signalling components

So, we have considered the most important plant responses to temperature in turn, treating them as separate pathways. But is there any evidence that we are close to revealing an upstream temperature sensor, or at least finding elements of temperature signalling networks that are common to several of the pathways? Are we to conclude that multiple independent temperature sensors individually regulate functionally distinct signal transduction networks specific for each response?

One obvious approach to this problem is to take mutants compromised in one temperature response and test them for phenotypes in another. In this way common components can be identified. For example, in both vernalization and the ambient temperature-promotion of flowering there is a role for changes in *FLC* expression. Experiments taking advantage of natural variation in temperature compensation of the circadian clock also revealed an unexpected role for FLC (Edwards *et al.*, 2006). At 27°C *flc-3* mutants are less likely to maintain a normal period length, possibly because of misregulation of the MYB transcription factor and clock component *LUX ARRYTHMO* (*LUX*; Edwards *et al.*, 2006; Hazen *et al.*, 2005). *Flc-3* mutants have a short period in free-running conditions compared with wild type. By contrast, mutants in the autonomous floral promotion pathway have a weak but significant lengthening effect on the circadian period, consistent with the large increase in FLC expression observed in these mutants. In *fca*, *fve* and *luminidependens* (*ld*) mutants, the period length can reach 25–26 d (Salathia *et al.*, 2006). This lengthening in period is only partly restored by loss of FLC, demonstrating that upregulation of *FLC* is only partly responsible for this affect. The fact that vernalization also shortens circadian period even in the absence of FLC suggests that a set of *FLC*-related genes may act to modulate the temperature signal to the circadian clock.

It is therefore tempting to place FLC action in a generalized temperature response pathway. Published transcriptomic data also shows that FLC activity quantitatively affects temperature-responsive gene expression. Figure 5a shows six genes whose

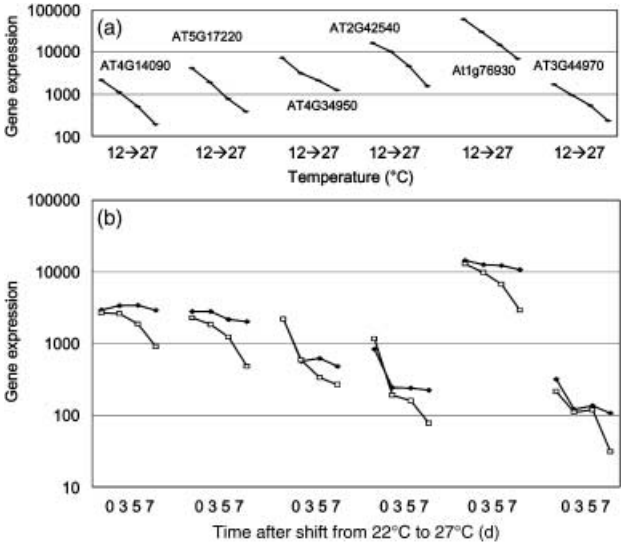

Fig. 5 Evidence for a general role for *FLOWERING LOCUS C* (*FLC*) in temperature responses, using publicly available Affymetrix ATH1 gene chip data downloaded from GENEVESTIGATOR (http://www.genevestigator.ethz.ch; Zimmerman *et al*., 2004). (a) The identification of six cold-inducible genes whose expression responds incrementally to decreasing temperature (27°C, 22°C, 17°C, 12°C). At2g42540 is the locus of the well-known *COLD-REGULATED 15a* (*COR15a*) gene. For this experiment seedlings were grown at 22°C for 7 d, then transferred to the indicated temperature for 2 d. RNA was then harvested once every 4 h for 24 h on the third day and then pooled for analysis. (b) The expression of the cold-regulated genes from (a) in wild type (circles) versus *flc-3* mutants (squares) after a temperature shift from 22°C to 27°C, as described in Balasubramanian *et al*. (2006). In *flc-3* all six genes show an exaggerated response to 27°C, demonstrating a general role for FLC in the normal plant response to this temperature.

