ANNUAL REVIEWS

Annual Review of Plant Biology

Temperature Sensing in Plants

Sandra M. Kerbler[1] and Philip A. Wigge[1,2]

[1]Leibniz Institute of Vegetable and Ornamental Crops (IGZ), Großbeeren, Germany; email: kerbler@igzev.de, wigge@igzev.de

[2]Institute of Biochemistry and Biology, University of Potsdam, Potsdam, Germany

Annu. Rev. Plant Biol. 2023. 74:341–66

First published as a Review in Advance on February 28, 2023

The *Annual Review of Plant Biology* is online at plant.annualreviews.org

https://doi.org/10.1146/annurev-arplant-102820-102235

Keywords

thermosensor, *Arabidopsis thaliana*, adaptation, heat stress, cold stress, thermomorphogenesis

Abstract

Temperature is a key environmental cue that influences the distribution and behavior of plants globally. Understanding how plants sense temperature and integrate this information into their development is important to determine how plants adapt to climate change and to apply this knowledge to the breeding of climate-resilient crops. The mechanisms of temperature perception in eukaryotes are only just beginning to be understood, with multiple molecular phenomena with inherent temperature dependencies, such as RNA melting, phytochrome dark reversion, and protein phase change, being exploited by nature to create thermosensory signaling networks. Here, we review recent progress in understanding how temperature sensing in four major pathways in *Arabidopsis thaliana* occurs: vernalization, cold stress, thermomorphogenesis, and heat stress. We discuss outstanding questions in the field and the importance of these mechanisms in the context of breeding climate-resilient crops.

Contents

1. INTRODUCTION

Plants have adapted to live in almost every ecological niche on earth, encompassing a remarkable range of temperatures from approximately −80°C to 70°C (81). The ability to thrive across such a wide range of climates is a reflection of their developmental plasticity and ability to adapt. Broadly, adaptation can be considered as passive or active. Passive mechanisms include hard-wired genomic traits such as amino acid composition and protein structure. Extremophiles, for example, have more rigid proteins with increased hydrogen bonds (17). Active mechanisms enable plants to adjust their growth and development to respond to sudden changes in temperature as well as anticipate seasonal change and future stresses. Some of these changes are visible on a macroscale, such as the opening of buds in the spring, while others are apparent only at a subcellular level. Such specific and targeted responses require both the perception and transmission of temperature information.

Temperature has wide-ranging effects on plants, influencing different aspects of metabolism, growth, and development. In general, low temperatures lead to reduced enzyme activity, rigidification of membranes, destabilization of protein complexes, stabilization of RNA secondary structures, accumulation of reactive oxygen species (ROS), impairment of photosynthesis, and leakage across membranes. Similarly, high temperatures cause proteins to unfold and aggregate, membranes to become more fluid, and changes that can be seen in the organization of cellular structures, including organelles and the cytoskeleton. Major protective responses to heat stress include enhanced production of phytohormones, such as abscisic acid, antioxidants, and other protective molecules, and transcriptional induction of a suite of genes encoding heat shock proteins (HSPs). While all plants respond to temperature, the most well-studied system is *Arabidopsis thaliana*, owing to its excellent tractability. *Arabidopsis* has four key temperature response pathways: (*a*) vernalization, the conferring of flowering competency in response to prolonged cold; (*b*) the cold stress pathway that protects plants against chilling stress (CBF induction); (*c*) thermomorphogenesis, the acceleration of growth and flowering in response to elevated ambient temperatures; and (*d*) the heat shock response to protect against cellular damage by high temperature stress. These pathways use temperature information to trigger major adaptive responses of both development and stress signaling to enhance adaptation.

Temperature is molecular motion and affects all components of the cell. Unlike many other stress and hormone responses, there is no distinct ligand to signal temperature, and so identifying thermosensors remains challenging. Likewise, temperature sensing may occur at multiple entry

Developmental plasticity: the modulation of plant form by integrating environmental information into the regulation of growth and developmental processes

Ambient temperature: temperature in a range that does not activate strong cold or heat stress responses in the plant (~12–27°C for *Arabidopsis thaliana*)

Molecular motion: the movement of molecules or constituent particles in a certain direction

Thermosensor: a molecular entity that receives and converts temperature stimuli into recognizable molecular signals

points within a signaling pathway. The broad effects of temperature on all cellular components also mean that any molecule in the cell can potentially act as a thermosensor. As further details of plant thermosensing mechanisms emerge, De Smet and colleagues (134) have proposed that putative plant thermosensors should obey the following three criteria: (*a*) temperature directly impacts the biochemical properties of the thermosensor, (*b*) the modified properties of the sensor play an important role in the signal transduction of the temperature response, and (*c*) these modifications lead to relevant changes in plant physiology and/or morphology.

Master temperature sensor: a thermosensor that is able to convey temperature information to multiple response pathways

As a consequence of the complexity and interconnectedness of temperature signaling, different models have been proposed for how temperature information is integrated into cellular responses. Conceptually, a master temperature sensor could sense and communicate temperature information to all downstream pathways. For example, in animals, transient receptor potential (TRP) channels are major temperature sensors that activate stimulus-responsive transcription factors and lead to the induction of delayed response genes (70, 126). An equivalent system in plants might enable multiple distinct pathways to be activated downstream of a given sensor. Conversely, temperature perception could reflect an integration of the activities of hundreds or thousands of different molecules in the cell responding individually to temperature. In this case, temperature perception is an emergent property from the broad behavior of cellular processes responding passively to changes in kinetic rates. As we learn more about temperature perception pathways in the cell, it is becoming apparent that a hybrid of these models exists. Molecules with very distinctive temperature-perceiving behaviors have been identified (i.e., thermosensors), indicating that multiple nodes throughout temperature response networks have evolved the ability to incorporate temperature information. Having distinct thermosensors is likely necessary since plants demonstrate considerable temperature sensitivity. For example, lettuce germination responds to a temperature change of as little as 1°C (6). Indeed, the described thermosensors exploit biophysical properties of proteins and RNA that feature cooperative responses that enable a small temperature difference to result in a significant molecular response—a sensitivity and switch-like behavior that is not available to most molecules in the biological temperature range. The evolution of multiple temperature-sensing nodes within a given pathway may represent a mechanism to make pathways robust against noise and provide a means to integrate temperature information.

In this review, we summarize the current knowledge of how plants sense and integrate temperature information in the context of the most well-studied temperature-controlled behaviors in *Arabidopsis*. We detail each of the established thermosensing mechanisms to date and highlight gaps in our knowledge. We conclude with a discussion of challenges and opportunities in the field.

2. AN OVERVIEW OF KEY TEMPERATURE PATHWAYS IN PLANTS

2.1. Vernalization

Many temperate plants employ a mechanism to avoid flowering during the winter months when frost could kill flowers and developing seeds. The ability to delay flowering until a prolonged cold period has been experienced (vernalization) is present in many *Arabidopsis* accessions, where this pathway has been extensively studied and is described by excellent reviews (117, 141). Vernalization in *Arabidopsis* is conferred by two key genes: *FRIGIDA* (*FRI*) and *FLOWERING LOCUS C* (*FLC*) (**Figure 1**). *FRI* encodes a plant-specific scaffold protein that forms a large transcription activator complex, composed of FRI, FRI-LIKE 1 (FRL1), FRI ESSENTIAL 1 (FES1), SUPPRESSOR OF FRI 4 (SUF4), and FLC EXPRESSOR (FLX), that is required for the transcriptional activation of *FLC* (28). *FLC* encodes a MADS-box transcription factor that represses flowering in a dose-dependent manner (3, 9). High levels of FLC repress the expression of floral integrator genes such as *FLOWERING LOCUS T* (*FT*), *FLOWERING LOCUS D* (*FD*), and

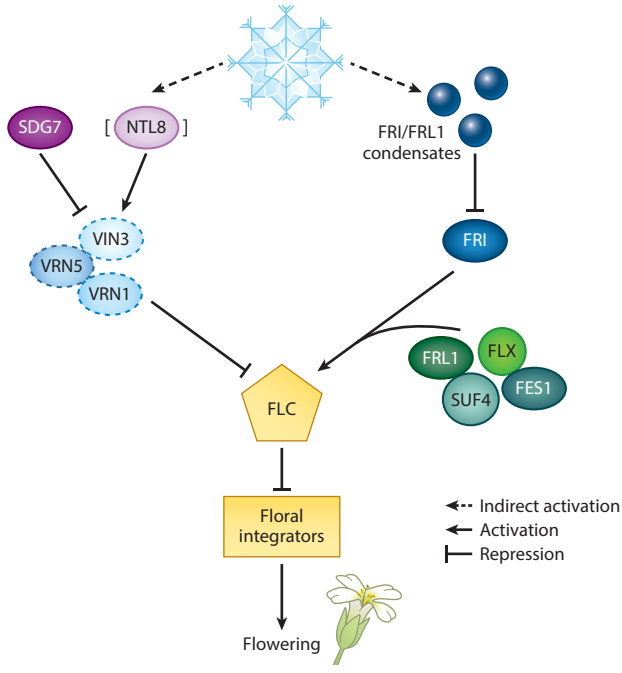

Figure 1

Long-term cold sensing during vernalization in *Arabidopsis*. Vernalization in *Arabidopsis* is conferred by two key genes: *FLC* and *FRI*. FRI encodes a plant-specific scaffold protein that forms a transcriptional activator complex composed of FRI, FRL1, FES1, SUF4, and FLX and is required for the transcriptional activation of *FLC*. Upon exposure to low temperatures, FRI and its interacting homolog, FRL1, undergo liquid–liquid phase separation to form nuclear condensates that do not colocalize with an active *FLC* locus. Prolonged cold also transcriptionally activates *VIN3*, one of many epigenetic modifying enzymes (*dashed circles*) that repress *FLC*. While SDG7 negatively regulates VIN3, accumulation of the NAC transcription factor NTL8 through growth promotes VIN3 accumulation at low temperatures. Collectively, these two pathways converge to repress *FLC* in the cold, relieving the repression of floral integrator genes such as *FT*, *FD*, and *SOC1*, and enable the activation of flowering. Abbreviations: FD, FLOWERING LOCUS D; FES1, FRI ESSENTIAL 1; FLC, FLOWERING LOCUS C; FLX, FLC EXPRESSOR; FRI, FRIGIDA; FRL1, FRI-LIKE 1; FT, FLOWERING LOCUS T; NTL8, NAC WITH TRANSMEMBRANE MOTIF1-LIKE 8; SDG7, SET DOMAIN-CONTAINING PROTEIN 7; SOC1, SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1; SUF4, SUPPRESSOR OF FRI 4; VIN3, VERNALIZATION INSENSITIVE 3; VRN, VERNALIZATION.

SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1 (*SOC1*), which in turn, results in delayed flowering (96, 118). In *Arabidopsis*, repression of *FLC* by prolonged cold is achieved by a number of genes involved in histone modification and include *VERNALIZATION 1* (*VRN1*), *VERNALIZATION 2* (*VRN2*), *VERNALIZATION 5* (*VRN5*), and *VERNALIZATION INSENSITIVE 3* (*VIN3*) (15, 48, 50, 87, 115, 140).

