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Perception and signalling of light and temperature cues in plants

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SUMMARY

Light and temperature patterns often correlate in natural plant growth conditions. In this review, we analyse the perception and signalling mechanisms shared by both of these environmental cues and discuss the functional implications of their convergence to control plant growth. The first point of integration is the phytochrome B (phyB) receptor, which senses light and temperature. Downstream of phyB, the signalling core comprises two branches, one that involves PHYTOCHROME INTERACTING FACTOR 4 (PIF4) and the other, CONSTITUTIVE PHOTOMORPHOGENIC 1 (COP1) and ELONGATED HYPOCOTYL 5 (HY5). The dynamics of accumulation and/or localization of each of these core signalling components depend on light and temperature conditions. These pathways are connected through COP1, which enhances PIF4 activity. The circadian clock modulates this circuit, since EARLY FLOWERING 3 (ELF3), an essential component of the evening complex (EC), represses *PIF4* gene expression and PIF4 transcriptional activity. Phytochromes are probably not the only entry point of temperature into this network, but other sensors remain to be established. The sharing of mechanisms of action for two distinct environmental cues is to some extent unexpected, as it renders these responses mutually dependent. There are nonetheless many ecological contexts in which such a mutual influence could be beneficial.

INTRODUCTION

Plant life is compromised by light and temperature conditions above or below an optimal range. In the absence of behavioural responses with which to elude extreme environmental conditions, plants have evolved complex mechanisms to perceive the informational cues

provided by changes in light and temperature; they use these signals to adjust their metabolism and body form to withstand unfavourable environments and thus to minimise damage. These processes are termed photo- and thermomorphogenesis.

Although many physiological processes respond to both light and temperature signals, the molecular mechanisms of photo- and thermomorphogenesis have often been analysed separately. Nonetheless, several studies focussed on temperature-mediated responses identified key players with a known role in light signalling (Heggie and Halliday, 2005; Franklin, 2009; Franklin *et al.*, 2014; Lorenzo *et al.*, 2016; Penfield, 2008). This review examines the patterns of cell elongation control by combined light and temperature cues, their molecular mechanisms, and their functional implications. Light and temperature conditions are not independent, which means that some combinations are more likely than others. One of the current challenges in molecular biology is to identify the mechanisms plants use to take advantage of such correlations, and reinforce or counteract the effect of a given environment.

Association between light and temperature cues

The temperature of plant tissues can be modelled as a function of air temperature, radiation, wind, and vapour deficit (Campbell and Norman, 2012). Although a proportion of the incoming radiation is reflected or transmitted, the photosynthetic pigments and other structures of green leaves strongly absorb intercepted radiation between 400 and 700 nm. Part of this energy is used in the photosynthetic process itself, and part can be re-emitted as fluorescence; the remainder generates heat that increases leaf temperature. Compared to tissues exposed to full sunlight, those shaded by neighbours experience not only reduced light signal input, but also reduced temperature (Figure 1). Besides this direct impact, radiation also makes an indirect contribution by raising air temperature and, in turn, plant tissue temperature. This is demonstrated by the correlation between the solar radiation that reaches the Earth's surface and oscillations in air temperature during daily, seasonal and even longer-term global fluctuations (Wang and Dickinson, 2013).

The link between light and temperature has often-neglected consequences. For instance, we applied a model to estimate the rate of hypocotyl elongation in open areas and beneath a canopy (Figure 1) (Legris *et al.*, 2016). If we consider only the light conditions (temperature was held constant at the values observed outside the canopy), the growth rate beneath the canopy was predicted to be eight-fold that observed outside of shade. If the actual temperature of the tissues under the canopy is considered, however, the growth ratio is reduced to four-fold; this is still significantly higher than in open places, but is half the ratio predicted when the real difference between the two conditions is not taken into account.

Other conditions can generate deviations from this general correlative trend. Cold wind, for example, can reduce temperature without affecting the light environment, whereas selective far-red light reflection by green tissues can generate changes in the light input signals without affecting temperature patterns. This adds further complexity to the prediction of elongation rates in seedlings grown in a natural habitat.

Overview of physiological outputs controlled by light and temperature

Changes in plant organ position in the vertical axis relative to the soil (height/depth), in the horizontal axis relative to neighbouring plants (distance), and in the temporal axis through the seasons cause large modifications in environmental conditions and can impose varied challenges on plants.

The positions of plant organs, buried in the soil or exposed to the aerial environment, involve different risks. For seeds, germination at a position deep within the soil increases the risk of reserve exhaustion before emergence, whereas germination above the soil increases desiccation risk. For seedlings, the etiolated developmental pattern (limited foliage expansion, rapid axis growth, and rudimentary synthesis of the photosynthetic and photoprotective machineries) facilitates their path through the soil but is a counterproductive strategy after emergence of aerial organs, which must initiate a photoautotrophic phase.

The distance and size of neighbouring plants determine the type of stress the plant will suffer. If a plant is exposed to intense neighbouring shade, it will receive limited light input for photosynthesis, but in open areas it is more likely to undergo heat and oxidative stress caused by the high radiation load, and to be exposed to greater wind impact.

Annual seasons can bring more favourable or more stressful weather conditions, including exposure to extreme temperatures, radiation load and/or drought. If seeds germinate in the wrong season, seedlings will be exposed to adverse conditions. Flowering time is also finely tuned, as the reproductive stage is a particularly sensitive developmental phase.

For each of these settings, both light and temperature patterns provide essential informational cues that guide plant adjustment to prevailing environmental conditions (Fig. 2, boxes). Light penetrates the soil very poorly, and the amplitude of temperature oscillation (difference between daily maximum and minimum temperatures) decreases with soil depth (Campbell and Norman, 2012); thus, light and temperature cues provide complementary data about position relative to the soil surface. Differences in incoming irradiance, spectral composition (red/far-red ratio), light direction and temperature supply information about the canopy cover. Photoperiod, irradiance and ambient temperatures provide information about the season.

