UNIQUE AND CONTRASTING EFFECTS OF LIGHT AND TEMPERATURE CUES ON PLANT TRANSCRIPTIONAL PROGRAMMES

Mai Jarad[1], Rea Antoniou-Kourounioti[2], Jo Hepworth[2], Julia I. Qüesta[1]

[1]Centre for Research in Agricultural Genomics (CRAG), CSIC-IRTA-UAB-UB, Campus UAB, Bellaterra, Barcelona, Spain.

[2]John Innes Centre, Norwich Research Park, Norwich NR4 7UH, UK.

Corresponding author: julia.questa@cragenomica.es, jo.hepworth@jic.ac.uk, rea.antoniou-kourounioti@jic.ac.uk

ABSTRACT

Plants have adapted to tolerate and survive constantly changing environmental conditions by reprogramming gene expression in response to stress or to drive developmental transitions. Among the many signals that plants perceive, light and temperature are of particular interest due to their intensely fluctuating nature which is combined with a long-term seasonal trend. Whereas specific receptors are key in the light sensing mechanism, the identity of plant thermosensors for high and low temperatures remains far from fully addressed. This review aims at discussing common as well as divergent characteristics of gene expression regulation in plants, controlled by light and temperature. Light and temperature signalling control the abundance of specific transcription factors, as well as the dynamics of co-transcriptional processes such as RNA polymerase elongation rate and alternative splicing patterns. Additionally, sensing both types of cues modulates gene expression by altering the chromatin landscape and through the induction of long non-coding RNAs (lncRNAs). However, while light sensing is channelled through dedicated receptors, temperature can broadly affect chemical reactions inside plant cells. Thus, direct thermal modifications of the transcriptional machinery add another level of complexity to plant transcriptional regulation. Besides the rapid transcriptome changes that follow perception of environmental signals, plant developmental transitions and acquisition of stress tolerance depend on long-term maintenance of transcriptional states (active or silenced genes). Thus, the rapid transcriptional response to the signal (Phase I) can be distinguished from the long-term memory of the acquired transcriptional state (Phase II – remembering the signal). In this review we discuss recent advances in light and temperature signal perception, integration, and memory in *Arabidopsis thaliana*, focusing on transcriptional regulation and highlighting the contrasting and unique features of each type of cue in the process.

KEYWORDS

Light and temperature sensing; plant transcriptional regulation; chromatin dynamics; long non-coding RNAs; epigenetic memory; stress memory

1. INTRODUCTION

1.1 LIGHT AND TEMPERATURE CUES INDUCE STRESS AND DEVELOPMENTAL RESPONSES IN PLANTS

Plants are constantly exposed to varying environmental cues that drive their growth and developmental processes. Some of the more prevalent abiotic stresses that plants are subjected to are drought (water scarcity), flooding (water overload), soil salinity, harmful radiation, toxic compounds in the soil, severe temperatures (freezing, cold, heat), and light fluctuations. Among them, light and temperature interact to control the timing of all the transitions of plant development, from germination, through the juvenile and floral transitions, to seed set and senescence.

Light may vary in intensity and wavelength and, like temperature, fluctuates widely in both predictable patterns (photoperiod) and unpredictable ones (cloud cover, shading from other plants). Plants have evolved the capacity to perceive all these complex combinations of light and temperature cues and utilise these signals to adjust their response. For instance, in the Brassicaceae and some winter cereals, spring flowering requires the combination of appropriate day length, memory of past prolonged low temperatures (vernalisation), and recent warm temperatures (thermomorphogenesis) [1–3]. Thus, the intertwining of both light and temperature sensing, together with the underlying signalling pathways, allow plants access to vital information that affect their growth and developmental responses. In addition, plants have developed mechanisms to counteract exposure to harmful radiation and to extreme temperatures, driving stress acclimation responses for survival.

Perception and integration of light and temperature signals, both developmental triggers and stressors, result in transcriptional reprogramming. Decades of work has deciphered numerous Transcription Factors (TFs, activators and repressors) that are responsive to light and/or temperature. However, recent efforts have started to shed light into other players such as the chromatin landscape, non-coding transcripts and components of the transcriptional machinery. In this review, we summarise the different levels of transcriptional regulation in plants and discuss how they can be affected by light and temperature cues. Our aim is to highlight common and distinct features of light and temperature mode of action on transcriptional regulation control in plants.

1.2. TRANSCRIPTIONAL REGULATION IN PLANTS

In eukaryotes, transcription of mRNAs, long non-coding RNAs (lncRNAs), miRNA precursors and enhancer RNAs (eRNAs) start with recruitment of the RNA POLYMERASE II (RNAPII) to proximal or distal regulatory regions of genes (promoters and enhancers, Figure 1, [4–6]). RNAPII recruitment and assembly at gene promoters located in chromatin accessible regions of the genome (Figure 1A) is generally facilitated by sequence specific TFs, as well as the regulatory role of enhancers (Figure 1B). In turn, binding of these "pioneer" TFs may also increase chromatin accessibility for RNAPII recruitment, and thus a feedback mechanism between chromatin and TFs is established. Once engaged, RNAPII switches to transcriptional elongation moving along the gene body (Figure 1C), the nascent transcript is co-transcriptionally spliced (Figure 1D), and transcription is terminated upon cleavage and polyadenylation at the 3' end of the gene (Figure 1E). Although research on transcriptional regulation has mostly focused on TFs triggering transcriptional initiation (Figure 1B), detailed mechanistic studies have revealed that transcription consists of multiple regulated steps including RNAPII pausing and stalling along genes, splicing, cleavage and polyadenylation [7]. The degree of chromatin accessibility adds another level of regulation, influencing not only TF binding dynamics [8] but also efficiency of the co-transcriptional processes such as splicing [9]. Together, these mechanisms determine the speed of transcript production, as well as the epigenetic state of genes: ACTIVE (ON) or INACTIVE (OFF) [10]. Post-transcriptional mechanisms including transcript stability, nuclear export and inactivation by RNA silencing pathways (for example the miRNA pathway) will determine transcript fate.

Plant transcriptional responses to environmental threats mostly happen rapidly and transiently upon sensing of the triggering signal. Initial perception is integrated into signalling pathways that confluence in the induction of a set of pioneer TFs. The function of those TFs is to reprogramme the transcriptome by activating or repressing genes (transcriptional activators and repressors, respectively). However, plant acclimation to stress and induction of developmental transitions require that the transcriptional status acquired by specific genes (ON or OFF) is maintained for prolonged periods of time even in the absence of the triggering signal. Epigenetic mechanisms, such as DNA methylation and histone post-translational modifications, have the capacity to lock genes in either the ON or OFF state, and perpetuate these states through DNA replication and cell divisions [11–14]. In the following sections we will

discuss the impact of light and temperature on different levels of transcriptional regulation, transiently and long-term.

