Review

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Tansley review

Epigenetic regulation of thermomorphogenesis and heat stress tolerance

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Summary

Many environmental conditions fluctuate and organisms need to respond effectively. This is especially true for temperature cues that can change in minutes to seasons and often follow a diurnal rhythm. Plants cannot migrate and most cannot regulate their temperature. Therefore, a broad array of responses have evolved to deal with temperature cues from freezing to heat stress. A particular response to mildly elevated temperatures is called thermomorphogenesis, a suite of morphological adaptations that includes thermonasty, formation of thin leaves and elongation growth of petioles and hypocotyl. Thermomorphogenesis allows for optimal performance in suboptimal temperature conditions by enhancing the cooling capacity. When temperatures rise further, heat stress tolerance mechanisms can be induced that enable the plant to survive the stressful temperature, which typically comprises cellular protection mechanisms and memory thereof. Induction of thermomorphogenesis, heat stress tolerance and stress memory depend on gene expression regulation, governed by diverse epigenetic processes. In this Tansley review we update on the current knowledge of epigenetic regulation of heat stress tolerance and elevated temperature signalling and response, with a focus on thermomorphogenesis regulation and heat stress memory. In particular we highlight the emerging role of H3K4 methylation marks in diverse temperature signalling pathways.

I. Introduction

Plants are sensitive to environmental perturbations and adjust to changing conditions continuously. This occurs especially for temperature cues that can change rapidly over the day and fluctuate in diurnal and seasonal rhythms (Chinnusamy *et al.*, 2007; Penfield, 2008; Legris *et al.*, 2016a; Quint *et al.*, 2016; Casal & Balasubramanian, 2019; Praat *et al.*, 2021). At the organism level, plant responses to temperature can be roughly separated into tolerance responses (ensure survival) and acclimation responses, associated with growth and physiological development, that facilitate optimal performance under suboptimal conditions. In general, tolerance responses are observed when plants are exposed to extreme temperatures such as heat stress (HS) and freezing stress. Acclimation responses are typically displayed upon exposure to milder temperature changes, such as chilling and high ambient

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temperatures (Thomashow, 1999; Sung *et al.*, 2003; Wahid *et al.*, 2007; Penfield, 2008; van Zanten *et al.*, 2014a; Hayes *et al.*, 2021; Praat *et al.*, 2021). Chilling stress for instance typically leads to reduced and compact growth, stimulating insulation (Hasdai *et al.*, 2006). Upon exposure to subzero temperatures, cryoprotective mechanisms such as accumulation of ice-binding proteins and plasma membrane thickening is induced to tolerate cellular damage resulting from ice crystals (Bredow & Walker, 2017). Conversely, HS can result in irreversible damage through protein denaturation and accumulation of reactive oxygen species (ROS), that can cause malfunctioning of organelles and the photosynthesis apparatus (Kotak *et al.*, 2007; Wahid *et al.*, 2007; Liu *et al.*, 2015). Typical symptoms of HS include growth inhibition, leaf senescence and abscission, sterility, and impaired seed vigour and germination (Kotak *et al.*, 2007). In general, two distinct tolerance mechanisms are discerned. The first one concerns the intrinsic capacity to withstand HS and is usually referred to as basal thermotolerance. Conversely, pre-exposure to mild HS can induce tolerance to a strong(er) HS event that would otherwise be lethal, in a process called acquired thermotolerance or HS priming. The latter implies memory of the previous HS episode (Yeh *et al.*, 2012; Bäurle, 2016).

The definition of mildly elevated temperature and HS depends on the natural habitat of the species under consideration (Yeh *et al.*, 2012). For *Arabidopsis thaliana*, mildly elevated temperature is loosely defined as a surpass of about 5–7°C (*c.* 27–29°C) above the standard growth temperature used in laboratories world-wide (20–22°C), a temperature above *c.* 30°C is referred to as HS and above *c.* 36°C as severe HS (Wahid *et al.*, 2007). Mildly elevated temperatures in general trigger acceleration of development, early flowering, and alter immunity (Hua, 2013; Verhage *et al.*, 2014; Capovilla *et al.*, 2015; Gangappa *et al.*, 2017). In diverse species such as tomato and Arabidopsis, a suite of morphological adaptations, collectively termed thermomorphogenesis, are induced to withstand suboptimal temperatures (Quint *et al.*, 2016; Casal & Balasubramanian, 2019). Typical thermomorphogenic responses are hypocotyl elongation, upward leaf movement (thermonasty), petiole elongation, reduced stomatal density and formation of smaller and thinner leaves (Koini *et al.*, 2009; Crawford *et al.*, 2012; van Zanten *et al.*, 2014a; Ibañez *et al.*, 2017; Casal & Qüesta, 2018). The open rosette structure that results from thermomorphogenesis contributes to the cooling capacity through transpiration and allows avoidance of solar heat flux (Crawford *et al.*, 2012; Bridge *et al.*, 2013; Park *et al.*, 2019).

In recent years, great progress has been made in our understanding of the molecular factors involved in thermosensing and signalling. The bHLH transcription factor PHYTOCHROME INTERACTING FACTOR 4 (PIF4) is considered a core thermomorphogenesis-signalling hub on which diverse temperature signalling pathways converge (Koini *et al.*, 2009; Sun *et al.*, 2012; Quint *et al.*, 2016; Gangappa *et al.*, 2017; Casal & Balasubramanian, 2019). However, PIF4-independent signalling pathways are now emerging (Vu *et al.*, 2021). Among many other responses, PIF4, and PIF7 alike (Chung *et al.*, 2020; Fiorucci *et al.*, 2020) directly stimulate auxin biosynthesis by binding and activating promoters of rate-limiting auxin biosynthesis genes (Franklin *et al.*, 2011; Sun *et al.*, 2012) that trigger thermomorphogenesis in a brassinosteroid-dependent manner (Martins *et al.*, 2017; Ibañez *et al.*, 2018).

Warm temperature is perceived through the photoreceptor phytochrome B (phyB) by the highly temperature-sensitive 'dark' reversion of active Pfr to the inactive Pr conformation. The rapid nuclear extrusion of phyB-Pr upon warmth releases PIF4 inhibition that subsequently initiates thermomorphogenesis (Jung *et al.*, 2016; Legris *et al.*, 2016b; Qiu *et al.*, 2019), a process that is attenuated at lower temperature conditions. Temperature also provides direct input to the PIFs, as translation of *PIF7* mRNA is enhanced by warmth through relaxation of the *PIF7* mRNA hairpin structure, resulting in PIF7 protein accumulation (Chung *et al.*, 2020). In addition, the temperature-dependent phase separation of the transcriptional repressor EARLY FLOWERING 3 (ELF3) into inactive condensates at warm temperatures also contributes to temperature sensing and thermomorphogenesis (Jung *et al.*, 2020).

Transcription of some heat shock protein (HSP) genes is regulated by mild warm temperatures and can even serve as a 'molecular thermometer' (Kumar & Wigge, 2010). However, these molecular chaperones and members of the heat shock transcription factors (HSFs) alike, are mostly associated with HS responses. In control temperature conditions HSPs bind to HSFs, maintaining them in an inactive state. During HS, HSPs are recruited to damaged proteins, facilitating their repair or removal (Scharf *et al.*, 2012; Li *et al.*, 2017; Ohama *et al.*, 2017). The consequent release of HSFs allows for their multimerisation and binding to heat shock elements (HSE) in the promoters of HSPs and other target genes. This activation boosts the production of even more HSPs to protect the cells. Simultaneously, the HSF–HSP balance is restored, which dampens the response.

While the direct transcriptional regulation of high temperature responses by means of regulators (e.g. PIFs, ELF3, phyB, HSFs and many others) is now well understood, increasing evidence supports a prominent role for epigenetic regulation in plant temperature signalling and response. The term epigenetics was first introduced in modern-day biology by embryologist Conrad Waddington in 1942, who defined epigenetics as 'the branch of biology which studies the causal interactions between genes and their products, which bring the phenotype into being' (Waddington, 1942). For a brief history please refer to Deichmann (2016). Later, a community consensus definition of the term epigenetics was reached at a Cold Spring Harbor meeting in 2008: 'An epigenetic trait is a stably heritable phenotype resulting from changes in a chromosome without alterations in the DNA sequence' (Berger *et al.*, 2009). In the broader sense, the term epigenetics is often used to describe 'chromatin modifications', that is chemical modifications of DNA or histone proteins placed around DNA that do not change the base sequence (Deichmann, 2016). As these modifications may or may not be stable and transmitted through cell division and subsequent generations, they do not necessarily need to conform to the narrow definition given above.

Typically, epigenetic mechanisms involve the regulation of gene transcription via different pathways including, but not limited to, DNA methylation, small RNAs, ATP-dependent chromatin

remodelling, histone variants, histone modifications, histone chaperones and long noncoding RNAs. Some of these epigenetic modifications and modifying proteins modulate expression of high temperature-responsive genes and function to prevent heat-related damage and/or to promote subsequent adaptation (He & Li, 2018). In addition, epigenetic processes are involved in priming and memory of HS, which promotes tolerance to recurring HS events within the same or in a subsequent generation (*trans*-generational memory).

In this review we provide a update on the current knowledge of epigenetic regulation of HS and mildly elevated temperature signalling and define topics for future research. We will focus primarily on thermomorphogenesis regulation and HS memory. We do acknowledge that epigenetic processes are a prominent component of many other temperature effects, including flowering time regulation, cold responsiveness (especially during vernalisation) and pollen development. Here, we do not cover these aspects as these have been the topic of excellent recent reviews (e.g. Hereme *et al.*, 2021; Pandey *et al.*, 2021; Luo & He, 2020; Chang *et al.*, 2020; Y. Chen *et al.*, 2016).