2.2. Cold Stress

Cold is a significant abiotic stress affecting crop production and the geographical distribution of plant species (13, 152). Plants generally encounter two forms of low temperature stress: chilling (0–15°C) and freezing (below 0°C) (**Figure 2**). When exposed to low, nonfreezing temperatures, plants can acquire the ability to cold acclimate, that is, "to increase tolerance to severe

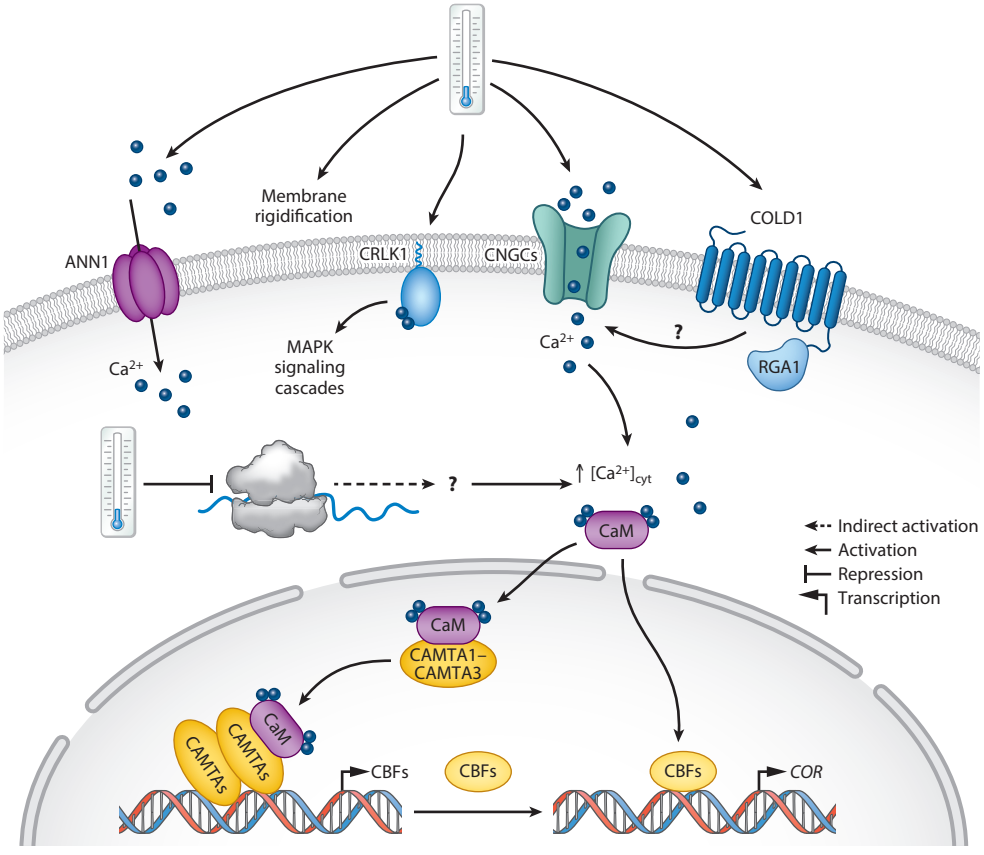

Figure 2

Low temperature stress–sensing mechanisms in plants. At the onset of cold stress, lipid membranes rigidify, which leads to the activation of membrane-associated proteins, including calcium channels (ANN1, CNGC channels) and the calcium/calmodulin-regulated receptor-like kinase CRLK1. Activation of these proteins may lead to a rapid influx of calcium into the cytoplasm ($[Ca^{2+}]_{cyt}$), which activates a range of calcium and MAPK signaling cascades. COLD1, a plasma membrane– and endoplasmic reticulum–located protein, has also been described as a cold sensor in rice. At low temperatures, COLD1 activates the GTPase activity of RGA1, which similarly triggers an influx of calcium into the cytosol; however, whether COLD1 is itself a calcium-permeable channel or interacts with other channels remains to be determined. In *Arabidopsis*, protein translation rate is also proportional to temperature and leads to a calcium influx via an unknown mechanism. CaM is a multifunctional intermediate calcium-binding messenger protein that binds to *CAMTA* transcription factors, which activate the CBF/DREB1 protein family. CBF transcription factors bind to the promoters of *COR* genes through CRT/DRE. Abbreviations: ANN1, ANNEXIN1; CaM, calmodulin; CAMTA, CALMODULIN-BINDING TRANSCRIPTION ACTIVATOR; CBF, C-repeat binding factor; CNGC, cyclic nucleotide-gated calcium; COLD1, CHILLING TOLERANCE DIVERGENCE 1; *COR*, cold-regulated; CRT, C-repeat; DRE, dehydration-responsive element; DREB1, dehydration-responsive element-binding1; GTPase, guanosine triphosphatase; MAPK, mitogen-activated protein kinase; RGA1, rice G protein α subunit 1.

cold (freezing) stress as a result of prior exposure to moderately suboptimal (chilling) temperatures" (113, pp. 36–37). Adaptation to low temperatures is controlled by the C-repeat binding factor (CBF)/dehydration-responsive element-binding1 (DREB1) protein family, which activates the promoters of cold-regulated (*COR*) genes through C-repeat (CRT)/dehydration-responsive

elements (DREs) (144). In *Arabidopsis*, *CBF1–CBF3* are rapidly induced in response to low temperature stress (95, 120).

2.3. Thermomorphogenesis

Plants adjust their morphology and development in response to elevated ambient temperatures. Collectively, this process is known as thermomorphogenesis and in *Arabidopsis* includes responses such as the elongation of hypocotyls, stems, petioles and roots; leaf hyponasty; and a reduction in leaf blade size (23, 110) (**Figure 3**). These responses lead to an open rosette structure that promotes efficient leaf cooling (32, 104). A central regulator of plant thermomorphogenesis is

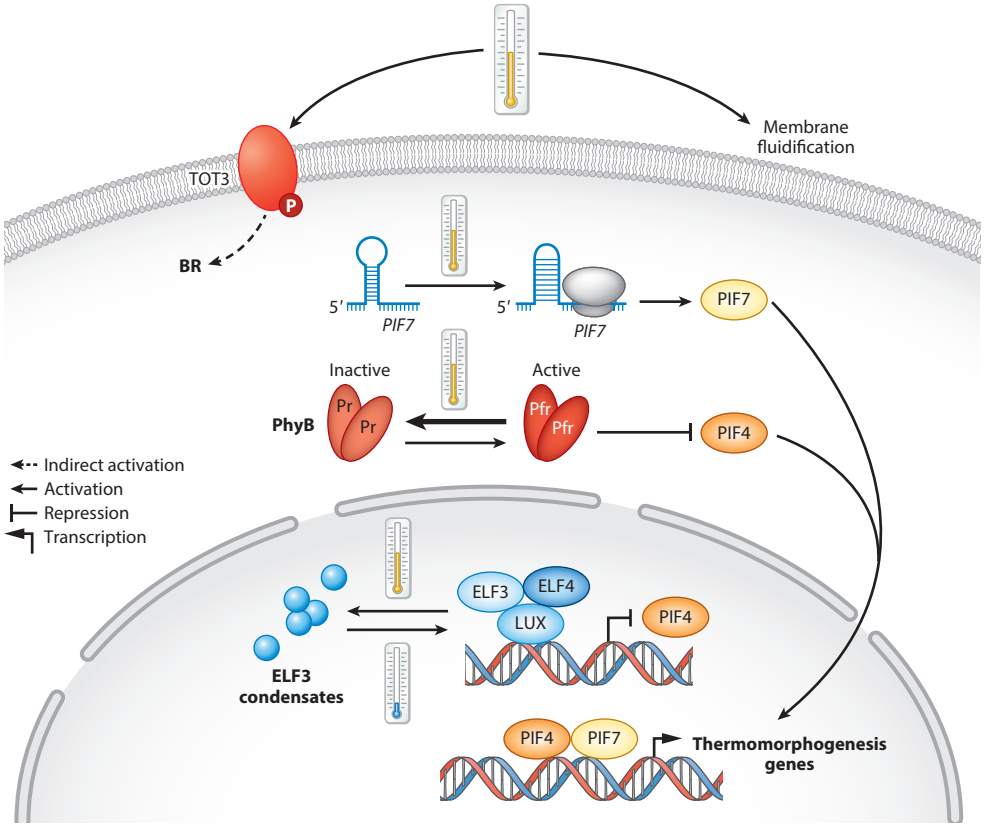

Figure 3

Temperature sensing during thermomorphogenesis. At elevated ambient temperatures, three main temperature-sensing mechanisms have been described. *PIF7* mRNA forms a hairpin structure within its 5′ UTR. Upon an increase in temperature, this structure partially unfolds to facilitate translation initiation leading to increased PIF7 protein. PhyB exists in two stable, interconvertible forms: a Pr state that is biologically inactive and a Pfr state that is active. Under red light, phyB is converted to a Pfr homodimer that promotes the inactivation of PIF transcription factors, including PIF4. High temperatures promote the reversion of phyB back to its inactive Pr state in a process known as thermal reversion, which enables PIF4 and PIF7 to activate thermomorphogenesis genes. Additionally, at low temperatures, ELF3, a component of the EC in addition to LUX and ELF4, represses the expression of PIF4. When temperatures increase, ELF3 forms liquid droplets through liquid–liquid phase separation that prevents its integration into the EC, which in turn relieves repression of PIF4. While unlinked to the above processes, TOT3 was found to be a regulator of the thermomorphogenic pathway that impinges on BR signaling. Arrow size indicates the propensity for a reaction to occur. Abbreviations: BR, brassinosteroid; EC, evening complex; ELF, EARLY FLOWERING; LUX, LUX-ARRHYTHMO; mRNA, messenger RNA; phyB, phytochrome B; PIF, PHYTOCHROME INTERACTING FACTOR; TOT3, TARGET OF TEMPERATURE 3; UTR, untranslated region.

the PHYTOCHROME-INTERACTING FACTOR 4 (PIF4) transcription factor (77). *PIF4* is temperature regulated both transcriptionally and posttranslationally (46, 58, 105, 148) and orchestrates reprogramming of the transcriptome in response to elevated ambient temperatures in *Arabidopsis* (65). While some components of the thermomorphogenic pathway may act independently of PIF4 (27, 75, 119, 135), the core signaling pathway is dominated by PIF4 and other factors that regulate plant growth and development in response to temperature and changing light conditions (35).

Priming: pre-exposure of plants to an eliciting factor that enables them to become more tolerant to later biotic or abiotic stress

2.4. Heat Stress

In all living organisms, temperatures above the optimum are perceived as heat stress (78) (**Figure 4**). High temperatures negatively affect seed germination, photosynthetic capacity, water use efficiency, cell growth and division, flowering, pollen viability, and plant productivity (55). To cope with heat stress, plants have evolved a variety of responses to protect cellular homeostasis and minimize damage. One such response is the upregulation of heat shock factors (HSFs) that are an integral part of the transcriptional regulation of heat protective genes, such as HSPs. HSPs are generally thought of as molecular chaperones; however, under stress, HSPs have also been shown to play wider roles in maintaining membrane integrity, ROS scavenging, and the production of antioxidants and osmolytes (72). Upon exposure to sublethal high temperatures (priming), many plant species adjust their stress response and metabolism to be able to cope with successive lethal temperatures. This ability is known as acquired thermotolerance, and understanding the molecular mechanism of this process is integral to improving plant performance at high temperatures.

3. MAJOR HYPOTHESES IN THE FIELD

3.1. Searching for an Elusive Master Temperature Sensor

Penfield (106, p. 615) noted that "one of the great unknowns in plant science is how temperature signals are perceived." As one of the most thermally sensitive macromolecular structures in the cell, the plasma membrane has long been proposed as a primary candidate for temperature sensing in plants (8, 99). Subtle changes in temperature can affect various properties of cellular membranes, including fluidity, thickness, permeability, and packing (99). While membrane lipids lack catalytic ability on their own, changes in their physical state strongly affect the folding, mobility, and activity of integral or membrane-associated proteins (59). These changes can have deleterious effects on cellular function but at moderate levels can serve as a mechanism for thermosensing (59).

To date, several membrane-associated proteins have been proposed as potential thermosensors. For example, as temperature stress is known to elicit rapid increases in cytosolic calcium (Ca^{2+}), researchers have proposed that calcium channels may act as plant thermosensors. Yang and colleagues (89) recently showed that the *Arabidopsis* Ca^{2+}-permeable transporter ANNEXIN1 (ANN1) mediates cold-triggered Ca^{2+} influx into the cytosol and the establishment of freezing tolerance (**Figure 1**). Interestingly, AtANN1 is also upregulated by heat stress (**Figure 4**) and has been shown to positively regulate heat-induced increases in cytosolic calcium as well as acquired thermotolerance (137). Furthermore, the potential role of cyclic nucleotide-gated calcium (CNGC) channels in temperature stress has been investigated, particularly at elevated temperatures. Goloubinoff and colleagues (42, 114) have proposed that *CNGCb* in *Physcomitrella patens* and *CNGC2/CNGC4* in *Arabidopsis* play a conserved role in activating the heat stress response via heat-induced cytosolic Ca^{2+} increases (**Figure 4**). Loss-of-function alleles in genes encoding these channels actually have a higher expression of heat shock genes, suggesting that the mechanism may be quite complex (42, 114). In rice, two closely related CNGC proteins, OsCNGC14