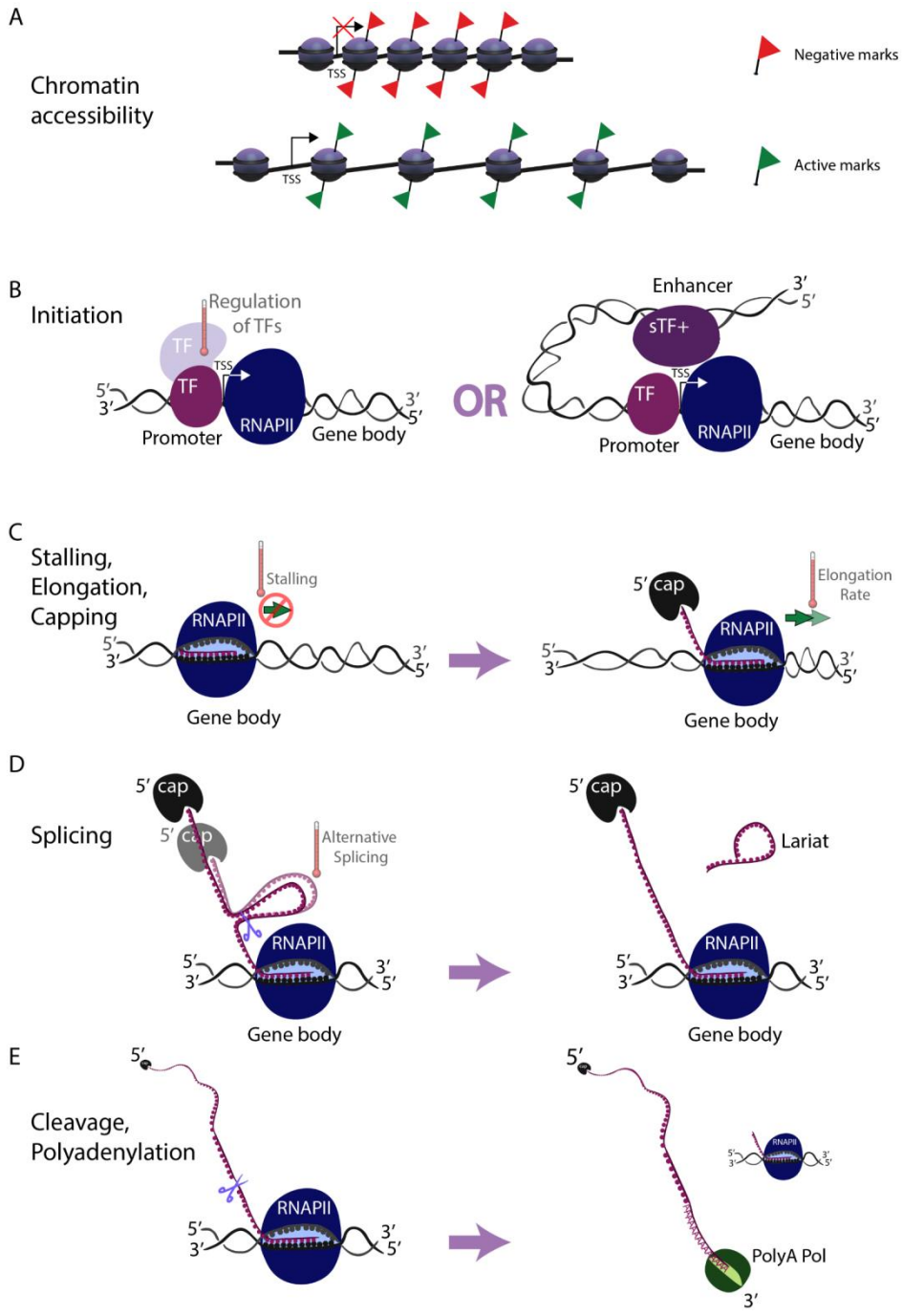

Figure 1. Transcription in eukaryotes

A, Chromatin accessibility affects the ability of the RNA Polymerase II (RNAPII) and other proteins to transcribe genes. B, RNAPII is recruited to the gene by transcription factors at promoters and enhancers for transcription initiation. C, RNAPII can stall at gene boundaries.

After release from promoter-proximal stalling, there is transcriptional elongation and 5' capping of the nascent transcript. The chromatin is directly affected by the activity of the RNAPII through changes in supercoiling ahead of and behind the polymerase. D, Splicing determines the sequence of the transcript, and often alternative transcripts can arise from the same gene as a result of this process. E, The transcript is released from RNAPII by cleavage and the template-independent Poly (A) Polymerase polyadenylates its 3' end.

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2. LIGHT AND TEMPERATURE CONTROL OF TRANSCRIPTION FACTORS

Light and temperature sensing triggers a cascade of transcription factors that control growth, acclimation and other responses. Many of these downstream factors are common to the two signals, thereby facilitating integration of environmental information. Furthermore, some of the photoreceptors have recently been found to also be sensitive to temperature. In the following sections we describe the known receptors of the two signals and the downstream pathways that control signalling, highlighting the interconnections between them.

2.1. LIGHT-TRIGGERED TRANSCRIPTIONAL RESPONSES

2.1.1. PLANT PHOTORECEPTORS

Relatively few reactions are light-sensitive within the spectra and intensity of light at the Earth's surface. Therefore, in order to sense light, plants have evolved to produce specific receptors for light of different wavelengths, most of which require a light-sensitive cofactor molecule for their activity. Five families of photoreceptors are known to date in Arabidopsis: the phytochromes (far red and red light), the cryptochromes and members of the Zeitlupe protein family (both blue and UV-A), phototropins (blue, UV-A and UV-B) and the UV-B photoreceptor UVR8 (Figure 2).

The phytochromes form a family of five proteins, phyA-phyE, whose activity depends on the light-responsive, wavelength-reversible conformation of a bilin cofactor. In the case of phyB, red light switches the phytochrome from its nascent inactive form (Pr) to its active form (Pfr) while far red light reverts Pfr to Pr again. In the active form, phytochromes move from the cytosol into the nucleus to mediate their effects through interaction with other proteins,

including other photoreceptors such as cryptochromes [15,16]. Although they are partially redundant, the different phytochromes react differently to control distinct responses depending on light intensity, wavelength and their reversion dynamics [16,17]. For example, in phyB, the active form 'relaxes' back to the inactive form in the absence of red light. This 'dark reversion' is temperature-sensitive, giving phyB the properties of a temperature sensor in conditions with low red light [18,19].

Like the phytochromes, the two cryptochromes are partially redundant. They require a flavin adenosine dinucleotide as cofactor. When activated by light, the cryptochromes undergo conformational change and protein homo-oligomerisation to activate binding to targets, which, like the phytochromes, includes direct interaction with target TFs [20,21].

Phototropins are serine/threonine kinases that also have flavin chromophores, binding flavin mononucleotides through two LIGHT, OXYGEN OR VOLTAGE (LOV) domains. Unlike the other photoreceptors, which are nuclear or cytosolic, the two phototropins are plasma membrane targeted, though not integral membrane proteins. Photoactivation by blue, UV-A and UV-B light results in autophosphorylation and phosphorylation of target proteins, leading to movement responses at the intracellular (chloroplast movement), cellular (stomatal opening) and whole plant level (phototropism) to maximise light reception [22,23]. In common with Arabidopsis phyB, the phototropins have also been implicated in temperature sensing (see thermosensors section).

A single LOV domain is present in the Zeitlupe family of three flavin-binding proteins: ZTL itself, FLAVIN-BINDING, KELCH REPEAT, F-BOX1 (FKF1) and LOV KELCH REPEAT PROTEIN 2 (LKP2). All three mediate blue light clues to the circadian clock via their F-box domains as part of E3 ubiquitin ligase complexes, marking target proteins, such as CYCLING DOF TFs, for degradation [24–26].

Most recently discovered is the UV-B photoreceptor UV RESISTANCE LOCUS 8 (UVR8) [27,28]. Ultraviolet photon absorption by UVR8 causes monomerisation from the inactive homodimer [29] leading to translocation to the nucleus and interaction with CONSTITUTIVE PHOTOMORPHOGENIC 1 (COP1) [28,30]. Uniquely, UVR8 does not require a chromophore

cofactor, with light sensitivity being provided by tryptophan residues within the protein itself [31].

In addition to these dedicated receptors, light reactions in the chloroplast lead to transcriptional responses both within the chloroplast itself and, by a variety of retrograde signalling pathways, in the nucleus. These signalling pathways include the direct outputs of photosynthetic function such as reactive oxygen species (e.g. single oxygen) [32,33], metabolites required for chloroplast function such as tetrapyrroles [34], and proteins from both nucleus and plastid, including transcription factors such as PHD-TYPE TRANSCRIPTION FACTOR WITH TRANSMEMBRANE DOMAINS (PTM) and the integrator TF ABSCISIC ACID INSENSITIVE4 (ABI4) [17,35–39]. Moreover, as the reactions of photosynthesis are inherently heat-sensitive, this retrograde signalling is also linked to temperature responses [40,41]. Similarly, circadian dynamics are a vital part of the modulation of light and temperature signalling, and are also controlled by light (e.g. the Zeitlupe family) [42,43].

2.1.2 TRANSCRIPTION FACTORS IN THE PRIMARY RESPONSE TO LIGHT

Light inputs from three of five photoreceptor families converge on the activity of a small number of transcription factors (Figure 2). In particular, two families of TFs dominate light responses: the basic Helix-Loop-Helix (bHLH) PHYTOCHROME INTERACTING FACTOR (PIF) family and the basic ZIPper (bZIP) TFs ELONGATED HYPOCOTYL5 (HY5) and its orthologue HY5 HOMOLOGUE (HYH). Light-induced changes in PIF and HY5 activity act through relief-from-repression mechanisms at different stages of the transduction route. Both families are key to plant development, particularly photomorphogenesis. Most information on light signalling, retrograde or otherwise, has been derived from study of this process, in which seedlings undergo major transcription, cellular and morphological changes from an autotropic etiolated ‘soil-grown’ state to green, phototropic, open-cotyledon phase.

HY5 and HYH generally act partially redundantly to promote transcription in response to red, blue and UV light signalling (Figure 2). HY5 itself lacks a transcriptional activation domain, and recent work has further supported the hypothesis that HY5 relies on interacting TFs to upregulate its targets, which trigger a cascade of indirect transcriptional responses [44]. In the dark, HY5 and HYH, along with several other factors that promote photomorphogenesis, are

ubiquitylated and marked for degradation by the 26S proteasome, through the action of the master light integrator, the E3 ligase COP1-SUPPRESSOR OF PHYA-105 (COP1/SPA) complex [45–47]. COP1-SPA complex action is in turn inhibited by the binding of activated (Pfr) phytochromes and cryptochrome oligomers to SPA proteins, resulting in the stabilisation of HY5 in the light and promotion of photomorphogenesis [17,20]. Active UVR8 monomers also bind to COP1 in the nucleus in a light-dependent manner, outcompeting COP1 binding of HY5 [27,28,30,48]. Although several different photoreceptors converge on HY5 via COP1, they do so in different manners and have different effects. Unlike PIF and CRY signalling, UV-B requires COP1 as a positive factor [49], with the resulting stabilisation of HY5 mediated through interplay between antagonistic E3 ligases downstream of UVR8 action [50]. UVR8 also binds to WRKY DNA-BINDING PROTEIN 36 (WRKY36) to inhibit its binding to and repression of the *HY5* promoter, resulting in increased *HY5* transcription [51]. In turn, CRYs antagonise UVR8 responses over the light spectra that both receptors share [52]. HY5 also directly promotes expression of its negative regulators *COP1, SPA1* and *SPA4* in a negative feedback loop [44].