The plant life cycle is closely coordinated with environmental conditions thanks to plant perception of the associated light and temperature cues. Processes such as seed germination (Casal and Sánchez, 1998; Footitt *et al.*, 2013), seedling de-etiolation (Arsovski *et al.*, 2012; Karayekov *et al.*, 2013), vegetative growth (Casal, 2013; Quint *et al.*, 2016), acclimation to low temperatures (Chew and Halliday, 2011; Lee and Thomashow, 2012) and flowering (Andrés and Coupland, 2012) respond to light and temperature signals, which optimise their timing and intensity (Fig. 2).

Growth responses to light and temperature

In the following sections, we will focus on a single physiological trait, the growth of the stem. Elongation of the embryonic stem (hypocotyl) is rapid in darkness and is inhibited by light during de-etiolation (Arsovski *et al.*, 2012), after which plants become particularly sensitive to the stem growth-promoting light signals of neighbouring plants (Casal, 2013). Increasing temperatures promote stem growth above chilling and below heat stress temperatures. The hypocotyl growth response to light and temperature cues is mediated by changes in cell expansion, rather than cell division. The stem growth rate varies significantly at different combinations of light (irradiance) and temperature conditions (Fig. 3). The close connection between the effects of these environmental cues is evident, as temperature in fact increases the response to light. The hypocotyl growth rate decreases at higher irradiances, with a steeper slope as temperature rises between 10 and 25ºC. Within this range, higher temperatures also increase growth rate at low irradiances (Fig. 3).

Moderately warm, constant ambient temperatures tend to antagonise light signals in the control of stem growth, but transient elevated temperatures (including heat shock) enhance the inhibition of light-induced hypocotyl growth (Karayekov *et al.*, 2013). When etiolated seedlings are exposed to daily pulses of warm temperatures in darkness, they become more light-sensitive at the time of day these heat events took place on previous days.

A surprising deviation from classical inhibition of hypocotyl elongation is observed when etiolated Arabidopsis seedlings are grown under continuous red light at 27°C. Rather than the typical linear negative relationship between hypocotyl length and log irradiance of red light at 17°C, growth shows a biphasic response at 27°C (Johansson *et al.*, 2014). At warm temperatures, growth decreases with irradiance to reach a minimum by 1 $\mu mol \cdot m^{-2} \cdot s^{-1}$ continuous red light, beyond which higher irradiance promotes growth. This phenomenon, termed photothermal switch (Johansson *et al.*, 2014), is not observed under continuous blue or white light (Johansson, 2013).

In addition to the average temperature, its daily pattern of variation is also important for control of stem growth. Days are normally warmer than nights, which results in a positive correlation between light and temperature. When plants are cultivated in conditions of negative difference between day and night temperatures (–DIF, night warmer than day), stem growth is reduced (Bours *et al.*, 2013; Bours *et al.*, 2015). In plants exposed to warm days and cold nights, hypocotyl growth occurs both day and night; in the opposite temperature regime, growth occurs mainly during the night, with little growth during the cold day.

PERCEPTION OF LIGHT AND TEMPERATURE CUES

Phytochrome B

Arabidopsis phytochrome B (phyB) was originally characterised as a photosensory receptor with a 1172-amino-acid apoprotein (PHYB, ~125 kDa) fused to a linear tetrapyrrole chromophore (phytochromobilin, PΦB) (Burgie and Vierstra, 2014). In Arabidopsis, the *PHYB* gene is expressed in all organs and at all developmental stages. PHYB is synthesized in the cytoplasm, and its spontaneous attachment to the chromophore gives rise to the inactive phyB holoprotein (Pr form). phyB Pr absorbs maximally in red light, which triggers the conformational change of Pr into the active Pfr form (k1, the rate constant for Pr to Pfr photoconversion; Fig. 4), the biologically active form that inhibits hypocotyl growth (among many other responses). phyB is thus very well suited to perceive the transition between full darkness (buried seedlings) and light that reaches open places (red light-rich) to trigger de-etiolation after shoot emergence from the soil.

The Pfr form absorbs maximally in far-red light, and excited Pfr relaxes into the Pr form (k2, the rate constant for Pfr to Pr photoconversion; Fig. 4). Green leaves reflect and transmit far-red more efficiently than red light, which is absorbed by photosynthetic pigments. Under the shade of neighbours, plants are therefore exposed to larger proportions of far-red light (low red/far-red ratio) than in open-field conditions. This light environment lowers the proportion of

Pfr and releases its inhibition of hypocotyl growth. phyB is thus very well suited to perceive the drop in the red/far-red ratio caused by the presence of neighbouring vegetation.

Arabidopsis phyB Pfr also reverts spontaneously to Pr in a reaction that does not require light, referred to as dark or thermal reversion (kr1, kr2; Fig. 4). Since thermal reversion counteracts Pfr formation, more light is needed to maintain a given level of the active form; that is, the system becomes more irradiance-dependent. In addition, the phyB Pfr level will gradually decrease in darkness due to continued thermal reversion in the absence of light reactions. phyB thus perceives changes in irradiance and length of night.

Early studies suggested a possible role for phytochrome in sensing temperature conditions (reviewed by Penfield, 2008). The phyB thermal reversion rate increases with temperature between 4 and 30°C (Jung *et al.*, 2016; Legris *et al.*, 2016) (Fig. 4). As a result, more light is needed to maintain a given Pfr level at warmer than at cooler temperatures during the day, and the Pfr level will decrease more rapidly during warm nights. phyB is therefore also a temperature sensor in light-grown plants (Jung *et al.*, 2016; Legris *et al.*, 2016).

phyB forms Pr-Pr or Pfr-Pfr homodimers or Pr-Pfr heterodimers *in vivo*. Recent studies involving spectroscopic measurements of phyB *in vivo*, combined with mathematical simulation, concluded that the thermal reversion rate of Pfr-Pfr to Pr-Pfr (kr2) is slow, but from Pr-Pfr to Pr-Pr (kr1) is substantially more rapid (Klose *et al.*, 2015). By modelling the relationship between the levels of different phyB pools and phyB inhibition of hypocotyl growth, the authors attributed phyB activity to the Pfr-Pfr dimer (Klose *et al.*, 2015).