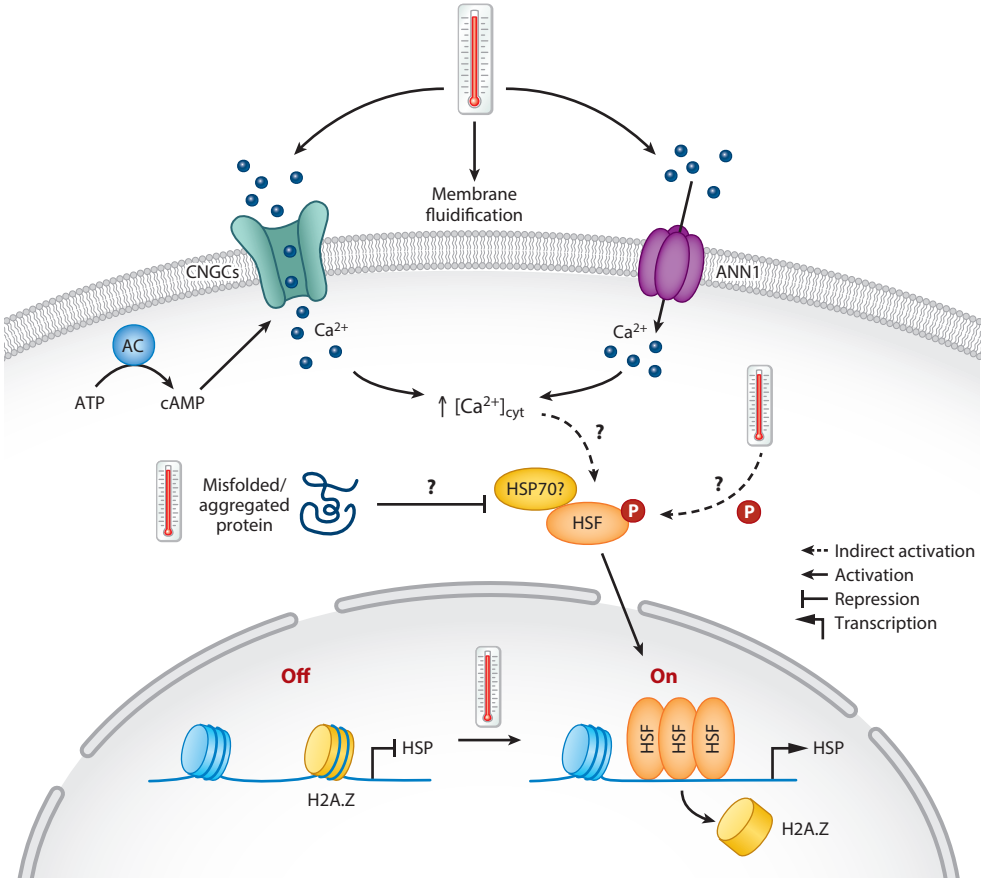

Figure 4

Sensing high temperatures as part of the heat stress response in *Arabidopsis*. At high temperatures, lipid membranes become more liquid, which leads to the activation of calcium channels, including ANN1 and CNGC channels. Activation of these proteins may lead to a rapid influx of calcium into the cytoplasm ($[Ca^{2+}]_{cyt}$). As cytosolic cAMP levels increase at elevated temperatures and are able to activate CNGCs, researchers have proposed that enzymes with AC activity may also act as membrane-associated temperature sensors. High temperatures promote the misfolding and aggregation of proteins. If left to accumulate to high enough levels, misfolded proteins can outcompete HSF proteins for binding with HSPs, such as HSP70. This triggers the release of the HSF, which promotes further expression of *HSP70* in an autoregulatory manner. Many temperature-responsive genes are bound by nucleosomes containing the histone variant H2A.Z, which is involved in stabilizing the +1 nucleosome to repress gene expression by reducing the accessibility of chromatin to transcriptional activators and RNA polymerases. H2A.Z is rapidly displaced from nucleosomes in response to increased ambient temperatures, and HSF binding at elevated temperatures is required to promote H2A.Z nucleosome depletion and transcriptional activation at heat-responsive genes. Abbreviations: AC, adenylyl cyclase; ANN1, ANNEXIN1; ATP, adenosine triphosphate; cAMP, 3′,5′-cyclic adenosine monophosphate; CNGC, cyclic nucleotide-gated calcium; HSF, heat shock factor; HSP, heat shock protein.

and OsCNGC16, were found to be important in generating cytosolic calcium signals in response to heat and cold, suggesting a potential overlap in the calcium signaling response to both high and low temperature stress (33). Furthermore, CNGC6 in *Arabidopsis* has been found to mediate heat-induced Ca^{2+} influx at the plasma membrane and facilitate the expression of *HSP* genes

(47). Interestingly, Gao et al. (47) observed that during mild heat shock, cytosolic 3′,5′-cyclic adenosine monophosphate (cAMP) levels increased, CNGC6 was activated by cytosolic cAMP, and exogenous cAMP promoted the expression of *HSP* genes. Such findings have led some to hypothesize that proteins with adenyl cyclase activity, rather than Ca^{2+} channels themselves, may act as membrane-associated temperature sensors (127) (**Figure 4**). Recently, an adenylyl cyclase [RPP13-like 3 (RPP13-LK3)] that is required for heat-induced cAMP synthesis and induction of HSP expression in maize has been identified (145).

In mammals, heat perception is mediated by both the TRP cationic channel family and the TWIK-RELATED POTASSIUM (TREK) channel family present in neurons, with elevated temperatures leading to depolarization and increased action potential firing of corresponding neurons (131, 132). While plants lack TRP homologs, the same criteria used to describe temperature sensors in animal cells have been used to identify CHILLING TOLERANCE DIVERGENCE 1 (COLD1), a plasma membrane– and endoplasmic reticulum–located protein in rice (92). Upon chilling, COLD1 activates the guanosine triphosphatase (GTPase) activity of rice G protein α subunit 1 (RGA1), which triggers an influx of calcium into the cytosol and enhanced cold tolerance (92) (**Figure 2**). It will be interesting to see if COLD1 is confirmed as a temperature sensor.

Many receptor-like protein kinases (RLKs), including two-component histidine kinases and G protein–associated kinases, have also been shown to play roles in temperature sensing at the plasma membrane. For example, it was discovered over a decade ago that a subset of A-type *Arabidopsis* response regulators (ARRs) involved in a multistep two-component signaling system involved in cytokinin signaling are significantly upregulated at low temperatures (63). As cold-inducible expression of A-type *ARR* genes is significantly reduced in *Arabidopsis* histidine kinase (AHK) mutants, *AHK2* and *AHK3* were postulated to be involved in mediating cold expression of A-type *ARR* genes independently of endogenous cytokinin levels (63). Additionally, the *Arabidopsis* calcium/calmodulin-regulated receptor-like kinase CRLK1 is also upregulated at low temperatures (146) (**Figure 2**). Genetic studies showed that CRLK1 positively regulates plant responses to chilling and freezing stress and interacts with MEKK1, a member of the MAP kinase kinase kinase family (146). Recently, TARGET OF TEMPERATURE 3 (TOT3), a MITOGEN-ACTIVATED PROTEIN KINASE KINASE KINASE KINASE (MAP4K), has been shown to localize to the plasma membrane and control warm temperature–responsive growth in plants (135) (**Figure 3**). TOT3 was found to be a regulator of the thermomorphogenic pathway that impinges on brassinosteroid signaling in *Arabidopsis*; however, TOT3 also plays a central role in the thermal response in wheat (135). Furthermore, another plasma membrane–localized protein kinase, COLD-RESPONSIVE PROTEIN KINASE 1 (CRPK1), has been shown to phosphorylate 14-3-3 proteins that cause their nuclear localization to downregulate CBF transcription factors (91).

Several lines of evidence therefore support a role of the plasma membrane in signaling in response to changing temperatures. Definitive proof of temperature sensing at a molecular level for these systems is challenging and typically requires structural information (26). Additionally, membrane fluidity is carefully adjusted under fluctuating temperature conditions to maintain a constant viscosity via the expression of genes encoding lipid desaturases (93), suggesting that membrane fluidity cannot convey absolute temperature information but only relative changes in temperature. Interestingly, perturbing biosynthesis of the phospholipid phosphatidylglycerol at cellular membranes of phloem companion cells has recently been shown to have direct consequences on temperature-responsive pathways, such as flowering (124). Therefore, determining if thermosensory behavior mediated by plasma membrane fluidity can be demonstrated mechanistically will be significant.

3.2. The Distributed Model

A plausible explanation for the reduced growth of plants at lower temperatures is that chemical reactions and metabolism are slower in the cold, leading directly to reduced growth rates. The discovery of multiple genotypes, however, where growth remains fast even at low temperature, for example, *della* loss-of-function mutants (80) as well as the *phyABCDE* mutant, which shows considerable elongation growth even at 12°C (68), indicates that plants control their growth rates to adapt to local temperature conditions. While extremes of temperature will always limit viability, it is clear that plants operate considerable control over growth and development within the ambient temperature range, presumably reflecting a means to anticipate and be primed for future conditions. Carbon balance within the plant is highly complex, reflecting a coordination between photosynthesis, sink–source partitioning, and growth, and it is also highly influenced by temperature (116), leading to the proposal that carbon balance may transmit temperature status in the plant. For example, modulating photosynthesis through changing light levels enhances the effects of high temperature under certain conditions, leading Vasseur et al. (130) to propose that carbon balance may be a temperature sensor. A final example of distributed temperature sensing is in the vernalization pathway, in which the perception of prolonged low temperatures may be mediated by growth rate. The model proposes that while cell division and growth slow down considerably at low temperatures, protein production rates remain high, leading to the accumulation of the regulator NTL8, which promotes expression of the vernalization gene *VIN3* (150) (**Figure 1**). Determining how widespread such distributed mechanisms are, and the extent to which they control temperature responses compared to discrete thermosensory molecules, will be interesting.

3.3. The Integrated Model

While no evidence has been found for a master sensor conveying temperature information to multiple pathways, the discovery of several thermosensing mechanisms suggests that pathways have acquired thermal responsiveness via particular nodes (often at the level of genes, transcripts, or proteins) becoming temperature dependent. As temperature is a physical parameter that influences cellular structures through simple thermodynamic effects (60), such a decentralized temperature-sensing system provides an opportunity to recruit multiple components to provide temperature information, which may enable rapid adaptation of new temperature traits as well as network robustness. Such a network architecture in *Escherichia coli*, where multiple thermosensory steps are coupled with feedback, enables robust responses to temperature (37). The ability to sense temperature in various cellular compartments (71) and across different temporal scales would lead to enhanced flexibility of stress-signaling pathways and also the ability to fine-tune temperature responses to achieve temperature resilience in energetically favorable ways. In the following section, we explore the main thermosensors and hypothesize how these mechanisms may potentially work together to achieve enhanced temperature tolerance in a changing environment.

3.3.1. Phytochromes. Crosstalk between light and temperature signaling is fundamental to plant growth and development (44). When activated by red light, phytochromes bind and promote the inactivation of the basic helix-loop-helix (bHLH) transcription factors known as PHYTOCHROME-INTERACTING FACTORS (PIFs). Conversely, in the presence of far-red light or elevated ambient temperatures, phytochrome function is reduced, which leads to the accumulation of active PIFs and the promotion of hypocotyl elongation through enhanced expression of auxin biosynthesis genes such as *YUCCA8* (*YUC8*) (68, 77, 85). Hypocotyl elongation is primarily driven by PIF4, PIF7, and, to some extent, PIF5 (29, 43, 77) and results in an open architecture that enhances leaf cooling (32).

Phytochrome B (phyB) is the main photoreceptor controlling the growth of *Arabidopsis* seedlings exposed to different shade conditions (22). Like other phytochromes, phyB is a homodimeric chromoprotein, with each subunit harboring a covalently bound phytochromobilin chromophore (85). The two stable, interconvertible forms of phyB are a red-light-absorbing biologically inactive Pr state and a far-red-light-absorbing biologically active Pfr state (18, 109) (**Figure 3**). While the Pr state arises upon assembly with bilin, formation of the active Pfr state requires red light, and levels are strongly influenced by the red:far-red ratio (85). In addition, phyB Pfr can spontaneously revert back to Pr in a light-independent process known as thermal reversion (112). Thermal reversion of phyB in the dark or low light is highly temperature dependent, occurring faster at 27°C than at lower ambient temperatures (68, 85). In this way, repression of elongation growth is rapidly released during nighttime at warm temperatures. The biophysical basis of the temperature dependence of thermal reversion is not known.

In recent years, strong connections between phyB and the PIF4-auxin pathway have been established. While thermal reversion was thought to occur most predominantly in the dark, recent work has shown that phyB also senses temperature in the light. In a study by Chen and colleagues (108), daytime temperature sensing of phyB required the participation of the transcriptional activator HEMERA (HMR), which was found to interact with PIF4 to induce the expression of PIF4 target genes and was necessary for the accumulation of PIF4 at elevated ambient temperatures. Similarly, four *SUPPRESSOR OF PHYA-105* (*SPA*) genes have been shown to act as positive regulators of thermomorphogenesis by controlling the phyB-PIF4 module (84). Although SPAs were necessary for stabilizing PIF4 in vitro and in vivo, SPAs promote destabilization of phyB at elevated temperatures (84). As PIFs have been shown to induce degradation of phyB at elevated ambient temperatures (86, 98), Lee et al. (84) propose that increased levels of PIF4 may support phyB degradation to fine-tune thermomorphogenesis. As phyB has the ability to undergo phase separation via a self-associating C terminus and a disordered N-terminal extension (25), determining if there is a relationship between protein phase change behavior and thermal reversion will be interesting.

The low temperature response is also influenced by phyB sensing as phytochromes are essential for the full development of cold acclimation in *Arabidopsis* (73, 122). Low red:far-red light ratio derepresses circadian-gated *CBF* transcription and increases the expression of *COR* genes at 16°C (45), suggesting a negative role of phyB in modulating CBF expression and the cold acclimation response. Yang and colleagues (64) showed that CBF transcription factors interact with PIF3 during cold stress to attenuate codegradation of PIF3-phyB. Cold-stabilized phyB acts downstream of CBFs to positively regulate freezing tolerance by modulating the expression of stress- and growth-related genes, including *PIF1*, *PIF4*, and *PIF5* (64). Therefore, the CBF-PIF3-phyB module may serve as a molecular hub to integrate cold- and phytochrome-mediated light-signaling networks to allow plants to adapt to low temperature stress (64).