The PIF family are key pioneer TFs for light signalling with critical roles in plant development, integrating light responses with temperature, hormone signalling and circadian dynamics [16,17]. In contrast to the HYs, PIFs act as negative regulators of photomorphogenesis by binding to G-box motifs in target promoters, antagonising HY5 action at shared targets [53]. In the dark, PIF proteins are stabilised by the binding of COP1 (PIF3 and PIF5 [54,55]) and DE-ETIOLATED 1 (DET1 [56]). DET1 is a classic negative regulator of photomorphogenesis, like COP1, and similarly provides substrate specificity to a CULLIN4 E3-ligase complex [57–60]. PIF repression of photomorphogenesis is relieved in light by phytochrome-triggered phosphorylation and degradation of PIFs, by the action of phyB in reducing PIF1 and PIF3 binding to DNA at target promoters, and by UVR8-mediated reduction in COP1-PIF5 binding, leading to PIF5 destabilisation [16,61–65]. Although partially redundant, different PIFs have different affinities for the various phytochromes, particularly the 'light labile' phyA, and for different heterodimerisation partner TFs [16,17,66]. As well as their interaction with phytochromes, CRYs also interact directly with PIF4 and PIF5 at the chromatin in limiting blue light and repress PIF transcriptional activity in high temperature responses [67,68].

As well as the PIFs and HYs, all the photoreceptors, except the phototropins, are known to interact directly with other TFs to mediate their specific responses. As a few examples: phyB

triggers the light-dependent degradation of EIN3 to reduce ethylene signalling [69]. UVR8, CRYs and possibly phyB interact directly with transcription factors such as BRI1-EMS-SUPPRESSOR 1 to integrate light signalling directly with the brassinosteroid signalling cascade [70–72]. Separately CRY2 binds with CRYPTOCHROME-INTERACTING bHLH1 (CIB1) to directly promote *FT* expression and flowering [73], while varying effects on flowering time due to different degradation targets characterises the separate members of the Zeitlupe family [74]. Some of the protein-protein interactions in these pathways may also be directly temperature sensitive [75]. As well as WRKY36, UVR8 also functions in root development via association with MYB73 and 77 [76].

In summary, although the various photoreceptors have distinct roles in development, they overlap in both their spectral sensitivities and target TFs. This results in a complicated crosstalk in natural, multispectra light conditions, which provides plants with the possibility of fine tuning their responses. Moreover, both photoreceptors and their interacting TFs additionally have roles in mediating temperature responses.

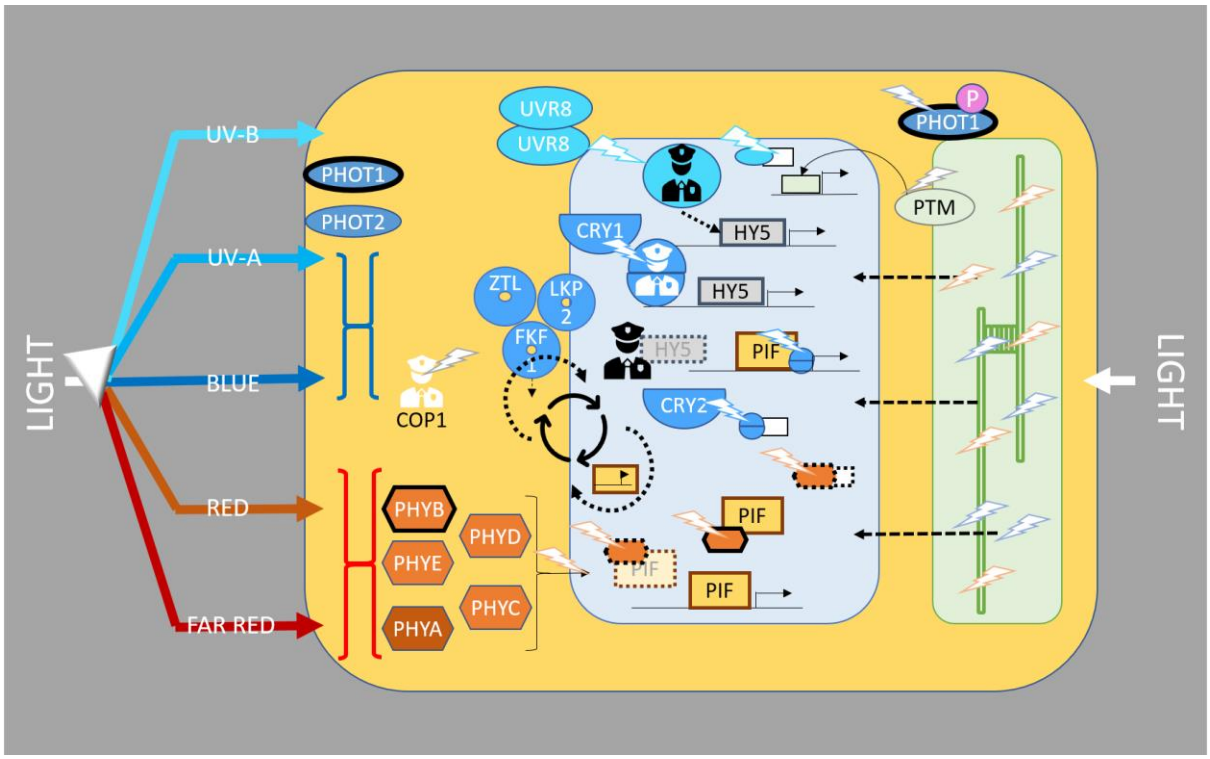

Figure 2. Multiple light signalling pathways are sensed by receptors and converge on transcription factors in the nucleus.

From the left, UV-B is received by UVR8 and triggers its monomerisation, association with COP1, and movement to the nucleus, which results in TF binding and HY5 stabilisation. Phototropins (blue, UV-A and UV-B) sit at the plasma membranes and light triggers their kinase activity. The members of the Zeitlupe family (ZTL, FKF1, LKP2) respond to blue light via changes in their affinities for different protein cofactors and their E3 ligase activity to affect the circadian clock (three-arrow circle), which has effects at all levels of cell activity, including the activity of PIFs. Blue and UV-A are sensed by CRYs, resulting in oligomerisation and TF binding in the nucleus as well as HY5 stabilisation. Phytochromes are activated and deactivated by red and far red light and respond by moving to the nucleus, destabilising PIF TFs and preventing PIF DNA binding. From the right: light fluence and quality activates the photosynthetic machinery, and results in biogenic and stress responses, communicated to the nucleus by a variety of routes. These include the cleavage of the chloroplast-envelope bound PHD transcription factor PTM, which moves to the nucleus itself to effect transcriptional responses. Key: Light split by a prism enters the cell (pale yellow rounded box), pale blue rounded box: nucleus, pale green rounded box: chloroplast. Lightning arrows: effect of light activation. Solid arrows: protein movement. Dotted lines: processes including multiple metabolites & proteins. Policeman black/white: COP1 active/inactive. Rectangular boxes in nucleus, grey/orange/green/white: TFs, HY5/PIFs/cleaved PTM/unspecified. Photoreceptors in thick black outlines are also defined as thermosensors.

2.2. INFLUENCE OF TEMPERATURE ON TRANSCRIPTIONAL INITIATION

2.2.1 PLANT THERMOSENSORS

A thermosensor detects changes in temperature cues by inducing changes in its structure and/or activity to adapt to the perceived signal. At the same time, temperature affects multiple processes in the plant and appropriate coupling of these temperature sensitivities can lead to compensation[77] or amplification[78] of the signal. As a thermosensor stands at the

core of any thermosensing mechanism, identifying specific thermosensors has been the target of much research effort [79].

Temperature fluctuations are known to affect cellular membrane fluidity, which in turn affects the activity of membrane bound proteins as well as their structure [80]. These changes in membrane fluidity are speculated to be the first step in the thermosensing process. Ca^{2+} channels are membrane bound proteins that exhibit changes due to cold temperatures, triggering Ca^{2+} influx. This influx is probably sensed via the Calmodulin Binding Transcription Activator (CAMTA) family of TFs to govern expression of the cold-responsive *C-REPEAT BINDING FACTOR* (*CBF*) genes. However, as of yet, there is no clear explanation of how Ca^{2+} influx is regulated under cold stress [81–84]. Heat stress has also been shown to affect Ca^{2+} channels and swiftly trigger Ca^{2+} influx to induce thermotolerance [85,86]. Further characterisation of the role of Ca^{2+} channels in the thermosensory mechanism will be needed in order for them to be considered as thermosensors themselves.