transcript abundance is negatively regulated by increasing temperature between 12°C and 27°C, using the data contained in NASCARRAYS experiment number 147 (http://affymetrix.arabidopsis.info/narrays; i.e. these genes are cold-induced). These genes can then be used to query transcriptome data from experiments involving *flc-3* mutants, such as that described in Balasubramanian *et al*. (2006; ArrayExpress E-MEXP-728). This experiment describes the transcriptome of whole *Arabidopsis* wild type or *flc-3* mutant plants shifted from 22°C to 27°C for 7 d (Fig. 5b). Note that in all six cases the expression of each cold-induced gene is lower in *flc-3* mutants after the shift to 27°C than in wild type. At 22°C wild type and *flc-3* mutants show similar transcript levels for all genes. It appears that *flc* mutants are hyper-responsive to the shift to 27°C, because cold-induced genes are generally lower expressed in *flc-3* mutants than in wild type. It is possible that this is a consequence of altered circadian clock function in *flc* mutants at the higher temperature, because it is at around this temperature that *flc* mutants begin to lose their ability to maintain wild-type clock rhythms (Edwards *et al*., 2006); furthermore, many of these cold-induced genes are subject to diurnal regulation (Gould *et al*., 2006). In general, *flc-3* mutants appear to 'believe' they are hotter than they really are, at least when transferred to higher temperatures. Because we know roughly how much these six marker genes change in transcript levels between 22°C and 27°C we can even estimate that at 27°C the temperature signalling output of *flc-3* mutants appears *c*. 2°C warmer than wild type. Therefore FLC may have a common role in multiple temperature signalling pathways, perhaps as a memory of the plant's previous experience of temperature, as in the vernalization response.

XV. Flowering time regulators with roles in cold acclimatization

A number of classical flowering time regulators have now been shown to have roles in cold acclimatization, or in freezing tolerance. The autonomous pathway component FVE also regulates cold-induced gene expression (Kim *et al*., 2004). *FVE* encodes a retinoblastoma-related protein required for the repression of *FLC* expression (Ausín *et al*., 2004). Kim *et al*. (2004) found that *fve* mutants showed increased *CBF3* and *COR15a* gene expression at higher ambient temperatures, suggesting common features of the two pathways. Activation of *COR15a* transcription in *fve* mutants exhibited a partial requirement for *FLC*, but *FLC* was not found to be strictly necessary. The role of *FLM* and *SVP* have not been tested. Interestingly it has recently been shown that CONSTANS (CO) and a NAC domain protein known as LONG VEGETATIVE PHASE 1 (LOV1) also link flowering time regulation to freezing tolerance. *LOV1* encodes a repressor of *CO* expression, and *LOV1* overexpressing lines and *co-2* mutants show similar photoperiod insensitive flowering phenotypes (Yoo *et al*., 2007). The authors also show that *co-2* mutants show increased freezing tolerance before and after cold acclimatization, whereas *lov1* mutants show hypersensitivity. This is consistent with the LOV1 effect being mediated by CO. Increased freezing tolerance in *co-2* and *35S:LOV1* was not associated with increased *CBF* transcription factor expression. Bizarrely, while CO is a negative regulator of freezing tolerance, GI appears to be a promoter (Cao *et al*., 2005). This is despite GI being known as a positive regulator of CO activity in flowering time control. Hence it is possible that GI has a CBF- and CO-independent role in maintaining freezing tolerance. All this work suggests a general role for the pathways well known as flowering time regulators in temperature responses. Yoo *et al*. (2007) also observed that *LOV1* transcript abundance is reduced in the *sensitive to freezing 6* (*sfr6*) mutant (Boyce *et al*., 2003), suggesting one mechanism through which LOV1 might interact with known cold response pathways. Further mutants exhibit both flowering time and cold acclimatization phenotypes. The *osmotically responsive genes 9* (*hos9*) mutant flowers late, and promotes freezing tolerance through a CBF-independent pathway (Zhu *et al*., 2004). *HOS9* encodes a homeodomain transcription factor, but its targets remain unknown. A final interesting mutant to consider is *hos1* (Ishitani *et al*., 1998; Lee *et al*., 2001). The *hos1-1* mutant is hypersensitive to applied cold and shows increased cold tolerance in the absence of an acclimatization period. These plants are also early flowering and the authors speculate that these plants may be constitutively vernalized. These phenotypes suggest that HOS1 has a wider role in the temperature response than simply the regulation of ICE1 and CBF activity. More interestingly, in *hos1-1* mutants cold-responsive gene expression was hypersensitive to the experience of cold temperatures up to but not exceeding 19°C (Ishitani *et al*., 1998). Note that this is roughly the temperature at which GI is not required for accurate maintenance of circadian period lengths (Gould *et al*., 2006).

