Role of chromatin in water stress responses in plants

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Abstract

As sessile organisms, plants are exposed to environmental stresses throughout their life. They have developed survival strategies such as developmental and morphological adaptations, as well as physiological responses, to protect themselves from adverse environments. In addition, stress sensing triggers large-scale transcriptional reprogramming directed at minimizing the deleterious effect of water stress on plant cells. Here, we review recent findings that reveal a role of chromatin in water stress responses. In addition, we discuss data in support of the idea that chromatin remodelling and modifying enzymes may be direct targets of stress signalling pathways. Modulation of chromatin regulator activity by these signaling pathways may be critical in minimizing potential trade-offs between growth and stress responses. Alterations in the chromatin organization and/or in the activity of chromatin remodelling and modifying enzymes may furthermore contribute to stress memory. Mechanistic insight into these phenomena derived from studies in model plant systems should allow future engineering of broadly drought-tolerant crop plants that do not incur unnecessary losses in yield or growth.

Key words: ABA, chromatin, drought, epigenetics, genome accessibility, water stress.

Introduction

Plant stress can be defined ‘Any unfavourable condition or substance that affects or blocks a plant’s metabolism, growth or development’ (reviewed by Lichtenthaler, 1998). Plants are exposed to a plethora of environmental stresses throughout their life. Drought attributable to climate change already causes water shortages in large parts of the world (Vorosmarty et al., 2010). Therefore, enhanced response to water deficit is an important trait for both crops and wild plant populations. Water is essential for plant metabolism, transport systems, and for generating the turgor pressure that allows an upright growth habit in herbaceous plants (reviewed by Des Marais and Juenger, 2010). It also adversely affects other aspects of plant growth, for example water stress reduces the rate of nitrogen fixation by legumes and their symbionts (Gil-Quintana et al., 2013). Due to their sessile nature, plants cannot escape from a water-deficient habitat. They instead need to adopt special strategies to cope with water limitation and to avoid substantial impacts on fitness, growth, and development (reviewed by Cramer et al., 2011; Less et al., 2011). The ability of the plant to display tolerance to water stress depends on transcriptional reprogramming (reviewed by Shinozaki and Yamaguchi-Shinozaki, 2007; Ahuja et al., 2010). For instance, factors involved in regulation of stress signal transduction as well as osmolytes and proteins that protect the cell from damage during water stress are induced in response to water deficit (reviewed by Shinozaki and Yamaguchi-Shinozaki, 2007).

In plants, water stress triggers the biosynthesis of the phytohormone abscisic acid (ABA) (reviewed by Xiong and Zhu, 2003). ABA binds to ABA receptors (PYR/PYL/RCAR) in a ternary complex with the clade A protein phosphatase 2C (PP2C) phosphatases, and this triggers a signal transduction cascade that leads to stomatal closure and transcriptional reprogramming (Ma et al., 2009; Park et al., 2009). Briefly, ABA sensing frees SnRK2-type kinases from inhibition by the PP2C phosphatases. SnRK2 autoactivates and subsequently phosphorylates downstream factors that promote...
drought tolerance; these include components of ion channels important in the stomatal response and transcription factors that induce stress response gene expression (Fujii et al., 2009; Lee et al., 2009; Umewata et al., 2009). The first components of this signalling and response pathway were identified in genetic screens for ABA-insensitive mutants more than a quarter of a century ago (Koornneef et al., 1984; Finkelstein, 1994; Finkelstein and Lynch, 2000; Lopez-Molina and Chua, 2000) and include dominant (constitutively active) clade A PP2C phosphatase mutants (abi1, abi2) and recessive loss-of-function mutants of transcriptional activators of the ABA response (abi3, abi4 and abi5) (Koornneef et al., 1984; Finkelstein, 1994; Finkelstein and Lynch, 2000; Lopez-Molina and Chua, 2000). Conversely, loss of function of these PP2Cs or gain of function of the transcription factors leads to ABA hypersensitivity (Parcy et al., 1994; Gosti et al., 1999; Soderman et al., 2000; Lopez-Molina et al., 2001; Merlot et al., 2001; Brocard et al., 2002; Kang et al., 2002; Fujita et al., 2005; Rubio et al., 2009).