3.3.2 Phase separation. The role of biomolecular condensates in forming membraneless compartments within cells is emerging as an important organizing and signaling concept (38). While plant cells possess a plethora of biomolecular condensates ranging from those with general descriptors such as cellular bodies, aggregates, and puncta to those of specific compartments such as the nucleolus, Cajal bodies, and stress granules, evidence suggests that many condensates form through a process termed liquid–liquid phase separation (38), in which a solute (e.g., a protein or nucleic acid) that is homogeneously distributed within a solution demixes into two (or more) distinct phases that stably coexist with one another (38). As phase separation is dependent on a number of parameters, including pH, concentration of the solute, and temperature (38), the formation of biomolecular condensates serves as an attractive mechanism to regulate cellular processes

Thermal reversion: temperature-regulated relaxation of a phytochrome molecule from an active to an inactive state

Biomolecular condensate: a non-membrane-bound and nonstoichiometric compartment made of one or more biological molecules concentrated relative to their surroundings

Liquid–liquid phase separation: a phenomenon whereby a solution spontaneously demixes into two or more distinct phases that stably coexist

in response to abiotic stresses, including temperature. Indeed, work from the Wigge group (67) has shown that EARLY FLOWERING 3 (ELF3), a component of the evening complex (EC) of the evening loop of the plant central oscillator, reversibly forms liquid droplets in response to increasing ambient temperatures (**Figure 3**). This biophysical response is conferred by a prion-like domain (PrD), which contains a polyglutamine repeat (polyQ) whose length correlates with thermal responsiveness (67). At 17°C, ELF3–green fluorescent protein (GFP) localizes to the nucleus with a diffuse signal. At elevated temperatures (27°C and 37°C), ELF3 condenses into multiple bright speckles—a behavior that is specific to the PrD (67). Interestingly, increasing the polyQ length also resulted in a greater tendency to form speckles (67). ELF3 is considered to be a thermosensor, as high ambient temperatures lead to condensation of the ELF3 protein, temporarily inactivating the EC and allowing increased transcription of downstream targets, such as *PIF4*.

Prion-like domain (PrD): intrinsically disordered regions within a protein that contain little amino acid diversity and are generally enriched in polar amino acids such as glutamine, asparagine, glycine, and serine

Similarly, it has recently been shown by Dean and colleagues (153) that during vernalization, plants are able to sense temperature change through the formation of FRI/FRL1 nuclear condensates that do not colocalize with an active *FLC* locus (**Figure 1**). In its condensed state, FRI is unable to activate *FLC*, leading to derepression of flowering after prolonged cold. This process is reversible during warm temperature spikes, which buffers *FLC* shutdown to prevent premature flowering (153). Interestingly, cold accumulation of FRI is influenced by specific cotranscriptional regulators and cold induction of a specific isoform of the antisense RNA, *COOLAIR* (153). The dynamic portioning of a transcriptional activator in response to natural temperature fluctuations is an intriguing mechanism to quickly remove a regulatory protein from its target, while maintaining the possibility to reinstate it when conditions become favorable again.

3.3.3 Nuclear localization. One of the earliest light responses, by either activation or deactivation of phyB, is the change in its subcellular localization (40). During dark-to-light transitions, photoactivated phyB translocates from the cytoplasm to the nucleus and initially localizes to many small foci (76, 143). After several hours, larger foci form, hereafter referred to as photobodies (PBs) (10, 76, 143). By contrast, during light-to-dark transitions, inactivation of phyB by thermal reversion triggers its disassembly from PBs back to small foci within the nucleoplasm (128). In a recent study by Chen and associates (54), investigation of phyB dynamics in relation to changing temperatures revealed that increasing ambient temperatures from 12°C to 27°C progressively reduced the number of PBs within *Arabidopsis* hypocotyl and cotyledon cells by stimulating phyB disassembly from selective thermo-unstable PBs. While PB formation is mediated by the C-terminal module of the phyB protein, it was found that the thermostability of PBs depends on the N-terminal photosensory module of phyB (54). Distinct PB forms were observed that either associated with nucleoli or occurred independently. The specific function of both nucleolar and nonnucleolar PBs has yet to be further elucidated; however, nonnucleolar PBs were shown to be the most thermoresponsive (54). These findings, in conjunction with a growing interest in phase separation, present a model whereby multiple temperature-sensing mechanisms may be used to distinguish between different environmental factors such as light and temperature and so fine-tune their ability to respond to a changing environment.

A regulator of phyB thermal reversion is PHOTOPERIODIC CONTROL OF HYPOCOTYL 1 (PCH1) (97). PCH1 acts to stabilize phyB PBs in their active Pfr state. Surprisingly, warm temperatures reduce *PCH1* gene expression and protein stability, suggesting that PCH1 may act to enhance the effect of thermal reversion on phyB (97). However, the effects of temperature on phyB nuclear bodies, and likely also on phyB activity, require PCH1. Therefore, phyB and PCH1 are both necessary to repress the warm temperature response during the night under mild temperatures, and elevated temperatures lower the activity of both (97). Controlling the expression and stability of PCH1 provides a mechanism to alter the thermal

responsiveness of phyB in a temporal and tissue-specific manner. These observations suggest that PCH1 may play a role as a modulator of the phyB thermosensor.

Elevated ambient temperatures are also known to trigger the nuclear import of CONSTITUTIVE PHOTOMORPHOGENIC 1 (COP1), a ubiquitin E3 ligase that conveys warm temperature signals to hypocotyl thermomorphogenesis (105). COP1 is a known master regulator of photomorphogenesis (82). In the dark, COP1 is transported from the cytoplasm into the nucleus, with such movement being antagonized by the photoactivation of some photoreceptors (133). Nuclear localization of COP1 promotes the degradation of its substrates via the ubiquitin-proteosome pathway (102), including the bZIP transcription factor, ELONGATED HYPOCOTYL 5 (HY5) (5). Also, *Arabidopsis* mutants impaired in skotomorphogenesis (the ability of a seedling to develop in the dark) do not exhibit hypocotyl elongation under elevated ambient temperatures (34), suggesting that the COP1-HY5 module may be involved in plant adaptation to heat. Park et al. (105) show that at 28°C, COP1 localizes to the nucleus, which leads to enhanced degradation of the thermomorphogenic repressor HY5 and alleviation of the suppression of hypocotyl growth. Nucleocytoplasmic trafficking of COP1 is also affected by various environmental stress conditions; for example, dark-grown seedlings subjected to heat shock have reduced nuclear COP1 (69). Furthermore, cold stress inhibits nuclear import of COP1 in the dark and is associated with enhanced freezing tolerance (24).

H2A.Z nucleosome: a basic unit of chromatin made of DNA and histone proteins containing a variant of the canonical H2A histone protein

3.3.4. Chromatin structure. Many temperature-responsive genes are bound by nucleosomes containing the histone variant H2A.Z (H2A.Z nucleosomes) at the +1 position as well as within the gene body (30). In a genetic screen for perturbation of *HSP70* expression, mutations in *ACTIN-RELATED PROTEIN 6* (*ARP6*), a component of the SWI2/SNF2-RELATED 1 (SWR1) complex necessary for H2A.Z deposition, were identified. Since *arp6* mutants confer constitutive expression of both heat shock and thermomorphogenesis, H2A.Z eviction in response to high temperature was proposed to be a general property of these nucleosomes, and chromatin state may confer temperature-dependent information (66). Follow-up experiments looking at the induction of *HSPs* showed that the induction of these genes and eviction of H2A.Z nucleosomes are dependent on the HSFA1 class of HSFs (31) (**Figure 4**). Similarly, induction of thermomorphogenesis genes containing H2A.Z nucleosomes requires the activity of PIFs (139). H2A.Z nucleosome eviction is an active process, requiring the Snf2 adenosine triphosphate (ATP)-dependent chromatin remodeling complex INOSITOL REQUIRING80-EIN6 ENHANCER (INO80-EEN) (122, 125). Specificity of H2A.Z eviction is provided by the PIFs that achieve H2A.Z removal at target genes through direct interaction with EEN, the *Arabidopsis* homolog of the chromatin remodeling complex subunit INO80 Subunit 6 (Ies6) (122). H2A.Z removal requires direct DNA binding of PIFs, as H2A.Z depletion is strongly attenuated in *pif4 pif5 pif7* triple mutants (122). These studies and others are consistent with a major role for H2A.Z nucleosomes as modulators of environmentally responsive gene expression. Analysis of promoter and enhancer regions have shown that H2A.Z is involved in stabilizing the +1 nucleosome to repress gene expression by reducing the accessibility of chromatin to transcriptional activators and RNA polymerase (25, 106). Moreover, work by Xue et al. (142) has also shown that the INO80 chromatin remodeling complex (INO80-C) directly interacts with WDR5a, a core component of the *Arabidopsis* H3K4me3 deposition complex COMPASS-like as well as the SPT4-2 transcription elongation factor. These genes play an important role in modulating RNA polymerase II elongation to facilitate efficient transcription as well as H3K4me3 deposition, an epigenetic mark generally associated with active transcription. As Xue et al. (142) similarly found that INO80 and EEN directly associate with PIF4 and that H3K4me3 levels were higher at PIF4 targets (which are also subsequently lost in *pif4*) under warm temperatures, this suggests that the INO80-C is required for warm

temperature–induced H3K4me3 deposition and transcription elongation at PIF4 targets. In addition, a range of transcription elongation factor mutants show impaired thermomorphogenesis phenotypes and have elevated H2A.Z levels similar to *ino80* and *pif4* mutants. This demonstrates that transcription elongation is essential for H2A.Z eviction at PIF4 target genes and suggests a mutual dependence of H2A.Z removal and active transcription during thermomorphogenesis. In summary, the INO80-C connects the eviction of H2A.Z with deposition of active histone modification H3K4me3 and transcription elongation to promote expression of temperature responsive genes.

Warm temperatures have also been associated with deacetylation of H3K9 at +1 nucleosomes of *PIF4* as well as *YUC8*, and POWERDRESS (PWR), a SANT domain–containing protein involved in regulating histone accessibility, is required for this response (125). PWR modifies acetylation status through its physical interaction with HISTONE DEACETYLASE 9 (HDA9), which results in histone deacetylation at specific loci across the genome (27, 75). Transcriptomic analysis of *pwr-2* mutants showed that there is a global misregulation of genes at elevated temperatures and significant overlap between these genes and those that are H2A.Z-enriched in their gene bodies (125), thus indicating a potential link between histone deacetylation and H2A.Z nucleosome dynamics in plants. While the interaction between histone deacetylation and H2A.Z nucleosome dynamics has been known for some time in yeast (138) and mammalian cells (53, 147), this interaction was only recently investigated in plants. Van Zanten and colleagues (129) have shown that HDA9-mediated H3K9K14 deacetylation leads to the eviction of H2A.Z nucleosomes at the *YUC8* locus, which, in turn, increases chromatin accessibility and activation of *YUC8* at warm ambient temperatures. While H2A.Z nucleosomes were depleted from the *YUC8* locus in response to high temperatures, this response was abolished in *hda9-1* mutants, demonstrating that the deacetylation of such nucleosomes in response to elevated temperatures is an important step in activating gene expression (129). Furthermore, acetylation changes in *hda9-1* seedlings were apparent at the transcriptional start site and gene body of *YUC8* at 27°C, while H2A.Z eviction was also observed upstream of the gene (129). Based on this observation, HDA9-mediated histone deacetylation is not likely to be directly causal for H2A.Z eviction, but may facilitate it. This model is supported by work in yeast, where the SWR1 histone replacement complex preferentially binds to acetylated nucleosomes, and so acetylation may, in turn, enhance the exchange of H2A for H2A.Z nucleosomes (4, 111). Interestingly, in a study that investigated the temperature responsiveness of three histone deacetylases, HDA15 had an opposite role in temperature-regulated gene expression in comparison to HDA9 and HDA19 (119). Mutant *hda15* seedlings displayed significantly longer hypocotyls compared to wild-type plants at 27°C than at 22°C, indicating that such plants were hyperresponsive to warm temperatures. Gene expression analyses of *hda15* also revealed upregulation of temperature-dependent genes, including *YUC8*, *HSP20*, *IAA3*, *IAA19*, and *IAA29* and, at a protein level, HDA15 interacts with LONG HYPOCOTYL IN FAR-RED 1 (HFR1) to downregulate gene expression (119). As HFR1 antagonizes the activity of PIF4 (61), HDA15 potentially controls thermomorphogenesis by repressing PIF4 activity (119). Moreover, HDA15 has been shown to interact with PIF3 and PIF5 to repress phyB-dependent seed germination, chlorophyll biosynthesis, and photosynthetic genes in etiolated seedlings (51, 90). Despite exhibiting similar warm temperature phenotypes, *hda9* and *hda19* mutants display little overlap in differentially expressed genes at 27°C, suggesting that related histone deacetylation proteins may function in different pathways involved in thermomorphogenesis (119).

