Interaction of light and temperature signalling

Keara A. Franklin1, Gabriela Toledo-Ortiz2, Douglas E. Pyott2 and Karen J. Halliday2,*

1 School of Biological Sciences, University of Bristol, Bristol BS8 1UG, UK
2 SynthSys, University of Edinburgh, C.H. Waddington Building, King’s Buildings, Edinburgh EH9 3JD, UK

* To whom correspondence should be addressed. E-mail: khalida@staffmail.ed.ac.uk

Received 11 November 2013; Revised 15 January 2014; Accepted 20 January 2014

Abstract

Light and temperature are arguably two of the most important signals regulating the growth and development of plants. In addition to their direct energetic effects on plant growth, light and temperature provide vital immediate and predictive cues for plants to ensure optimal development both spatially and temporally. While the majority of research to date has focused on the contribution of either light or temperature signals in isolation, it is becoming apparent that an understanding of how the two interact is essential to appreciate fully the complex and elegant ways in which plants utilize these environmental cues. This review will outline the diverse mechanisms by which light and temperature signals are integrated and will consider why such interconnected systems (as opposed to entirely separate light and temperature pathways) may be evolutionarily favourable.

Key words: Circadian clock, cold acclimation, flowering, HY5, light, photoreceptors, phytochrome, phytochrome-interacting factors, signalling, temperature.

Introduction

Light signalling in plants is mediated by discrete protein receptors which undergo a conformational shift following absorption of specific wavelengths of light. Previously, analysis of plant phototransduction was confined to phytochromes, which absorb red and far-red light, and the phototropins, cryptochromes, and members of the zeitlupe family, which absorb in blue/UV-A wavelengths (Franklin and Quail, 2010). More recently, UVR8 (UV RESISTANCE LOCUS 8) has been added to the list of bona fide photoreceptors responsible for mediating responses to UV-B light (Wu et al., 2012). This array of photoreceptors with different wavelength specificities allows plants to react to subtle changes in the quality, quantity, and direction of light. An enormous body of work has provided a remarkably detailed understanding of the structure, photochemistry, and signalling components regulated by these photoreceptors. For a detailed discussion of these areas, the reader is directed to recent review articles (Christie, 2007; Fankhauser and Ulm, 2011; Ulijasz and Vierstra, 2011; Casal, 2013; Tilbrook et al., 2013).

In contrast to the relatively well-defined light signalling pathways, molecular characterization of temperature perception and signalling has been harder to resolve. This is largely due to the complex and ubiquitous effects of temperature on cellular responses. Indeed, if one were loosely to define a temperature receptor as a cellular component that exhibits temperature-dependent activity, then the list of putative temperature receptors would be extensive, as temperature affects most, if not all, enzymatic processes (Laidler, 1997). As a consequence, research on temperature perception has traditionally taken a reductionist approach; examining specific responses to temperature and identifying components that are necessary and sufficient. For instance, CNGC2 (a calcium-conducting cyclic nucleotide-gated channel) has been shown to have a thermosensory role in the induction of HEAT SHOCK PROTEIN (HSP) expression in response to elevated temperature (Finka et al., 2012). Other studies have shown that cold treatment activates calcium-permeable channels in Arabidopsis mesophyll cells (Carpaneto et al., 2007). However, in these instances,
the primary temperature-sensing event(s) has yet to be uncovered. Another study has identified a central molecular process through which gene expression can be tuned by relatively moderate changes in ambient temperature (Kumar and Wigge, 2010). Specifically, this mechanism involves the nucleosomes containing the histone variant H2AZ which exhibit temperature-sensitive binding to promoter sequences. H2AZ nucleosome occupancy at specific promoters has been shown to prevent gene expression. Elevated temperature leads to the expulsion of H2AZ nucleosomes, opening up access for transcription factors that activate gene expression. While these studies provide important insights into the different modes of temperature signalling, further study is required to elucidate the primary biochemical events that react to temperature.

There is now a growing recognition that light and temperature signalling are connected. Indeed there is an expanding catalogue of cross-talk and nodes at which light and temperature signals converge. This review highlights the pathways that operate at the confluence of light and temperature signalling and examines the adaptive value of signal integration in a changing environment.

**Temperature modification of photoreceptor activity**

**Phytochrome—dark reversion**

Temperature directly affects the properties of phytochrome. Among the earliest demonstrations of this phenomenon were the pioneering ‘flip flop’ experiments of Harry Borthwick and colleagues, showing red/far-red reversibility in the promotion of lettuce seed germination (Borthwick et al., 1952). In this study, the authors observed that the effectiveness of red light pulses was temperature dependent, with significantly greater germination recorded at 20 °C than at 30 °C. They concluded that germination was controlled by two opposing pigments, with accumulation of the inactive ‘dormant’ pigment favoured at higher temperatures (Borthwick et al., 1952). We now know this process to be the thermally sensitive non-photochemical or ‘dark reversion’ of phytochrome from the biologically active Pfr form to the biologically inactive Pr form.

Dark reversion has been observed in multiple phytochromes, in multiple species, with PfrPr heterodimers showing significantly faster reversion than PfrPfr homodimers (Brockman and Schafer, 1987; Hennig and Schafer, 2001). This process is strongly temperature dependent, with accelerated dark reversion recorded at increased temperatures (Schafer and Schmidt, 1974; Eichenberg et al., 1999; Hennig and Schafer, 2001). The thermoinhibition of lettuce seed germination at high temperature can be reduced by increasing the frequency of red light treatments, effectively counteracting accelerated dark reversion of Pfr in these conditions (Saini et al., 1989). Genetic screens have identified multiple mutants with altered phytochrome dark reversion. Examples include the light-hypersensitive phyB-401 mutant and the light-hyposensitive phyB-101 mutant (Eich and Chory, 1997; Kretsch et al., 2000). More recently, it has been shown that the dark reversion of phyB involves targeted phosphorylation of a specific serine residue at the N-terminus of the PHYB protein (Medzihradzsky et al., 2013). Furthermore, it has been demonstrated that altering the dark reversion process by targeted mutagenesis can be a useful tool for manipulating plant photomorphogenesis (Zhang et al., 2013). The role of phytochrome dark reversion in plant temperature responses has, however, yet to be fully explored.