II. Epigenetic regulation of thermomorphogenesis

1. Histone variant H2A.Z is evicted at warm temperatures

Chromatin remodelling represents an important level of regulation in temperature sensing and response mechanisms. In eukaryotes, the DNA is organised within nucleosomes where it is wrapped around histone proteins. Canonical histones and histone variants are highly conserved globular proteins whose N-terminal tails are exposed on the surface of the nucleosome octamer for chemical modifications, including methylation and acetylation (Kouzarides, 2007). In addition, ATP-dependent chromatin remodellers can modify histone–DNA interactions, providing accessibility to transcriptional regulators (Ho & Crabtree, 2010). Histone proteins are usually deposited into nucleosomes during the S-phase of the cell cycle (Liu *et al.*, 2015). Together with the incorporation of canonical histones, nonallelic histone variants can also be integrated during the entire cell cycle (Kamakaka & Biggins, 2005). Histone variants can alter nucleosome stability and structure, thereby affecting chromatin accessibility and transcription.

The first solid evidence of histone variants regulating thermoresponsiveness was presented by the Wigge laboratory (Kumar & Wigge, 2010). They identified ACTIN-RELATED PROTEIN 6 (ARP6) as mediator of temperature responses in Arabidopsis (Fig. 1). *Arp6* mutants display elongated hypocotyls already at low temperatures, indicative of a constitutive warm temperature phenotype. ARP6 is a subunit of the Snf2 ATPase remodelling complex SWR1-C, responsible for the exchange of the permissive canonical histone variant H2A with repressive H2A.Z. The *arp6* mutant showed higher *HSP70* expression and de-repression of many other thermo-responsive genes due to the inability to include H2A.Z nucleosomes at temperature-regulated loci primarily at the +1 site. Chromatin immunoprecipitation (ChIP) followed by real-time PCR revealed depletion of H2A.Z occupancy at the *HSP70* locus in the *arp6* mutant, indicating that higher *HSP70* expression is correlated with changes in chromatin structure (Fig. 1). Further analyses showed that H2A.Z depletion scaled with temperature, as at 17°C H2A.Z occupancy at the *HSP70* locus was greater than at 27°C, when *HSP70* expression is increased (Kumar & Wigge, 2010).

Later, H2A.Z eviction at elevated temperatures was observed for a specific cluster of environment-responsive genes, occurring at different chromatin regions and not exclusively around transcription start sites (Cortijo *et al.*, 2017). To investigate whether H2A.Z depletion was required for transcriptional activation or was instead a consequence, H2A.Z occupancy was measured at different timepoints during high temperature exposure. H2A.Z occupation was lost within minutes from activation, suggesting that H2A.Z-containing nucleosomes may be required to maintain gene repression during moderate temperature conditions. Furthermore, binding of the HSFA1a transcription factor to heat-responsive genes, including *HSP70*, appeared critical to promote H2A.Z eviction and hence gene expression (Cortijo *et al.*, 2017) (Fig. 1). Altogether, this indicates that H2A.Z-containing nucleosomes are not temperature sensors *per se*, but that their occupancy depends on the presence/absence of transcriptional regulators, allowing a switch-like chromatin re-organisation in response to environmental cues to induce appropriate, timely and specific (transcriptional) responses to cope with the changing temperature conditions.

In line with H2A.Z, other variants like H2A.W, which controls heterochromatin organisation and coordinates DNA methylation levels together with the histone linker H1, are promising subjects of future studies on plant temperature responses (Yelagandula *et al.*, 2014; Bourguet *et al.*, 2021).

2. Role of histone (de)methylation in responses to elevated temperatures

Histone epigenetic modifications occur at specific histone tails residues that extrude from the nucleosomes, primarily on lysine (K) and arginine (R). The position and timing of placement or removal of these covalent modifications are essential for appropriate responsiveness to environmental cues and cause either an increase or decrease in transcription (Perrella & Kaiserli, 2016).

FLOWERING CONTROL LOCUS A (FCA) is an RNA binding protein that is involved in chromatin silencing by promoting histone demethylation of *FLOWERING LOCUS C* (Tian *et al.*, 2019). Interestingly, *fca* mutants display hyperelongated hypocotyls when exposed to 28°C (Lee *et al.*, 2014). FCA directly interacts with PIF4 and, at 28°C, PIF4 recruits FCA to the chromatin of growth-promoting target genes, including *YUCCA8* (*YUC8*) (Lee *et al.*, 2014). *YUC8* encodes a rate-limiting enzyme in auxin biosynthesis that is critical for thermomorphogenesis (Franklin *et al.*, 2011; Sun *et al.*, 2012) (Fig. 2a). FCA binding triggers histone H3K4me2 demethylation (an activating mark). In addition, FCA modulates PIF4 dissociation from the *YUC8* locus and attenuates *YUC8* expression (Lee *et al.*, 2014). Thereby, high temperatures-induced hypocotyl elongation seizes (Fig. 2a).

Correspondingly, binding of Jumonji C (JmjC) demethylases JMJ14 and JMJ15 to target genes was enhanced at 27°C, suggesting

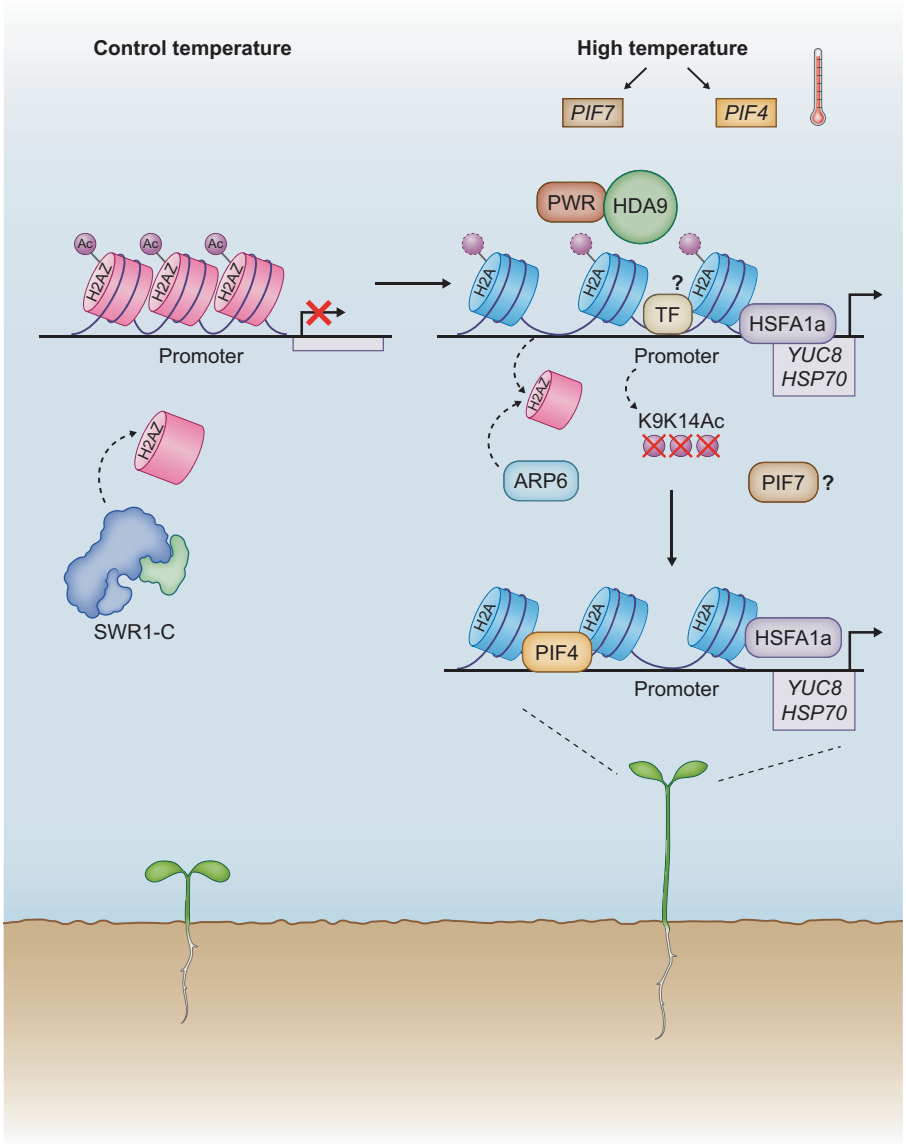

Fig. 1 HDA9-dependent H2A.Z eviction at high ambient temperatures. (Left) At control temperatures, the SWR1 multiprotein complex (SWR1-C) containing ACTIN-RELATED PROTEIN 6 (ARP6) is responsible for the incorporation of the histone variant H2A.Z (pink) that suppresses thermo-responsive genes. (Right) At high temperatures, HEAT STRESS TRANSCRIPTION FACTOR A1a (HSFA1a) is involved in H2A.Z eviction. In addition, the POWERDRESS-HISTONE DEACETYLASE 9 (PWR-HDA9) complex deacetylates H3K9K14Ac at the *YUCCA8* (*YUC8*) and *HEAT SHOCK PROTEIN 70* (*HSP70*) loci, thereby proposedly impeding ARP6-dependent H2A.Z incorporation. As a result, these loci contain relatively high levels of permissive H2A (blue) and relatively low levels of suppressive H2A.Z. This chromatin state facilitates the binding of promoter regions by PHYTOCHROME INTERACTING FACTOR 4 (PIF4) and possibly PIF7, resulting in transcriptional activation of genes required for thermomorphogenesis responses (e.g. *YUC8* to stimulate auxin biosynthesis) such as hypocotyl elongation and thermonasty. How HDA9 is recruited by transcription factors (TF) to its target genes in response to elevated temperatures remains to be investigated. Black dashed and solid arrows, respectively, indicate positioning/removal of the indicated molecular factor or stimulation of the indicated process.

active recruitment of these H3K4me3 (considered an activating mark) demethylases at high temperatures (Cui *et al.*, 2021) (Fig. 2b). However, in apparent contradiction to *fca* mutants, in a *jmj14* mutant combined with a mutation in its cofactor-producing enzyme cytosolic isocitrate-dehydrogenases (cICDH), expression of several auxin-related genes, such as *YUC8*, was suppressed and, accordingly, thermomorphogenesis capacity was reduced as well. This suggest that opposite to a suppressive role for FCA, JMJ14 and the redundant JMJ15 and JMJ18 act as positive regulators of high temperature-mediated changes in gene expression and thermomorphogenesis (Cui *et al.*, 2021) (Fig. 2b).