3.3.5. Transcriptional regulation. The EC is a transcriptional repressor complex that is a key component of the plant circadian oscillator. The EC contains the transcription factor LUX-ARRHYTHMO (LUX), the scaffold protein ELF3, and ELF4. In addition to regulating

oscillations of core clock gene expression, the EC is also involved in temperature and light entrainment, thus acting as an important environmental sensor that conveys information to growth and development pathways (100). For example, *PIF4* transcription is repressed by the EC (16). Within the EC, only LUX has an MYB DNA-binding domain, which binds to the *PIF4* promoter and represses its transcription (16). *ELF3* was identified by quantitative trait locus (QTL) mapping of *Arabidopsis* multiparent advanced-generation intercross (MAGIC) lines as a major locus controlling thermomorphogenesis (16). It was found that the genome-wide DNA-binding activity of the EC is temperature dependent, with binding at 27°C being greatly reduced (39). Consistent with these findings, in vitro assays have shown that the EC binds more strongly to DNA at 4°C than at 27°C (121). The temperature-dependent nature of the EC binding to DNA is controlled by the thermosensor ELF3 (68). Intriguingly, phyB has been observed to co-occur at multiple sites where the EC is bound genome wide (39), suggesting that these regulators may integrate different aspects of environmental information.

Alternative splicing (AS): a cellular process in which exons from the same pre-mRNA are joined in different combinations, leading to related but different mRNA transcripts

3.3.6. Alternative splicing. Temperature affects alternative splicing (AS) in both animals and plants. In *Arabidopsis*, approximately 870 genes are alternatively spliced under high temperature conditions (65); however, how temperature regulates AS remains poorly understood. One well-documented example of AS under different temperature conditions includes *FLOWERING LOCUS M* (*FLM*) and SHORT VEGETATIVE PHASE (SVP), which are repressors of *FT* and are regulated upon changes in ambient temperature. *FLM* is alternatively spliced, producing two dominant splice variants: *FLM-β* and *FLM-δ*, which are formed by the mutual exclusion of exons. Exon 2 is maintained in *FLM-β*, while exon 3 is maintained in *FLM-δ* (83, 107). Low ambient temperatures (16°C) favor the expression of the repressive isoform *FLM-β*, and its expression decreases with increasing ambient temperatures (27°C) (83, 107). *FLM-β* forms a complex with SVP, a MADS-domain transcription factor that actively represses flowering by binding to flowering genes such as *FT* and *SOC1* (107). While it was initially thought that *FLM-δ* may play an important role in the control of flowering through direct competition with *FLM-β* at higher temperatures, follow-up research has revealed a less important role for *FLM-δ* (66). In addition to the two dominant splice variants of *FLM*, additional splice variants are also generated at elevated ambient temperatures (21, 123). These splice variants have been observed to harbor premature termination codons (PTCs), which are targeted for degradation by nonsense-mediated decay (NMD) and, as a consequence, lead to a decrease in the number of transcripts available for translation into the functional *FLM-β* isoform at higher temperatures (66). At the same time, SVP is degraded by the 26S proteasome, which limits the activity of the SVP-FLM-β repressor complex at elevated temperatures and promotes flowering (123). Furthermore, a comparison of *flm* loss-of-function mutants showed that expression of *FLM-δ* alone is not sufficient to promote flowering at elevated temperatures, while *FLM-β* alone delays flowering (21). Taken together, these data suggest that temperature-induced AS regulates the level of *FLM-β*, which plays a role in flowering time regulation.

Additionally, regulators of the spliceosome, or the spliceosome itself, may be targeted by temperature-dependent AS. In mammals, CDC-like kinases (CLKs) are known to directly phosphorylate serine/arginine (SR) proteins, which are key components of the spliceosome in human cells. SR proteins contain one or two RNA-binding domains, as well as a serine-/arginine-rich domain, that bind to the exonic splicing enhancer to facilitate recognition of the 5′ splice site by the U1 small nuclear ribonucleoprotein (U1 snRNP) (19). The phosphorylation status of SR proteins plays a critical role in splicing (49). Interestingly, mammalian CLKs expressed in vitro display extreme sensitivity to temperature change. Using kinase activity assays, CLK activity decreased 75% during an increase in temperature from 33°C to 37°C, with a complete loss of activity at 40°C (56).

Temperature sensitivity of the protein is conferred by the kinase activation domain at the carboxyl end of the protein, which undergoes subtle conformational changes that are temperature dependent and reversible when proteins are exposed to lower temperatures (56). Furthermore, a CLK homolog in *Arabidopsis*, *FUS3-COMPLEMENTING GENE 2* (*AFC2*), has recently been revealed to participate in temperature-responsive AS control and negatively regulate thermomorphogenesis in a PIF4-dependent manner (88). AFC2 directly phosphorylates the SR-rich protein splicing factor (SF) RSZ21, and activity of the protein decreases with increasing ambient temperature (88). In addition to regulatory factors involved in AS, the splicing machinery itself is also affected by both low- and high-ambient-temperature-induced AS. In a study by Nimmo and colleagues (62), RNA-binding splicing factors including POLYPYRIMIDINE TRACT BINDING PROTEIN 1 (PTB1) and U2AF65A undergo cold-induced AS isoform switching, such that the balance between functional and nonfunctional transcripts is temperature dependent. Mutant studies suggested that PTB1-U2AF65A-SUA (SUPPRESSOR PG ABI3-5; the *Arabidopsis* homolog of RNA binding motif protein 5) represents part of a network involved in the perception and transduction of prevailing temperature fluctuations to the plant central oscillator via splicing of the 5′ UTR region of *LATE ELONGATED HYPOCOTYL* (*LHY*) (62). It appears that splicing of the 5′ UTR of *LHY* has characteristics of a molecular thermostat, as the ratio of transcript isoforms is sensitive to temperature changes as modest as 2°C and is scalable over a wide dynamic range of temperature. Moreover, the putative splice regulator PORCUPINE (PCP) was identified in a strand-specific RNA sequencing assay conducted in Col-0 seedlings grown at 16°C, 23°C, and 27°C (20). *PCP* is downregulated in response to elevated ambient temperatures, and its temperature-dependent expression could be confirmed via real-time quantitative polymerase chain reaction (RT-qPCR). Phenotypic analysis of *pcp-1* mutants at 16°C revealed defects at the shoot apical meristem and failure to develop properly formed lateral organs. When the same plants were grown at 23°C, however, plants grew similarly to the wild type and only displayed subtle phenotypes at low frequency (20). Shifting *pcp-1* mutants grown initially at 23°C to 16°C leads to an arrest in plant growth and the production of male sterile flowers, while shifting such plants back to 23°C restored mutants to a status like that of the wild type (20). Lastly, in a recent study that showed that PIF4 and HOOKLESS1 (HLS1) form a coregulatory module that leads to a large number of genes being either differentially expressed or alternatively spliced at elevated temperatures, further comparative analyses observed that HLS1/PIF4 coregulated differentially expressed genes and alternatively expressed genes and exhibited almost no overlap, suggesting that high temperature triggers two distinct strategies to control plant thermomorphogenesis (65).

Of the *Arabidopsis* genes known to undergo AS due to elevated temperatures, approximately 96% contain a histone H3 lysine 36 trimethylation (H3K36me3)-enriched region within their gene body (103). A lack of histone methyltransferases involved in the deposition of H3K36me3 marks leads to altered AS upon a temperature shift from 16–25°C, and mutants defective in writing, reading, and erasing H3K36me3 marks show altered elevated temperature-induced flowering (103). These results demonstrate that epigenetic marks play a significant role in regulating ambient temperature–induced AS of biological significance, and further studies are required to investigate the overlap of these two mechanisms.

3.3.7. Protein translation. RNA secondary structures regulate many processes and are sensitive to environmental change. In prokaryotes, messenger RNA (mRNA) stem-loop structures have been shown to be thermosensory, facilitating ribosome binding and translation (79). In a ribosome sequencing (ribo-seq) experiment in *Arabidopsis*, *PIF7* was identified as a transcript that was more highly translated at 27°C (29). Analysis showed that the *PIF7* transcript forms a hairpin structure within the 5′ UTR, which partially unfolds at elevated ambient temperatures to

facilitate translation initiation (29) (**Figure 3**). As this high temperature response was reversible when plants were placed back at lower temperatures and artificial disruption of the *PIF7* hairpin could not complement the thermoresponse of *pif7* mutants, this is consistent with *PIF7* being a functional thermosensor (29). Interestingly, similar hairpin sequences were found in several other high temperature–responsive genes, including *HSFA2* and *WRKY22* (29). Though it needs to be confirmed if these genes similarly form stem-loop structures, it is important to note that regulation of *PIF7* translation is not simply due to the presence or absence of a hairpin. While mutations that strengthen the hairpin do indeed block translation, mutations that disrupt the hairpin were also seen to reduce translation rates, indicating that the role of mRNA secondary structures in the regulation of translation is dynamic and not fully understood.

In *E. coli*, it has long been established that the rate of translation elongation is proportional to temperature (41). Now this phenomenon has also been observed in plants. In the work of Guillaume-Schöpfer et al. (52), it was observed that translation rate in *Arabidopsis* is indeed proportional to temperature and that a reduction in translation rate due to low temperature stress or cycloheximide (CHX) treatment is sufficient to rapidly induce *COR* genes (**Figure 2**). Importantly, the response to CHX was specific to the immediate CBF regulon as later-responding cold genes such as *COR15a* were not induced. Furthermore, reduced translation rates also triggered a rise in intracellular free calcium independent of the canonical calcium spike seen in early onset chilling (52). This delayed calcium signal activated CALMODULIN-BINDING TRANSCRIPTION ACTIVATOR (CAMTA) transcription factors that are able to directly activate cold-induced gene expression (**Figure 2**). Although further work is required to investigate the contribution of translation rate to temperature sensing, considering the dual function that key cellular processes such as translation may play during changing temperature conditions is of interest.

3.3.8. Protein stability. The plant heat stress response is a multifactorial trait that functions to protect macromolecular structures, restore cellular homeostasis, and prevent damage (59). Cellular defense mechanisms are activated by monitoring the accumulated damage of DNA, protein, and membranes (8, 36, 99). One highly conserved response to elevated temperatures is the activation of HSPs that act as molecular chaperones to promote the correct folding of proteins and counteract aggregation of proteins. When HSPs bind to misfolded and aggregated proteins, HSF transcription factors are released and bind to heat shock elements (HSEs) of genes to activate their transcription under stress (2) (**Figure 4**). In plants, HSPs also contribute to the thermomorphogenic response. For example, HSP90 has been shown to stabilize the auxin receptor TRANSPORT INHIBITOR RESPONSE 1 (TIR1), suggesting an overlap in temperature signaling between the heat stress response and thermomorphogenesis (136). HSP90 has also been recently implicated in the induction of *HIGH EXPRESSION OF OSMOTICALLY RESPONSIVE GENES1* (*HOS1*) under warm temperatures (57). HOS1 is a protein with E3 ubiquitin ligase activity that also acts as a transcriptional regulator. Stabilization of the protein by HSP90 enables HOS1-mediated upregulation of DNA repair and improved thermotolerance (57). As HOS1 negatively regulates the transcriptional activity of PIF4, it has also been proposed to repress thermomorphogenic growth under heat stress (74).

At present, there is only limited information on how protein stability and expression of the plant chaperone network is influenced by ambient temperature in plants. It has recently been shown that translation of *HSFA2* is enhanced at elevated temperatures, similar to *PIF7* (29). Like *PIF7*, *HSFA2* was also identified as having a hairpin structure within its 3′ UTR and so may similarly undergo relaxation of the hairpin at warm temperatures to promote *HSP* expression in response to heat. Autoregulatory feedback is another possible mechanism that may be responsible for temperature-induced *HSP* expression. In yeast, HSF1 associates with the chaperone HSP70 at normal growth

temperatures (151). Under heat shock, misfolded proteins accumulate and outcompete HSF1 for binding with HSP70. This triggers the release of HSF1, which in turn promotes the expression of *HSP70*. Newly synthesized HSP70 once again competes for HSF1 binding, and a new equilibrium is established (151).

Protein thermostability: how resistant the unique structure and chemical properties of polypeptide chains are under extreme temperatures

While such mechanisms have not yet been demonstrated in plants, new techniques such as proteome-wide analyses of protein thermostability provide a new perspective on the effects of temperature on plant metabolism. Protein thermostability describes the effect of increased temperature on protein folding (14). It is now possible, through advances in protein mass spectrometry, to determine protein thermostability on a proteomic scale. Termed thermal proteome profiling, this technique has been successfully used to probe the thermostability of proteins in both *E. coli* (94) and human cells (11). How such techniques will be applied to plant proteins and the impact of thermal proteome profiling in discovering new temperature-sensing mechanisms will be significant to witness.