XVI. A general role for the circadian clock in temperature signal transduction

Circadian clocks are temperature-sensitive, and therefore have a key property required of a plant temperature sensor. In the fungus *Neurospora crassa*, temperature regulates the alternate splicing of the mRNA encoded by the Frequency locus, a central clock component (Liu *et al*., 1997). There is also evidence for a role for the plant circadian clock in mediating temperature signalling itself. For example, the circadian clock regulates *CBF* expression and the magnitude of their transcriptional activation by cold. *CBF* expression is increased when cold is experienced 4-h after dawn, compared with a similar experience 4-h after dusk (Fowler *et al*., 2005). This phenomenon, known as 'gating', is well known in light response pathways. Important targets of the CBF regulon are also circadian-regulated at ambient temperatures, including

COR15a and *COR15b* (Gould *et al.*, 2006), underlining the potential of the circadian clock to regulate cold responses in plants. More evidence that the circadian clock has a role in temperature signal transduction comes from analyses of GI function in low temperature responses. It has been reported that *GI* transcripts are elevated at low temperatures, and that *gi* mutants are defective in freezing tolerance (Cao *et al.*, 2005); GI also has a role in temperature-regulated flowering time in *Medicago truncatula* (Paltiel *et al.*, 2006). I can also show that GI plays a central role in the promotion of seed germination by low-temperature, while LHY and CCA1 also have roles in temperature-regulated germination (S. Penfield and A. Hall, unpublished). Therefore, GI and the circadian clock may have a general role in plant temperature signalling.

In chestnut it has been shown that the experience of winter of a low temperature treatment stalls the rhythmic expression of the circadian clock components LHY and TOC1 (Ramos *et al.*, 2005). Warming the plants was sufficient to restart the clock, but this effect did not depend on the dormancy of the buds (Ramos *et al.*, 2005). So it is possible that the circadian clock also has a role in the temperature-regulation of bud dormancy in trees.

XVII. Cytosolic calcium may integrate temperature and circadian signalling

One of the earliest known plant responses to temperature shifts is an elevation in cytosolic free calcium levels, and it has been hypothesized that calcium oscillations may integrate temperature and circadian information (Hotta *et al.*, 2007). Our knowledge of these calcium spikes depends on the use of proteins that emit light in the presence of calcium and a substrate, such as aequorin or aequorin containing polyproteins (Knight *et al.*, 1991). Calcium spikes can be measured within seconds or minutes of cold application and are associated with membrane depolarization. Furthermore, they have a characteristic waveform that depends both on the magnitude of the temperature shift, and on the absolute temperature (Knight *et al.*, 1996; Plieth *et al.*, 1999). It is also interesting that magnitude of the calcium spikes is also dependent on the plant's previous experience of temperature: repeated application of a low temperature produces an ever more dampened reaction (Plieth *et al.*, 1999). Therefore, plants appear to have a calcium signature memory of earlier temperature experiences. Most of the calcium appears to enter the cytosol from an extracellular source, but a second smaller influx has been noted which is sensitive to inhibitors of tonoplastic calcium channels (Knight *et al.*, 1996).

In *Arabidopsis* a circadian rhythm of cytosolic calcium concentration has been demonstrated (Love *et al.*, 2004). In shorter days the peak cytosolic free calcium concentration occurs at dawn, precisely when the circadian clock allows maximum *CBF* gene transcription in response to cold (Fowler *et al.*, 2005). Moreover, the application of a cold stimulus and time of day have additive affects on the size of the calcium spike, suggesting a possible mechanism to explain cold signal gating (Dodd *et al.*, 2006). The best evidence that these spikes in calcium concentration play a role in temperature signal transduction comes from pharmacological experiments in which calcium levels are altered. Application of lanthanum, an inhibitor of calcium channels, or ethylene glycol-*bis*(beta-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA), a calcium chelating agent, resulted in reduced cold responsive expression of *KIN1*, a frequently used marker of the cold acclimatization response (Knight *et al.*, 1996). Furthermore, inhibitors of calcium release, or inhibitors of calcium-dependent protein kinases (CDPKs) and calmodulin all impaired freezing tolerance in *Arabidopsis*, and affected *COR* gene induction after cold treatment (Tahtiharju *et al.*, 1997). However, inducible overexpression of one calmodulin isoforms repressed rather than induced *COR* gene expression (Townley & Knight, 2002), so the role of these genes in cold acclimatization remains unclear. Genetic evidence for a role for calcium oscillations in temperature responses is still sorely lacking. However, cold inducible calcium spikes have been observed in some putative low-temperature signalling mutants, suggesting that if they do play a role it is likely upstream of these genes (Carpaneto *et al.*, 2007).