Another histone methylation mark that correlates with an increase in temperature (from 15°C to 25°C) is H3K36me3. In particular, H3K36me3 enrichment was associated with Differentially Spliced (DiS) events upon temperature changes (Pajoro *et al.*, 2017). DiS regions involve transcripts previously reported to undergo temperature-induced Alternative Splicing (AS), such as *FLOWERING LOCUS M*, *MADS AFFECTING FLOWERING 2*, as well as clock components such as *PSEUDO-RESPONSE REGULATOR 3* (*PRR3*) and *PRR7*. Transcriptome analyses of mutants of the histone H3K36 methyltransferases *SET DOMAIN-CONTAINING GROUP 8* (*SDG8*) and *SDG26* revealed that most DiS events were diminished in these mutants, suggesting that H3K36me3 is indeed required for such events. In addition, measurement of transcription rates in Arabidopsis seedlings subjected to various temperatures revealed that H2A.Z gene body occupancy and H3K27me3 levels were associated with a more thermostable transcription rate (Sidaway-Lee *et al.*, 2014).

3. Histone deacetylases affect responsiveness to elevated temperatures

Histone acetylation and deacetylation are characterised by the addition and removal of acetyl groups to lysine residues on H3 and H4 histone tails. This is catalysed by histone acetyltransferases (HATs) and histone deacetylases (HDACs), respectively, which

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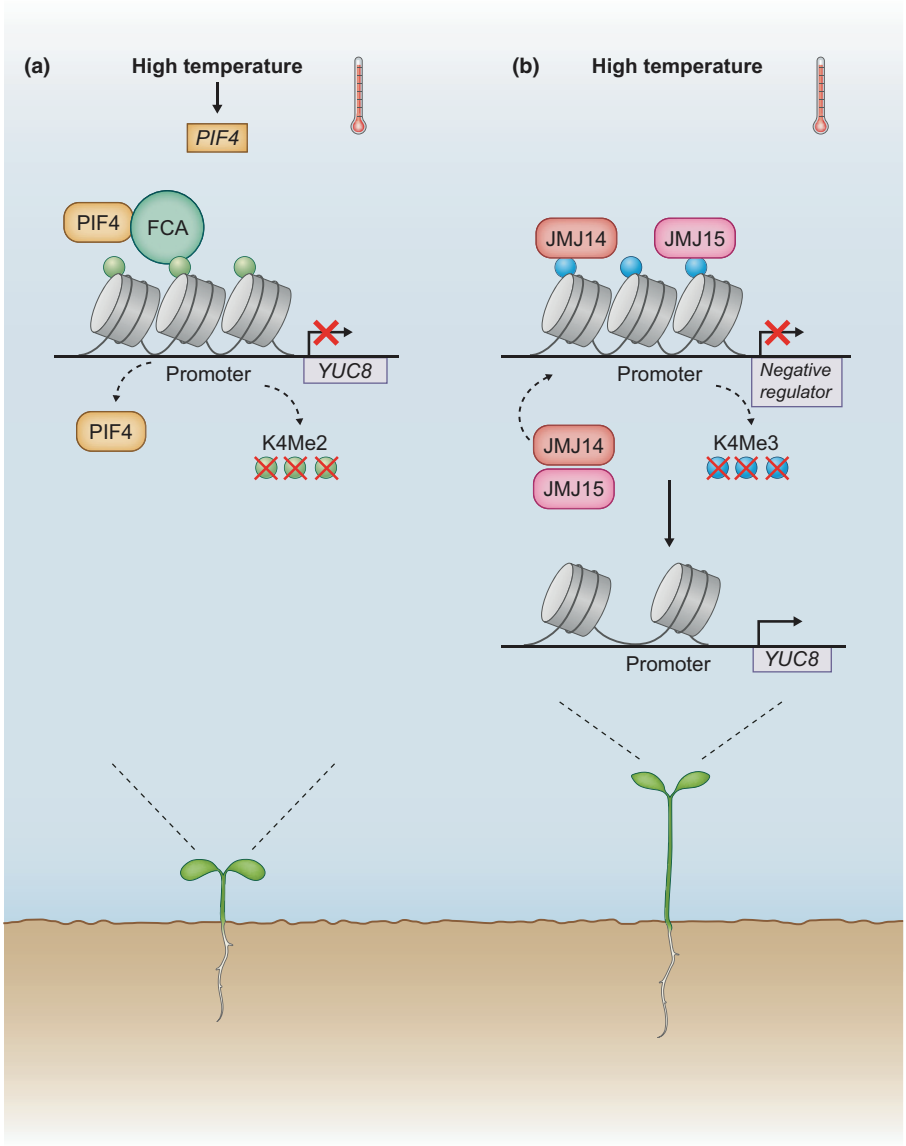

Fig. 2 Histone modification regulation associated to thermomorphogenesis. (a) High temperature triggers the expression of transcription factor *PIF4*. Accumulated PIF4 protein activates transcription of genes required for thermomorphogenesis responses, such as *YUC8*, to stimulate thermomorphogenesis. PIF4 also directly interacts with FLOWERING CONTROL LOCUS A (FCA) that is recruited to the chromatin where it allows H3K4me2 demethylation of the *YUC8* promoter region and triggers PIF4 dissociation from the DNA, thereby reducing *YUC8* expression to attenuate thermomorphogenesis. (b) In a different scenario, high temperature triggers the recruitment of the histone demethylases Jumonji (JMJ) 14 and 15 that mediate the removal of H3K4me3 marks that could affect the expression of a negative regulator. In addition, *YUC8* transcript level is increased, causing hypocotyl elongation. Black dashed and solid arrows indicate, respectively, positioning/removal of the indicated molecular factor or stimulation of the indicated process.

ultimately modulate chromatin accessibility for transcription factors, other regulatory proteins and the transcription machinery. This 'opening' and 'closure' of specific chromatin regions by HATs and HDACs can affect gene expression in response to environmental stimuli (Asensi-Fabado *et al.*, 2017).

Early work from the Luschnig laboratory showed that the reactivation of a β-*GLUCURONIDASE* transgene after HS was correlated with an increase in H3K9K14ac. This reactivation was more pronounced in the *HISTONE DEACETYLASE 6* (*hda6*) mutant (Lang-Mladek *et al.*, 2010). More recently, a connection between histone deacetylation and H2A.Z has been revealed (Fig. 1). In a forward genetics screen for mutants displaying impaired thermomorphogenesis a mutation in POWERDRESS (PWR), a SANT domain protein known to interact with HDA9, was identified (Tasset *et al.*, 2018). *Pwr* and *hda9* mutants display similar temperature-dependent phenotypic traits, such as impaired hypocotyl elongation in response to warmth and a compact rosette morphology. However, some responses were retained in *hda9* compared with *pwr*. For instance, *hda9* mutant seedlings displayed a wild-type-like *HSP70* induction at high temperature, while in *pwr* this temperature marker gene was not induced (Tasset *et al.*, 2018; van der Woude *et al.*, 2019; de Rooij *et al.*, 2020). Similarly, unlike PWR, HDA9 is seemingly not involved in *PIF4* transcriptional induction in response to elevated temperatures. Strikingly, HDA9 functions independently of phyB thermosignalling and light quality signalling (van der Woude *et al.*, 2019), setting it apart from the shade avoidance syndrome, a suite responses similar to those induced by warmth (Ballaré & Pierik, 2017). Transcriptome analyses demonstrated that induction of auxin-related genes, including *YUC8*, was hindered in *hda9* and *pwr* mutants at warm temperatures. Subsequent ChIP-PCR analysis revealed that the *YUC8* locus was indeed hyperacetylated in these mutants at 27°C, thereby hinting that histone deacetylation is necessary for *YUC8* induction. Interestingly, *hda9* mutants displayed high levels of H2A.Z occupancy at the *YUC8* locus and a reduced ability for PIF4 binding at warm temperatures. Altogether, this suggests that

HDA9-dependent histone deacetylation is required for net H2A.Z eviction from *YUC8*'s nucleosomes at warm temperatures, which subsequently allowed PIF4 binding to the *YUC8* promoter, followed by *YUC8* transcriptional activation, auxin accumulation and thermomorphogenesis (Fig. 1). Whether PIF7 binding to chromatin is also modulated by HDA9-dependent histone deacetylation and H2A.Z eviction remains to be studied (Fig. 1).

In another study, *hda15*, *hda9* and *hda19* mutants were shown to have partly opposite temperature responses, as *hda15* mutant seedlings displayed longer hypocotyls than the wild-type at 27°C, while *hda9* and *hda19* hypocotyls were shorter (Shen *et al.*, 2019). HDA15, HDA9 and HDA19, therefore, have distinct functions in temperature signalling. Gene expression analysis of *hda15* seedlings revealed upregulation of temperature-dependent genes including *YUC8*, *HSP20*, *INDOLE-3-ACETIC ACID INDUCIBLE 3* (*IAA3*), *IAA19* and *IAA29*. Furthermore, HDA15 was found to interact with LONG HYPOCOTYL IN FAR-RED (HFR1) to downregulate gene expression. Because HFR1 interacts with PIF4 and antagonises its activity (Hornitschek *et al.*, 2009), HDA15 proposedly controls thermomorphogenesis by repressing PIF4 activity (Shen *et al.*, 2019). Moreover, HDA15 interacts with PIF1 and PIF3 to repress phyB-dependent seed germination, chlorophyll biosynthesis and photosynthetic genes in etiolated seedlings (Liu *et al.*, 2013; Xu *et al.*, 2017). Together, this hints that HDA15 – and possibly other HDAC's alike – have a role in governing transcriptional networks that translate environmental cues in appropriate functional responses, in which HDA9 apparently has a more specific role in temperature signalling, because *hda9* mutants are not disturbed in responsiveness to light quality cues (van der Woude *et al.*, 2019).

Interestingly, *hda9* and *hda19* mutants, despite exhibiting similar high temperature phenotypes, displayed little overlap in differentially expressed genes. This suggests that these related HDAC proteins might function in different pathways leading to thermomorphogenesis. ChIP-PCRs indicated that both HDA15 and HDA19 directly bind to promoters of stress responsive genes, while no direct DNA binding was detected for HDA9 (Shen *et al.*, 2019). Whether HDA15 and HDA19 also regulate H2A.Z occupancy of their target genes remains to be determined.