**Phytochrome—functional hierarchy**

It is well established that phyA is the most abundant phytochrome in dark-grown seedlings (Clough et al., 1995) while, in de- etiolated plants, phyB abundance is highest (Sharrock and Clack, 2002). Unsurprisingly, phyB dominates phytochrome signalling in the light. This is demonstrated by the obvious elongated architecture and early flowering phenotype of phyB-deficient plants when compared with other single phytochrome mutants (Franklin and Quail, 2010). The relative functional hierarchies of different phytochrome family members can be altered by growth temperature at multiple stages of the plant life cycle. Heschel and colleagues (2007) used Arabidopsis mutants deficient in different combinations of phytochrome to investigate their individual roles in promoting germination at different temperatures. At low temperature (10 °C), phyE assumed the most dominant role, followed by phyB. An involvement of phyA was observed, but only after prolonged cold. At high temperature (28 °C), phyB was the principal regulator, followed by phyA and phyE. At an intermediate temperature (19 °C), loss of phyA, phyB, and phyE had little effect on seed germination, suggesting a novel role for phyC and/or phyD. The authors suggest that different phytochrome family members may differentially regulate gibberellin (GA) signalling at different temperatures. Alternatively, dark reversion and/or the threshold level of Pfr required to break dormancy may differ between phytochrome family members at different temperatures (Heschel et al., 2007).

An example of temperature altering the functional hierarchy of phytochromes in adult plants is provided by the work of Halliday and colleagues who observed that Arabidopsis phyB mutants displayed accelerated flowering at 22 °C, but not at 16 °C (Halliday and Whitelam, 2003). As with seed germination, a dominant role for phyE was observed at the cooler temperature, although no difference in PHYE abundance was detected between plants grown at 16 °C and 22 °C (Halliday et al., 2003). The molecular events that underlie these temperature-mediated alterations in functional hierarchy are not yet known. However, flowering responses at both temperatures correlated with transcript abundance of the floral activator FLOWERING LOCUS T (FT) (Halliday et al., 2003). Elongated petioles were observed in phyB mutants at both temperatures, suggesting the existence of discrete pathways controlling architectural and flowering responses.

**Phytochrome—signalling**

In addition to flowering, Arabidopsis leaf area responses to a low red:far-red ratio (R:FR) have been shown to display
temperature dependence. At 22 °C, low R:FR-grown plants exhibited characteristic shade avoidance phenotypes, including reduced leaf size, thickness, and biomass. At 16 °C, however, this response was reversed, with low R:FR-grown plants displaying considerably greater leaf area and thickness than high R:FR-grown controls (Patel et al., 2013). Low R:FR treatment at 16 °C, but not at 22 °C, also resulted in an increase in soluble sugars and expression of the C-REPEAT BINDING FACTOR (CBF) regulon of cold acclimation genes (see later), suggesting that temperature and photoreceptor signalling are tightly integrated (Franklin and Whitelam, 2007; Patel et al., 2013).

**Cryptochromes and phototropins**

**Cryptochrome**

Cryptochrome has been shown to act redundantly with phytochrome to regulate plant architecture at warmer temperatures. When grown at temperatures >20 °C, cry1 can compensate for phyB loss by repressing internode elongation in Arabidopsis rosettes (Mazzella et al., 2000). In the presence of cry1, phyB acts redundantly with phyA and phyE to mediate this response (Devlin et al., 1999). At cooler temperatures, however, internode growth is arrested, even in severely photoreceptor-deficient mutants (Mazzella et al., 2000; Halliday and Whitelam, 2003). Temperature dependency of cry function has also been observed in the regulation of flowering time. Mutants deficient in cry1 displayed wild-type flowering behaviour at 23 °C, but were significantly late flowering at 16 °C (Blazquez et al., 2003). In addition, the late flowering phenotype of cry2 (Guo et al., 1998) was significantly exaggerated at 16 °C (Blazquez et al., 2003). Analyses of multiple photoreceptor-deficient mutants showed that the temperature sensitivity of the cry2 phenotype results, in part, from reduced phyA activity at cooler temperature.

**Phototropin**

Few reports exist describing the integration of temperature and phototropin signalling. In ferns, the low-temperature-mediated relocation of chloroplasts from periclinal to anticlinal cell walls was shown to require phot2 (Kodama et al., 2008). No cold-mediated reorientation of chloroplasts was, however, observed in summer-green ferns or Arabidopsis. The authors propose that low temperature chloroplast positioning may be a survival mechanism in some wintering plants (Kodama et al., 2008). The dimerization of Arabidopsis phot1 has been observed to show temperature dependency in vitro, but the physiological significance of these findings remains unexplored (Nakasako et al., 2008).

**Shared signalling components**

Given that light and temperature signals regulate many of the same responses, it is perhaps not surprising that both signals converge on shared downstream regulators. Some of the key molecular components involved in integrating photoreceptor and temperature signalling pathways are discussed below.