Bioactive Pfr-Pfr levels are affected by temperature both during the day (Legris *et al.*, 2016) and the night (Jung *et al.*, 2016), but the mechanisms involved in each case are not exactly the same. For the night period, the Pfr-Pfr achieved at the end of the day will revert to Pr-Pfr at a kr2-dependent rate. Since the Pr-Pfr heterodimeric form reverts rapidly to Pr-Pr (kr1), the Pfr-to-Pr reversion rate in darkness is twice the kr2 value. During the night, phyB temperature perception depends on the effect of temperature on kr2; as a result, Pfr, which interacts with the

promoter regions of selected target genes, reduces this association in plants grown at warm temperatures during the night (Jung *et al.*, 2016).

The thermal reversion rate that dominates in darkness (kr2) is too slow to compete with light reactions. Under continuous irradiation, however, Pfr steady-state levels are lower than predicted based on photoconversion (k1, k2) and the slow thermal reversion rate (kr2). This difference has been explained by a faster reversion rate when Pfr is part of a Pr-Pfr heterodimer (Klose *et al.*, 2015). In darkness, this pool has a negligible contribution, as it is rapidly depleted to Pr-Pr. In the light, the heterodimeric pool is more important, as it is continuously reconstituted by light. phyB temperature perception during the day therefore depends mainly on its impact on kr1 (Legris *et al.*, 2016). Temperature effects on the Pfr level in the light were measured spectroscopically for full-length phyB synthesised *in vitro* and bound to the native PΦB chromophore and are also reproduced *in vivo* in etiolated seedlings that overexpress phyB and lack phyA, to preclude interference with the phyB signal (Legris *et al.*, 2016).

phyB abundance is affected by the dark-to-light transition during de-etiolation (Ni *et al.*, 2014). Nonetheless, no temperature effects are observed on total phyB levels in light-grown seedlings (Jung *et al.*, 2016). Whereas Pr is synthesised in the cytosol, Pfr migrates to the nucleus, where it localises to the nucleoplasm and/or nuclear granules, termed nuclear bodies or photobodies (van Buskirk *et al.*, 2012). Nuclear localisation is necessary for phyB function (Huq *et al.*, 2003), and phyB activity correlates with the formation of large nuclear bodies during de-etiolation (van Buskirk *et al.*, 2012) and shade avoidance (Trupkin *et al.*, 2014). Mutation of the universally conserved Tyr residue that associates with the bilin chromophore yields constitutively active phyB, which localises to nuclear bodies even in darkness (Wu and Lagarias, 1997). Mutant variants of phyB that do not form photobodies likewise have impaired responses to red light (Zhang *et al.*, 2013). Large phyB nuclear bodies are proposed as sites of transcriptional activity regulation (Kaiserli *et al.*, 2015). The average size of phyB nuclear bodies is enhanced by light of high irradiance and an elevated red/far-red ratio (Legris *et al.*,

2016). The average size of phyB nuclear bodies shows an optimum in its response to temperature as the rate of Pfr-Pfr incorporation into nuclear bodies is more rapid at higher temperatures, but the Pfr-Pfr level is itself reduced by thermal reversion (Legris *et al.*, 2016).

To date, no differences in phyB thermal reversion among Arabidopsis accessions (indicate natural variation) have been reported. The phyB amino acid sequence is important, however, as several laboratory mutants show changes in physiological outputs that can be attributed to changes in thermal reversion (Elich and Chory, 1997; Ádám *et al.*, 2011). For example, a set of amino acid substitutions in the phyB chromophore binding pocket alters Pfr stability, which highlights the relevance of this N-terminal portion of the protein (Zhang *et al.*, 2013; Burgie *et al.*, 2014). Other components are also important, as Ser86 phosphorylation accelerates phyB thermal reversion (Medzihradszky *et al.*, 2013), whereas interaction with ARABIDOPSIS RESPONSE REGULATOR 4 (ARR4) reduces thermal reversion *in vivo* (Sweere *et al.*, 2001). Thermal reversion is suggested to be slower for phyB in nuclear bodies, due to the phyB interaction with other factors that co-localise to these photobodies (Rausenberger *et al.*, 2010; Klose *et al.*, 2015). PHOTOPERIODIC CONTROL OF HYPOCOTYL 1 (PCH1) is necessary for phyB photobody stability in the dark, and the underlying mode of action might involve reduced thermal reversion (Huang *et al.*, 2016).

Despite the role of phyB in temperature sensing, *phyB* mutants do not necessarily respond less to temperature than wild type. In absolute terms, the *phyB* mutation (either alone or in combination with other mutations that affect photosensory receptor genes) often leads to an increased response to temperature (Mazzella *et al.*, 2000; Halliday and Whitelam, 2003). This indicates that phyB is not the only temperature sensor in plants, and that the effect of additional sensors is stronger in the absence of phyB. Several pathways work redundantly to control hypocotyl elongation, and growth promotion by higher temperatures might be facilitated when *phyB* mutation releases growth from its imposed brake. A similar redundancy phenomenon is observed for the blue-light receptor cryptochrome 1 (cry1), which inhibits rosette stem (Mazzella

et al., 2000) and hypocotyl elongation (Ma *et al.*, 2016) in response to high temperatures. Modelling approaches have been developed to address the specific phyB contribution to temperature sensing (Legris *et al.*, 2016). This type of analysis indicates that the phyB contribution depends on irradiance levels and is in the same quantitative range as the phyB-independent temperature effects. Ignoring the contribution of temperature effects on phyB status reduces the capacity of the model to predict growth in different light and temperature regimes.

Additional light and temperature sensors

Other photoreceptors could also be involved in temperature sensing. The quintuple *phyA phyB phyC phyD phyE* mutant is severely impaired in its long-term response to temperature (final hypocotyl length), a defect not observed in the *phyB* mutant (Jung *et al.*, 2016). For example, the phytochrome pool (presumably phyA) of etiolated *Cucurbita pepo* seedlings responds to temperature in darkness due to thermal reversion (Schäfer and Schmidt, 1974). In contrast, no detectable phyA thermal reversion is observed in Arabidopsis Columbia-0, Landsberg *erecta* and Wassileskija accessions, whereas RLD phyA shows thermal reversion, although its amino acid sequence is identical to that of Columbia-0. This finding suggests that additional genetic components contribute to defining the cell context (Hennig *et al.*, 1999; Eichhenberg *et al.*, 2000).