4. The role of the INO80 complex in responses to elevated temperatures

A recent report demonstrated that the Snf2 ATP-dependent chromatin remodelling complex INO80–EIN6 ENHANCER (EEN) is required for thermomorphogenesis by mediating H2A.Z eviction (Xue *et al.*, 2021) (Fig. 3a). INO80 and EEN directly associate with PIF4 to activate transcription of auxin-related genes, including *YUC8*, under elevated temperatures. In addition, constitutive induction of PIF4 target genes by *PIF4* overexpression was suppressed in the *ino80* mutant background. Moreover, PIF4 is required for INO80 complex (INO80-C) recruitment to PIF4 target loci, to facilitate local H2A.Z eviction at warm temperatures and H2A.Z eviction was compromised in both *pif4* and *ino80* mutants. Yeast-two-hybrid screening and subsequent confirmations indicated that INO80-C interacts with the H3K4me3 deposition complex COMPASS-like core component WDR5a and the SPT4-1 and SPT4-2 transcription elongation factors. These modulate RNA Pol II elongation to facilitate efficient transcription and H3K4me3 deposition, an epigenetic mark mainly associated with active transcription. Indeed, H3K4me3 levels were elevated at PIF4 targets at warm temperatures and this was lost in the *pif4* mutant. This suggests that INO80-C is required for warm temperature-induced H3K4me3 deposition and transcription elongation at PIF4 target genes (Fig. 3a). Moreover, mutants in diverse transcription elongation factors exhibited impaired thermomorphogenesis phenotypes and had elevated H2A.Z levels, similar to *ino80* and *pif4* mutants. This demonstrates that efficient Pol II elongation facilitates thermomorphogenesis and is seemingly required for H2A.Z removal at PIF4 targets, and therefore that H2A.Z eviction and active transcription are associated together (Xue *et al.*, 2021). Given the requirement of HDA9 and PWR1 for PIF4-mediated induction of auxin biosynthesis under warm temperatures (Tasset *et al.*, 2018; van der Woude *et al.*, 2019), and because *hda9* mutants display enhanced H3K4me2/3 levels under salt stress (Zheng *et al.*, 2020), it seems possible that HDA9 and PWR1 also associate with INO80-C.

In an independent study, Willige *et al.* (2021) demonstrated that PIF7 binding to promoters is required for local H2A.Z removal within minutes after exposure to a low ratio of red to far-red light (Willige *et al.*, 2021) (Fig. 3b). PIF7 was proposed to mediate H2A.Z removal through its interaction with the EEN subunit of INO80-C. Therefore, similar to warm temperature conditions (Xue *et al.*, 2021), PIFs are seemingly needed for H2A.Z removal and simultaneous activation of their target genes under shade (Willige *et al.*, 2021). Given the involvement of HDAC activity in H2A.Z incorporation/eviction dynamics (van der Woude *et al.*, 2019) on the one hand, and the demonstrated HDA15 interaction with PIF1 and PIF3 (Liu *et al.*, 2013; Xu *et al.*, 2017) and the light signalling components HFR1 (Hornitschek *et al.*, 2009), on the other hand, it would be interesting to test for possible contributions by HDA15 to H2A.Z dynamics in both shade and high temperature conditions. This possibility is further supported by the observation that HDA15 interacts with NUCLEAR FACTOR-Y, subunit C (NF-YC) proteins (Tang *et al.*, 2017) that induce H2A.Z deposition in a light-dependent manner to inhibit photomorphogenesis (Zhang *et al.*, 2021).

5. Integrating the role of histone modifications and modifying enzymes

Combined with the observation that *PIF7* mRNA functions as a thermosensor (Chung *et al.*, 2020), it is tempting to propose the existence of a short temperature signalling cascade. When temperatures rise, *PIF7* expression is induced and translation of *PIF7* mRNA is enhanced through mRNA hairpin relaxation (Chung *et al.*, 2020). PIFs then bind to the *G-box* element of their target genes and mediate H2A.Z depletion in concert with the INO80–EEN complex, possibly in association with PWR and HDA9. This net H2A.Z removal then possibly relieves target gene repression, followed by transcriptional activation and induction of thermomorphogenesis. Despite being a tempting hypothesis, some

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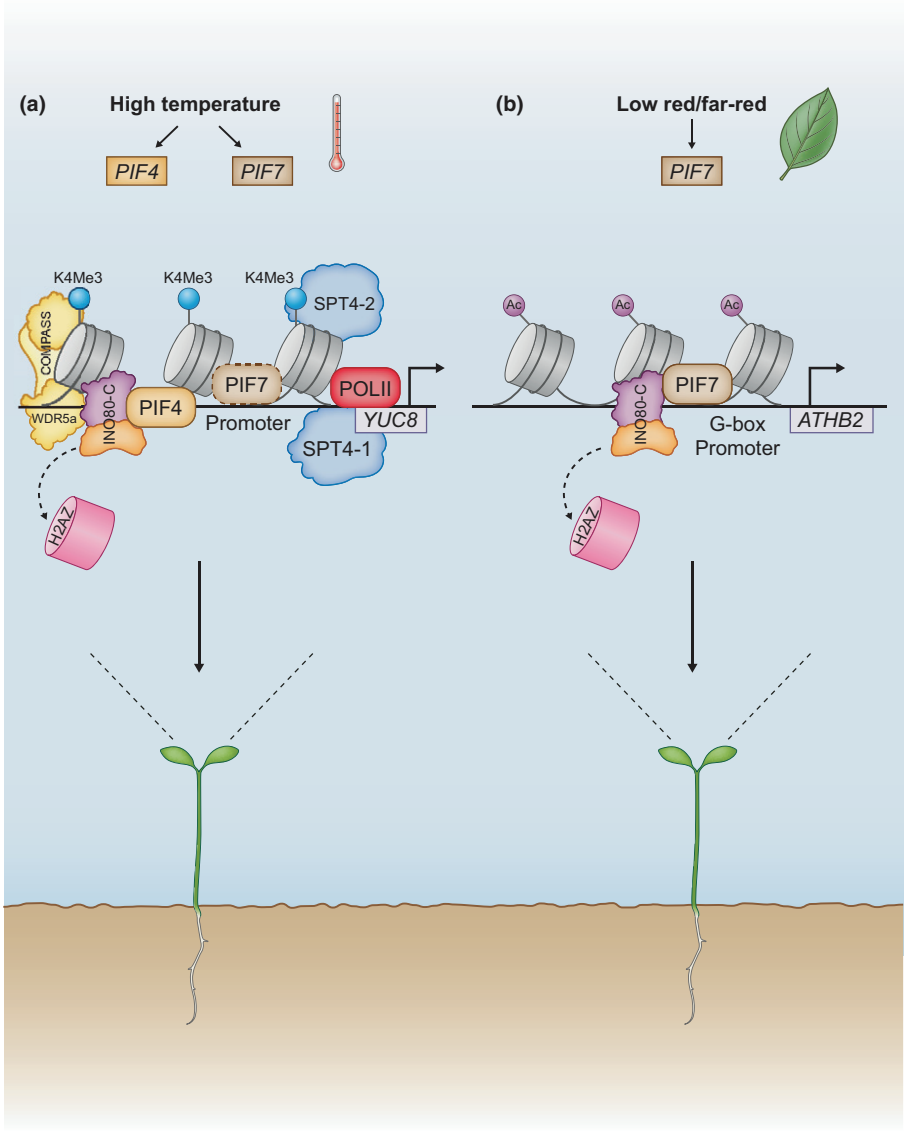

Fig. 3 The role of the INO80-C complex in elongation responses. (a) At high temperatures *PIF4* and *PIF7* transcription is induced. The INO80 complex (INO80-C) can directly associate with PIF4 protein to induce the downstream expression of auxin-related genes (e.g. *YUC8*). INO80-C also interacts with members of the H3K4me3 deposition complex COMPASS-WDR5a, which triggers H3K4me3 methylation and with RNA POLYMERASE II (POLII) elongation factors SPT4-1 and SPT4-2, which in turn are responsible for H2A.Z removal and initiation of transcription. Whether PIF7 plays a role in this elevated temperature signalling mechanism, remains to be elucidated. However, (b) upon exposure to a low red/far-red light ratio, PIF7 interacts with the INO80-C remodelling complex and binds to the *G-box* elements of target genes. This interaction mediates H2A.Z eviction and induces expression of *ATHB2*. In addition, PIF7 binding to the *G-box* allows an increase of histone acetylation marks. Black dashed and solid arrows indicate, respectively, positioning/removal of the indicated molecular factor or stimulation of the indicated process.

apparent conflicting findings emerge that may point to divergence of high temperature and light signalling at the level of H2A.Z occupancy regulation. First, PIF7 binding to *G-boxes* triggers H3K9 hyperacetylation of regulatory regions under low red to far-red light exposure (Willige *et al.*, 2021) (Fig. 3b). No involvement of PIF4 was however observed in global (genome-wide) hyper-acetylation under warmth (van der Woude *et al.*, 2019). Second, whereas HDA9 is required for warm temperature signalling, it is not clearly involved in light signalling (van der Woude *et al.*, 2019). Furthermore, whereas HDA9-mediated histone deacetylation (H3K9K14Ac) associates with H2A.Z eviction from *YUC8* nucleosomes at warm temperature conditions, the opposite was found during low red to far-red light signalling, in which enhanced H3K9 acetylation of PIF7 target genes correlates with H2A.Z removal and target gene induction (Willige *et al.*, 2021). Whether PWR also regulates nucleosome exchange in response to light quality signals remains to be determined. However, as also indicated above, PWR has a more pleiotropic role than HDA9 (de Rooij *et al.*, 2020). Moreover, PWR affects thermo-inhibition of seed germination by mediating H3K9 deacetylation and H2A.Z deposition at the *SOMNUS* (*SOM*) locus, a negative regulator of phyB-dependent seeds germination (Yang *et al.*, 2019). HDA9 also has a role in establishing seed dormancy, seed longevity and germination (van Zanten *et al.*, 2014b). It would therefore be interesting to study whether HDA9 confers the temperature dependency of these processes.