Increasing evidence shows that transcriptional reprogramming in stress-responsive gene expression, proper resource allocation to growth versus stress responses, acclimation, and long-term stress memory are at least in part attributable to changes in the chromatin organization (reviewed by Chinnusamy and Zhu, 2009; Mirouze and Paszkowski, 2011; Gutzat and Mittelsten Scheid, 2012). This is not surprising given that chromatin has long been viewed as the interface between the environment and the genome (reviewed by Badeaux and Shi, 2013; Johnson and Dent, 2013; Suganuma and Workman, 2013). In the eukaryotic nucleus, the genome is packaged into the fundamental unit of chromatin, the nucleosome, which is comprised of 147 bp of DNA wrapped around a histone octamer (Luger et al., 1997). The histone octamer consists of two copies each of the histones H2A, H2B, H3, and H4. Nucleosomal arrays are further condensed into higher-order chromatin structures that incorporate the linker histone H1 (Luger et al., 1997). The compaction of the genome in the context of chromatin physically restricts the accessibility of the genomic DNA to regulatory proteins such as transcription factors and RNA polymerase II (reviewed by Petesch and Lis, 2012). Genomic DNA accessibility in the context of chromatin can be altered by various mechanisms including incorporation of histone variants, post-translational modifications of the histones or the DNA, and non-covalent alteration of the positioning or occupancy of the nucleosome (reviewed by Bell et al., 2011). Here, we will review the roles of various mechanisms that affect chromatin organization in water stress responses. We explore the link between water stress perception and modulation of chromatin regulator activity and discuss resource allocation to diverse survival programmes by chromatin regulators. Finally, we consider the role of chromatin in transient or long-term stress memory. Another important mechanism for modulation of gene expression in response to environmental stress relies on non-coding RNAs, several classes of which are critical for changes in chromatin compaction. As there are extensive recent reviews on non-coding RNA-directed gene silencing (reviewed by Wang and Chang, 2011; Wierzbicki, 2012; Castel and Martienssen, 2013) and their roles in development (reviewed by Chen, 2012; Zhang and Chen, 2013) and in abiotic stress responses (reviewed by Conteras-Cubas et al., 2012; Khraiwesh et al., 2012; Ding et al., 2013), we will not cover this topic in the current review.

**Chromatin changes induced by water stress**

In this section of the review, we will briefly discuss each of the different mechanisms that increase or decrease the accessibility of the genomic DNA in the context of chromatin as well as the available evidence that links each mechanism to water stress responses.

**Histone modifications**

Certain amino acids of histones, for example in their N-terminal tails, are frequently post-translationally modified via acetylation, methylation, phosphorylation, ubiquitination, sumoylation, or ADP-ribosylation (reviewed by Bannister and Kouzarides, 2011; Zentner and Henikoff, 2013). These modifications are dynamically established or erased by specialized enzymes called ‘writers’ or ‘erasers’, respectively (reviewed by Bannister and Kouzarides, 2011). The functional outcome of these changes in histone modifications is either alteration of the strength of the DNA histone interaction or recruitment of non-histone proteins, the so-called ‘readers’, to the chromatin (reviewed by Bannister and Kouzarides, 2011; Patel and Wang, 2013; Zentner and Henikoff, 2013).

Typically, histone acetylation is correlated with more open chromatin and hence more active transcription, whereas the converse is true for histone deacetylation (reviewed by Zentner and Henikoff, 2013). By contrast, histone methylation can affect different transcriptional outcomes, depending on the amino acid modified and the degree of modification (mono-, di-, or trimethylation) (reviewed by Li et al., 2007). For example, H3K4 and H3K36 trimethylation are found at actively transcribed genes, whereas methylation of H3K27 and H3K9 are well-known marks for repressed loci and heterochromatin, respectively (reviewed by Zentner and Henikoff, 2013). Histone arginine residues can be methylated by protein arginine methyltransferases (PRMTs). Different PRMT family members can catalyse mono-methylarginine, asymmetric di-methylarginine, and symmetric di-methyl-arginine, which direct either gene activation or repression (reviewed by Ahmad and Cao, 2012).