The UV-B photoreceptor UV RESISTANCE LOCUS 8 (UVR8) perceives UV-B via specific tryptophan residues in the protein, whose mutation causes complete loss of UV-B absorption (Rizzini *et al.*, 2011). UV-B perception induces dissociation of dimeric UVR8 into its active monomeric form. Active UVR8 reverts to the inactive dimeric state in a non-light-dependent reaction regulated by the WD40 proteins REPRESSOR OF UV-B PHOTOMORPHOGENESIS 1 (RUP1) and RUP2 (Heijde and Ulm, 2013). Studies in natural daylight conditions showed that steady state UVR8 dimer/monomer levels are influenced by temperature, with lower temperatures (8-10ºC) reducing the monomer-to-dimer reversion rate (Findlay and Jenkins,

2016). Whether temperature effects on UVR8 status figure in the control of stem growth remains to be established.

The blue light photoreceptor zeitlupe (ZTL) promotes hypocotyl elongation, as the *ztl* mutant is short in continuous red light, and ZTL overexpressers have an elongated phenotype in red, blue or white light. ZTL promotion of warm temperature hypocotyl elongation is thought to be mediated via physical interaction with phyB, to release PIF4 inhibition (Miyazaki *et al.*, 2015).

It should be noted that not all temperature sensors are necessarily involved in light perception. After a far-red light pulse followed by darkness to minimise photosensory receptor activity, seedlings of the *phyB* mutant still respond to temperature (Legris *et al.*, 2016). Changes in membrane fluidity and histone modification are suggested to participate in temperature sensing (Penfield, 2008) and probably mediate this light-independent response.

SIGNALLING DOWNSTREAM OF phyB

Overview of the signalling network

We can define two interconnected branches that act downstream of phyB in the light- and temperature-mediated control of hypocotyl growth (Fig. 5). One branch involves the transcription factor PHYTOCHROME INTERACTING FACTOR 4 (PIF4), while the other involves CONSTITUTIVE PHOTOMORPHOGENIC 1 (COP1) and the transcription factor ELONGATED HYPOCOTYL 5 (HY5). These branches link a core set of genes necessary for normal control of hypocotyl growth in response to light and temperature. The list is by no means comprehensive. In darkness, under shade, or at warm temperatures, PIF4 and COP1 activities are high (due in part to reduced phyB activity) and HY5 abundance is eventually reduced (due partially to enhanced COP1 activity). This scenario is reversed by light or low temperatures, which increase phyB activity.

The PIF4 branch

PIF1 (also termed PIF3-LIKE 5, PIL5), PIF3, PIF4, PIF5 (PIL6), and PIF7 are a set of basic helix-loop-helix (bHLH) transcription factors with an active phyB-binding (APB) domain,

necessary and sufficient for their interaction with light-activated phyB (Leivar and Quail, 2011). PIF1 and PIF3 also bind the Pfr conformer of phyA through an additional active phyA-binding (APA) domain. These interactions facilitate the phosphorylation of PIF, which are partially degraded in the 26S proteasome and/or affected in their binding capacity to target gene promoters (Leivar and Quail, 2011; Park *et al.*, 2012). The PIF proteins in turn regulate phyB abundance by recruiting the LIGHT RESPONSE BTB (LRB) E3 ubiquitin ligases into the PIF-phyB complex during de-etiolation (Ni *et al.*, 2014).

The rapid stem growth characteristic of skotomorphogenesis is mediated by several PIF (Shin *et al.*, 2009). Shade-avoidance responses require mainly PIF4, PIF5 and PIF7 (Lorrain *et al.*, 2008; Li *et al.*, 2012), whereas growth promotion by warm temperatures requires mainly PIF4 (Koini *et al.*, 2009; Stavang *et al.*, 2009); PIF4 is therefore shared by light and temperature growth responses. PIF4 protein stability is increased by darkness, shade (Nozue *et al.*, 2007; Lorrain *et al.*, 2008), or elevated temperatures (Foreman *et al.*, 2011). Elevated ambient temperature also enhances *PIF4* gene expression during late night (Koini *et al.*, 2009). Figure 6 illustrates the *pPIF4::PIF4::GFP* nuclear abundance response to shade and/or warm temperatures.

Auxin is essential for hypocotyl growth in response to shade (Tao *et al.*, 2008) and warm temperatures (Gray *et al.*, 1998). In addition to direct activation of cell wall-degrading enzymes needed for cell expansion, PIF modulate the expression of several auxin-related genes and play a central role in auxin synthesis control (de Lucas and Prat, 2014). During shade avoidance, PIF4, PIF5 and PIF7 bind the promoter of the auxin biosynthetic *YUCCA* genes, activate their transcription, and upregulate auxin levels (Hornitschek *et al.*, 2012; Li *et al.*, 2012). Temperature-dependent selective binding of PIF4 to the *TAA1*, *CYP79B2* and *YUC8* promoters also leads to high auxin levels in response to warm temperatures (Franklin *et al.*, 2011; Sun *et al.*, 2012).

Control of PIF4 activity by DELLA

A common mechanism to antagonize PIF-mediated growth promotion is their sequestration into inactive complexes unable to bind DNA. The finding that DELLA proteins inhibit PIF4 and PIF3 transcriptional activity by binding the DNA recognition domains of these factors identified the link between light and gibberellin signalling in the regulation of hypocotyl growth (De Lucas *et al.*, 2008; Feng *et al.*, 2008). DELLA proteins were also recently reported to promote PIF degradation in the ubiquitin-proteasome system via an unknown E3 ligase (Li *et al.*, 2016). Whereas DELLA-mediated promotion of PIF degradation occurs in darkness as well as in light (Li *et al.*, 2016), control by sequestration is more important during the day, when DELLA are more abundant (Arana *et al.*, 2011). The abundance of DELLA proteins is reduced by darkness (Achard *et al.*, 2007), shade (Djakovic-Petrovic *et al.*, 2007), and high temperatures (Stavang *et al.*, 2009), which contributes to PIF4 release.