Temperature fluctuations affect protein activity and/or structure, therefore thermosensing can occur through protein conformational changes under the influence of temperature. A good example is phyB. Red light induces the transition from active Pr to inactive Pfr by altering the structure of its chromophore, with warm temperatures inducing the relaxation of the chromophore back to the Pr state[18,19]. Other phytochromes have also been proposed as thermosensors since it has been observed that temperature-induced hypocotyl elongation in the quintuple phytochrome mutant (*phyabcde*) is lost [18,19]. In addition, the blue-light photoreceptor phototropin has been shown to sense cold signals in the liverwort *Marchantia polymorpha* through its temperature dependent reversion dynamics, and is required for the cold-avoidance movement of chloroplasts [87,88]. This temperature sensitive role may be mediated by PHOT2 in Arabidopsis[88]. Further investigation is required to determine the viability of phototropin as a thermosensor in plants.

Moreover, high temperatures can lead to protein misfolding. Under the influence of high temperatures, HEAT SHOCK PROTEINs (HSPs) bind misfolded proteins, triggering in turn the release of *HEAT STRESS TRANSCRIPTION FACTORs* (*HSFs*) that regulate the thermotolerance response through a set of *HEAT STRESS RESPONSIVE* (*HSRs*) genes [89,90]. In addition to proteins, RNA molecules can also be sensitive to conformational changes upon temperature variation. Recent work has provided an example of an RNA thermoswitch in plants. The 5’-UTR of the

PIF7 RNA folds into a hairpin structure, which adopts two distinct conformations in a temperature-dependent manner [91]. This molecular switch adopts a more relaxed, yet distinct conformation at warmer temperatures, resulting in enhanced translation.

Pioneering work on the effect of temperature on chromatin dynamics proposed the histone variant H2A.Z as a potential thermosensor [92]. In *Arabidopsis thaliana*, nucleosomes containing H2A.Z are evicted from chromatin as ambient temperature rises [92]. The question of whether temperature could directly affect H2A.Z nucleosomes remained open for several years and led to much speculation. However, follow-up work demonstrated that H2A.Z nucleosome eviction requires the function of the HSFA1 clade of the Arabidopsis HSFs [93]. Indeed, in Brassica rapa, a close relative of Arabidopsis, H2A.Z controls transcriptional response to temperature but in the opposite direction, as H2A.Z levels increase with increasing temperature [94].

A new physical mechanism of temperature sensing has recently been described, whereby the relative abundance of a protein changes in response to temperature, but without an equivalent change in expression or stability. The transcription factor NTL8 accumulates over long periods of cold through a dilution-mediated mechanism, due the temperature-dependence of growth [95]. Whether similar mechanisms underlie other temperature responses is unknown. Despite this recent progress, plant thermosensors, unlike light sensors, remain largely uncharacterised.

2.2.2. TEMPERATURE-RESPONSIVE TRANSCRIPTION FACTORS

Several signalling pathways have been proposed to regulate cellular responses to both elevated and low environmental temperatures. Heat stress affects several processes during plant growth and development, such as hypocotyl and petiole elongation [96], while elevated ambient temperature has a positive effect on flowering [2,97] and a negative effect on stomata formation [98]. Several genes contribute to thermotolerance. Li and colleagues systematically analysed the transcriptional regulatory network in Arabidopsis plants after exposure to Heat Shock (HS) at 37°C using RNA sequencing (RNA-seq) [99]. They determined that HEAT SHOCK FACTOR A1 family proteins (HSFA1s), and the circadian clock proteins REVEILLE4 (RVE4) and REVEILLE8 (RVE8), are among the primary transcription factors that mediate the first wave of

HS-induced gene expression – providing insight into how the circadian clock helps plants adapt to high temperatures during the day. During HS, HSFA1s regulate transcriptional responses leading to thermotolerance (Figure 3A-B). However, their activity is suppressed under normal temperature environments through the action of HEAT SHOCK PROTEIN 70 (HSP70) and HSP90 (Figure 3A-B) [100–102]. Although not considered HS, high temperatures between ~29-36°C (supraoptimal) independently impact plant developmental responses. One example of this is inhibition of seed germination at supraoptimal high temperatures (thermoinhibition) [103]. High temperatures during seed imbibition activate expression of *SOMNUS* (*SOM*), which prevents seed germination by increasing ABA seed content and reducing GA biosynthesis [104]. Similarly, although warm temperatures accelerate flowering (see below), supraoptimal temperatures delay it through upregulation of *FLOWERING LOCUS C,* a MADS-box TF that represses flowering. This upregulation is due to higher protein stability of the chromatin modifier JUMONJI 30 (JMJ30) at 29°C, which prevents the accumulation of H3 lysine 27 di- and trimethylation (H3K27me2/3) at *FLC* [105]. These joint mechanism of delay of flowering and germination (thermoinhibition) by supraoptimal conditions may have important implications to seed set and survival when germinating under adverse environmental conditions.

The developmental response to warm temperatures, such as longer hypocotyls and petioles and more rapid flowering (thermomorphogenesis, ~20-28°C), is a separate process to HS (>37°C) and the supraoptimal high temperature response. *PIF4*, *PIF5*, and *PIF7,* discussed above for their role in light signalling, play a vital role in the positive regulation of plant thermomorphogenesis [106,107]. Rapid induction of *PIF4* expression has been observed to occur during early evenings due to warm temperatures and it is negatively regulated by the evening complex EARLY FLOWERING 3 (ELF3), ELF4, and LUX ARRHYTHMO (LUX) (Figure 3C) [108]. Another set of negative regulators of *PIF4* are POWERDRESS (PWR) and its interactor HISTONE DEACETYLASE 9 (HDA9), which control thermomorphogenesis through the induction of H3 deacetylation (Figure 3C) [109]. On the other hand, SHORT HYPOCOTYL UNDER BLUE 1 (SHB1) is recruited by the core components of the circadian clock CIRCADIAN CLOCK-ASSOCIATED 1 (CCA1) and LATE ELONGATED HYPOCOTYL (LHY) to positively regulate the expression of *PIF4* (Figure 3C) [110]. Further, the transcription factors TEOSINTE BRANCHED 1/CYCLOIDEA/PCF 5

(TCP5) and BRASSINAZOLE-RESISTANT 1 (BZR1) have been recently demonstrated to play a role in the positive regulation of *PIF4* in the thermomorphogenic response (Figure 3C) [111,112].

In addition, the UV-B receptor UVR8 and the light signalling TF *HY5* have separately been demonstrated to impair the thermomorphogenic response through negative regulation of *PIF4* expression (Figure 3C) [113,114]. During hypocotyl thermomorphogenesis, COP1 is imported into the nucleus where it reduces the negative regulation of HY5 on hypocotyl growth (Figure 3D). *cop1* mutants have been shown to be defective in *PIF4* expression and warmth-induced hypocotyl elongation [114,115]. Another key light-signaling component that plays a role in thermomorphogenesis is DET1. Together with COP1, DET1 is essential for the transcriptional regulation of *PIF4* through HY5 (Figure 3D) [116]. PIF4 then regulates hypocotyl elongation through auxin biosynthesis genes (e.g. *TAA1*, *CYP79B2* [117] and *YUC8* [118]) and auxin responsive proteins (e.g. IAA19, IAA29 [119]). Auxin biosynthesis in response to PIF4 occurs primarily in the epidermis of cotyledons and auxin acts as a mobile signal, travelling to the hypocotyl where it induces elongation in combination with local signals [120,121].

HY5 expression can also be induced by cold stress where it plays a positive regulatory role in the expression of the *COLD REGULATED* (*COR*) genes via the function of the Z-box and other cis-acting elements in their promoters (Figure 3E) [122]. The expression of *HY5* under cold stress is independent of the other major cold-stress pathway [122]. This latter involves the three *CBF* genes, *CBF1*, *CBF2*, and *CBF3*, and their transcriptional regulatory pathway (Figure 3F) [123,124]. Low temperatures bring on the swift induction of *CBF* gene expression and the consequential activation of *COR* gene expression. Another transcription factor that plays a role in cold acclimation is the bHLH transcription factor INDUCER OF CBF EXPRESSION 1 (ICE1) and its homolog ICE2, with both of them playing a role in the response to deep freezing through a *CBF*-dependent pathway in *Arabidopsis thaliana* plants (Figure 3F) [125,126]. The role of ICE1 in freezing tolerance is modulated through its phosphorylation by OPEN STOMATA 1 (OST1) [127]. OST1 is a SNF1-related protein kinase that positively regulates freezing tolerance in plants through inhibition of the interaction between the E3 ubiquitin ligase HIGH EXPRESSION OF OSMOTICALLY RESPONSIVE GENE1 (HOS1) and ICE1, leading to a more stable form of ICE1 and therefore a sustained freezing tolerance response [127,128]. Furthermore, OST1 plays a role in improving the protein stability of CBF proteins through the phosphorylation of BASIC TRANSCRIPTION FACTOR 3 proteins (Figure 3F) [129].

Other factors also play a role in plant mechanistic responses to cold stress h. For instance, cold stress has been found to activate several regulatory responses, such as the CYTOPLASMIC RECEPTOR-LIKE KINASE 1 (CRPK1) phosphorylation of 14-3-3 proteins, which induces their interaction with and stabilisation of CBFs [130]. Phosphorylation of ICE1 by the MAP kinases MPK3 and MPK6, as well as by BRASSINOSTEROID INSENSITIVE 2 (BIN2), is also induced by cold stress, leading to negative regulation of *CBF* expression [131,132]. Nonetheless, these extensive investigations of cold responses so far fall short of providing a comprehensive understanding of the full pathway from cold-sensing to response in plant cold and freezing acclimation.