At the protein level HDA9 accumulates shortly after germination in response to warmth (Fig. 1), mainly in the root and root–hypocotyl junction, while becoming less abundant during seedling establishment. This suggests that HDA9 should be considered an early regulator of seedling responsiveness to temperature and that this function is 'replaced' by phyB-mediated thermosignalling when the cotyledons develop and become photoautotrophic (Stavang *et al.*, 2009; Bellstaedt *et al.*, 2019). PIF7-mediated histone modifications might occur temporarily after HDA9's task has been fulfilled. Furthermore, the functional characterisation of

HDA9 as transcriptional activator (by facilitating H2A.Z eviction) during thermomorphogenesis induction revealed a very unusual role for a HDAC, as these enzymes are typically considered transcriptional co-repressors (Fig. 1). In support of this, HDA9 preferentially associates with transcriptionally active regions and its binding to chromatin is required for gene expression initiation (Kang *et al.*, 2015; Kim *et al.*, 2016; X. Chen *et al.*, 2016; Mayer *et al.*, 2019; van der Woude *et al.*, 2019).

6. Involvement of chromatin remodelling factors in thermomorphogenesis

SEUSS (SEU) is a homologue of LGN-binding domain (LBD) proteins in mammals in which they recruit transcription factors to form higher-order complexes (Agulnick *et al.*, 1996). Together with LEUNIG (LEU), SEU forms a complex to transcriptionally repress a subsets of plant genes (Fig. 4). Through T-DNA insertion line screening, a specific mutant for *SEU*, named *enhanced photomorphogenic2* (*epp2*) was identified that displayed short hypocotyls in response to red, far-red and blue light, as well as at elevated temperatures (Huai *et al.*, 2018). Genetic analysis of *pif4 seu* double mutants indicated that PIF4 acts via SEU to respond to temperature stimuli (Fig. 4). Furthermore, SEU and PIF4 proteins interact and activate expression of growth-promoting genes, including *IAA19* and *YUC8*, by binding to their promoters. In addition, H3K4me3 levels at both loci were greatly reduced in *seu* mutants. Therefore, SEU-PIF4 activates auxin biosynthesis and integrates environmental cues to influence plant growth (Fig. 4). SEU can also interact with phyB and SUMO E3 ligase SAP AND MIZ1 DOMAIN-CONTAINING LIGASE1 (SIZ1) (Zhang *et al.*, 2020). Posttranslational modifications, particularly SUMOylation, knowingly regulate environmental responses (Sadanandom *et al.*, 2015) and SUMOylation of SEU, via interaction with SIZ1, is essential for its function. Site-directed mutagenesis of putative SEU SUMOylation sites greatly affected histone methylation levels at the *IAA19* locus and henceforth gene expression. Interestingly, the SEU–PIF4 interaction is partially affected by SUMO modifications, therefore affecting PIF4 degradation in the light (Zhang *et al.*, 2020).

The CHD-chromatin remodelling factor PICKLE (PKL) modulates the interaction between DNA and the histone octamer, allowing the transcriptional complex to position correctly (Kwon & Wagner, 2007). Mutations in PKL affect H3K27me3. Moreover, PKL interacts with transcription factors such as HY5, PIF3, and DELLAs to regulate the expression of growth-promoting genes (Jing *et al.*, 2013; Zhang *et al.*, 2014). *PKL* expression is induced in response to elevated temperatures, while *pkl* mutant seedlings display much shorter hypocotyls than the wild-type at 28°C (Zha *et al.*, 2017) (Fig. 4). Interestingly, *in vitro* assays revealed that circadian clock components such as CIRCADIAN CLOCK ASSOCIATED1 (CCA1) can associate with the *PKL* promoter and induce its expression. Furthermore, *cca1* mutant and overexpression lines displayed a thermomorphogenesis phenotype, placing the clock at the verge of ambient temperature responses (Zha *et al.*, 2017).

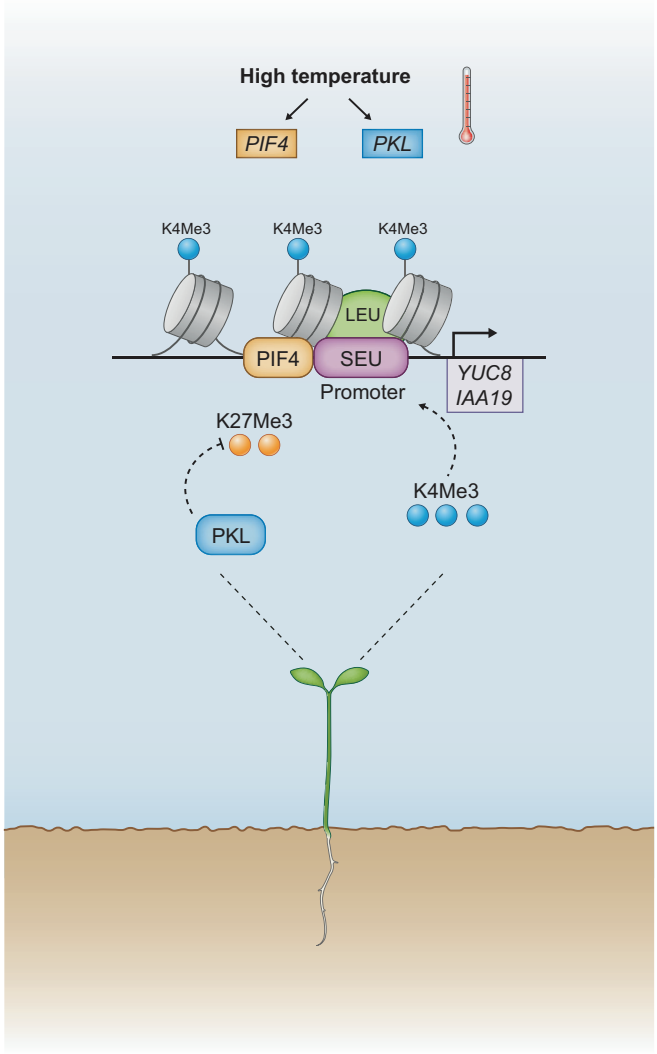

Fig. 4 The role of SEUSS, LEUNIG and PICKLE in promoting thermomorphogenesis. High temperature induces the expression of *PIF4* and *PICKLE* (*PKL*) genes. Accumulated PIF4 protein can then interact with the SEUSS (SEU)–LEUNIG (LEU) complex and activates the expression of growth-promoting genes such as *YUC8* and *INDOLE-3-ACETIC ACID INDUCIBLE 19* (*IAA19*) by promoting H3K4me3 methylation. The induction of these transcripts is correlated with the PKL–dependent removal of the repressive mark H3K27me3, leading to enhanced hypocotyl elongation. Black dashed and solid arrows indicate, respectively, positioning of the indicated molecular factor or stimulation of the indicated process. Blunted-end dashed line indicates inhibition of the indicated process.

In addition to PKL, also members of the SWI/SNF complex are crucial components of temperature-mediated transcriptional regulation. In particular, SWI/SNF factor BAF60 negatively regulates hypocotyl elongation under light and temperature conditions (Jégu *et al.*, 2017). BAF60 represses gene expression by binding *G-box* motifs of growth-promoting genes. In darkness BAF60 competes with PIF4 over the same regulatory regions (Jégu *et al.*, 2017).

Altogether this shows that different chromatin remodelling factors convene to modulate active and repressive histone marks on key temperature-responsive genes. How the dynamics of these modifications take place remains an open question.

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7. Roles of DNA methylation and RNAs in elevated temperature signalling

Exposure of plants to high temperature, either in the short term or to prolonged heat, can lead to the reactivation of silenced transgenes, endogenous DNA repeats and heterochromatic genomic regions. Intriguingly these changes in gene activation do not require alteration of the DNA methylation status (Lang-Mladek *et al.*, 2010; Pecinka *et al.*, 2010; Tittel-Elmer *et al.*, 2010). Conversely, post-transcriptional gene silencing (PTGS) of the chimeric receptor-like kinase *NOVEL RESISTANCE GENE 1* (*NRG1*) causes a dwarfed phenotype that can be alleviated by exposing the plants to 30°C (Zhong *et al.*, 2013). This radical change in morphology was due to a release of PTGS, associated with changes in DNA methylation of the *BRASSINOSTEROID-INSENSITIVE 1* (*BRI1*) locus (Zhong *et al.*, 2013). However, correlation between DNA methylation and PTGS, at least for this locus, was not clear nor consistent. Further evidence supporting a putative role for DNA methylation in responsiveness to HS came from a study in which different epigenetic mutants were tested for tolerance to HS. In particular, plants deficient in *NRPD2*, the second-largest subunit of RNA polymerases IV and V and part of the RNA-dependent DNA methylation pathway, were hypersensitive to HS (42°C) (Popova *et al.*, 2013). The effect of mildly elevated temperature on the above-mentioned mutants remains to studied.

Elevated temperatures can also affect a small subset of short interfering RNAs (siRNAs) (21–24 nt) and long noncoding RNAs (lncRNAs) (Gyula *et al.*, 2018; Severing *et al.*, 2018). In this context, pioneering work was done in the Paszkowski laboratory, where an siRNA pathway responsible for the response to HS was discovered. They showed that a copia-like retransposon (named *ONSEN* after the Japanese 'hot spring') became transcriptionally active in response to heat and was able to pass on heat-responsiveness to neighbouring genes (Ito *et al.*, 2011). The responsiveness, however, was not transmitted to the progeny of plants treated with HS, highlighting the importance of resetting mechanisms in the next generation.

Recently, high-throughput sequencing of sRNAs from different Arabidopsis tissues allowed for the identification of *c.* 50 temperature-dependent microRNAs (miRNAs). Another 48 were discovered using degradome libraries (Gyula *et al.*, 2018). Interestingly, the miRNA family miR169 was found to target NF-Y transcription factors that bind to the promoters of the flowering regulator *FLOWERING LOCUS T* (*FT*) as well as *YUCCA2* (*YUC2*) that is involved in petiole elongation under warm temperature conditions (Gyula *et al.*, 2018). Further analysis revealed the presence of siRNAs and DNA methylation upstream of the *YUC2* promoter, which was diminished in response to elevated temperature. This suggests that different epigenetic mechanisms converge at *YUC2*.