4. FUTURE CHALLENGES AND OPEN QUESTIONS

Significant progress has been made in the last decade in identifying both thermosensors and the underlying pathways connecting them to developmental and stress response pathways. These studies highlight the molecular plasticity of plants and their inherent ability to rapidly sense, integrate, and respond to temperature information across a wide temporal range. Despite these advances, many open questions remain.

The extent of temperature sensing at the plasma membrane has been actively proposed for many decades. While the role and mechanism of action of thermosensory TRP channels in mammalian cells have been demonstrated, the extent of temperature sensing at the plasma membrane in plants is less clear. Therefore, determining whether a clear genetic role can be shown for the activity of a major temperature response pathway being controlled via plasma membrane signaling will be very interesting.

Plants can grow to a large size and have complex root and aboveground tissues that are exposed to different temperature conditions. This raises the question of how local temperature information is sensed and integrated, and whether there are systemic temperature signals within the plant. Interestingly, it appears that roots sense temperature independently of shoots in *Arabidopsis*, and known shoot thermomorphogenesis regulators, phytochromes and ELF3, are not primarily involved in the response (1, 12). In aerial tissues, the heat shock response is particularly strongly induced in the shoot apical meristem, suggesting that specific cell types have different responses to temperature (101). A common factor in the enhanced root growth in response to temperature is auxin. The extent to which temperature signals across the plant are integrated and whether there are systemic temperature signals remain open questions.

In addition to the spatial scale, plants are exposed to differences in temperature from seconds to months to years. On shorter timescales, observing the molecular changes at the level of cellular behavior, such as phase change and phytochrome thermal reversion, which occur at a comparable time span, is often possible. Longer timescale responses require more stable examples of cellular acclimation or memory. One of the best examples of this is the vernalization response in *Arabidopsis*, where silencing of the floral repressor *FLC* by repressive histone marks plays an important long-term effect in remembering winter. On a shorter timescale, plants display priming responses to both heat and cold stress, which can often remain for several days (7).

The earth is currently undergoing an unprecedented period of global heating, which is also increasing the frequency of extreme weather events. Since many staple crops are particularly vulnerable to temperature stress, this is concerning, with historical data indicating that crop yields

decline significantly for every 1°C increase in temperature (149). However, as plants demonstrate remarkable plasticity and have the ability to thrive across a wide range of temperatures, considerable genetic variation exists to enable adaptation of crops to future climates. A major challenge will be advancing our understanding of the mechanisms of temperature perception and adaptation to different climates with sufficient depth so that we can engineer these responses into crop plants to enhance their thermal resilience. Such an endeavor will require considerable advances in our understanding of all the thermosensory mechanisms in plants. While the mechanisms underlying thermomorphogenesis are becoming more clearly understood, major questions still surround the activation of heat and cold stress responses and how this is modulated at different temperatures. A detailed molecular understanding of thermosensory mechanisms and how they act, particularly to temperature extremes, will represent a key resource for breeding climate-resilient crops.

SUMMARY POINTS

1. Temperature is a significant environmental factor that affects all aspects of plant metabolism, development, and growth.
2. As temperature is molecular motion and can influence all components of the cell, identification of plant thermosensors remains challenging.
3. Different models have been proposed to determine how temperature information is sensed and integrated into downstream responses, including a master temperature sensor, the distributed model, and the integrated model.
4. While researchers have yet to identify a master sensor that is able to convey temperature information to multiple pathways, recent findings indicate that plants integrate the temperature response of a broad range of cellular processes, including lipid metabolism, light signaling, phase separation of proteins, cellular localization, altered chromatin structure, transcriptional and translational regulation, alternative splicing, protein stability, and growth.

FUTURE ISSUES

1. What is the extent of temperature sensing at the plasma membrane and membranes in general?
2. How widespread are distributed thermosensing mechanisms, and to what extent do they control temperature responses in comparison to discrete thermosensory molecules?
3. How is local temperature information sensed and integrated? Are there systemic temperature signals generated by plants?
4. How do temperature perception mechanisms differ at various timescales, and how is temperature information integrated over time?
5. Can temperature-sensing processes be manipulated to produce climate-resilient crops?

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

S.M.K. and P.A.W. are supported by a European Research Council (ERC) Advanced Grant TIPTOP (ERC Adv. 101021246). P.A.W.'s department is supported by the Leibniz Association.

LITERATURE CITED

1. Ai H, Bellstaedt J, Bartusch KS, Eschen-Lippold L, Babben S, et al. 2022. Auxin-dependent acceleration of cell division rates regulates root growth at elevated temperature. bioRxiv 2022.06.22.497127. **https://doi.org/10.1101/2022.06.22.497127**
2. Åkerfelt M, Morimoto RI, Sistonen L. 2010. Heat shock factors: integrators of cell stress, development and lifespan. *Nat. Rev. Mol. Cell Biol.* 11(8):545–55
3. Alexandre CM, Hennig L. 2008. *FLC* or not *FLC*: the other side of vernalization. *J. Exp. Bot.* 59(6):1127–35
4. Altaf M, Auger A, Monnet-Saksouk J, Brodeur J, Piquet S, et al. 2010. NuA4-dependent acetylation of nucleosomal histones H4 and H2A directly stimulates incorporation of H2A.Z by the SWR1 complex. *J. Biol. Chem.* 285(21):15966–77
5. Ang L-H, Chattopadhyay S, Wei N, Oyama T, Okada K, et al. 1998. Molecular interaction between COP1 and HY5 defines a regulatory switch for light control of *Arabidopsis* development. *Mol. Cell.* 1(2):213–22
6. Argyris J, Truco MJ, Ochoa O, Knapp SJ, Still DW, et al. 2005. Quantitative trait loci associated with seed and seedling traits in *Lactuca*. *Theor. Appl. Genet.* 111(7):1365–76
7. Balazadeh S. 2022. A 'hot' cocktail: the multiple layers of thermomemory in plants. *Curr. Opin. Plant Biol.* 65:102147
8. Balogh G, Péter M, Glatz A, Gombos I, Török Z, et al. 2013. Key role of lipids in heat stress management. *FEBS Lett.* 587(13):1970–80
9. Bastow R, Mylne JS, Lister C, Lippman Z, Martienssen RA, Dean C. 2004. Vernalization requires epigenetic silencing of *FLC* by histone methylation. *Nature* 427(6970):164–67
10. Bauer D, Vicziaén A, Kircher S, Nobis T, Nitschke R, et al. 2004. Constitutive photomorphogenesis 1 and multiple photoreceptors control degradation of phytochrome interacting factor 3, a transcription factor required for light signaling in Arabidopsis. *Plant Cell.* 16(6):1433–45
11. Becher I, Andrés-Pons A, Romanov N, Stein F, Schramm M, et al. 2018. Pervasive protein thermal stability variation during the cell cycle. *Cell* 173(6):1495–507.e18
12. Bellstaedt J, Trenner J, Lippmann R, Poeschl Y, Zhang X, et al. 2019. A mobile auxin signal connects temperature sensing in cotyledons with growth responses in hypocotyls. *Plant Physiol.* 180(2):757–66
13. Berry JA, Raison JK. 1981. Responses of macrophytes to temperature. In *Physiological Plant Ecology I: Responses to the Physical Environment*, ed. OL Lange, PS Nobel, CB Osmond, H Zeigler, pp. 277–338. Berlin: Springer-Verlag
14. Bischof JC, He X. 2006. Thermal stability of proteins. *Ann. N. Y. Acad. Sci.* 1066(1):12–33
15. Bond DM, Dennis ES, Finnegan EJ. 2009. Hypoxia: a novel function for VIN3. *Plant Signal. Behav.* 4(8):773–76
16. Box MS, Huang BE, Domijan M, Jaeger KE, Khattak AK, et al. 2015. *ELF3* controls thermoresponsive growth in *Arabidopsis*. *Curr. Biol.* 25(2):194–99
17. Brininger C, Spradlin S, Cobani L, Evilia C. 2018. The more adaptive to change, the more likely you are to survive: protein adaptation in extremophiles. *Semin. Cell Dev. Biol.* 84:158–69
18. Burgie ES, Vierstra RD. 2014. Phytochromes: an atomic perspective on photoactivation and signaling. *Plant Cell* 26(12):4568–83
19. Cáceres JF, Kornblihtt AR. 2002. Alternative splicing: multiple control mechanisms and involvement in human disease. *Trends Genet.* 18(4):186–93
20. Capovilla G, Delhomme N, Collani S, Shutava I, Bezrukov I, et al. 2018. PORCUPINE regulates development in response to temperature through alternative splicing. *Nat. Plants.* 4(8):534–39
21. Capovilla G, Symeonidi E, Wu R, Schmid M. 2017. Contribution of major FLM isoforms to temperature-dependent flowering in *Arabidopsis thaliana*. *J. Exp. Bot.* 68(18):5117–27

22. Casal JJ. 2013. Photoreceptor signaling networks in plant responses to shade. *Annu. Rev. Plant Biol.* 64:403–27
23. Casal JJ, Balasubramanian S. 2019. Thermomorphogenesis. *Annu. Rev. Plant Biol.* 70:321–46
24. Catalá R, Medina J, Salinas J. 2011. Integration of low temperature and light signaling during cold acclimation response in *Arabidopsis*. *PNAS* 108(39):16475–80
25. Chen D, Lyu M, Kou X, Li J, Yang Z, et al. 2022. Integration of light and temperature sensing by liquid-liquid phase separation of phytochrome B. *Mol. Cell* 82:3015–29.e6
26. Chen H, Deng J, Cui Q, Chanda B, Henzler-Wildman K. 2021. Mapping temperature-dependent conformational change in the voltage-sensing domain of an engineered heat-activated K^+ channel. *PNAS* 118(14):e2017280118
27. Chen X, Lu L, Mayer KS, Scalf M, Qian S, et al. 2016. POWERDRESS interacts with HISTONE DEACETYLASE 9 to promote aging in *Arabidopsis*. *eLife* 5:e17214
28. Choi K, Kim J, Hwang H-J, Kim S, Park C, et al. 2011. The FRIGIDA complex activates transcription of *FLC*, a strong flowering repressor in *Arabidopsis*, by recruiting chromatin modification factors. *Plant Cell* 23(1):289–303
29. Chung BYW, Balcerowicz M, Di Antonio M, Jaeger KE, Geng F, et al. 2020. An RNA thermoswitch regulates daytime growth in *Arabidopsis*. *Nat. Plants* 6(5):522–32
30. Coleman-Derr D, Zilberman D. 2012. Deposition of histone variant H2A.Z within gene bodies regulates responsive genes. *PLOS Genet*. 8(10):e1002988
31. Cortijo S, Charoensawan V, Brestovitsky A, Buning R, Ravarani C, et al. 2017. Transcriptional regulation of the ambient temperature response by H2A.Z nucleosomes and HSF1 transcription factors in *Arabidopsis*. *Mol. Plant* 10(10):1258–73
32. Crawford AJ, McLachlan DH, Hetherington AM, Franklin KA. 2012. High temperature exposure increases plant cooling capacity. *Curr. Biol.* 22(10):R396–97
33. Cui Y, Lu S, Li Z, Cheng J, Hu P, et al. 2020. CYCLIC NUCLEOTIDE-GATED ION CHANNELs 14 and 16 promote tolerance to heat and chilling in rice. *Plant Physiol*. 183(4):1794–808
34. Delker C, Sonntag L, James GV, Janitza P, Ibañez C, et al. 2014. The DET1-COP1-HY5 pathway constitutes a multipurpose signaling module regulating plant photomorphogenesis and thermomorphogenesis. *Cell Rep*. 9(6):1983–89
35. Delker C, van Zanten M, Quint M. 2017. Thermosensing enlightened. *Trends Plant Sci*. 22(3):185–87
36. Ding Y, Shi Y, Yang S. 2020. Molecular regulation of plant responses to environmental temperatures. *Mol. Plant* 13(4):544–64
37. Dufour YS, Sneddon MW, Emonet T. 2011. Thermal robustness: lessons from bacterial chemotaxis. *Curr. Biol.* 21(12):R465–68
38. Emenecker RJ, Holehouse AS, Strader LC. 2021. Biological phase separation and biomolecular condensates in plants. *Annu. Rev. Plant Biol.* 72:17–46
39. Ezer D, Jung J-H, Lan H, Biswas S, Gregoire L, et al. 2017. The evening complex coordinates environmental and endogenous signals in *Arabidopsis*. *Nat. Plants* 3(7):17087
40. Fankhauser C, Chen M. 2008. Transposing phytochrome into the nucleus. *Trends Plant Sci*. 13(11):596–601
41. Farewell A, Neidhardt FC. 1998. Effect of temperature on in vivo protein synthetic capacity in *Escherichia coli*. *J. Bacteriol.* 180:4704–10
42. Finka A, Cuendet AFH, Maathuis FJM, Saidi Y, Goloubinoff P. 2012. Plasma membrane cyclic nucleotide gated calcium channels control land plant thermal sensing and acquired thermotolerance. *Plant Cell* 24(8):3333–48
43. Fiorucci A-S, Galvão VC, Ince YÇ, Boccaccini A, Goyal A, et al. 2020. PHYTOCHROME INTERACTING FACTOR 7 is important for early responses to elevated temperature in Arabidopsis seedlings. *New Phytol*. 226(1):50–58
44. Franklin KA. 2009. Light and temperature signal crosstalk in plant development. *Curr. Opin. Plant Biol.* 12(1):63–68
45. Franklin KA, Whitelam GC. 2007. Light-quality regulation of freezing tolerance in *Arabidopsis thaliana*. *Nat. Genet.* 39(11):1410–13