Control of PIF4 activity by EARLY FLOWERING 3 (ELF3)

The *elf3* mutant has an elongated hypocotyl in the light and a limited response to warm temperatures (Thines and Harmon, 2010; Zagotta *et al.*, 1996; Reed *et al.*, 2000; McWatters *et al.*, 2000). Natural variation of *ELF3* alleles affects shade (Coluccio *et al.*, 2011; Jiménez-Gómez *et al.*, 2010) and thermal responses (Raschke *et al.*, 2015; Box *et al.*, 2015). The role of ELF3 in light and temperature control of hypocotyl elongation relies at least in part on its control of PIF4 via two distinct molecular mechanisms (Fig. 7). The relative quantitative contribution of these mechanisms to the control of light and ambient temperature responses is thus far little understood.

The evening complex (EC), which consists of the ELF3, ELF4 and LUX ARRHYTHMO (LUX) proteins, is an essential component of the circadian clock, necessary to maintain circadian periodicity (Nusinow *et al.*, 2011). The EC binds the *PIF4* and *PIF5* gene promoters (among many others) via the LUX GARP transcription factor and suppresses expression of these genes during early night (Nusinow *et al.*, 2011). ELF3 association to its target promoters is attenuated

at warm temperature by a still-unknown mechanism; this leads to partially inhibited EC function and therefore, upregulation of its targets, especially during early night (Box *et al.*, 2015; Mizuno *et al.*, 2014). ELF3 thus affects light and temperature responses by modifying *PIF4* expression and PIF4 control of auxin synthesis (Box *et al.*, 2015; Raschke *et al.*, 2015). In addition, ELF3 interacts with PIF4 independently of EC and, similar to the DELLA sequestration mechanism, prevents PIF4 from activating its transcriptional targets (Nieto *et al.*, 2015).

ELF3 protein levels are severely reduced in the *phyB* mutant, which suggests that light perceived by phyB stabilises ELF3 (Nieto *et al.*, 2015). The mechanisms involved in temperature effects on ELF3 association to its target promoters (Box *et al.*, 2015; Mizuno *et al.*, 2014) remain to be elucidated. PIF4 overexpression reduces ELF3 protein abundance (Nieto *et al.*, 2015), probably through negative feedback regulation of phyB levels (Ni *et al.*, 2014); darkness, shade and warm temperatures might thus affect ELF3 activity by enhancing PIF4 abundance. In this way, ELF3 integrates light and temperature cues to the clock by modulating expression of core clock components as well as clock output genes (Huang and Nusinow, 2016).

Control of PIF4 activity by brassinosteroids

The bHLH transcription factors BRASSINAZOLE-RESISTANT 1 (BZR1) and BZR2 have a central role in the activation of growth-promoting genes in response to brassinosteroids (Belkhadir and Jaillais, 2015). If brassinosteroid levels are reduced, BZR1 and BZR2 are phosphorylated by the kinase BRASSINOSTEROID-INSENSITIVE 2 (BIN2) and marked for degradation by the proteasome (Belkhadir and Jaillais, 2015). Brassinosteroids inhibit BIN2 activity, which leads to nuclear accumulation of BZR1 and BZR2 and brassinosteroid-regulated gene activation. Promotion of hypocotyl growth in darkness (Li *et al.*, 1996), shade (Luccioni *et al.*, 2002), or warm temperature (Stavang *et al.*, 2009) requires normal brassinosteroid synthesis, not only for BZR1 and BZR2 accumulation, but also to stabilize PIF4 (Bernardo-García *et al.*, 2014). BIN2 phosphorylates and marks PIF4 for proteasome degradation, and

BIN2 inactivation by brassinosteroid thus promotes PIF4 activity. In turn, PIF4 and BZR1 interact to synergistically co-regulate expression of various genes with roles in cell elongation (Oh *et al.*, 2012) by binding to conserved E-box elements in their promoters. DELLA proteins also inhibit BZR1 and BZR2 activity via a sequestration mechanism like that described for PIF (Bai *et al.*, 2012; Gallego-Bartolomé *et al.*, 2012). BZR1 is needed for hypocotyl elongation at elevated temperatures, although in contrast to PIF4, BZR1 levels are not markedly affected by temperature (Oh *et al.*, 2012). The brassinosteroid synthesis inhibitor propiconazole (PPZ) impedes growth promotion at warm temperatures, and inhibition is reduced in the constitutive *bzr1-1D* mutant. Nonetheless, *pifq bzr1-1D* mutants do not respond to ambient temperature, which underscores a pivotal role for both BZR1 and PIF4 in this response.

The COP1-HY5 branch

COP1 is a RING E3 ligase that targets a number of proteins involved in photomorphogenesis for degradation in the 26S proteasome, including the transcription factor HY5 (Osterlund *et al.*, 2000), which is needed to inhibit hypocotyl growth (Oyama *et al.*, 1997). Although COP1 can ubiquitinate targets on its own *in vitro*, *in vivo* it forms a complex with SUPPRESSOR OF PHYA-105 1 (SPA1), SPA2, SPA3 and/or SPA4, which enhance COP1 activity (Zhu *et al.*, 2008). The COP1-SPA core acts in turn as a substrate adaptor for a multimeric CULLIN 4 (CUL4)-DAMAGED DNA BINDING PROTEIN 1 (DDB1) E3 ligase (Lau and Deng, 2012). DE-ETIOLATED 1 (DET1) and COP10 form a different multimeric CUL4-DDB1 ligase that apparently reinforces activity of the CUL4-DDB-COP1-SPA multimeric complex (Lau and Deng, 2012). DET1 also functions in chromatin regulation (Benvenuto *et al.*, 2002) and as a transcriptional corepressor (Lau *et al.*, 2011).

The *cop1*, *det1*, *cop10*, *cul4* and *spa1 spa3 spa4* mutants show reduced hypocotyl growth in darkness (Deng *et al.*, 1992; Pepper *et al.*, 1994; Wei *et al.*, 1994; Bernhardt *et al.*, 2006; Laubinger and Hoecker, 2003), as well as in warm temperatures (Delker *et al.*, 2014). Shade avoidance is also significantly impaired in *cop1* and *spa1 spa3 spa4* mutants (Rolauffs *et al.*,

2012). Conversely, the *hy5* mutant shows long hypocotyls in the light (Oyama *et al.*, 1997) and increased temperature-induced hypocotyl elongation (Delker *et al.*, 2014).