LncRNAs are noncoding RNA molecules longer than 200 bp (Statello *et al.*, 2021). Of the different lncRNAs currently identified by genomewide studies, only a handful have been characterised (Csorba *et al.*, 2014). Recently, *FLOWERING LONG INTERGENIC NON CODING RNA* (*FLINC*) expression has been reported to decrease responsiveness to high temperatures (Severing *et al.*, 2018). Mutant *flinc* seedlings showed a more pronounced early flowering phenotype when cultivated at 25°C, pointing to a role for FLINC in flowering time control in response to high temperatures. However, its putative function in this pathway and whether it contributes to thermomorphogenesis is yet to be confirmed.

III. Epigenetic regulation of HS memory

Plants respond to HS with acute acclimation to high temperatures, referred to as acquired thermotolerance, or HS priming (Yeh *et al.*, 2012; Bäurle, 2016; Ohama *et al.*, 2017). HS also has prominent effects on nuclear organisation. As indicated above, long-term HS can result in the alleviation of transcriptional gene silencing (TGS) (Lang-Mladek *et al.*, 2010; Pecinka *et al.*, 2010; Tittel-Elmer *et al.*, 2010). This loss of TGS was accompanied by a severe heat-induced decompaction of chromocentres (Pecinka *et al.*, 2010). Chromocentres are subnuclear structures that contain condensed heterochromatin, consisting of the (peri)centromeric part of the chromosomes rich in repeats and transposable elements (Fransz & de Jong, 2011). Although the biological role of chromocentre decondensation and its connection with TGS in HS responses is not entirely clear, it appears to be critical for basal heat tolerance in Arabidopsis, as mutants that are disturbed in *HEAT-INTOLERANT 4* (*hit4-1*) do not exhibit heat-induced chromocentre decondensation and display impaired basal (but not acquired) thermotolerance. Moreover, HIT4 associates with chromocentres in the nucleus and is required for heat reactivation of various silenced loci (Wang *et al.*, 2013).

For HS-induced changes in chromatin organisation and noncoding RNAs, it is often unknown whether they persist after they return to normal growth temperatures. Therefore, it remains unclear whether they conform with the narrow definition of epigenetics given in the Introduction. The involvement of chromatin organisation factors during heat signalling and the effects of extremely high temperatures on epigenetic modifications have been reviewed extensively, and not covered here (Liu *et al.*, 2015; Ohama *et al.*, 2017; Zhao *et al.*, 2020).

1. Two types of transcriptional memory after HS

In addition to acquisition of thermotolerance *per se*, a genetically separable mechanism maintains the duration of acquired thermotolerance after a HS event seizes. This is referred to as maintenance of acquired thermotolerance or HS memory (Charng *et al.*, 2006, 2007; Yeh *et al.*, 2012; Stief *et al.*, 2014). Events of extremely high temperatures in the natural environment are often temporally clustered, and HS memory is therefore considered an adaptive mechanism allowing plants to respond more rapidly to recurrent HS events. Emerging research from the last few years indicated that HS memory is partly based on epigenetic mechanisms. In Arabidopsis, at the whole plant level, HS memory lasts for up to 5 d and results in increased survival after a HS that is lethal to a naïve (nonprimed) plant (Charng *et al.*, 2006; Stief *et al.*, 2014; Friedrich *et al.*, 2021). At the level of gene expression, this is reflected by two types of transcriptional memory (Fig. 5a) (Oberkofler *et al.*, 2021). Type I describes sustained induction of a set of HS-induced genes that exceeds the duration of HS by several days (Stief *et al.*, 2014).

Type II describes enhanced re-induction of a subset of HS-induced genes upon a recurrent HS event after a stress-free period, during which the expression of HS-induced genes returned to baseline levels (Lämke *et al.*, 2016). This type II enhanced re-induction was caused by faster transcriptional reactivation (Fig. 5a), suggesting that the locus remained in a state of elevated transcriptional competence without being transcriptionally active (Liu *et al.*, 2018). In Arabidopsis, the sets of genes that showed type I and type II memory are partially overlapping. Examples of type I genes include *HEAT SHOCK-ASSOCIATED32* (*HSA32*) and *ACORBATE PEROXIDASE2* (*APX2*), examples of type II genes include *HSP22.0* as well as *APX2*. The quantification of un-spliced transcripts confirmed that both types of transcriptional memory are likely to operate at the level of transcription. This is in line with recent findings that, globally, transcript stability is in the range of minutes to hours (Hetzel *et al.*, 2016; Crisp *et al.*, 2017; Chantarachot *et al.*, 2020; Szabo *et al.*, 2020) and further supports the notion that HS memory effects occur at the level of transcription, rather than stabilisation of existing RNAs molecules. As detailed below, the mechanisms that mediate both types of transcriptional memory are partially but not fully overlapping.

2. The role of HSFA2 in HS memory

The transcription factor HSFA2 is a key regulator of HS memory and is required for both types of transcriptional memory (Charng *et al.*, 2007; Lämke *et al.*, 2016; Liu *et al.*, 2018) (Fig. 5a,b). *HSFA2* expression is strongly induced by HS through HSFA1 isoforms (Liu & Charng, 2013). In *hsfa2* mutants HS priming is largely normal, however, these mutants are specifically defective in the sustained induction of a set of genes that are initially activated by HSFA1s (type I). Accordingly, *hsfa2* mutants are unable to maintain acquired thermotolerance and are highly susceptible to a strong HS after a recovery of 2 d following a priming HS event (Charng *et al.*, 2007). At the molecular level, the separation of HS priming and HS memory is explained by the regulation of these processes by different HSF family members (HSFA1s vs HSFA2/HSFA3). This allowed chromatin features that are induced by the acute HS response and by HS memory to be investigated separately. Such comparative analyses pinpointed histone modifications that are linked to HS memory, but do not depend on the initial transcriptional activation (Lämke *et al.*, 2016). Most importantly, type I memory genes displayed histone H3K4 hypermethylation that lasted for at least 3 d after seizing of HS (Lämke *et al.*, 2016; Liu *et al.*, 2018). By contrast, HS-induced genes that did not show type I memory/sustained induction (such as *HSP70, HSP101*), did not display H3K4 hypermethylation. In *hsfa2* mutants, the H3K4 hypermethylation of HS memory genes is lost, despite the initial transcriptional activation being intact (which is mediated by HSFA1s). Both H3K4 dimethylation (H3K4me2) and trimethylation (H3K4me3) were induced with slightly different dynamics. H3K4me2 increased after H3K4me3, while H3K4me3 started to decline earlier than H3K4me2, after *c.* 2 d (Lämke *et al.*, 2016). The later peak accumulation may indicate that H3K4me2 plays a role in transcriptional memory (Fig. 5b). Whether both marks are equally active in the induction and maintenance of memory, or whether there is a functional difference, remains to be investigated. Histone acetylation is more tightly connected with transcription than H3K4 methylation. H3K9Ac levels increase after HS priming, and similarly in HS-induced genes and HS memory genes (Lämke *et al.*, 2016).

As described above, HSFA2 is required for H3K4 hypermethylation at HS memory genes. Simultaneously, HSFA2 binds to its target loci in a hit-and-run mode (Lämke *et al.*, 2016), meaning that the strongest binding was observed shortly after HS and decreased rapidly thereafter. The induction of a histone modification that is maintained in the absence of the transcription factor provides a tentative mechanism for the sustained gene induction memory. Indeed, in various organisms, increased H3K4 methylation has been proposed to act as a marker of recent transcriptional activation that may mediate enhanced transcriptional reactivation (Ng *et al.*, 2003; D'Urso *et al.*, 2016). A recent example comes from yeast, in which transcriptional memory of the *Inositol-3-phosphate synthase* (*INO1*) gene was correlated with enhanced H3K4 methylation and stalled RNA polymerase II (D'Urso *et al.*, 2016).

Interestingly, a positive feedback loop between HSFA2 and the H3K27me3 Jumonji histone demethylase RELATIVE OF EARLY FLOWERING 6 (REF6) mediated a transgenerational memory of HS that drove early flowering, while dampening pathogen responses (Lu *et al.*, 2011; Liu *et al.*, 2019). A recent publication also reported a further connection between HS memory and H3K27 methylation (Yamaguchi *et al.*, 2021). The authors showed that the removal of H3K27me3 after a priming HS event of the HS-induced *HSP22* and *HSP17.6C* genes prepared these genes for faster reactivation after a recurrent HS. H3K27me3 was actively removed at these loci in a HS-dependent manner by a set of JMJ histone demethylases. It remains to be investigated how JMJ proteins are recruited to these two specific loci.