As well as cold stress and freezing, plants also sense low temperatures over long periods as a seasonal signal, in a process termed vernalisation (Figure 3G). Vernalisation is a prevalent trait of plants in temperate zones and it means that exposure to long periods of cold promotes the induction of flowering [133]. In *Arabidopsis,* the regulation of vernalisation occurs through the action of the floral transcriptional repressor *FLC* which inhibits *FT*, a component of the flowering-inducing factor florigen [134,135]. *FLC* expression is repressed by long-term cold, and this process needs the PHD protein VERNALIZATION INSENSITIVE3 (VIN3), which is quantitatively upregulated during cold. *VIN3* seems to be regulated at the level of transcriptional initiation by three independent temperature inputs, a fast temperature response (minutes-hours), a short-term response to warmth (hours-days) and a slow response to cold (weeks) [136,78]. The latter was recently found to be mediated by NTL8 protein accumulation in long periods of cold [95], but the faster thermosensors remain unknown. *FLC* shutdown promotes flowering thermomorphogenesis, driven by both *FT* and other flowering regulating genes that are activated [137,138].

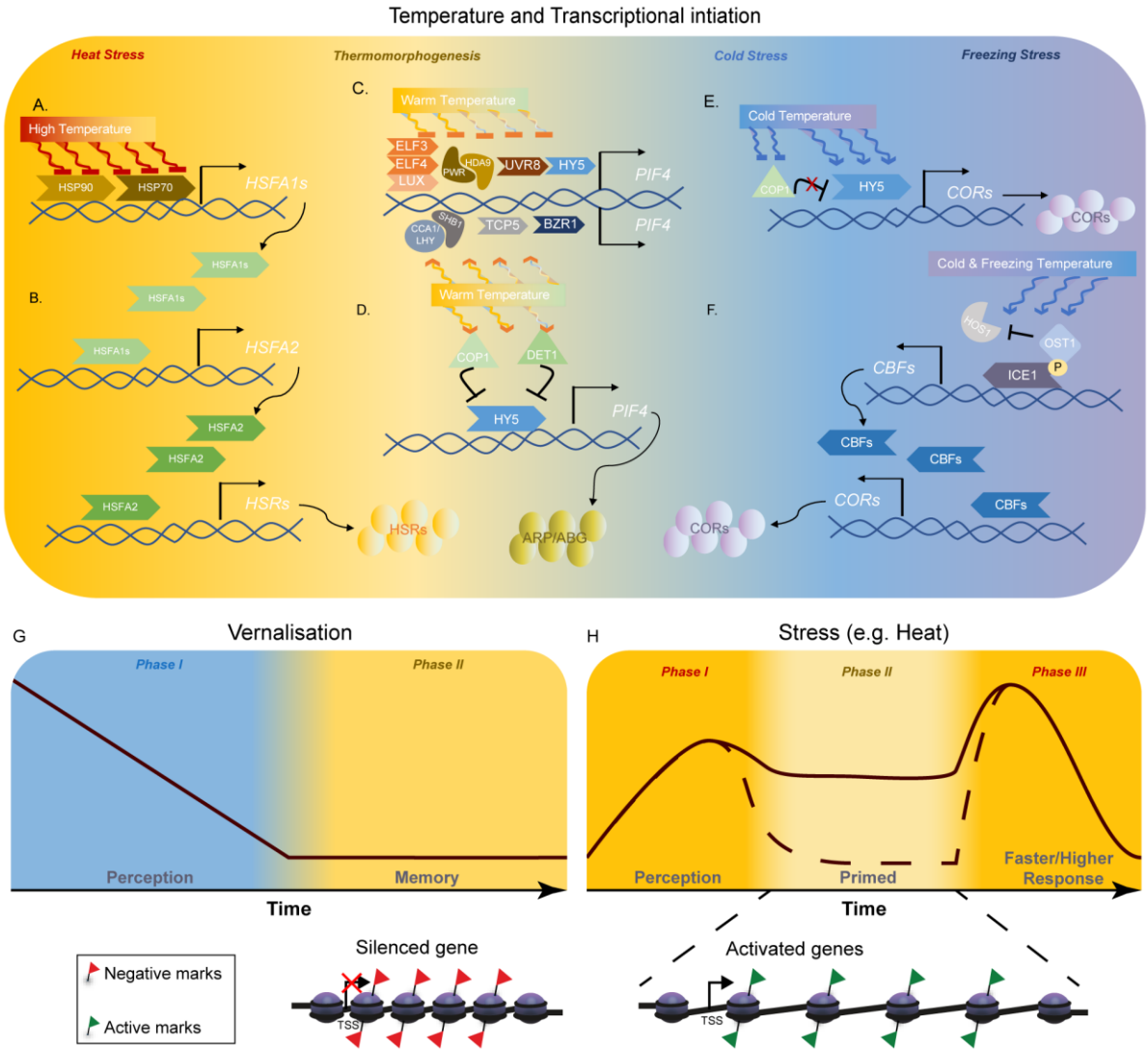

Figure 3. Temperature driven transcription initiation.

A, Heat stress halts the inhibitory activity of HSP70 and HSP90 on *HSFA1s*, leading to their disassociation from HSP70 and HSP90 and subsequent activation. B, Following the activation of HSFA1s they in turn regulate the transcriptional initiation of several *HSR* genes such as *HSFA2* that trigger thermotolerance. C, Warm temperatures induce the expression of *PIF4* by inhibiting the expression of several of its negative regulators such as the evening complex (ELF3, ELF4, LUX), PWR/HAD9, UVR8 and HY5. Warm temperatures also lead to the positive regulation of *PIF4* expression under the influence of several positive regulators such as CCA1/LHY/ SHB1, TCP5, and BZR1. D, Warmth causes both COP1 and DET1 to actively attenuate HY5 stability leading to its degradation and the subsequent induction of expression of *PIF4*. PIF controls auxin responsive proteins (e.g. IAA19) as well as auxin biosynthesis genes

(e.g. YUC8), indicated as ARP and ABG respectively in the schematic. E, Cold temperatures induce the expression of *HY5* and increase its stabilisation through nuclear depletion of COP1 and in turn initiate cold stress responses. F, Under cold and freezing stress, ICE1 is phosphorylated and stabilised by the protein kinase OST1 which interrupts ICE1 ubiquitination and degradation by the E3 ligase HOS1. Stabilisation of ICE1 leads to the initiation of the transcription of several *COR* genes leading to the triggering of cold acclimation and freezing tolerance. G, During vernalisation, perception of winter cold temperatures (Phase I) triggers the quantitative inactivation of *FLC* transcript (green solid line). In the absence of the cold signal during spring, epigenetic memory (Phase II) maintains the *FLC* at low levels. *FLC* silent state correlates with the accumulation of the H3K27me3 repressive mark at *FLC* chromatin. H, Transcriptional memory of Heat Stress (HSt) can also be divided into distinct phases. Perception of high temperatures induces activation of HSt memory genes (Phase I). Upon removal of the heat signal (Phase II or primed state), HSt memory genes can exhibit sustained (green solid line) or baseline (green dotted line) expression levels, with their chromatins coated by the H3K4me2/3 active marks. HSt memory genes show higher or faster activation following recurrent heat stress (Phase III) conferring on the plant the ability to prevent damaging effects.

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3. DIVERGENT EFFECTS OF LIGHT AND TEMPERATURE ON PLANT TRANSCRIPTION

Light sensing is translated into both cytoplasmic and chloroplast retrograde signalling pathways that converge in specific TFs to regulate transcriptional initiation. Temperature-induced transcriptional regulation diverges from light because it will not only influence the set of genes being turned ON or OFF by specific TFs, but may also directly affect the physical properties of nascent RNA transcript processing and RNAPII kinetics. In the following sections, we summarise the direct and indirect effects of temperature and light, respectively, on the regulation of co-transcriptional processes in plants.

3.1. DIRECT EFFECT OF TEMPERATURE ON CO-TRANSCRIPTIONAL PROCESSES

It has been recently proposed that temperature sensing in plants is distributed across regulatory networks, with multiple temperature inputs combined to control each process [78]. Such a temperature sensing system could be controlling the abundance of the TFs described previously, but it could also be directly affecting mechanisms relating to transcription, following transcriptional initiation. Co-transcriptional processes such as elongation, RNAPII stalling at gene borders, splicing, termination and polyadenylation may also be sensitive to temperature (Figure 1B-D). In warm-blooded mammals, it has been proposed that cooling stabilises secondary structures of pre-mRNAs affecting splice site selection [139]. In plants, given their sessile nature, physical perturbations on macromolecules caused by temperature variation are likely to be even more dramatic.