During de-etiolation, light-activated cryptochromes (Lian *et al.*, 2011) and phytochromes (Sheerin *et al.*, 2014) reduce COP1 activity by interfering with its interaction with SPA1 in the nucleus. In addition, COP1 nuclear levels are reduced rapidly during de-etiolation (Pacín *et al.*, 2014) and re-accumulate when plants are exposed to shade (Pacín *et al.*, 2013). The control of COP1 dynamics by temperature is complex because in rosette leaves, the expression of the *COP1* gene increases with warmer temperatures while the stability of the COP1 protein decreases (Jang *et al.*, 2015). In the root, low temperatures reduce COP1 abundance in the nucleus, likely via a nuclear exclusion mechanism (Catalá *et al.*, 2011).

Light, which reduces COP1 activity, enhances HY5 stability during de-etiolation (Osterlund *et al.*, 2000). HY5 stability is also enhanced by cold temperature in dark-grown roots (Catalá *et al.*, 2011), with no difference in HY5 levels observed in seedlings grown at 17ºC or 27°C under continuous red light (Johansson *et al.*, 2014). Light and temperature also control *HY5* gene expression. *HY5* is induced by transitions from dark to light (Oyama *et al.*, 1997) and shade to light (Sellaro *et al.*, 2011); it is reduced at 28ºC compared to 20°C (Delker *et al.*, 2014) and at 20ºC compared to 4°C in the presence of light (Catalá *et al.*, 2011).

PIF4 and COP1 pathway interconnections

The two branches of the signalling core are interconnected at several points. For instance, COP1 and DET1 activities are both necessary to maintain PIF stability, as very low PIF protein levels are observed in *cop1* and *det1* mutants (Bauer *et al.*, 2004; Dong *et al.*, 2014). Increased *PIF4* expression is also observed in the *hy5* mutant (Delker *et al.*, 2014).

An important link is established by LONG HYPOCOTYL IN FAR-RED (HFR1), which encodes an atypical bHLH protein that forms heterodimers with PIF4 (and other PIF) and prevents PIF4 from binding to DNA to activate gene expression (Hornitschek *et al.*, 2009). HFR1 stability is reduced by darkness (Duek *et al.*, 2004) or shade (Pacín *et al.*, 2016) in a

COP1-dependent manner, and COP1 hence reinforces PIF4 activity. Under blue light photoperiods, HFR1 protein stability is increased by warm temperature (Foreman *et al.*, 2011), which indicates that the negative correlation between light and temperature signalling is broken at this point. The *hfr1* mutant has enhanced temperature-induced hypocotyl elongation under blue light/dark cycles, where cry1 inhibits growth, but displays normal temperature responses under red, far-red or white light photoperiods (Foreman *et al.*, 2011; Delker *et al.*, 2014). *HFR1* expression is increased by shade (Sessa *et al.*, 2005) and by warm temperature (Foreman *et al.*, 2011).

PHYTOCHROME RAPIDLY REGULATED 1 (PAR1) also inhibits PIF4-mediated gene activation by forming a non-DNA binding heterodimer, and reduces the stem growth response to shade and warm temperatures (Roig-Villanova *et al.*, 2007; Hao *et al.*, 2012). PAR1 is also stabilized by light, which could involve COP1, although this remains to be established (Hao *et al.*, 2012). COP1 can also target ELF3 for degradation (Fig. 5) (Wang *et al.*, 2015), but apparently not in all contexts (Jang *et al.*, 2015).

Another point of interaction between the two branches arises from the competitive occupancy of G-box elements by PIF4 and HY5, which form a dynamic activation-suppression module in the control of photosynthetic pigment accumulation by light and temperature (Toledo-Ortiz *et al.*, 2014). In the case of hypocotyl growth, HY5 antagonizes PIF4 activity at low temperatures (Johansson *et al.*, 2014). This could result from competition for co-targeted promoters and/or negative regulation of *PIF4* gene expression (Delker *et al.*, 2014).

Signalling in photothermal switch conditions

At 17ºC, increasing irradiances of continuous red light up to 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ reduce final hypocotyl length; conversely, at 27ºC, growth inhibition is observed up to 1 $\mu mol \cdot m^{-2} \cdot s^{-1}$, while growth is promoted at higher irradiances (Johansson *et al.*, 2014). Mathematical modelling based only on the phyB-PIF signalling module predicts that hypocotyl length should remain relatively stable at irradiances >1 $\mu mol \cdot m^{-2} \cdot s^{-1}$. On its own, this module is therefore insufficient

to describe this complex interaction between red light and temperature, known as photothermal switch. The model requires the incorporation of X to inhibit growth at 17ºC and Y to promote growth at 27ºC. X and Y are temperature- and irradiance-dependent factors that control PIF activity (Johansson *et al.*, 2014). HY5 fulfils the requirements for X, as it inhibits growth at 17ºC and high irradiance, and reduces PIF4 activity. The identity of Y has not been established, but it is predicted to enhance PIF activity and expression of PIF-dependent auxin response genes (Johansson *et al.*, 2014). One possibility is that warm temperatures reduce the abundance of the H2A.Z histone variant in the nucleosomes, facilitating PIF access to the target promoters, as reported for *FLOWERING LOCUS T (FT)* (Kumar *et al.*, 2012). Another option is that the activity of transcription factors that show cooperative interactions with PIF increase with irradiance at 27ºC. The switch is controlled by a specific gene set that includes auxin pathway genes (Johansson *et al.*, 2014); this photothermal switch is absent under blue light, possibly due to a cry1-mediated decrease in PIF4 activity, which is observed at warm temperatures (Ma *et al.*, 2016).

Signalling dynamics under differential day and night temperatures

Hypocotyl and petiole elongation are inhibited when plants are grown in conditions in which days are cooler than nights (negative day/night temperature difference; -DIF), an artificial setting used in horticulture to produce more compact plants. Inhibition by -DIF is not observed in *phyB*, *pif4*, *pif5* and *pif3* mutants, suggesting that the phyB-PIF pathway is involved in this response (Bours *et al.*, 2013).