3. Role of other HSF proteins in HS memory

While the role of HSFA2 in promoting HS memory has long been known, it remained unclear whether any of the additional 20 HSF family members were also involved in HS memory (Scharf *et al.*, 2012; Ohama *et al.*, 2017). HSFA1 isoforms contribute to type II transcriptional memory, but on their own they are not sufficient to promote enhanced transcriptional re-induction upon recurrent HS (Liu *et al.*, 2018) (Fig. 5b). Very recently, it was shown that HSFA1a, HSFA1b and HSFA1d interacted with HSFA2, providing a tentative mechanism for their involvement in HS memory (Friedrich *et al.*, 2021). HSF transcription factors act as trimers or even hexamers (Chan-Schaminet *et al.*, 2009; Li *et al.*, 2017), but the *in vivo* composition remained unclear. A forward genetic screen for expression modifiers of the type I memory gene *HSA32* has identified a second HSF; *FORGETTER 3* (*FGT3*), in addition to HSFA2, that was specifically required for the sustained induction of gene expression after a priming HS (Friedrich *et al.*, 2021). These *forgetter 3* (*fgt3*) mutants are deficient in the sustained induction of several type II memory genes, resembling *hsfa2* mutants. *FGT3* is allelic to *HSFA3* and was found to interact with HSFA2 and HSFA1s (Fig. 5b). Like HSFA2, but different from HSFA1 isoforms, HSFA3 is specifically required for sustained induction, but not initial transcriptional

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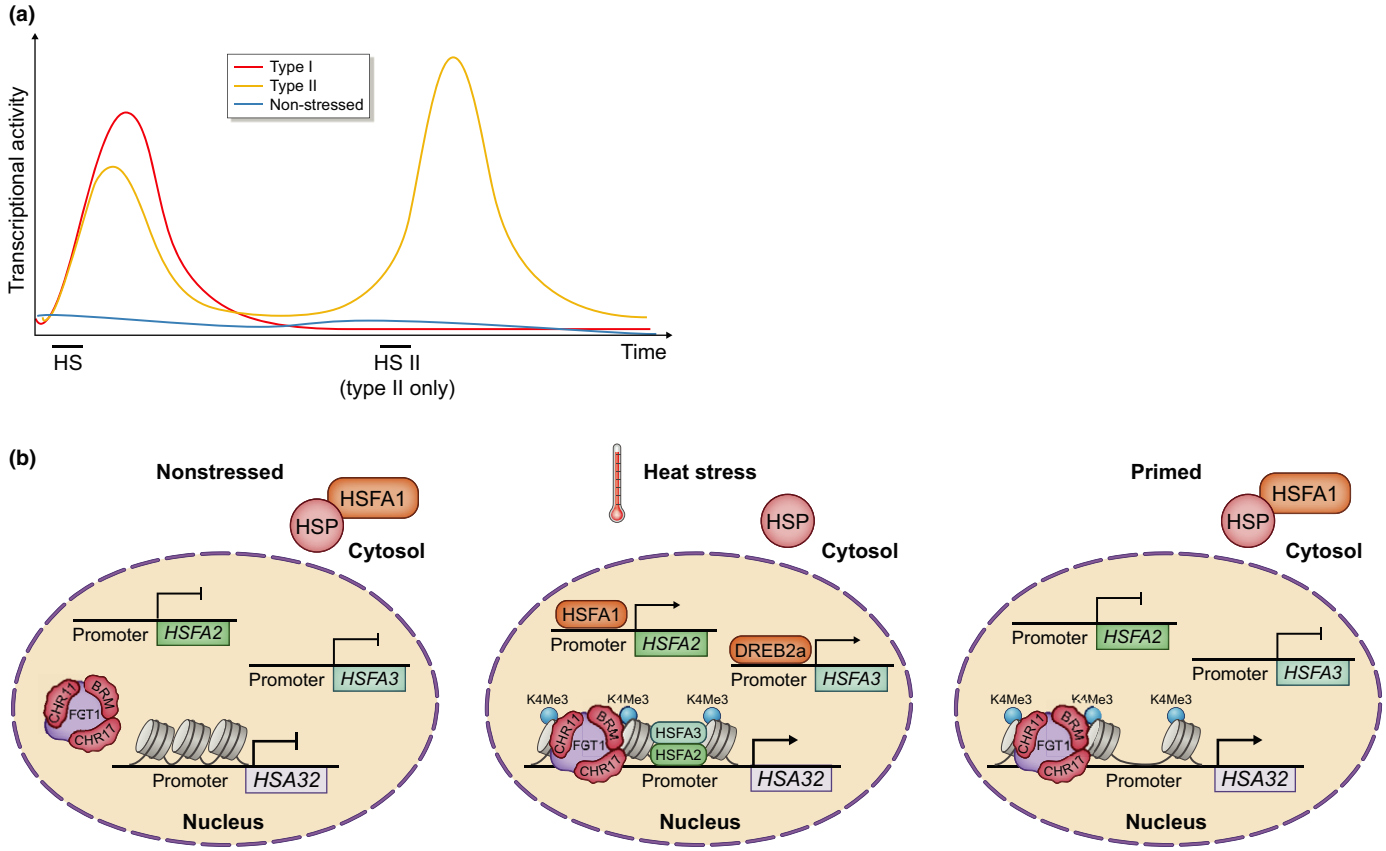

Fig. 5 Epigenetic and transcriptional memory of heat stress (HS) events. (a) Following HS, plants can display two different types of transcriptional HS memories. Compared to the nonstressed situation (blue line), Type 1 (red line) can be described as a sustained transcriptional induction due to a stress event that exceeds the stress by several days. Type II (yellow line) indicates an enhanced induction of HS genes caused by a recurrent HS that occurs after a stress-free time period. (b) Graphical representation of HS memory mechanisms. (Left) Under nonstressed conditions, HSFA1 is retained in the cytosol by HSP chaperone proteins. In the nucleus the memory gene *HSA32* is not active, neither are *HSFA2* and *HSFA3*. In addition, the chromatin remodelling complex that includes FGT1, BRM, CHR11 and CHR17 is in 'standby' mode. (Centre) In the presence of HS, HSFA1 is released from HSP proteins and migrates to the nucleus where, together with DREB2A, it activates the expression of *HSFA2* and *HSFA3*. Subsequent HSFA2 and HSFA3 binding to *HSA32* triggers H3K4me3 methylation, while the FGT1, BRM, CHR11 and CHR17 chromatin remodelling complex reduces nucleosome occupancy, thereby inducing *HSA32* expression. (Right) Several days after the HS events, the *HSA32* locus is primed. The chromatin of the locus is still enriched in H3K4me3 methylation, and the chromatin remodellers still ensure a reduction in nucleosome occupancy. Therefore, the *HSA32* maintains a sustained expression. Black arrows and blunted-end head arrows, respectively, indicate stimulation and inhibition of the indicated process.

activation after HS. This suggests that only complexes that contain both HSFA2 and HSFA3 are fully memory active/competent and are able to facilitate sustained H3K4 hypermethylation. This also indicated that certain unidentified structural properties of the HSFA2 and HSFA3 proteins contribute to placing H3K4 hypermethylation on HS memory genes loci and that these features are absent in HSFA1 proteins. As HSFA3 is activated by DEHYDRATION-RESPONSIVE ELEMENT-BINDING PROTEIN 2A (DREB2A) (Fig. 5b), which in turn is activated also by HSFA1s (Sakuma *et al.*, 2006; Schramm *et al.*, 2008; Yoshida *et al.*, 2008), the combinatorial action of both transcription factors may ensure that HS memory is activated only in certain environments.

4. Nucleosome occupancy during HS memory

Similar to their role in thermomorphogenesis (see previous section), positioning and overall occupancy of nucleosomes has emerged as a determinant of gene expression regulation during HS (Teves *et al.*, 2014; Lai & Pugh, 2017). In addition to H3K4 hypermethylation, nucleosome positioning regulates type I transcriptional memory after HS. From the mutagenesis screen mentioned above, *FORGETTER1* (*FGT1*) was identified and found to be required for sustained induction of HS memory genes (Brzezinka *et al.*, 2016) (Fig. 5b). *FGT1* encodes a protein with two helicase domains and a PHD domain that ensures binding to histone H3. FGT1 interacts directly with chromatin remodelling proteins of the SWI/SNF and ISWI families. Like FGT1, the SWI/SNF chromatin remodeller BRAHMA (BRM) and the ISWI remodellers CHR11 and CHR17 (Bezhani *et al.*, 2007) are required for physiological HS memory (Brzezinka *et al.*, 2016). Moreover, FGT1 mediates reduced nucleosome occupancy at HS memory loci throughout the memory phase. This suggests that low nucleosome occupancy promotes sustained induction of HS memory genes, likely by removing obstacles to transcribing RNA polymerase II in the body of FGT1 target genes.

An additional link between priming, H3K4 hypermethylation and nucleosome positioning was provided by the finding that