Recent studies have started to elucidate distinct features of nascent transcription in plants [140–144] with particular attention given to how cooling influences co-transcriptional processes [145]. A detailed time series during cold acclimation in Arabidopsis revealed dynamic changes in expression of several genes, including induction of previously characterised cold-inducible genes, as well as massive changes in alternative splicing (AS) patterns [146]. Over a quarter of genes whose expression changes significantly in the cold also undergo AS. Among the differentially spliced genes identified, some had previous evidence of involvement in cold responses, such as *REGULATOR OF CBF EXPRESSION 1* (*RCF1*), *PIF7* and *PHYB*. In addition to protein coding genes, hundreds of long non-coding RNAs (lncRNAs) are differentially expressed and alternatively spliced in response to cold [147]. Variation in gene splicing patterns occurs rapidly upon cold exposure and can be sensitive to temperature reductions as small as 2°C [146]. AS makes a major contribution to changes in the transcriptome, amplifying the ability of temperate plants to grow in a wide range of fluctuating temperatures, as influencing the spliceform generated from each locus increases the potential transcriptomic variation that a signal cascade can induce. Temperature-induced AS could be caused by either a direct effect on the splicing components and the secondary structure of the nascent RNA, or the result of the variation of RNAPII kinetics as temperature decreases. Indeed, the application of a NET-seq approach in Arabidopsis (plaNET-seq) has shown transient molecular adaptations of RNAPII transcription in response to cold [145,148]. RNAPII kinetics are modulated as temperature decreases leading to changes in transcriptional elongation and termination. Interestingly, the

dynamics of the splicing reaction are initially affected during early stages of cold acclimation but later recover to normal levels, reinforcing the influence of AS in rapid responses to cold.

Elevated ambient temperature and heat stress can also impact co-transcriptional processes in plants. Temperature-induced differential accumulation of two *FLOWERING LOCUS M* (*FLM*) splice variants, FLMβ and FLMδ, regulates Arabidopsis floral transition [149]. Whereas low ambient temperature favours accumulation of FLMβ that actively represses flowering, rising temperature increases the FLMδ/FLMβ ratio to release floral repression. In addition to splicing, *FLM* levels are also reduced by the Nonsense-Mediated mRNA Decay (NMD) pathway under elevated temperatures resulting in floral induction [150]. Heat stress also has a strong effect on mRNA splicing. The U5-snRNP-interacting protein STABILIZED1 (STA1) is a heat inducible gene involved in the splicing of heat shock factors (HSF) and HSP transcripts during high temperature stress [151]. Interestingly, STA1 had originally been found to regulate the splicing of cold responsive genes such as *COR15A* under low temperature stress [152].

Widely present in mammals and metazoans, the process of RNAPII stalling over gene boundaries was recently demonstrated in plants [140,143]. Engaged RNAPII initially transcribes 20–60 nucleotides before undergoing promoter-proximal (5' end) pausing, a regulatory checkpoint for execution of transcription programmes [153]. In Arabidopsis, promoter-proximal stalling of RNAPII transiently increases at early stages of cold acclimation to adjust transcription to low temperatures [145]. In addition to promoter-proximal stalling, RNAPII stalls near 3' ends of Arabidopsis genes [141–143]. Cold exposure significantly decreases the 3' end peak of RNAPII observed when the polymerase is stalled downstream of the Polyadenylation Site (PAS), suggesting a major change in transcription dynamics associated with termination in response to low temperatures [145].

3.2. PHOTORECEPTOR-MEDIATED EFFECTS ON CO-TRANSCRIPTIONAL RESPONSES

Light also controls co-transcriptional processes such as choice of Transcription Start Site (TSS), pre-mRNA- and alternative- splicing of large numbers of genes, with mechanisms distinct from those controlling transcriptional responses [148,154–163]. In the case of light-induced alternative TSS use, this leads to changes in protein fate. Ushijima and colleagues found that red-light activated phyB alters TSS usage for over 2000 loci, enriched in plastid-targeted proteins, and the resulting changes in N-terminal signal sequence leads to changes in

subcellular protein localisation for nearly a fifth of these genes [163]. In contrast, Kurihara et al. investigated blue light, finding that a small (~200) subset of target genes have TS sites both upstream and downstream of short open reading frames in the 5'UTR of the main coding sequence [155]. These upstream open reading frames can trigger transcript degradation through the NMD pathway, or interfere with translation itself, both routes leading to reduced protein output [164]. Blue light promotes use of the downstream, protein-producing TSS in most cases, a response partially mediated via HY5, which has binding sites within many of the target promoters [155]. Like the red-light response, the blue light target set is enriched in light responsive genes, and both responses modulate the photorespiration pathway to cope with effects of fluctuating light on the photosynthetic machinery [155,163].

Like temperature, light also induces alternative splicing, in the majority of cases promoting production of protein-encoding splice variants over those with NMD features [165]. At least two sets of photoreceptors are known to mediate this response. One reacts to white light intensity via chloroplast retrograde signalling in response to Photosystem II function [158,165,166]. The other route requires red-light sensitive phytochrome action and is conserved across the land plants [159,161,162,165,167–169]. These routes are independent but not mutually exclusive, and converge on serine/arginine-rich (SR) splicing factors such as *RS31* [160,161].

Phytochrome-dependent AS is critical for phy-mediated photomorphogenesis and regulates several transcription factors, such as *PIF3, PIF6* and *ELF3*, and negative regulators, such as *SPA3,* required for light-signalling itself [159,161,162,169,170]. The mechanism of phytochrome-dependent AS appears to be through direct interaction of phyB with the pre-mRNA splicing factors REDUCED RED-LIGHT RESPONSES IN CRY1CRY2 BACKGROUND1 (RRC1) and SPLICING FACTOR FOR PHYTOCHROME SIGNALING (SFPS) [162,167,169]. In Arabidopsis, the interaction between RRC1 and phyB is light-induced but independent of the Pr/Pfr conformation of phyB [162], whereas in the moss *Physcomitrella patens*, interaction between PpPhy4, a phyB homologue, and the splicing regulator PphnRNP-F1 is red-light specific, and induces interaction of PphnRNP-F1 with other spliceosome components [171,172]. In Arabidopsis, *RRC1* is itself alternatively spliced in an SFPS-dependent response to light, providing a positive feedback loop [162,165,167].

At least part of the light-driven AS response seems to occur due to enhanced RNAPII elongation in response to light [166]. It is unlikely that light will directly modulate the physical properties of RNAPII and RNA transcripts as has been suggested for temperature [145]. Instead,

while the expression of the transcription elongation factor *TFIIS* itself is not affected, members of the plant POLYMERASE ASSOCIATED FACTOR COMPLEX (PAF1-C), including *EARLY FLOWERING7*, are transcriptionally upregulated in response to light and may provide part of the mechanism [166]. More directly, genetic evidence links stress-induced singlet oxygen in the chloroplast to the activity of TOPOISOMERASE VI, which binds a subset of high-light induced target promoters directly and may also enhance elongation [33]. Recent work has demonstrated a specific mechanism for integration of light into circadian-clock regulated transcriptional activation via the NIGHT LIGHT-INDUCIBLE AND CLOCK-REGULATED (LNK) proteins [173]. LNKs are transcriptionally upregulated in response to light, probably via phytochrome signalling [174] and recruit RNPII and the transcript elongation FACT complex to their targets via interaction with the MYB TF RVE8 [173]. Interestingly, RVE8 is also involved in temperature responses [99].

Questions remain, however, on which responses are direct and which due to secondary signalling [156]. Many of the light-responsive AS genes are splicing factors themselves, leading to a secondary cascade of AS throughout the plant [160–162]. The presence of the AS response in non-irradiated, chloroplast-lacking roots indicates the role of a secondary, non-cell-autonomous system [158], possibly mediated via sugar signalling [165]. As a result light, like temperature, can affect genome-wide co-transcriptional processes at the whole plant level, via secondary signalling.

In conclusion, both light and temperature generate genome-wide cascades of co-transcriptional effects. However, while in the case of light these are generally triggered through distinct receptor pathways and subsequent upregulation of the transcriptional machinery, temperature can modulate transcription kinetics directly.

3.3 INDUCTION OF LONG NON-CODING TRANSCRIPTS

A common phenomenon observed in plant responses to light and temperature is the induction of lncRNAs. During long cold periods, plants undergo massive transcriptional reprogramming, which also results in the activation of lncRNAs. The *FLC* locus has provided a highly informative system for studying the effects of these transcripts. *COOLAIR* lncRNAs, antisense to *FLC* locus, significantly increases in expression levels in the first couple of weeks of winter temperature [175,176]. Transcription from the antisense strand may inhibit RNAPII firing

from the *FLC* sense strand, as suggested by the mutually exclusive expression of *COOLAIR* and *FLC* nascent transcript at single loci resolution [177]. Although the precise mechanism by which *COOLAIR* suppresses nascent *FLC* expression during cold remains unknown, modulation of chromatin dynamics is likely implicated in this process. Arabidopsis transgenic lines with reduced *COOLAIR* function fail to remove the H3K36me3 mark at the *FLC* locus, resulting in less efficient reduction of *FLC* nascent transcript during cold [175]. This observation suggests that cold-induced non-coding transcripts at *FLC* are required to set the chromatin landscape for transcriptional inactivation.