Growth inhibition in –DIF conditions is caused by reduced cell elongation during the cold photoperiod, and auxin application is able to restore impaired responses of *pif4* and *pif5* mutants, but not that of *pif3* (Bours *et al.*, 2015). In normal growth conditions (+DIF), auxin levels as well as the expression of auxin synthesis genes (*YUC8*, *YUC5),* the expression of auxin-responsive genes (*SMALL AUXIN UP-REGULATED 19 (SAUR19), SAUR21, SAUR22, SAUR23*, *SAUR24, IAA29*) and the activity of a DR5-luciferase reporter are elevated during the

day and decline during the night (Bours *et al.*, 2015). In -DIF conditions, all these variables remain constitutively low, at or below the levels observed in +DIF at night (Bours *et al.*, 2015). This pattern is also reproduced by application of the ethylene precursor ACC, which likewise restores hypocotyl growth under –DIF and thus indicates that auxin and ethylene are linked in this response. Growth inhibition in –DIF conditions is indeed impaired in the *ethylene-insensitive 2* (*ein2*) signalling mutant and in the biosynthesis *1-aminocyclopropane-1-carboxylic acid synthase (acs)*-octuple loss-of-function mutant. Whereas auxin application does not rescue the -DIF phenotype of these mutants, ACC application restores hypocotyl length to that of +DIF conditions. This places ethylene downstream of auxin signalling; in accordance with this finding, *ACS* gene expression is reduced in –DIF and auxin application restores its expression levels (Bours *et al.*, 2015).

ACC-induced hypocotyl elongation in the light depends on transcriptional activation of *PIF3*; *pif3* mutants notably do not respond to auxin or ACC application under either +DIF or -DIF conditions (Bours *et al.*, 2015). *PIF3* promoter activity is in fact upregulated by auxin and ACC under -DIF conditions, which suggests that PIF3 regulates hypocotyl length downstream of auxin and ethylene signalling, whereas PIF4 and PIF5 act upstream of the auxin and ethylene signalling cascade (Bours *et al.*, 2015). Moreover, *PIF3*ox, *PIF5*ox and *phyB-9* seedlings are unaffected by –DIF, but *PIF4*ox lines respond to –DIF, suggesting that thermoperiodic conditions have a stronger effect on PIF4 activity.

Additional mechanisms are probably important for modifying growth based on day/night temperature patterns. In pea stems, a gibberellin deactivation gene is proposed to mediate thermoperiodic stem elongation (Stavang *et al.*, 2005). SPATULA mediates the repression of rosette growth only by cool daytime temperatures, with little effect under warmer conditions or in response to temperature during the night (Sidaway-Lee *et al.*, 2010).

Signalling dynamics in response to transiently elevated temperatures

When dark-grown seedlings are exposed daily to transiently elevated temperatures, hypocotyl growth becomes more light-sensitive at the time of day these heat shocks occurred on previous days (Karayekov *et al.*, 2013). No synergism between light and transient high temperatures is observed in the *phyB* mutant, and it is reduced in the *pif3 pif4*, *pif4 pif5*, *cop1, hy5, elf3, elf4* and several clock gene mutants. This indicates that the response requires components of both signalling branches downstream of phyB (Fig. 5) (Karayekov *et al.*, 2013), the COP1-HY5 pathway and the PIF4 pathway (which is controlled by ELF3 and clock-related genes). Heat shocks delay formation of phyB nuclear bodies when seedlings are exposed to light; this would not explain the enhanced light sensitivity, however, as the formation of large nuclear bodies correlates with enhanced phyB activity. Heat shocks reduce the nuclear COP1 levels and enhance HY5 stability (Karayekov *et al.*, 2013), which is consistent with greater inhibition of hypocotyl growth by light. Heat shocks transiently increase *PSEUDO-RESPONSE REGULATOR7 (PRR7)* and *PRR9* expression and that of their targets *LATE ELONGATED HYPOCOTYL (LHY)* and *CIRCADIAN CLOCK ASSOCIATED1 (CCA1)*; these, in turn, are needed for high *PIF4* and *PIF5* expression. Low PIF4 and PIF5 levels in response to heat shock enhance light sensitivity (Karayekov *et al.*, 2013). Continuous warm temperatures increase COP1 and PIF4 nuclear abundance and reduce that of HY5, which appears to contradict post-heat shock patterns. At the termination of the heat shock, temperature decreases to pre-elevation values; there might thus be an overcompensatory response to temperature decrease after heat shock.

FUNCTIONAL SIGNIFICANCE

The degree of overlap between factors involved in sensing and transducing light and temperature cues has come as a surprise. It was predictable that light and temperature pathways might converge in the final signalling steps that control a physiological process; but we now know that even a receptor shares these functions. We must therefore consider the

functional significance of simultaneous control of growth by light and temperature. One axis of this analysis is defined by the environmental input, as changes in light and temperature might correlate or not; the second axis is defined by the physiological output, since the effects of light and temperature can be antagonistic or synergistic. This scenario defines four possibilities (Figure 8).

(a) Correlated input, antagonistic output. This would be the case of a seedling whose shoot has just emerged from the soil. The stronger the irradiance input, the higher the temperature of the soil and plant tissues. Although strong light input inhibits hypocotyl growth, this effect would be balanced by warm temperatures, which would promote growth and push foliage upward, farther from the risk of heat stress near the bare soil. Increased inter-leaf and leaf-soil separation caused by stimulated stem growth might enhance the transpiration rate and evaporative cooling capacity (Crawford *et al.*, 2012; Bridge *et al.*, 2013).

(b) Correlated input, synergistic output. Average values as well as environmental fluctuation patterns are important for growth control. Seedlings grown in the darkness of the soil can be exposed daily to transient warm temperatures as they approach the sunlight-heated surface. This temperature cue prepares the seedling for more efficient de-etiolation in response to subsequent light signals received by aerial organs as they emerge (Karayekov *et al.*, 2013).

(c) Independent input, antagonistic output. A seedling shaded by neighbours in winter will have slower stem growth and thus, less efficient shade avoidance than in warmer conditions (Patel *et al.*, 2013). During the cold season, however, photosynthesis is often limited by the enzyme kinetics of the Calvin cycle; a relatively high light input thus cannot be channelled through photosynthesis and there is some risk of reactive oxygen species generation. In this case, a weaker shade-avoidance reaction would reduce the chance of exposure to full sunlight and oxidative stress.