**PIF4**

Plants grown at high temperature display phenotypes reminiscent of the shade avoidance syndrome (Casal, 2012). These include elongated hypocotyls and petioles, leaf elongation (hyponasty), and early flowering (Gray et al., 1998; Koini et al., 2009). Such responses are thought to facilitate leaf cooling and accelerate seed set in unfavourable conditions (Gray et al., 1998; Crawford et al., 2012; Bridge et al., 2013). The signalling pathways regulating shade avoidance and high temperature acclimation converge on a shared transcriptional regulator, PHYTOCHROME INTERACTING FACTOR 4 (PIF4) (Lorrain et al., 2008; Koini et al., 2009; Stavang et al., 2009). The PIFs are a subgroup of basic helix–loop–helix (bHLH) transcription factors that physically interact with photochrome (Toledo-Ortiz et al., 2003). Unlike shade avoidance, where redundancy between multiple PIFs is observed, PIF4 dominates in the regulation of high temperature acclimation (Lorrain et al., 2008; Koini et al., 2009; Stavang et al., 2009; Li et al., 2012). PIF4 was originally isolated in a screen for mutants displaying hypersensitivity to red light (Huq and Quail, 2002). In these conditions, photochrome binds PIF4, leading to proteosomal degradation of the transcription factor and hypocotyl growth inhibition. During shade avoidance, low R:FR stabilizes PIF4, driving the elongation growth of plant stems (Lorrain et al., 2008). PIF4 transcript levels display transient elevation following transfer to high temperature (Koini et al., 2009; Stavang et al., 2009; Sun et al., 2012), but mixed reports exist as to whether significant increases in PIF4 protein levels result. These discrepancies may reflect differences in experimental conditions and whether measurements reflect dynamic or steady-state levels (Stavang et al., 2009; Foreman et al., 2011; Kumar et al., 2012; Yamashino et al., 2013). Phosphorylation of PIF4 has been shown to increase at high temperature, suggesting that post-translational modification may contribute to the enhanced PIF4 activity observed in these conditions (Foreman et al., 2011).

The mechanisms through which PIF4 regulates shade avoidance and high temperature acclimation have recently started to emerge. Both processes require elevated auxin biosynthesis (Gray et al., 1998; Tao et al., 2008). Roles for PIF4 and PIF5 in auxin signalling were initially suggested based on transcriptomic analyses (Nozue et al., 2011). It was subsequently demonstrated that at high temperature, PIF4 displays enhanced binding to three genes involved in tryptophan-dependent auxin biosynthesis (TAA1, CYP79B2, and YUCCA8), resulting in enhanced transcript abundance, auxin levels, and elongation growth (Franklin et al., 2011; Sun et al., 2012). Mutants deficient in PIF4 are unable to up-regulate auxin at high temperature and display significantly reduced architectural responses in these conditions (Franklin et al., 2011; Sun et al., 2012). In high R:FR, mutants deficient in PIF4 and PIF5 display a dwarfed architecture. This phenotype is exacerbated in shaded conditions. During shade avoidance, PIF4, PIF5, and PIF7 act redundantly to promote auxin biosynthesis, although the dominance of each appears to depend on the experimental conditions used (Hornscheidt et al., 2012; Li et al., 2012).
In addition to auxin, the hormones GA and brassinosteroids (BRs) are involved in PIF4-mediated light and temperature signalling. DELLAs are growth-repressing proteins that physically bind PIFs, preventing the activation of target genes (de Lucas et al., 2008; Feng et al., 2008). GA degrades DELLAs, promoting PIF-mediated elongation growth (see later). Functional GA and BR signalling is required for PIF4-mediated elongation growth at high temperature (Gray et al., 1998; Stavang et al., 2009). More recently, PIF4 has been shown to bind the BR-activated transcription factor, BZR1, providing a molecular mechanism integrating BR, auxin, and GA signalling during plant adaptation to light and temperature signals (Oh et al., 2012).

A further example of PIF4 integrating light and temperature signals involves the regulation of Arabidopsis flowering time. Overexpression of PIF4 dramatically accelerates flowering, suggesting that PIF4 stabilization in low R:FR may contribute to the early flowering component of the shade avoidance syndrome (Lorrain et al., 2008; Casal, 2012). In short days, PIF4 performs a dominant role in the high temperature-mediated acceleration of flowering (Kumar et al., 2012). In these conditions, PIF4 displays enhanced binding to the floral promoter, FT, elevating transcript abundance (Kumar et al., 2012). The authors propose that PIF4 binding to the FT promoter is facilitated at high temperature by increased accessibility, resulting from decreased H2A.Z occupancy (see above).

HFR1

LONG HYPOCOTYL IN FAR RED (HFR1) performs an important dual function, limiting PIF-mediated elongation growth in low R:FR and high temperature conditions. The hfr1 mutant was initially identified in a screen for seedlings displaying hyposensitivity to far-red (Fairchild et al., 2000; Fankhauser and Chory, 2000). In de-etiolated plants, HFR1 transcript levels increase in low R:FR (Sessa et al., 2005). Mutants deficient in HFR1 were shown to display exaggerated elongation growth in these conditions, suggesting that HFR1 operates as a negative regulator of shade avoidance. This was confirmed by subsequent studies showing that HFR1 binds PIF4 and PIF5, limiting their DNA binding and transcriptional activity (Galstyan et al., 2011; Hornitschek et al., 2012). HFR1 protein levels also increase at elevated temperature in blue light (Foreman et al., 2011). Here, hfr1 mutants displayed exaggerated elongation responses, suggesting that HFR1 imposes significant restrictions on PIF4 activity in warm environments. The importance of multiple photoreceptors in controlling growth at high temperature was further demonstrated by the extreme elongation and reduced biomass observed in phyBcry1 double mutants in these conditions (Foreman et al., 2011).