Another cold-induced lncRNA also represses *FLC* via modulation of local chromatin architecture. Expression of the sense lncRNA COLDWRAP from the sense *FLC* inactive promoter induces the formation of an intragenic gene loop within the *FLC* locus to perpetuate gene inactivation [178]. Finally, the intronic sense lncRNA *COLDAIR* mediates PHD-PRC2 (PLANT HOMEODOMAIN- POLYCOMB REPRESSIVE COMPLEX 2) recruitment to *FLC* for stable epigenetic silencing [179]. Another vernalisation-linked lncRNA, that does not map to the *FLC* gene, is the *MADS AFFECTING FLOWERING 4* (*MAF4*) *antisense* (*MAS*) transcript [180]. *MAS* is transcribed during cold exposure [180] mimicking the expression pattern of *MAF4* [181]. Induction of MAS seems to play a crucial role in the recruitment of COMPASS-like complexes to MAF4 chromatin to activate its expression [180]. In all cases, it remains to be determined what pioneer TFs trigger transcription of all these cold-induced lncRNAs.

Temperature-induced non-coding transcription has been shown to affect transcription termination. Cold-induced transcription of the Arabidopsis *CBF* locus triggers expression of a cascade of antisense transcripts that fine-tunes the level of *CBF1* mRNA [182]. The lncRNA *SVALKA*, located downstream of the *CBF1* gene, is transcribed from the antisense strand. *SVALKA* transcription terminates downstream of the *CBF1* locus in plants growing under warm temperatures. During cold acclimation, however, RNAPII read-through transcription of *SVALKA* results in a cryptic lncRNA overlapping *CBF1* on the antisense strand, termed *asCBF1*. The *SVALKA*-*asCBF1* system limits the production of maximal *CBF1* mRNA levels by a RNAPII collision mechanism in the 3'-end of *CBF1* [182]. Thus, the interplay of two lncRNAs tightly regulating *CBF1* mRNA levels allows plants to appropriately acclimate to low temperatures.

Increasing evidence shows that non-coding transcription also leads to the formation of R-loops in the plant genome. R-loops are created by the invasion of the DNA duplex by complementary RNA molecules, generating an RNA-DNA helix and leaving a single-stranded section of complementary DNA. At the single gene scale, R-loops appear to be important for transcriptional regulation [183–186]. R-loop stabilisation at the promoter of *COOLAIR* is required for correct expression of both *COOLAIR* and *FLC* [187]. Invasion of the lncRNA *APOLO* (*AUXIN REGULATED PROMOTER LOOP*) into a number of genomic loci, including auxin responsive genes, results in R-loop formation and subsequent displacement of Polycomb repressive activity leading to gene activation [188]. However, construction of a genome-wide R-loop atlas by the Sun lab revealed that R-loop presence does not strongly correlate with mRNA transcript abundance [189]. Indeed, genome wide R-loop patterns seem to be largely conserved through many environmental perturbations, although developmental phase changes such as germination and flowering have more noticeable effects [189]. Exposure to long term heat stress results in a slight increase in antisense R-loops localised to the TSS. A subset of these show correlation of expression with the expression with known R-loop regulators during this process, hinting at a regulatory role. However RNAPIII-transcribed genes, such as small nucleolar RNAs and tRNAs, are consistently associated with higher R-loop levels in germinated seedlings than germinating or dark-grown seedlings, raising the possibility that R-loop dynamics may be particularly important for light regulation of RNAPIII transcription [189]. Further work on individual gene dynamics and R-loop regulators will shed more light on the mechanistic basis of these responses and their biological role.

Similar genome-wide studies of lncRNAs in response to light have focused on natural antisense transcripts (NATs) in de-etiolating seedlings [190]. A majority of light-induced 'concordant' NAT (those changing expression in the same direction as the coding transcript) are principally expressed in cotyledons and include members of the light-sensing pathway such as *SPA1* and *HYH*. Light also induces a number of long intergenic non-coding RNAs of unknown functional significance [165]. Further detailed work is required to functionally characterise the mechanism and roles of these light-induced lncRNAs.

3.4 BOTH LIGHT AND TEMPERATURE IMPACT PLANT CHROMATIN DYNAMICS

Plants monitor temperature changes both diurnally and seasonally. Temperature may physically alter the interaction of DNA with nucleosomes and thus have a direct effect on chromatin compaction [191]. Not surprisingly, plant genes may display unique chromatin signatures in response to temperature cues [192]. As mentioned above, elevated temperatures trigger a universal response which involves the rapid upregulation of heat shock proteins (HSPs) [193]. Heat stress, which for Arabidopsis usually means exposing plants to temperatures above 36°C, induces substantial changes to plant nuclear architecture, including decrease of heterochromatin compaction [194] and decondensation of chromocentres [195]. DNase-seq studies revealed that the degree of chromatin accessibility changes dynamically upon heating [196]. The most highly accessible genes following heat shock encode heat shock proteins and several heat-stress-related TFs, which correlates with their rapid activation. Similarly, heat shock results in the reduction of nucleosome density, mediated by HEAT INTOLERANT 4 (HIT4) [197].

Moving from dark to light also results in major changes in nuclear organisation such as decondensation of chromocentres and decompaction of heterochromatin, especially during photomorphogenesis [192,198–201]. As well as the large-scale transcriptional reprogramming associated with the developmental aspects of photomorphogenesis, high light is a cellular stress, and UV irradiation causes DNA damage. Responses to all these aspects are united by the multifunctional roles of DET1 [60]. As well as its role in stabilisation of PIF proteins [56], DET1 also has roles in the repair of UV-induced DNA damage through the global genome repair pathway [59]. In addition, DET1 binds directly to non-acetylated tails of histone H2B [202] and is required for the global increase in H2B ubiquitination that is induced by light [203], via DET1-mediated degradation of a deubiquitination module of the SAGA complex [204]. Correct H2Bub is required for light-induced transcription change and seems particularly important for genes that respond especially rapidly [203].

Besides heat shock, more moderate variation in ambient temperature also impacts plant chromatin and transcription. In fact, temperature changes as little as 1°C may lead to gene reprogramming, and can also affect crop yield [205]. As part of the thermomorphogenic response, in *Arabidopsis thaliana* nucleosomes containing the histone variant H2A.Z are

evicted from the chromatin of thermo-responsive genes when temperature rises from 22°C to 27°C [92] resulting in transcriptional change dependent on the locus. H2A.Z nucleosomes may act to enhance the transcriptional dynamic range by keeping genes transcriptionally repressed under non-inductive conditions via restriction of promoter and gene accessibility. Histone deacetylation by HDA9 also facilitates H2A.Z nucleosome eviction from the promoter of the thermo-responsive gene *YUC8* under warmer temperature [206] resulting in transcriptional activation. This process, likely occurring downstream of HSFs, extends our view of histone deacetylation, which is generally associated with transcriptional repression. In addition, histone H3 lysine 36 methylation (H3K36me3) affects temperature-induced alternative splicing, potentially by facilitating the recruitment of splicing factors to the chromatin during transcription [207]. Whether H3K36me3 dynamics could be acting as a thermosensor needs further validation. More generally, the ability of any chromatin structure to directly sense a temperature change to control thermal responses remains an open question [79].

Increases in histone acetylation appear to be a general part of light-induced chromatin changes that are associated with the transcriptional upregulation of many genes, including HY5 and HYH [208]. Indeed, addition and removal of acetylation at HY5 and PIF target genes may form part of the antagonistic actions of these light regulators on transcription. For example, Peng et al. find that PIF recruits HISTONE ACETYL TRANSFERASEs (HATs) to regulate shade response [209], while Yang et al. find HY5 recruits HDA9 to repress target genes involved in autophagy in the dark [210]. HISTONE DEACETYLASE 15 (HDA15) is also recruited to targets by both PIF1 and PIF3 to mediate repression by deacetylation of target genes [211,212].

PIF1 additionally recruits another form of histone modifier to its targets: the SWI/SNF complex member BRAHMA (BRM) [213]. BRM acts with PIF1 to reduce H3K4 methylation and expression of chlorophyll biosynthesis genes in the dark, preventing photobleaching during subsequent irradiation. PIF1 itself is under chromatin regulation, with normal *PIF1* expression requiring the H3K36 methylase SDG8 [214].

Downstream of light-regulated transcription itself, several chromatin modifiers have been characterised as necessary for light response. Among the chromatin modifiers themselves transcriptionally regulated in response to light are several Jumonji-domain proteins. *JMJ20*

and *JMJ22* are upregulated in response to red light, and enhance seed germination by demethylating histone H4 arginine residues and thereby derepressing gibberellin synthesis loci [215]. The H3K27me3 demethylase *JMJ30/JMJD5*, which acts at *FLC* in response to high temperatures, is also alternatively spliced in response to light, although the functional significance of this is unknown [160]. JMJ30 is involved in temperature compensation of the circadian clock [216], potentially providing another route for light and temperature integration at the chromatin level. In addition, the ATP-dependent chromatin remodelling factor PICKLE (PKL) acts downstream of both light and temperature signalling to promote Arabidopsis growth [217,218] thus providing further integration of light and temperature signals through chromatin regulation.