(d) Independent input, synergistic output. This would be the case of a plant exposed to shade during the warm season. The optimum temperature for the mitochondrial respiration rate

is normally higher than that for photosynthesis. Rapid growth driven by the combination of shade plus warm ambient temperatures would increase the chance of overtopping the canopy, and avoid a condition where high respiration coincides with low photosynthesis due to limited light.

CONCLUSIONS

The mechanisms of perception and signalling of light and temperature cues share pivotal molecular components, and the output of these signals is therefore strongly interdependent. The well-established photoreceptor phyB was recently also identified as a temperature sensor, as the reversion rates from Pfr-Pfr to Pr-Pfr dimers and from Pr-Pfr to Pr-Pr dimers respectively confer night- and daytime thermal dependency to phyB activity. Phytochromes are not the only entry point for light or temperature cues, however; PIF4, COP1 and HY5 are core components of light and temperature signalling downstream of phyB. The ELF3 protein regulates PIF4 activity at different points, and ELF3 natural genetic variability confers the capacity for distinct responses to light and temperature. Nonetheless, the degree of control of ELF3 activity by these two cues has not been fully elucidated. This interdependence of light and temperature control of growth could be an advantage in specific situations. Studies dealing simultaneously with light and temperature inputs are needed to determine how this signalling links the complex environment of plants to their growth control.

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FIGURE LEGENDS

Figure 1. Canopy shade simultaneously modifies light and temperature patterns, and the shade-avoidance response depends on the combined action of both inputs. Pots containing *Arabidopsis thaliana* plants at the rosette stage were placed under full sunlight or under a green canopy and allowed to equilibrate for 1 h. Radiation reaching each plant was recorded by placing a spectroradiometer remote probe (Ocean Optics USB4000-UV-VIS spectrometer configured with a DET4-200-850 detector and QP600-2-SR optical fibre) on top of the rosettes. Rosette temperature was measured with an infrared thermometer (Protomax VA6520). Photosynthetically active radiation (400 - 700 nm) was 1719 ± 75 and 13 ± 1 $\mu mol.m^{-2}.s^{-1}$ outside and beneath the canopy, respectively; the red to far-red photon fluence rate ratio was 1.11 ± 0.00 and 0.10 ± 0.01 outside and beneath the canopy. Data are shown as mean ± SE of four measurements. The hypocotyl growth rate (mm/h) was estimated using a model (Legris *et al.*, 2016) with real light and temperature conditions as input (first two seedlings from left), or with canopy light and the temperature of unshaded seedlings, i.e., ignoring cooler conditions under the canopy (seedling in brackets).

Figure 2. Position relative to the soil surface and to neighbouring plants, as well as the season, all generate light and temperature cues that affect growth and developmental programmes throughout the plant life cycle. The developmental processes (germination, seedling de-etiolation, shade avoidance, flowering) are illustrated (centre). The boxes show how position in space and time (depth in soil, top; height within the canopy, right; and season, left) affects light and temperature cues. The arcs extend over the developmental processes affected by each space or time condition (arc colour matches border colour for each condition).

Figure 3. Combined effect of light and temperature on hypocotyl growth. (a) Hypocotyl growth rate plotted against log irradiance for different temperatures. *Arabidopsis thaliana* (Columbia-0) seedlings were grown under 50 $\mu mol.m^{-2}.s^{-1}$ white light (photoperiod 10 h, 20°C, 3 d). One hour after the beginning of day 4, seedlings were transferred to the indicated irradiance and

temperature. Increase in hypocotyl length was measured after 9 h. (b) Slope of the growth - log irradiance response for distinct temperatures. (c) Growth at log irradiance = 0 (intercept). Drawn after Legris *et al.* (2016).

Figure 4. phyB dynamics is affected by light and temperature cues. k1 increases with red light, k2 increases with far-red light, kr1 and kr2 increase with temperature. Pr: red light-absorbing, inactive form of phyB. Pfr: far-red-absorbing, active form of phyB.

Figure 5. Core signalling components shared by light and temperature cues that control growth. The scheme highlights key components of two interconnected signalling branches that act downstream of phyB. To focus on components shared by light and temperature, additional photosensory receptors, important for light perception, are not represented; the temperature signal also has additional unidentified entry points.

Figure 6. Synergistic enhancement of PIF4 nuclear abundance by shade and warm temperature. Seeds bearing a *pPIF4::PIF4-GFP* transgene were sown on agar-water, stratified, exposed for one day to white light (simulated sunlight, photoperiod 10 h) at 22ºC followed by three days of simulated sunlight at 22ºC, simulated shade at 22ºC, simulated sunlight at 28ºC or simulated shade at 28ºC 22ºC. Three to four h after the beginning of the third day of treatments, the seedlings were analysed by confocal microscopy. Experimental methodology was as described (Pacín *et al.*, 2016). (a) Confocal microscopy images of representative hypocotyls. Chlorophyll fluorescence is shown (red). Bar, 50 μm. (b) Mean ± SE of 12 seedlings. Factorial ANOVA indicates significant effects of light and temperature ($P < 0.01$).

Figure 7. ELF3 is important in light and temperature growth responses, as it negatively regulates *PIF4* gene expression (Nusinow *et al.*, 2011) and PIF4 protein activity (Nieto *et al.*, 2015). The mechanism by which light (L) and temperature (T) cues affect ELF3 activity is little understood. The scheme shows that ELF3 binding to the PIF4 promoter is reduced by warm temperatures (Box *et al.*, 2015); it remains to be determined whether this is also the case for light, or whether any of these cues affects ELF3-PIF4 protein interaction.

Figure 8. Functional consequences of simultaneous light and temperature control of growth. Four cases are shown in which light and temperature cues are independent or correlated, and their outputs are antagonistic or synergistic. In each case, the seedlings show the effect of the combined action of light and temperature cues, and dashed shapes show the output if controlled only by the light signal. Labels for dashed shapes indicate the stress that this condition would cause. ROS: reactive oxygen species.