DELLAs

As discussed previously, the inactivation of key transcription factors in light and temperature responses is also achieved by the binding of DELLA proteins. Targets include PIF3 (Feng et al., 2008), PIF4 (de Lucas et al., 2008), and the BR-induced transcription factors, BZR1 and BES1, placing DELLAs as a node of cross-talk between GA and BR signalling (Bai et al., 2012; Gallego-Bartolome et al., 2012; Li et al., 2012). In Arabidopsis, there are five DELLA proteins, GAI, RGA, RGL1, RGL2, and RGL3 (Cao et al., 2005; Hauvermale et al., 2012). These accumulate in the light and are degraded in prolonged low R:FR (Achard et al., 2007; Djakovic-Petrovic et al., 2007). DELLAs regulate photomorphogenesis throughout the plant life cycle, from the timing of seed germination, through to reproductive development (Cao et al., 2006). During de-etiolation, DELLA-mediated transcriptional repression acts to suppress hypocotyl elongation and promote chlorophyll and carotenoid biosynthesis (Achard et al., 2007; Cheminant et al., 2011), although a temporally regulated growth promotion effect of DELLAs has been observed (Stewart Lilley et al., 2013).

In addition to regulating high temperature signalling, DELLAs act at low temperature to inhibit the growth and flowering of Arabidopsis rosettes. Achard and colleagues showed that these phenotypes could be mimicked at warmer temperature by overexpression of CBF1, a transcription factor involved in cold acclimation and the acquisition of freezing tolerance (Achard et al., 2008). They further demonstrated that increased CBF1 expression in the cold up-regulates GA2-oxidase enzymes, inactivating GA and stabilizing DELLAs to retard development. The flexible modulation of plant growth and development by DELLAs is thought to confer adaptive significance in natural environments, possibly via the reallocation of resources toward stress tolerance (Achard et al., 2008).

HYS

ELONGATED HYPOCOTYL 5 (HYS) is a bZIP transcription factor with a central role in promoting plant photomorphogenesis (Lau and Deng, 2012). Mutants deficient in hy5 display impaired de-etiolation, with significantly elongated hypocotyls and reduced pigment accumulation (Ang and Deng, 1994). HY5 is degraded in the dark by the E3 ubiquitin ligase CONSTITUTIVE PHOTOMORPHOGENESIS 1 (COP1) and SUPPRESSOR OF PHYA-105 (SPA) complex of WD40-domain proteins (Lau and Deng, 2012). In the light, COP1 inactivation by phytochrome and cryptochrome stabilizes HY5 which promotes photomorphogenesis. Transcriptomic studies have shown that HY5 directly binds >9000 genes, affects the expression of >11000, and integrates multiple hormone signalling pathways (Lee et al., 2007; Lau and Deng, 2010; H. Zhang et al., 2011). HY5 additionally modulates phyA signalling through negatively regulating transcript abundance of the phyA nuclear transport proteins FAR-RED ELONGATED HYPOCOTYL 1 (FHY1) and its homologue FHY1-LIKE (FHL) (Li et al., 2010). In UV-B, HY5 acts redundantly with a closely related protein, HY5 HOMOLOG (HYH), to control both photomorphogenic and protective responses (Brown and Jenkins, 2008). In contrast to classical photomorphogenesis, where COP1 and HY5 act antagonistically, COP1 and HY5 act together to promote UV-B signalling (Favory et al., 2009). In these conditions,
HY5 positively regulates COP1 transcription, providing a positive feedback loop (Huang et al., 2012).

In addition to photomorphogenesis, HY5 performs a central role in low temperature signalling. Transcriptomic profiling of Arabidopsis wild-type and hy5 mutants exposed to 4 °C showed HY5 to regulate ~10% of cold-induced genes (Catala et al., 2011). Fewer than half of the cold-induced targets were also light regulated, suggesting that HY5 regulates both distinct and overlapping regulons. HY5 levels increased at low temperature, through both elevated transcription and protein stabilization resulting from the nuclear exclusion of COP1 (Catala et al., 2011). Freezing tolerance assays showed hy5 mutants to display a higher LT 50 (the temperature at which 50% lethality is observed), confirming that HY5 acts as a positive regulator of cold acclimation. The sensitivity of hy5 mutants to freezing temperatures was attributed, in part, to reduced anthocyanin production, leading to elevated levels of reactive oxygen species (ROS) (Catala et al., 2011; Y. Zhang et al., 2011). Anthocyanins have been suggested to enhance plant freezing tolerance in multiple species, but the underlying mechanisms remain unclear (Chalker-Scott, 1999). Zhang and colleagues further demonstrated that GA can reduce low temperature-induced anthocyanin production (Y. Zhang et al., 2011). In the cold, HY5 acts redundantly with HYH1 to up-regulate GA2ox1, thereby reducing GA levels and enhancing protective anthocyanin accumulation alongside growth restraint (Achard et al., 2008; Y. Zhang et al., 2011).

The circadian clock

Circadian clocks are internal time-keeping machines that generate rhythms with a periodicity of ~24h, allowing the synchronization of signalling and physiological events to favourable times of the day (reviewed by Nagel and Kay, 2012; Troncoso-Ponce and Mas, 2012). The circadian clock comprises three interlocking feedback loops (Zhang and Kay, 2010). The so-called morning loop involves two Myb-related transcription factors CIRCADIAN CLOCK ASSOCIATED (CCA1) and LONG ELONGATED HYPOCOTYL (LHY) that activate their repressors PSEUDO RESPONSE REGULATORS PRR9, PRR7, and PRR5. Evening loop genes include TIMING OF CAB EXPRESSION (TOC1), GIGANTEA (GI), ZEITLUPE (ZTL), and the evening complex (EC) formed by LUX ARRTHMO (LUX), EARLY FLOWERING 3 (ELF3), and EARLY FLOWERING 4 (ELF4) (Pokhilko et al., 2012). This loop is driven through mutual repression between TOC1/GI and EC. The current Arabidopsis circadian clock model proposes a central repressilator structure that connects the morning and evening loops through the sequential repression (represented by the symbol |–): CCA1/LHY |– PRR |– EC |– CCA1/LHY, whereby each component represses the expression of preceding components and is repressed by subsequent components (Pokhilko et al., 2012).