3.5 LONG TERM MEMORY OF ENVIRONMENTAL STIMULI PERCEPTION

So far, we have discussed how perception of specific cues results in transient transcriptional regulation. However, many transcriptional reprogramming events, as is the case for acquisition of stress tolerance and developmental switches, should persist for prolonged periods of time even in the absence of the triggering signal [219,220]. In those cases, two distinct phases can be distinguished in the response (Figure 3G-H): initial and rapid up- and down-regulation of genes induced by perception and integration of a specific cue from the surroundings (Phase I); and perpetuation of the acquired ACTIVE or INACTIVE potential of those genes for extended periods of time by epigenetic mechanisms, in the absence of the inductive cue (Phase II).

The best characterised example of such an epigenetic process is the silencing of the gene *FLC* in vernalisation, and the memory of the silencing following winter (Figure 3G). The *FLC* gene is highly expressed in vernalisation-requiring accessions of Arabidopsis, until the plants experience prolonged cold [221]. Increasing weeks of cold temperature trigger the inactivation of *FLC* transcription (Phase I) and this is accompanied by the build-up of the epigenetic silencing apparatus at *FLC* chromatin. Subsequently, Phase II secures the maintenance of the *FLC* silent state once the cold signal has disappeared (Figure 3G).

During Phase I, exposure to cold temperatures induces epigenetic silencing of *FLC* by the action of lncRNAs (COOLAIR, COLDAIR, COLDWRAP, as discussed above), by the sequence-specific transcriptional repressors (VAL1 and VAL2 proteins, [222,223]) and by the PRC2 combined

with Plant Homeodomain (PHD) proteins (PHD-PRC2 complex; [224,225]). PHD-PRC2 assembles at *FLC* chromatin during cold and deposits the H3K27me3 histone repressive mark at individual *FLC* loci [226–230] while histone demethylases remove the chromatin active marks (H3K36me3 and H3K4me3) that were present in those nucleosomes [231].

After the cold signal is gone, the individually silenced *FLC* copies remain transcriptionally inactive allowing the plants to remember the cold period and quantify its duration (Phase II, Figure 3G). Upon return to warm temperatures, H3K27me3 spreads from the nucleation region to cover the *FLC* locus (Figure 3G)[225,226,228–230].

Cold-sensing is not just a signature for floral induction. Plants are able to remember cold stress to enhance survival when they are exposed to low temperatures a second time [232,233]. Before cold stress, H3K27me3 coats the genomic region of a subset of early-cold inducible genes required to trigger cold acclimation [234–236]. Therefore, PRC2-mediated repression likely prevents activation of these loci under non-stress conditions [236]. However, H3K27me3 does not seem to control short-term cold-stress memory [236] as it does for the long-term maintenance of *FLC* silencing during vernalisation. Future work will reveal the chromatin components underlying cold stress transcriptional memory.

A well-documented example of stress memory is thermotolerance, where perception of moderate HS allows plants to subsequently withstand high temperatures that would otherwise be lethal [237]. A number of genes, including *HSP22.0* and *ASCORBATE PEROXIDASE 2* (*APX2*), are initially activated during the priming HS and their expression levels are either sustained or downregulated to basal levels in the absence of the inductive cue [238] (Figure 3H). Either way, they show an increased response after a recurring HS days after the primary HS, consistent with the definition of transcriptional memory (Figure 3H)[239]. HS transcriptional memory at the memory-related loci *HSP22.0* and *APX2* depends on the sustained accumulation of H3K4me3 and H3K4me2 [239]. Both HS transcriptional memory and the sustained H3K4me3 and H3K4me2 depend on the HEAT SHOCK TRANSCRIPTION FACTOR A2 (HSFA2) [239]. In addition, the Arabidopsis homologue of the metazoan Strawberry Notch protein FORGETTER 1 (FGT1) is required for HS memory. FGT1 associates with the promoters of HS memory-related genes to ensure proper nucleosome remodelling during the acquisition of thermotolerance [240]. By analogy to vernalisation, plant HS memory can also be divided into

two phases (Figure 3H) corresponding to “perception” (Phase I) and “memory” or “primed state” (Phase II). Additionally, stress memory exhibits a unique Phase III that correlates with the rapid or altered transcriptional responses to the recurrence of the triggering signal (Figure 3H).

Another example of transcriptional stress memory occurs during recurring dehydration stresses [241]. In this case, not only does H3K4me3 act as a persistent epigenetic mark, but in addition stalling of the phosphorylated serine 5 form of RNAPII (Ser5P) is associated with transcriptional memory [241]. Interestingly, light signalling via phytochromes is required for transcriptional memory of the salt- and dehydration-stress inducible gene *Δ1-PYRROLINE-5-CARBOXYLATE SYNTHASE 1* (*P5CS1,* [242]). HY5 and HYH are not required for initial upregulation of *P5CS1,* but HY5 binding to the promoter is necessary for maintenance of H3K4me3 levels at the locus for enhanced response to repeated stimuli [242].

Undoubtedly, chromatin dynamics play a key role in plant adaption to light fluctuations [192,200,243]. However, the existence of mechanisms of transcriptional stress memory in the field of light stress responses remains an open question. Exposure to high light results in priming, or photoacclimation, as well as in the induction of a systemic acquired response that renders non-exposed leaf tissues less susceptible to recurrent exposure to light stress [244–247]. However, efforts to try to link epigenetic modifications, e.g. DNA methylation, with the acquisition of a “primed state” following excess light stress have not been successful [247,248]. Follow up studies will be necessary to reveal what are the chromatin components taking part in these mechanisms that prime plants to rapidly respond to harmful irradiation.

7. CONCLUDING REMARKS

Light and temperature are the two most important environmental signals to determine seasonal progression and correct timing of development for most plants. At the same time, they are linked to the plants ability to produce energy, capture CO_2 and survive other stresses in direct ways, as well as being potential stressors themselves. Therefore, it is not surprising that plants have a complex way of responding to each of these signals separately, and that they also become integrated in many responses. The pathways of environmental signalling have multiple stages, starting with signal perception by “sensors” (Phase I), followed by downstream transcriptional cascades and in some cases long-term memory of the signal

(Phase II/III). The presence and duration of this memory will depend on the nature of the signal and the desired response. For example, in stress response, the experience of a stress now is likely to indicate that the same or a related stress will occur again in the near future. However, the opposite is true in more long-term signalling that determines seasonal progression such as vernalisation, which tells the plant that winter has passed and is unlikely to occur again for a long time.

In the perception of environmental signals, it is very hard to distinguish direct, sensor-mediated responses and indirect, downstream transcriptional cascades, and so the search to identify the primary sensors has been a difficult one. For light sensors, which have more distinctive properties, many have been known for a long time. Temperature instead has more direct effects in biochemistry and so most processes can contribute to its perception, making temperature sensing a question of integration of (often subtle) inputs from multiple "thermosensors". Thus, plant thermosensors, unlike light sensors, remain largely unexplored. Furthermore, though the mechanism of its significance is still unknown for most cases, the fluctuating nature of the temperature signal has a strong effect on its perception [136,249,250]. This highlights the pressing need for further knowledge on the subject, considering the important role of thermosensing in plant growth, development and temperature stress acclimation. Under a scenario of global temperature increase, in combination with an increase in extreme events, knowledge on how to modulate plant thermosensing mechanisms would provide great advantages for crop breeding and growth.

Regarding transcriptional regulation, light and temperature have similar yet divergent effects. Following perception, the underlying signalling pathways converge in the function of numerous TFs, some of which are common to both cues. In addition, temperature affects the photosensitive reactions of several of the known light receptors, thus signalling is integrated from the start. The application of novel transcriptomic technologies has now allowed to shift the focus from TFs to the analysis of effects on splicing, RNAPII elongation rate and stalling in plants. In the case of light, thus far these effects seem to be mediated through TF control of the components of the transcriptional and splicing complexes. However, this has not yet been found in analyses of temperature effects. These initial key findings will pave the way for future studies to determine whether the components of the transcriptional machinery (RNAPII, spliceosome, nascent RNA) are themselves thermosensors. Given the highly structured

nature of RNA molecules, the possibility remains that nascent RNAs may adopt dynamic conformational changes directly modulating co-transcriptional processes. Despite advances in recent years, research on RNA *in vivo* structures in plants is still in its early days. Efforts in this direction should focus on investigating perturbations to RNA molecules in plants growing in natural habitats, to ensure their biological relevance.

Both light and temperature signalling converge in modulation of chromatin states. These dynamic changes in chromatin conformation become themselves part of the signalling process by controlling expression of numerous genes both short and long term. Chromatin changes that follow light perception are very likely a consequence of the action of photoreceptor-induced TFs. Conversely, temperature may directly perturb physical conformations of chromatin, but conclusive *in vivo* examples of such processes are still missing. Work is required to define to what extent nuclear chromatin is responsive to temperature variation. In parallel, possible thermal effects on the kinetics of the catalytic activities of chromatin "readers" and "writers" should also be evaluated.

In this context, precise, quantitative analysis of the responses to temperature and light levels are required to dissect the contribution of each mechanism to the overall response. Moreover, the sheer complexity of these interacting, simultaneous processes will likely require mathematical and computational approaches to elucidate non-intuitive results. Such approaches, very informative in dissecting the mechanism of photomorphogenesis [251], have already begun to bear fruit in the identification of the elusive plant thermosensors.

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DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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