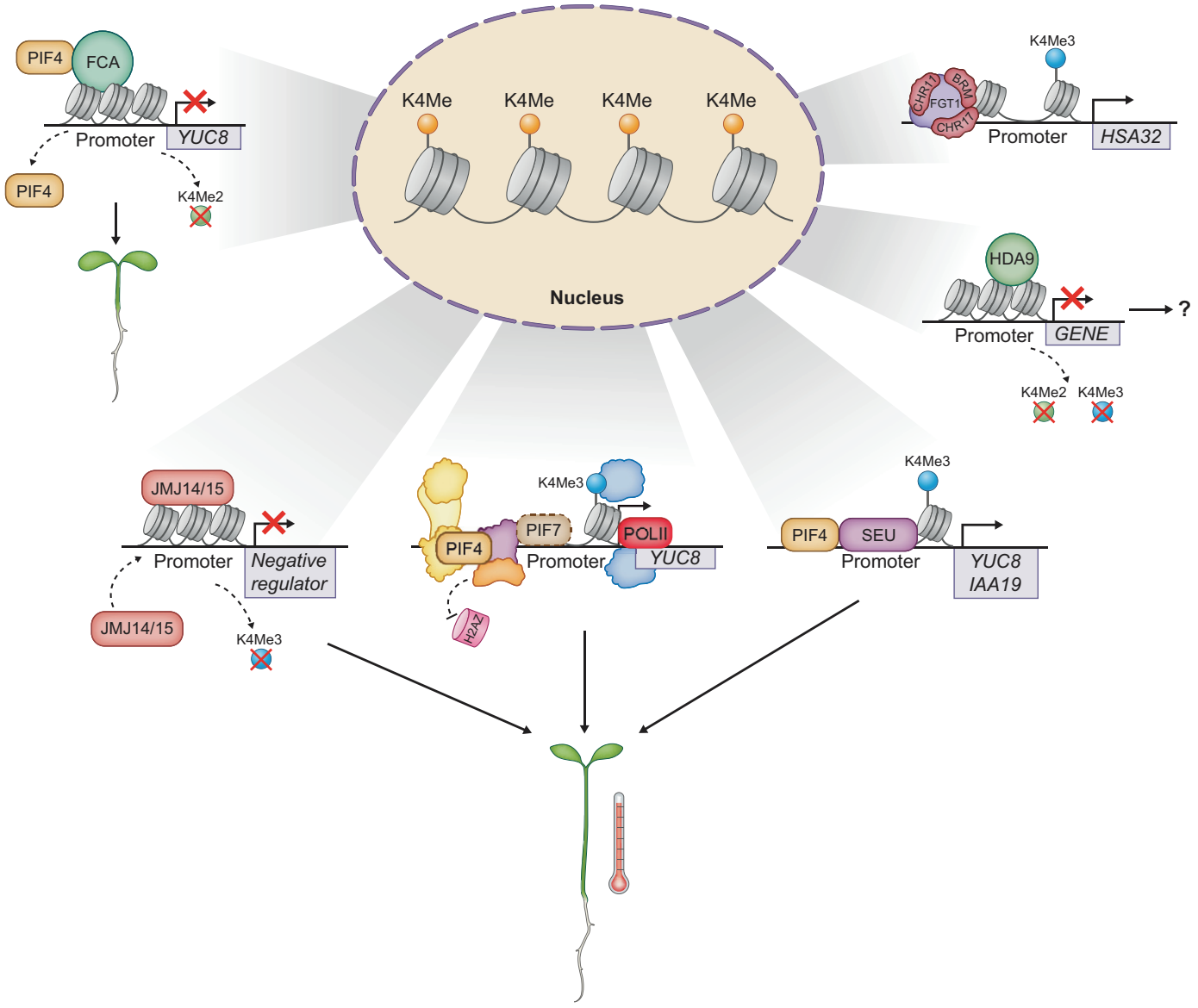

Fig. 6 H3K4me is a signalling hub in temperature signalling networks. Graphical representation of the central role of H3K4 methylation marks in diverse temperature signalling pathways discussed in this Tansley review. H3K4me2 demethylation through FCA mediates PIF4 dissociation and reduces hypocotyl elongation (Fig. 2a). High temperature recruits JMJ14 and JMJ15 that remove H3K4me3 from negative regulators, facilitating thermomorphogenesis (Fig. 2b). Similarly, PIF4 can mediate H3K4me3 methylation and therefore promoting the expression of auxin-related genes, either by associating with the INO80-EEN chromatin remodelling complex and/or by interacting with SEU (Figs 3a, 4). HDA9 plays a role in H3K4me2/3 demethylation under salt stress, however whether this mechanism is conserved under elevated temperatures, remains to be assessed. During Type 1 HS memory the '*Memory gene*' locus is enriched in H3K4me3 methylation even several days after the heat stress (HS) event has occurred (Fig. 5b). Black dashed and solid arrows indicate, respectively, positioning/removal of the indicated molecular factor or stimulation of the indicated process.

mutants in the chromatin assembly factor 1 (CAF-1) histone chaperone are constitutively primed against pathogen responses (Mozgova *et al.*, 2015). This priming was associated with elevated H3K4 methylation levels and low nucleosome occupancy. CAF-1 is required for depositing H3H4 tetramers onto newly replicated DNA (Kaya *et al.*, 2001; Hoek & Stillman, 2003). This may hint towards a mechanism for inheritance of priming-related histone modifications over cell divisions.

Interestingly, ANTI-SILENCING FUNCTION 1 (ASF1) histone chaperone was implicated in transcriptional activation after HS of a subset of HS-responsive genes, including *HSFA2* and several of its target genes (Weng *et al.*, 2014). Recruitment of ASF1 to target gene chromatin correlated with low nucleosome occupancy, high RNA polymerase II occupancy and histone acetylation (Weng *et al.*, 2014). Another hint for the open question of how HS-induced histone modifications are inherited across replication, came from the finding that the BRUSHY1 (BRU1)/TONSOKU/MGOUN3 protein was required for HS memory at the physiological and gene expression levels (Guyomarc'h *et al.*, 2004; Takeda *et al.*, 2004; Suzuki *et al.*, 2005; Brzezinka *et al.*, 2019). Previously,

BRU1 was found to be involved in the faithful inheritance of chromatin states over DNA replication and cell division. Originally, BRU1 was identified as a factor required for copying repressive chromatin states during transcriptional silencing (Takeda *et al.*, 2004; Suzuki *et al.*, 2005). The *bru1* mutant has similar developmental phenotypes as mutants in CAF-1 components. However, *caf-1* mutants were not required for HS memory at the physiological level (Brzezinka *et al.*, 2019). BRU1 orthologues from mammals bind to single-stranded DNA and newly incorporated nucleosomes after replication, allowing postulation of the exciting hypothesis that BRU1 is directly involved in copying epigenetic marks onto newly replicated DNA (Saredi *et al.*, 2016; Huang *et al.*, 2018).

The role of chromatin organisation in marking loci for sustained induction and altered re-induction after recurrent HS has been shown in several studies (Brzezinka *et al.*, 2016; Lämke *et al.*, 2016; Friedrich *et al.*, 2021; Olas *et al.*, 2021). Other work suggested that a component of HS memory may be mediated by mechanisms independent of chromatin and transcription, such as protein stability (Sedaghatmehr *et al.*, 2016) and membrane dynamics (Urrea Castellanos *et al.*, 2022). It remains to be investigated whether and how diverse cellular mechanisms are integrated to produce a coherent outcome.

IV. Conclusions and outlook

If high temperatures persist, plants need to make a 'decision' on how to balance costs (e.g. energy and resource investments) with benefits (e.g. growth, life-cycle completion and stress memory) to respond swiftly to the current stress and its possible recurrence. For instance, an elongated (thermomorphogenic) phenotype to enhance evaporative cooling may be beneficial when temperatures remain high, but not necessarily during the cooler period that may follow. This may relate to fitness costs that come with tissue weakening, such as increased risk of pathogen infection and potential lodging of elongated stem. In Arabidopsis, HS memory has benefits at the physiological level that last for *c.* 5 d (Friedrich *et al.*, 2021). Type II transcriptional memory lasts for *c.* 6 d (Liu *et al.*, 2018) and therefore is an example of somatic stress memory (Lämke & Bäurle, 2017) (Fig. 5a). The limited duration agrees with the idea that it provides an adaptation against recurrent HS, rather than a long-term memory that extends throughout the life cycle or even into the next generation. The mechanisms that limit the duration of HS memory remain to be investigated but may very well involve epigenetic mechanisms.

In nature, plants are exposed to assaults from several stresses with different levels of severity. It therefore may be beneficial to limit the duration of memory against individual stressors to compromise negative effects on, for example, growth and to be able to allocate energy and resources to face future – or simultaneous occurring – stresses during their life cycle. Even if priming provides a smaller fitness cost than constitutive acclimation, it may ultimately be more advantageous to re-acclimate after a certain stress-free period. Whether there is a truly transgenerational HS memory with physiological benefits that is active over at least one intermittent stress-free generation, and what epigenetic mechanisms are involved, remains to be investigated. Also, whether transgenerational inheritance of thermomorphogenesis traits exists is, to the best of our knowledge, not known. In any case, both transgenerational HS memory and possible thermomorphogenesis memory would most likely follow a different mechanism than the somatic HS memory mechanisms summarised in this review. The general feasibility of transgenerational effects that are induced by HS can be seen from the transgenerational activation of retrotransposition of the *ONSEN* copia-like transposable element (Ito *et al.*, 2011). Here, lack of DNA polymerase IV generates a sensitised background in which sustained activation of *ONSEN* retrotransposition can be triggered by HS. The long terminal repeats of *ONSEN* and related elements contain binding sites for HSFA2 and HSFA1s, therefore enabling a mechanism in which new insertions of *ONSEN* confer HS responsiveness to nearby genes (Ito *et al.*, 2011; Cavrak *et al.*, 2014; Baduel *et al.*, 2021).

While DNA methylation changes in response to HS events are relatively well understood, we are still beginning to discover the putative function of DNA methylation during mildly elevated temperature events. We therefore recommend detailed high-throughput bisulfite or MSAP sequencing approaches, preferably on the organ- and single cell-specific level, at which plants undergo thermomorphogenesis. From a more generalising perspective, detailing the genome-wide landscape of different epigenetic marks in response to mildly elevated temperatures would contribute to a better understanding of thermomorphogenesis regulation and fitness benefits and costs. Of particular interest would be a systematic assessment of the H3K4 epigenome landscape. As detailed above, this residue appears important for many temperature signalling pathways (Fig. 6). First, PIF4 dissociation from the *YUC8* locus is mediated by H3K4me2 demethylation through FCA, to supress hypocotyl elongation (Lee *et al.*, 2014) and JMJ14 and JMJ15 demethylases are recruited to remove H3K4me3 under warm temperature conditions to trigger gene expression and thermomorphogenesis (Cui *et al.*, 2021) (Fig. 2a,b). Second, H3K4me3 levels increased at PIF4 target genes under elevated temperatures, which depended on INO80-C interaction with the H3K4me3 deposition complex COMPASS-like component WDR5a (Xue *et al.*, 2021) (Fig. 3a). Third, *hda9* mutants display enhanced H3K4me2/3 levels under salt stress (Zheng *et al.*, 2020). Given the intricate role HDA9 plays in thermomorphogenesis regulation via auxin biosynthesis (Tasset *et al.*, 2018; van der Woude *et al.*, 2019; de Rooij *et al.*, 2020) (Fig. 1) a(n) (indirect) role for HDA9 in regulating H3K4me2/3 levels under elevated temperature conditions seems plausible. Fourth, PIF4 acts via SEU; H3K4me3 levels at the *IAA19* and *YUC8* loci were reduced in the *seu* mutant (Huai *et al.*, 2018) (Fig. 4), thereby supporting that regulation of H3K4me3 levels is important for auxin biosynthesis. During HS, type I memory genes exhibit high histone H3K4 levels that persist for at least 3 d after HS diminishment (Lämke *et al.*, 2016) (Fig. 5b), suggesting a recent event of transcriptional activation (Ng *et al.*, 2004; D'Urso *et al.*, 2016). Moreover, H3K4 hypermethylation contributes to the priming of gene expression in HS and to pathogen defence responses (Jaskiewicz *et al.*, 2011; Mozgova *et al.*, 2015; Friedrich *et al.*, 2021). Strikingly, H3K4 methylation has a role in cold signalling and vernalisation as well

(reviewed in Luo & He, 2020; Pandey *et al.*, 2021). Therefore, H3K4 is seemingly at the nexus of temperature signalling networks across the temperature spectrum from freezing to HS (Fig. 6). Studying H3K4 methylation on a genome-wide level, under a range of temperature conditions, is therefore an important next step to improving the understanding of temperature signalling mechanisms and functional responses in an integrated manner.

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