One of the hallmarks of the circadian clock is its ability to operate robustly when subject to unpredictable variations in temperature (McClung and Davis, 2010). This buffering against environmental variations contrasts with the need to remain sensitive to light and temperature changes to entrain the clock accurately. This section explores how the clock is able to cope with these apparently conflicting requirements.

Clock temperature compensation

Biochemical reaction rates are temperature sensitive, yet the speed of the circadian clock is remarkably stable over a broad temperature range (Gardner and Feldman, 1981; Mattern et al., 1982; Edwards et al., 2005). This property of the clock is widely known as temperature compensation (McClung and Davis, 2010). Part of this compensating mechanism is counterbalance control of morning and evening loop components (Gould et al., 2006). At higher physiological temperatures, the amplitude of LHY expression decreases, but this is balanced by a complementary rise in TOC1. Interestingly, the cca1 mutation was shown to have a larger impact on CAB::LUC rhythmicity at 12 °C compared with higher temperatures, suggesting that CCA1 had a more prominent role in regulating target genes at cooler temperatures.

Genetic analysis of the morning loop has aided our understanding of temperature compensation. PRR9 and PRR7 act as transcriptional repressors of CCA1 and LHY that respond preferentially to warm temperatures (Nakamichi et al., 2010; Salome et al., 2010). This is evident in the prr7;prr9 double mutant where, in contrast to the wild type, the period of CCA1::LUC lengthens dramatically with increasing temperature in the 12–27 °C range (Salome et al., 2010). Depletion of either CCA1 or LHY by artificial micro RNA (amiRNA) knockdown partially restores periodicity to prr7;prr9 plants. This illustrates that PRR7 and PRR9 suppression of CCA1 and LHY is required to maintain a stable period when temperatures rise. Another temperature-buffering mechanism was uncovered by analysing the effects of protein kinase CK2 that phosphorylates CCA1 in the modulation of its activity (Sugano et al., 1998; Daniel et al., 2004; Portoles and Mas, 2010). High temperatures have been shown to enhance CCA1 binding to the morning genes PRR9 and PRR7, and the evening genes TOC1 and LUX. However, CK2 can counteract CCA1 activity by reducing the ability of CCA1 to bind to target promoters (Salome et al., 2010).

Light is implicated in temperature compensation

A recent systems biology study demonstrated a prominent role for light receptors in temperature buffering of the clock. Statistical analysis of an extensive data set that quantified the impact of genetic, light quality, and temperature perturbation on period length identified strong coupling between the temperature and light input pathways to the clock (Gould et al., 2013). Model analysis supported the hypothesis that light and temperature signalling converge at common clock parameters. In this study, red and blue light photoreceptors were shown to act in opposition to control period length in warmer conditions. CRY1 was identified as the principal blue light receptor regulating the circadian period at 27 °C. A non-intuitive prediction that LHY protein levels rose with temperature was validated experimentally. As LHY is an activator of PRR9, this finding provided a potential explanation for an observed rise in PRR9...
transcript levels at higher temperature. This work illustrates that the collective photoreceptor action is required to maintain period length at higher temperatures and it identifies key temperature-activated processes in the clock. Interestingly, a recent theoretical study using balance equations indicates that buffering of the free-running period to light or temperature arises as a consequence of phase buffering (Domijan and Rand, 2011). As temperature compensation is often studied under constant conditions, this work illustrates the relevance of thermal buffering to clock function under more natural diurnal conditions.

Clock sensitivity to light and temperature signals

While it is imperative that the circadian clockwork is resistant to temperature change, elements of sensitivity to incoming light and temperature signals are necessary for entrainment across the seasons. The main entraining photoreceptors are the phytochromes, the cryptochromes, and the family of LOV-domain F-box proteins including ZTL (Somers et al., 1998; Devlin and Kay, 2000; Yanovsky et al., 2000; Imaizumi et al., 2003; Kim et al., 2007; Baudry et al., 2010). Light has multiple entry points to the oscillator (Pokhilko et al., 2012). It modulates the clock through transcriptional control of CCA1, LHY, PRR9, GI, and ELF4 (Kikis et al., 2005; Nakamichi et al., 2005; Ito et al., 2007; McWatters et al., 2007; Yamashino et al., 2008; Wenden et al., 2011; Hemmes et al., 2012). PRR5, PRR7, and TOC1 protein levels are subject to strong diurnal regulation with increased abundance during the daytime (Mas et al., 2003; Farre and Kay, 2007; Kiba et al., 2007; Fujiwara et al., 2008). The stability of PRR5 and TOC1 has been shown to be modulated directly by ZTL that mediates their proteolytic degradation in darkness (Mas et al., 2003; Kiba et al., 2007; Fujiwara et al., 2008). ELF3 has been implicated in the gating light input to the circadian oscillator (McWatters et al., 2000). The stability of the EC protein ELF3 is controlled by CONSTITUTIVELY PHOTOMORPHOGENIC 1 (COP1) E3 ligase, while levels of GI are modulated by the COP1–ELF3 complex (Yu et al., 2008). These studies, which are by no means exhaustive, illustrate that the clock is strongly regulated by light through transcriptional and post-transcriptional mechanisms.