29. Shows that temperature-dependent translational enhancement of *PIF*7 mRNA is mediated by an RNA hairpin.

38. Comprehensively reviews biomolecular condensates in plants.

46. Gangappa SN, Kumar SV. 2017. DET1 and HY5 control PIF4-mediated thermosensory elongation growth through distinct mechanisms. *Cell Rep*. 18(2):344–51
47. Gao F, Han X, Wu J, Zheng S, Shang Z, et al. 2012. A heat-activated calcium-permeable channel—Arabidopsis cyclic nucleotide-gated ion channel 6—is involved in heat shock responses. *Plant J*. 70(6):1056–69
48. Gendall AR, Levy YY, Wilson A, Dean C. 2001. The *VERNALIZATION 2* gene mediates the epigenetic regulation of vernalization in *Arabidopsis*. *Cell* 107(4):525–35
49. Golovkin M, Reddy ASN. 1999. An SC35-like protein and a novel serine/arginine-rich protein interact with *Arabidopsis* U1-70K protein. *J. Biol. Chem.* 274(51):36428–38
50. Greb T, Mylne JS, Crevillen P, Geraldo N, An H, et al. 2007. The PHD finger protein VRN5 functions in the epigenetic silencing of *Arabidopsis FLC*. *Curr. Biol.* 17(1):73–78
51. Gu D, Chen C-Y, Zhao M, Zhao L, Duan X, et al. 2017. Identification of HDA15-PIF1 as a key repression module directing the transcriptional network of seed germination in the dark. *Nucleic Acids Res*. 45(12):7137–50
52. Guillaume-Schöpfer D, Jaeger KE, Geng F, Doccula FG, Costa A, et al. 2020. Ribosomes act as cryosensors in plants. bioRxiv 2020.12.07.414789. **https://doi.org/10.1101/2020.12.07.414789**
53. Gursoy-Yuzugullu O, Ayrapetov MK, Price BD. 2015. Histone chaperone Anp32e removes H2A.Z from DNA double-strand breaks and promotes nucleosome reorganization and DNA repair. *PNAS* 112(24):7507–12
54. Hahm J, Kim K, Qiu Y, Chen M. 2020. Increasing ambient temperature progressively disassembles *Arabidopsis* phytochrome B from individual photobodies with distinct thermostabilities. *Nat. Commun.* 11(1):1660
55. Haider S, Iqbal J, Naseer S, Yaseen T, Shaukat M, et al. 2021. Molecular mechanisms of plant tolerance to heat stress: current landscape and future perspectives. *Plant Cell Rep*. 40(12):2247–71
56. Haltenhof T, Kotte A, De Bortoli F, Schiefer S, Meinke S, et al. 2020. A conserved kinase-based body-temperature sensor globally controls alternative splicing and gene expression. *Mol. Cell* 78(1):57–69.e4
57. Han S-H, Park Y-J, Park C-M. 2020. HOS1 activates DNA repair systems to enhance plant thermotolerance. *Nat. Plants* 6(12):1439–46
58. Han X, Yu H, Yuan R, Yang Y, An F, Qin G. 2019. *Arabidopsis* transcription factor TCP5 controls plant thermomorphogenesis by positively regulating PIF4 activity. *iScience* 15:611–22
59. Hayes S, Schachtschabel J, Mishkind M, Munnik T, Arisz SA. 2021. Hot topic: thermosensing in plants. *Plant Cell Environ*. 44:2018–33
60. Hemantaranjan A, Patel PK, Singh R, Srivastava AK. 2012. Heat stress responses of wheat and other plants. In *Advances in Plant Physiology*, ed. A Hemantaranjan, pp. 279–313. Jodhpur, India: Sci. Publ.
61. Hornitschek P, Lorrain S, Zoete V, Michielin O, Fankhauser C. 2009. Inhibition of the shade avoidance response by formation of non-DNA binding bHLH heterodimers. *EMBO J*. 28(24):3893–902
62. James AB, Calixto CPG, Tzioutziou NA, Guo W, Zhang R, et al. 2018. How does temperature affect splicing events? Isoform switching of splicing factors regulates splicing of *LATE ELONGATED HYPOCOTYL* (*LHY*). *Plant Cell Environ*. 41(7):1539–50
63. Jeon J, Kim NY, Kim S, Kang NY, Novák O, et al. 2010. A subset of cytokinin two-component signaling system plays a role in cold temperature stress response in *Arabidopsis*. *J. Biol. Chem.* 285(30):23371–86
64. Jiang B, Shi Y, Peng Y, Jia Y, Yan Y, et al. 2020. Cold-induced CBF–PIF3 interaction enhances freezing tolerance by stabilizing the phyB thermosensor in *Arabidopsis*. *Mol. Plant* 13(6):894–906
65. Jin H, Lin J, Zhu Z. 2020. PIF4 and HOOKLESS1 impinge on common transcriptome and isoform regulation in thermomorphogenesis. *Plant Comm.* 1(2):100034
66. John S, Olas JJ, Mueller-Roeber B. 2021. Regulation of alternative splicing in response to temperature variation in plants. *J. Exp. Bot.* 72(18):6150–63
67. Jung J-H, Barbosa AD, Hutin S, Kumita JR, Gao M, et al. 2020. A prion-like domain in ELF3 functions as a thermosensor in *Arabidopsis*. *Nature* 585:256–60
68. Jung J-H, Domijan M, Klose C, Biswas S, Ezer D, et al. 2016. Phytochromes function as thermosensors in *Arabidopsis*. *Science* 354(6314):886–89
69. Karayekov E, Sellaro R, Legris M, Yanovsky MJ, Casal JJ. 2013. Heat shock–induced fluctuations in clock and light signaling enhance phytochrome B–mediated *Arabidopsis* deetiolation. *Plant Cell* 25(8):2892–906

67. Shows that a prion-like domain–containing protein forms condensates in response to temperature fluctuations.

68. Demonstrates that the rate of phytochrome B inactivation is proportional to temperature in the dark.

70. Kashio M, Tominaga M. 2022. TRP channels in thermosensation. *Curr. Opin. Neurobiol.* 75:102591
71. Kerbler SM, Taylor NL, Millar AH. 2019. Cold sensitivity of mitochondrial ATP synthase restricts oxidative phosphorylation in *Arabidopsis thaliana*. *New Phytol.* 221(4):1776–88
72. Khan Z, Shahwar D. 2020. Role of heat shock proteins (HSPs) and heat stress tolerance in crop plants. In *Sustainable Agriculture in the Era of Climate Change*, ed. R Roychowdhury, S Choudhury, M Hasanuzzaman, S Srivastava, pp. 211–34. Cham: Springer Int. Publ. 1st ed.
73. Kim H-J, Kim Y-K, Park J-Y, Kim J. 2002. Light signalling mediated by phytochrome plays an important role in cold-induced gene expression through the C-repeat/dehydration responsive element (C/DRE) in *Arabidopsis thaliana*. *Plant J.* 29(6):693–704
74. Kim J-H, Lee H-J, Jung J-H, Lee S, Park C-M. 2017. HOS1 facilitates the phytochrome B-mediated inhibition of PIF4 function during hypocotyl growth in *Arabidopsis*. *Mol. Plant.* 10(2):274–84
75. Kim YJ, Wang R, Gao L, Li D, Xu C, et al. 2016. POWERDRESS and HDA9 interact and promote histone H3 deacetylation at specific genomic sites in *Arabidopsis*. *PNAS* 113(51):14858–63
76. Kircher S, Kozma-Bognar L, Kim L, Adam E, Harter K, et al. 1999. Light quality–dependent nuclear import of the plant photoreceptors phytochrome A and B. *Plant Cell* 11(8):1445–56
77. Koini MA, Alvey L, Allen T, Tilley CA, Harberd NP, et al. 2009. High temperature-mediated adaptations in plant architecture require the bHLH transcription factor PIF4. *Curr. Biol.* 19(5):408–13
78. Kotak S, Larkindale J, Lee U, von Koskull-Döring P, Vierling E, Scharf K-D. 2007. Complexity of the heat stress response in plants. *Curr. Opin. Plant Biol.* 10(3):310–16
79. Kudla J, Becker D, Grill E, Hedrich R, Hippler M, et al. 2018. Advances and current challenges in calcium signaling. *New Phytol.* 218(2):414–31
80. Kumar SV, Lucyshyn D, Jaeger KE, Alós E, Alvey E, et al. 2012. Transcription factor PIF4 controls the thermosensory activation of flowering. *Nature* 484(7393):242–45
81. Lancaster LT, Humphreys AM. 2020. Global variation in the thermal tolerances of plants. *PNAS* 117(24):13580–87
82. Lau OS, Deng XW. 2012. The photomorphogenic repressors COP1 and DET1: 20 years later. *Trends Plant Sci.* 17(10):584–93
83. Lee JH, Ryu H-S, Chung KS, Posé D, Kim S, et al. 2013. Regulation of temperature-responsive flowering by MADS-box transcription factor repressors. *Science* 342:628–32
84. Lee S, Paik I, Huq E. 2020. SPAs promote thermomorphogenesis by regulating the phyB-PIF4 module in *Arabidopsis*. *Development* 147(19):dev189233
85. Legris M, Klose C, Burgie ES, Rojas CCR, Neme M, et al. 2016. Phytochrome B integrates light and temperature signals in *Arabidopsis*. *Science* 354(6314):897–900
86. Leivar P, Monte E, Al-Sady B, Carle C, Storer A, et al. 2008. The *Arabidopsis* phytochrome-interacting factor PIF7, together with PIF3 and PIF4, regulates responses to prolonged red light by modulating phyB levels. *Plant Cell* 20(2):337–52
87. Levy YY, Mesnage S, Mylne JS, Gendall AR, Dean C. 2002. Multiple roles of *Arabidopsis VRN1* in vernalization and flowering time control. *Science* 297(5579):243–46
88. Lin J, Shi J, Zhang Z, Zhong B, Zhu Z. 2022. Plant AFC2 kinase desensitizes thermomorphogenesis through modulation of alternative splicing. *iScience* 25(4):104051
89. Liu Q, Ding Y, Shi Y, Ma L, Wang Y, et al. 2021. The calcium transporter ANNEXIN1 mediates cold-induced calcium signaling and freezing tolerance in plants. *EMBO J.* 40(2):e104559
90. Liu X, Chen C-Y, Wang K-C, Luo M, Tai R, et al. 2013. PHYTOCHROME INTERACTING FACTOR3 associates with the histone deacetylase HDA15 in repression of chlorophyll biosynthesis and photosynthesis in etiolated *Arabidopsis* seedlings. *Plant Cell* 25(4):1258–73
91. Liu Z, Jia Y, Ding Y, Shi Y, Li Z, et al. 2017. Plasma membrane CRPK1-mediated phosphorylation of 14-3-3 proteins induces their nuclear import to fine-tune CBF signaling during cold response. *Mol. Cell* 66(1):117–28.e5
92. Ma Y, Dai X, Xu Y, Luo W, Zheng X, et al. 2015. *COLD1* confers chilling tolerance in rice. *Cell* 160(6):1209–21
93. Martinière A, Shvedunova M, Thomson AJW, Evans NH, Penfield S, et al. 2011. Homeostasis of plasma membrane viscosity in fluctuating temperatures. *New Phytol.* 192:328–37