XVIII. The role of phytochrome signalling in temperature responses

A close relationship between light and temperature signalling was first proposed in the 1950s and 1960s, after experiments with seed germination. These studies showed that the range of temperatures that permit germination could depend on light quality, particularly those wavelengths detected by phytochrome (Toole *et al.*, 1955). At least two early studies concluded that phytochrome itself was responsible for the transduction of the temperature signal (Roth-Bejerano *et al.*, 1966; Scheibe & Lang, 1965). Their results can be summarized by the notion that red light and low temperature have similar effects on seeds, whereas far-red light and high temperature effects are also similar. These observations have also been made on *Arabidopsis* seedling growth: high temperatures or far-red light both result in similar responses, inducing petiole elongation, reduced chlorophyll accumulation and early flowering (Halliday & Whitelam, 2003). In the 1960s it was hypothesized that the interconversion rate of the red absorbing (Pr) and far-red absorbing (Pfr) forms of phytochrome in the absence of light depended on temperature, and that the equilibrium Pr : Pfr ratio could encode temperature information.

Arabidopsis contains five phytochromes (phyA–E), and genetic and physiological studies have shown a strong interaction between loss of individual phytochromes and growth temperature. In vegetative tissues phytochrome is important in regulating leaf morphology and metabolism, and the timing of the floral transition. At 22°C the monogenic *phyB* mutant

exhibits early flowering compared with wild type, whereas this phenotype is absent at 16°C (Halliday *et al.*, 2003). By contrast, *phyA* mutants flower with the same number of leaves when grown at 16°C or 22°C (Blazquez *et al.*, 2003). The *phyA phyB phyD* triple mutant exhibits an extreme early flowering phenotype at 22°C: yet, even this is lost if the temperature is reduced to 16°C, suggesting that phyB and phyD action is restricted to the higher temperature. However, loss of phyE is important at 16°C, as *phyA phyB phyE* triple mutants flower early at the high and low temperature (Halliday *et al.*, 2003). One possible interpretation is that there is functional substitution of phytochrome isoforms at different temperatures, with phyE most important at 16°C, phyB and phyD most important at 22°C, and phyA important at all temperatures. Another explanation is that phytochromes themselves are temperature sensors, or may regulate temperature sensors, with each specialized for different temperatures. In this scenario their absence would lead to derepression of the floral transition at the temperatures for which each is most important. Arguing against this is the observation of temperature responses in multiple phy-deficient lines (Halliday & Whitelam, 2003). Placing *Arabidopsis* phytochrome quadruple mutants (*phyABDE* mutants) at high temperature produces an extreme additive affect on internode elongation, with plants even showing internode elongation between each leaf. This type of experiment suggests that the temperature signal is transduced independently of phytochrome.

The role of each phy species in temperature responses has also been recently investigated in seed germination control. Interestingly, this work raises parallels with Halliday and Whitelam's earlier work on the role of phytochromes in the control of the floral transition. In germinating *Arabidopsis* seeds phyE appears to be most important in responding to lower temperatures, because *phyE* mutants show reduced germination only at temperatures below 16°C (Heschel *et al.*, 2007). Loss of phyA or phyB compromises germination either at 16°C or 22°C. By contrast, seed germination at 19°C was virtually unaffected by loss of any combination of phyA, phyB and phyE. This suggests that either phyD or phyC are important at this temperature, or that at 19°C temperature signalling is uniquely uncoupled from the light-signalling pathway. This temperature is close to that at which GI is not required to maintain the period of the circadian clock (Gould *et al.*, 2006), suggesting there may be a link between the circadian clock and phytochrome action in temperature response pathways. I propose that this temperature is an important threshold in plant temperature signalling, and that plants measure temperature not absolutely, but relative to this value.

It has recently been found that *CBF* gene expression can be promoted at nonchilling temperatures by manipulating light quality (Franklin & Whitelam, 2007). These authors found that under far-red rich light regimes the CBF regulon can be activated in *Arabidopsis* plants growing at 16°C in the absence of chilling (Franklin & Whitelam, 2007). This activation depends on the regulation of *CBF* transcription by the circadian clock, such that the role of far-red light greatly increases the amplitude of the normal circadian regulation of CBF transcription. Strikingly, a low red : far-red ratio or phytochrome deficiency can even confer freezing tolerance on plants that have not been cold-acclimated.

XIX. Conclusions

Despite two decades of molecular plant science temperature sensors have still not been identified. Yet we now have a good grasp of the complex signalling networks regulating plastic plant responses to the environment, and components of many of these participate in more than one response to temperature. Future analyses of these signalling networks will be able to localize the sources of temperature inputs, and the use of genetics combined with new model systems will continue to identify further components of temperature signalling pathways. In the next decade we will develop computational models of these signalling processes and this will enable comprehensive approaches to hypothesis generation and testing. These combined approaches are close to revealing the molecular nature of plant temperature sensors.

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