An important feature of the clock is that it can be entrained by either light or temperature cycles (Salome and McClung, 2004). In terms of thermocycle entrainment cues, high temperatures appear to be equivalent to daylight and low temperatures are interpreted as darkness (McClung, 2006). This suggests that light and temperature signals may converge at some shared entrainment mechanisms. Indeed, PRR7, PRR9, ELF3, and ZTL, that are known to be light regulated, have also been implicated in the maintenance of periodicity by thermal cycles (McWatters et al., 2000; Kevei et al., 2006; Thines and Harmon, 2010; James et al., 2012b).

Temperature-activated isoform switching

Alternative splicing is another mechanism through which environmental shifts in temperature are perceived (Filichkin and Mockler, 2012; James et al., 2012a; Steager and Brown, 2013). In the clock, the CCA1 gene is subject to alternative splicing, resulting in two splice variants: full-length CCA1α, and CCA1β that makes a protein lacking the MYB DNA-binding domain. CCA1β acts as a dominant-negative regulator interfering with the formation of functional CCA1α and LHY homodimers, as well as CCA1α–LHY heterodimers and preventing DNA binding (Park et al., 2012; Seo et al., 2012). High (37 °C) temperature elevated levels of CCA1β transcript compared with CCA1α, while exposure to cold (4 °C) boosted CCA1α and suppressed CCA1β. The cold repression of CCA1β was shown to be important to permit CCA1α accumulation and transcriptional control of CBF genes that promote freezing tolerance (see later). LHY and PRR7 also appear to be subject to temperature-dependent alternative splicing. Shifts from 20 °C to 4 °C led to the production of non-functional variants and nonsense-mediated transcript decay (James et al., 2012a). Another study has demonstrated that alternative splicing is a widespread phenomenon amongst clock genes (Filichkin and Mockler, 2012). For CCA1 and LHY, generation of transcripts with in-frame premature stop codons was shown to be temperature dependent, suggesting that alternative splicing may be involved in temperature compensation.

Temperature-activated alternative splicing has also been reported for the central clock gene FREQUENCY (FRQ) in Neurospora crassa. At cool temperatures, a short form (s-FRQ) predominates, while in warmer conditions a long form (l-FRQ) is more abundant (Liu et al., 1997; Diernfellner et al., 2005). In this way, the thermosensitive isoforms s-FRQ and l-FRQ are proposed to maintain clock function across temperatures. Modelling has shown that this relatively simple counterbalance mechanism is an effective means of achieving control over a broad temperature range (Akman et al., 2008). It is evident from work on Arabidopsis that temperature-activated isoform switching is a widely exploited mechanism that accomplishes a range of signalling responses to temperature (Kalyna et al., 2012; Syed et al., 2012).

Central splicing components integrate light–temperature signals

PRMT5 encodes a type II protein arginine methyltransferase that targets spliceosomal proteins, modulating a range of splicing events. While PRMT5 participates in extensive splicing regulation, it also has a specific role in the circadian clock, at least in part through controlling PRR9 splicing (Hong et al., 2010; Sanchez et al., 2010). PRMT5 may provide a portal through which light and temperature can regulate alternative splicing as PRMT5 expression is sensitive to both these external signals (Hong et al., 2010). Additionally, the splicing factor SNW/SKI INTERACTING PROTEIN (SKIP) appears to be required for temperature and light input to the clock. SKIP has been implicated in a variety of wide-ranging splicing events, including the alternative splicing of PRR7 and PRR9 (Wang et al., 2012).

Circadian gating of light and temperature cues

Cold acclimation

Due to circadian control, stimuli of the same strength applied at different times of the day can result in different magnitudes
of response, a phenomenon known as circadian ‘gating’ (Mas and Yanovsky, 2009). Light and low temperature are amongst the wide spectrum of responses that are gated by the clock (Mas, 2008). Cold acclimation allows plants to increase their freezing tolerance following a priming period of low temperatures (Thomashow, 1999; Browse and Xin, 2001; Miura and Furumoto, 2013). Exposure to cold leads to the rapid induction of CBF1, CBF2, and CBF3 followed by CBF-targeted genes (the CBF regulon) (Gilmour et al., 1998; Browse and Xin, 2001; Thomashow, 2001, 2010; Medina et al., 2011). However, the extent to which cold induces CBF expression varies according to the time of day at which the plants were exposed to low temperature (Fowler et al., 2005). A number of studies have shown that CBF genes are themselves regulated by the clock (Salome and McClung, 2005; Pruneda-Paz and Kay, 2010; Maibam et al., 2013). Instrumental in this process are LHY and CCA1 that directly bind to CBF promoters (Dong et al., 2011). Consistent with this regulation, the prr5;prr7;prr9 triple mutant has elevated levels of cold stress genes (i.e. DREB1, LTI1, LTI30, ATGOL3, and ADS2) and increased freezing tolerance (Nakamichi et al., 2009). Recent mathematical modelling has further identified that the EC and TOC1 act as regulators of CBF3 expression, in addition to the transcriptional activator INDUCER OF CBF EXPRESSION 1 (ICE1) (Chinnusamy et al., 2003; Zhou et al., 2011; Keily et al., 2013).

Photoperiod and light quality are instrumental regulators of the cold acclimation pathway (Kim et al., 2002; Lee and Thomashow, 2012). When grown in daily light/dark cycles, expression of the CBF regulon genes peaks at ~8h, but the amplitude of this peak is greatly increased in short days relative to long days (Lee and Thomashow, 2012). Interestingly, the action of phyB, PIF4, and PIF7 is required to repress the peak of expression in long days. In this way freezing tolerance is enhanced in short days relative to long photoperiods. Other studies have shown that light quality can have a dramatic impact on the CBF response, but, similarly to cold temperature induction, this is dependent on the clock. Exposure to bursts of low R:FR light that deactivate phytochrome significantly increases the amplitude of the CBF rhythm (Franklin and Whitelam, 2007). This dramatic response depends upon phyB and phyD inactivation. Furthermore, plants grown under low R:FR significantly increased freezing tolerance, illustrating that light quality can have a substantial impact on the priming of cold acclimation.