85. Shows that the thermal reversion rate of phytochrome B is accelerated at warm temperatures.

94. Mateus A, Bobonis J, Kurzawa N, Stein F, Helm D, et al. 2018. Thermal proteome profiling in bacteria: probing protein state *in vivo*. *Mol. Syst. Biol.* 14(7):e8242
95. Medina J, Bargues M, Terol J, Pérez-Alonso M, Salinas J. 1999. The Arabidopsis *CBF* gene family is composed of three genes encoding AP2 domain-containing proteins whose expression is regulated by low temperature but not by abscisic acid or dehydration. *Plant Physiol.* 119:463–70
96. Michaels SD, Himelblau E, Kim SY, Schomburg FM, Amasino RM. 2005. Integration of flowering signals in winter-annual Arabidopsis. *Plant Physiol.* 137(1):149–56
97. Murcia G, Enderle B, Hiltbrunner A, Casal JJ. 2021. Phytochrome B and PCH1 protein dynamics store night temperature information. *Plant J.* 105(1):22–33
98. Ni W, Xu S-L, Tepperman JM, Stanley DJ, Maltby DA, et al. 2014. A mutually assured destruction mechanism attenuates light signaling in *Arabidopsis*. *Science* 344(6188):1160–64
99. Niu Y, Xiang Y. 2018. An overview of biomembrane functions in plant responses to high-temperature stress. *Front. Plant Sci.* 9:915
100. Nusinow DA, Helfer A, Hamilton EE, King JJ, Imaizumi T, et al. 2011. The ELF4-ELF3-LUX complex links the circadian clock to diurnal control of hypocotyl growth. *Nature* 475(7356):398–402
101. Olas JJ, Apelt F, Annunziata MG, John S, Richard SI, et al. 2021. Primary carbohydrate metabolism genes participate in heat-stress memory at the shoot apical meristem of *Arabidopsis thaliana*. *Mol. Plant* 14(9):1508–24
102. Osterlund MT, Hardtke CS, Wei N, Deng XW. 2000. Targeted destabilization of HY5 during light-regulated development of *Arabidopsis*. *Nature* 405(6785):462–66
103. Pajoro A, Severing E, Angenent GC, Immink RGH. 2017. Histone H3 lysine 36 methylation affects temperature-induced alternative splicing and flowering in plants. *Genome Biol.* 18(1):102
104. Park Y-J, Lee H-J, Gil K-E, Kim JY, Lee J-H, et al. 2019. Developmental programming of thermonastic leaf movement. *Plant Physiol.* 180(2):1185–97
105. Park Y-J, Lee H-J, Ha J-H, Kim JY, Park C-M. 2017. COP1 conveys warm temperature information to hypocotyl thermomorphogenesis. *New Phytol.* 215(1):269–80
106. Penfield S. 2008. Temperature perception and signal transduction in plants. *New Phytol.* 179(3):615–28
107. Posé D, Verhage L, Ott F, Yant L, Mathieu J, et al. 2013. Temperature-dependent regulation of flowering by antagonistic FLM variants. *Nature* 503(7476):414–17

108. Demonstrates that phytochrome B plays an equally critical role in temperature sensing during the day.

108. Qiu Y, Li M, Kim RJ-A, Moore CM, Chen M. 2019. Daytime temperature is sensed by phytochrome B in *Arabidopsis* through a transcriptional activator HEMERA. *Nat. Commun.* 10(1):140

109. Quail PH, Boylan MT, Parks BM, Short TW, Xu Y, Wagner D. 1995. Phytochromes: photosensory perception and signal transduction. *Science* 268(5211):675–80
110. Quint M, Delker C, Franklin KA, Wigge PA, Halliday KJ, van Zanten M. 2016. Molecular and genetic control of plant thermomorphogenesis. *Nat. Plants* 2(1):15190
111. Ranjan A, Mizuguchi G, FitzGerald PC, Wei D, Wang F, et al. 2013. Nucleosome-free region dominates histone acetylation in targeting SWR1 to promoters for H2A.Z replacement. *Cell* 154(6):1232–45
112. Rockwell NC, Su Y-S, Lagarias JC. 2006. Phytochrome structure and signaling mechanisms. *Annu. Rev. Plant Biol.* 57:837–58
113. Ruelland E, Vaultier M-N, Zachowski A, Hurry V. 2009. Cold signalling and cold acclimation in plants. *Adv. Bot. Res.* 49:35–150
114. Saidi Y, Finka A, Muriset M, Bromberg Z, Weiss YG, et al. 2009. The heat shock response in moss plants is regulated by specific calcium-permeable channels in the plasma membrane. *Plant Cell* 21(9):2829–43
115. Searle I, He Y, Turck F, Vincent C, Fornara F, et al. 2006. The transcription factor FLC confers a flowering response to vernalization by repressing meristem competence and systemic signaling in *Arabidopsis*. *Genes Dev.* 20(7):898–912
116. Seydel C, Kitashova A, Fürtauer L, Nägele T. 2022. Temperature-induced dynamics of plant carbohydrate metabolism. *Physiol. Plant.* 174(1):e13602
117. Sharma N, Geuten K, Giri BS, Varma A. 2020. The molecular mechanism of vernalization in Arabidopsis and cereals: role of Flowering Locus C and its homologs. *Physiol. Plant.* 170(3):373–83
118. Sheldon CC, Finnegan EJ, Dennis ES, Peacock WJ. 2006. Quantitative effects of vernalization on *FLC* and *SOC1* expression. *Plant J.* 45(6):871–83

119. Shen Y, Lei T, Cui X, Liu X, Zhou S, et al. 2019. Arabidopsis histone deacetylase HDA15 directly represses plant response to elevated ambient temperature. *Plant J.* 100(5):991–1006
120. Shinwari ZK, Nakashima K, Miura S, Kasuga M, Seki M, et al. 1998. An *Arabidopsis* gene family encoding DRE/CRT binding proteins involved in low-temperature-responsive gene expression. *Biochem. Biophys. Res. Commun.* 250:161–70
121. Silva CS, Nayak A, Lai X, Hutin S, Hugouvieux V, et al. 2020. Molecular mechanisms of Evening Complex activity in *Arabidopsis*. *PNAS* 117(12):6901–9
122. Soitamo AJ, Piippo M, Allahverdiyeva Y, Battchikova N, Aro E-M. 2008. Light has a specific role in modulating *Arabidopsis* gene expression at low temperature. *BMC Plant Biol.* 8:13
123. Sureshkumar S, Dent C, Seleznev A, Tasset C, Balasubramanian S. 2016. Nonsense-mediated mRNA decay modulates FLM-dependent thermosensory flowering response in *Arabidopsis*. *Nat. Plants* 2(5):16055
124. Susila H, Jurić S, Liu L, Gawarecka K, Chung KS, et al. 2021. Florigen sequestration in cellular membranes modulates temperature-responsive flowering. *Science* 373(6559):1137–42
125. Tasset C, Yadav AS, Sureshkumar S, Singh R, van der Woude L, et al. 2018. POWERDRESS-mediated histone deacetylation is essential for thermomorphogenesis in *Arabidopsis thaliana*. *PLOS Genet.* 14(3):e1007280
126. Thiel G, Lesch A, Rubil S, Backes TM, Rössler OG. 2018. Regulation of gene transcription following stimulation of transient receptor potential (TRP) channels. *Int. Rev. Cell Mol. Biol.* 335:167–89
127. Thomas L, Marondedze C, Ederli L, Pasqualini S, Gehring C. 2013. Proteomic signatures implicate cAMP in light and temperature responses in *Arabidopsis thaliana*. *J. Proteom.* 83:47–59
128. Van Buskirk EK, Reddy AK, Nagatani A, Chen M. 2014. Photobody localization of phytochrome B is tightly correlated with prolonged and light-dependent inhibition of hypocotyl elongation in the dark. *Plant Physiol.* 165(2):595–607
129. van der Woude LC, Giorgio P, Snoek BL, van Hoogdalem M, Novák O, et al. 2019. HISTONE DEACETYLASE 9 stimulates auxin-dependent thermomorphogenesis in *Arabidopsis thaliana* by mediating H2A.Z depletion. *PNAS* 116(50):25343–54
130. Vasseur F, Pantin F, Vile D. 2011. Changes in light intensity reveal a major role for carbon balance in *Arabidopsis* responses to high temperature. *Plant. Cell Environ.* 34(9):1563–76
131. Viatchenko-Karpinski V, Ling J, Gu JG. 2018. Characterization of temperature-sensitive leak K^+ currents and expression of TRAAK, TREK-1, and TREK2 channels in dorsal root ganglion neurons of rats. *Mol. Brain* 11:40
132. Voets T, Droogmans G, Wissenbach U, Janssens A, Flockerzi V, Nilius B. 2004. The principle of temperature-dependent gating in cold- and heat-sensitive TRP channels. *Nature* 430(7001):748–54
133. von Arnim AG, Deng X-W. 1994. Light inactivation of Arabidopsis photomorphogenic repressor COP1 involves a cell-specific regulation of its nucleocytoplasmic partitioning. *Cell* 79(6):1035–45
134. Vu LD, Gevaert K, De Smet I. 2019. Feeling the heat: searching for plant thermosensors. *Trends Plant Sci.* 24(3):210–19
135. Vu LD, Xu X, Zhu T, Pan L, van Zanten M, et al. 2021. The membrane-localized protein kinase MAP4K4/TOT3 regulates thermomorphogenesis. *Nat. Commun.* 12(1):2842
136. Wang R, Zhang Y, Kieffer M, Yu H, Kepinski S, Estelle M. 2016. HSP90 regulates temperature-dependent seedling growth in *Arabidopsis* by stabilizing the auxin co-receptor F-box protein TIR1. *Nat. Commun.* 7(1):10269
137. Wang X, Ma X, Wang H, Li B, Clark G, et al. 2015. Proteomic study of microsomal proteins reveals a key role for *Arabidopsis* annexin 1 in mediating heat stress-induced increase in intracellular calcium levels. *Mol. Cell. Proteom.* 14(3):686–94
138. Watanabe S, Radman-Livaja M, Rando OJ, Peterson CL. 2013. A histone acetylation switch regulates H2A.Z deposition by the SWR-C remodeling enzyme. *Science* 340(6129):195–99
139. Willige BC, Zander M, Yoo CY, Phan A, Garza RM, et al. 2021. PHYTOCHROME-INTERACTING FACTORs trigger environmentally responsive chromatin dynamics in plants. *Nat. Genet.* 53(7):955–61
140. Wood CC, Robertson M, Tanner G, Peacock WJ, Dennis ES, Helliwell CA. 2006. The *Arabidopsis thaliana* vernalization response requires a polycomb-like protein complex that also includes VERNALIZATION INSENSITIVE 3. *PNAS* 103(39):14631–36

121. Shows that the full EC acts as a direct thermosensor with stronger binding in the cold.

124. Demonstrates that low temperatures facilitate FT sequestration in cellular membranes, thus reducing florigen availability and delaying flowering.

134. Proposes a set of selection criteria for the identification of plant thermosensors.

141. Xu S, Chong K. 2018. Remembering winter through vernalisation. *Nat. Plants* 4(12):997–1009
142. Xue M, Zhang H, Zhao F, Zhao T, Li H, Jiang D. 2021. The INO80 chromatin remodeling complex promotes thermomorphogenesis by connecting H2A.Z eviction and active transcription in *Arabidopsis*. *Mol. Plant* 14:1799–813
143. Yamaguchi R, Nakamura M, Mochizuki N, Kay SA, Nagatani A. 1999. Light-dependent translocation of a phytochrome B-GFP fusion protein to the nucleus in transgenic *Arabidopsis*. *J. Cell Biol.* 145(3):437–45
144. Yamaguchi-Shinozaki K, Shinozaki K. 1994. A novel *cis*-acting element in an Arabidopsis gene is involved in responsiveness to drought, low-temperature, or high-salt stress. *Plant Cell* 6:251–64
145. Yang H, Zhao Y, Chen N, Liu Y, Yang S, et al. 2021. A new adenylyl cyclase, putative disease-resistance RPP13-like protein 3, participates in abscisic acid-mediated resistance to heat stress in maize. *J. Exp. Bot.* 72(2):283–301
146. Yang T, Ali GS, Yang L, Du L, Reddy ASN, Poovaiah BW. 2010. Calcium/calmodulin-regulated receptor-like kinase CRLK1 interacts with MEKK1 in plants. *Plant Signal. Behav.* 5(8):991–94
147. Yu Y, Deng Y, Reed SH, Millar CB, Waters R. 2013. Histone variant Htz1 promotes histone H3 acetylation to enhance nucleotide excision repair in Htz1 nucleosomes. *Nucleic Acids Res.* 41(19):9006–19
148. Zhang B, Holmlund M, Lorrain S, Norberg M, Bakó L, et al. 2017. BLADE-ON-PETIOLE proteins act in an E3 ubiquitin ligase complex to regulate PHYTOCHROME INTERACTING FACTOR 4 abundance. *eLife* 6:e26759
149. Zhao C, Liu B, Piao S, Wang X, Lobell DB, et al. 2017. Temperature increase reduces global yields of major crops in four independent estimates. *PNAS* 114(35):9326–31
150. Zhao Y, Antoniou-Kourounioti RL, Calder G, Dean C, Howard M. 2020. Temperature-dependent growth contributes to long-term cold sensing. *Nature* 583(7818):825–29
151. Zheng X, Krakowiak J, Patel N, Beyzavi A, Ezike J, et al. 2016. Dynamic control of Hsf1 during heat shock by a chaperone switch and phosphorylation. *eLife* 5:e18638
152. Zhu J-K. 2016. Abiotic stress signaling and responses in plants. *Cell* 167(2):313–24
153. Zhu P, Lister C, Dean C. 2021. Cold-induced *Arabidopsis* FRIGIDA nuclear condensates for *FLC* repression. *Nature* 599(7886):657–61

153. Shows that cold promotes the formation of FRI condensates that do not colocalize with an active *FLC* locus.