**External coincidence—interactions between clock outputs and light/temperature**

Interactions between environmental signals and circadian clock outputs form the basis of the phenomenon known as external coincidence, first introduced by Erwin Bunning (Pittendrigh, 1972). In plants, external coincidence has been most extensively described in terms of the photoperiodic control of developmental responses such as hypocotyl elongation and floral induction. PIF4 and PIF5 have recently emerged as key components for the external coincidence control of hypocotyl elongation (Nozue et al., 2007; Kunihiro et al., 2011; Nomoto et al., 2012, 2013; Yamashino et al., 2013). When *Arabidopsis* plants are grown in short-day conditions, *PIF4/PIF5* transcript abundance is rhythmic, with a peak occurring at the end of the long night (Nozue et al., 2007; Kunihiro et al., 2011; Nomoto et al., 2013). This peak coincides with a fall in activity of the EC (ELF3–ELF4–LUX) that binds directly to PIF promoters via the LUX-binding domain to suppress transcription at the start of the night (Nusinow et al., 2011). Light promotes the degradation and inactivation of PIFs. PIF4 and PIF5 are thought to regulate diurnal hypocotyl elongation by up-regulating the expression of auxin-associated genes (GH3.5, IAA19, and IAA29), GA-associated genes (GA1), BR-associated genes (*BR60x2*), ethylene-associated genes (*ACS8*), cytokinin-associated genes (*CKX5*), and genes encoding cell wall-modifying enzymes (*XTR7*) (Nomoto et al., 2012). Hence hypocotyl expansion occurs just before dawn in short days, when the intrinsic rhythm of *PIF4/5* expression coincides with darkness. In long days, the peak in *PIF4/5* expression occurs during the light period, where degradation by phyB prevents the induction of hypocotyl growth (Kunihiro et al., 2011). Recently, this external coincidence model has been extended to account for the observation that high temperatures induce hypocotyl expansion in a *PIF4* dependent manner, even in long days. To account for this, a ‘dual coincidence’ model has been proposed whereby high temperatures can shift the peak of *PIF4* expression to coincide with darkness in long-day conditions (Yamashino et al., 2013). Hence, temperature is effective at modulating the interactions between light and circadian clock outputs in external coincidence signalling. An interesting parallel to this phenomenon is the PIF4-mediated thermal induction of flowering in short days. *Arabidopsis* is a facultative long-day plant, meaning that flowering is accelerated by lengthening photoperiods. This relies upon an external coincidence mechanism driving the expression of the *FT* inducer, *CONSTANS* (*CO*), in long days. Persistent warm growth temperatures can, however, over-ride this long-day requirement to induce flowering in short days. As described previously, the thermal induction of flowering involves increased binding of PIF4 to the *FT* promoter at high temperatures (Kumar et al., 2012). This once again demonstrates that temperature signalling can alter responses driven by external coincidence between light and the clock.

**Independent actions of light and temperature on flowering control**

**Vernalization—floral competence**

The transition from vegetative to reproductive growth is a critical event which could be considered the most important stage of a flowering plant’s life cycle. Accordingly, floral induction is precisely tuned to the environment to maximize reproductive success, and light and temperature signalling play key roles in this process. Previously we described how light and temperature signals converge on the common component, PIF4, to regulate expression of *FT* (see above). This
provides a highly tuneable system, crucial for coupling the flowering response to the current environment. Certain species (termed ‘winter annuals’) require a prolonged period of cold to reach floral competence, followed by warming temperatures and lengthening photoperiods to induce flowering (Henderson and Dean, 2004). This represents an additional level at which light and temperature are integrated to control flowering, only this time acting through distinct pathways.

The phenomenon of cold-induced floral competence is known as vernalization and has long been recognized as an epigenetic response (Gendall et al., 2001; Bastow et al., 2004; Schmitz and Amasino, 2007). In vernalization-sensitive Arabidopsis plants, the response involves the de-repression of key genes required for floral development by the epigenetic silencing of the MADS box transcriptional suppressor FLC (FLOWERING LOCUS C) (Searle et al., 2006). FLC silencing is triggered by a period of prolonged cold and is mediated by several independent mechanisms which occur at the apical meristem (Lang, 1965). An early response to chilling involves the expression of non-coding transcripts at the FLC locus including a sense transcript named ‘COOLAIR’ and an antisense transcript called ‘COOLAIR’ (Swiezewski et al., 2009). It has recently been shown that the initiation of COOLAIR transcription involves cold-induced disruption of an inhibitory gene loop (Crevillen et al., 2013). A proposed function of these non-coding transcripts is to recruit the histone-modifying enzyme FLD (FLOWERING LOCUS D), which removes active histone marks at FLC by de-methylation of the H3 histone tail at Lys4 (H3K4) (Liu and Mara, 2010). In parallel to this, chilling promotes the transcription of a DNA-binding protein, VIN3 (VERNALISATION INSENSITIVE 3). VIN3 localizes to the promoter and first exon of the FLC locus (Sung and Amasino, 2004). When in position, VIN3 recruits additional chromatin-remodelling factors such as VRN2, VRN5, and SWINGER, which together comprise a plant homeodomain polycomb-repress complex (PHD–PRC2) capable of adding the transcriptionally repressive H3K27me3 histone marks to the FLC locus by tri-methylation of Lys27 of histone 3. Importantly, during the chilling period, these H3K27me3 marks only appear at the nucleation site on the FLC locus, where VIN3 initially binds (Angel et al., 2011). A return to warm temperatures is required for the H3K27me3-mediated silencing to spread across the FLC locus to ensure stable silencing of FLC transcription, a process shown to be dependent on VRN2 and VRN5 (Gendall et al., 2001).

Furthermore, the ‘epigenetic memory’ of the vernalization is extremely sophisticated as it allows the duration of the chilling period into the response to the effect that longer cold periods result in more extensive FLC silencing upon return to warm temperatures (Shindo et al., 2006; Angel et al., 2011; Coustham et al., 2012). It was recently shown, through a combination of experimental and modelling approaches, that the duration of the cold period is measured by a population-averaging process of cells at the apical meristem (Angel et al., 2011). In brief, longer periods of cold result in a higher proportion of cells initiating FLC silencing. As histone marks can propagate through mitotic divisions, growth of the meristem amplifies the initial conditions of the proportion of cells with FLC silencing, allowing this to be used as an accurate measure for the duration of the cold period (Angel et al., 2011; Song et al., 2012).

Floral induction

While vernalization represents a very precise and elegant temperature-sensing mechanism involved in the flowering control of certain species, it is only necessary but not sufficient to induce flowering. This is because it removes the inhibition of flowering but does not actively induce positive floral regulators per se. For this, inputs from separate light and temperature signalling pathways are required. These include: photoperiodic control of FT via external coincidence; induction of FT by light and temperature via PIF4; and the activation of GA signalling to enhance expression of floral-inducing genes such as LFY (LEAFY) and SOC1 (SUPPRESSOR OF CO1). These responses occur primarily in leaves, whereas vernalization occurs at the shoot apex (Lang, 1965). The various inputs to floral induction are summarized in Fig. 1. Importantly, these processes are all distinct from vernalization as they are separated both temporally and spatially and share few common components. This parsing of light and temperature signalling can be viewed as an evolutionarily adaptive trait as it allows the two pathways to act in a permissive manner; light and warm temperature-mediated induction is ineffective without prior competence conferred by vernalization. Hence, the dual inputs of light and temperature ensure that flowering (in vernalization-requiring species) is tightly controlled to occur only at precisely the most optimal time of year and is not falsely initiated by environmental fluctuations such as an early ‘cold snap’ or by thermal- or photo-inductive conditions when the plant is too young. Interestingly, the legume Medicago truncatula has a different vernalization mechanism from that of Arabidopsis and does not appear to contain any FLC homologues in its genome. Genetic studies have, however, indicated that vernalization acts independently of floral induction (Jaudal et al., 2013). Hence, even in species with evolutionarily distinct mechanisms of vernalization, there appears still to be a clear divide between thermally induced floral competence and light and temperature inductive processes, supporting the notion that this separation of signalling is an adaptive trait.

Summary

It is logical that light and temperature responses are associated, as darkness is normally accompanied by cooler temperature and light by warmth. It could be argued, therefore, that one possible evolutionary driver for the convergence of light and temperature signalling is signal redundancy. For example, diurnal light and temperature cycles in nature will often occur concurrently (namely warm and light during the day, and cold and dark at night). However, the association between light and temperature is not simple when considering seasonal responses, latitudinal effects, or daily fluctuations in light/temperature. A second advantage of integrating light...
and temperature signals is that it allows for a finely tuned response to be achieved. Given that minor changes in light and temperature can dramatically alter the physiology of plants, it is important that responses to each signal are modified by the other. This is illustrated by the fact that the outcome of photoperiodic responses such as hypocotyl extension or flowering induction by long days is completely dependent on the ambient temperature. Additionally, light and temperature can improve the fidelity of responses by signalling through distinct pathways to control a common output. This could be considered a form of ‘environmental licensing’ where, in the flowering example given, vernalization results in competence to respond to photoinductive signals. Based on the mounting evidence for a close association between light and temperature signalling, along with the growing appreciation for taking a systems (rather than reductionist) approach of studying biology, we speculate that advances in signal integration will be central to achieving a comprehensive understanding of plant growth and survival in a changing environment.

Acknowledgements
GT-O is supported by FP7-CIG (PCIG11-GA-2012-321649) and the UK Biotechnology and Biological Sciences Research Council (BBSRC) grant BB/F005237/1 (ROBuST project) awarded to KJH. DEP is supported by the BBSRC EastBio DTP award BB/J01446X/1. KAF is supported by a Royal Society University Research Fellowship.

References


Brown BA, Jenkins GI. 2008. UV-B signaling pathways with different fluence-rate response profiles are distinguished in mature Arabidopsis leaf tissue by requirement for UVR8, HYS, and HYH. Plant Physiology 146, 576–588.


Delvin PF, Kay SA. 2000. Cryptochromes are required for photchrome signaling to the circadian clock but not for rhythmicity. The Plant Cell 12, 2490–2510.


Kikis EA, Khanna R, Quail PH. 2005. ELF4 is a phytochrome-regulated component of a negative-feedback loop involving the central oscillator components CCA1 and LHY. The Plant Journal 44, 300–313.


Sun J, Qi L, Li Y, Chu J, Li C. 2012. PIF4-mediated activation of YUCCA8 expression integrates temperature into the auxin pathway in regulating arabidopsis hypocotyl growth. The Plant Journal 8, e1002594.


Troncoso-Ponce MA, Mas P. 2012. Newly described components and regulatory mechanisms of circadian clock function in Arabidopsis thaliana. Molecular Plant 5, 545–553.