Several reports in plants have shown that drought sensing or treatment with the stress hormone ABA induces changes in histone modifications (reviewed by Kim et al., 2010a; Yuan et al., 2013). In one study, a short pulse of ABA or salt stress was sufficient to induce global H3S10 phosphorylation and H4K14 acetylation in cultured Arabidopsis and tobacco cells (Sokol et al., 2007). In 15-d-old Arabidopsis seedlings, H3K9, H3K23, and H3K27 acetylation was enriched at...
coding regions of drought stress-responsive genes after short drought treatment, which was correlated with gene activation. H3K4me3 enrichment with gene activation was similar to H3K9 acetylation (Kim et al., 2008). Genome-wide analysis in 4-week-old rosette Arabidopsis leaves under dehydration stress revealed a modest change in H3K4me2 and H3K4me1 levels at a subset of known stress response genes, but the H3K4me3 abundance over gene bodies changed more dramatically at genes whose transcript levels increased or decreased during dehydration (van Dijk et al., 2010). Recent genome-wide analysis in 25-d-old rice seedling also uncovered a positive correlation between H3K4me3 accumulation and the expression levels of some drought-responsive genes during dehydration. This correlation could be extended to genes involved in stress-related metabolite and hormone-signalling pathways (Zong et al., 2013). As changes in transcription direct changes in histone modifications (reviewed by Zentner and Henikoff, 2013), further studies are needed to elucidate whether the observed alterations in post-translational histone modifications are a cause or a consequence of the transcriptional changes triggered by water stress.

**Histone (de)acytelyases**

More direct evidence for a role of histone modifications in water stress responses comes from studies of mutants lacking histone-modifying enzymes. Several studies from rice and Arabidopsis have shown that the expression of histone deacetylases is regulated by drought and/or ABA (Sridha and Wu, 2006; Luo et al., 2012). In Arabidopsis, expression of the plant-specific HD2 histone deacetylases is repressed by ABA and NaCl (Sridha and Wu, 2006; Luo et al., 2012). Plants overexpressing AtHD2C exhibited ABA hyposensitivity (Sridha and Wu, 2006), whilst hdc2 mutants displayed ABA hypersensitivity during germination (Luo et al., 2012). The gene expression changes reported for these mutants are inconsistent with the phenotypes of mutants lacking components of ABA signalling pathway (Gosti et al., 1999; Merlot et al., 2001), and may therefore be an indirect consequence thereof. Mutations in either one of the genes coding two RPD3-type histone deacetylases, HDA6 and HDA19, in Arabidopsis also cause ABA hypersensitivity (Chen et al., 2010; Chen and Wu, 2010; Zhou et al., 2013). Several embryonic genes including 7S1, LEC2, 2S2, CRA1, FUS3, and LEC1 were de-repressed in hda19 seedlings (Zhou et al., 2013) in agreement with a role of histone acetylation in activation of these genes (Ng et al., 2006). Similar phenomena were observed in HDA6 RNA interference lines (Tanaka et al., 2008) and in wild-type plants treated with a histone deacetylase inhibitor (Tanaka et al., 2008). HDA19 associates with the regulatory regions of the above-mentioned embryonic genes (Zhou et al., 2013). It remains to be seen whether failure to directly repress embryonic genes is also observed during germination and whether depression of such genes causes the germination defects and ABA hypersensitivity of germinating hda19 mutants. Histone acetyltransferase complex components have also been linked to altered water stress responses. A loss-of-function mutant of ADA2b, a component of the GCN5-containing histone acetyltransferase complex, leads to increased drought tolerance (Vlachonasios et al., 2003, 2011). It is not yet known which gene expression changes are directly triggered by this complex and cause the observed phenotype.

**Histone lysine methyltransferases**

Loss of function of Arabidopsis trithorax-like factor ATX1, which trimethylates histone H3 at lysine 4 (H3K4me3), results in decreased dehydration tolerance compared with wild-type seedlings. ATX1 directly regulates transcription of N Ced3, which encodes a key ABA biosynthesis enzyme. Activation of N Ced3 transcription upon dehydration or ABA treatment is greatly reduced in atx1 mutants, suggesting that ATX1-mediated H3K4 methylation is required for N Ced3 induction and possibly ABA accumulation caused by water stress (Ding et al., 2011).

Trithorax group proteins act in opposition to polycomb group proteins (reviewed by Simon and Kingston, 2013). H3K27me3 marks established by the polycomb repressive complex 2 (PRC2) induce a persistent silent state of the transcription of the target locus (reviewed by Simon and Kingston, 2013). In Drosophila, PRC1 recognizes H3K27me3 and plays a role in the stable maintenance of gene repression (reviewed by Simon and Kingston, 2013). Whilst PRC2 complex components are conserved in plants and metazoans, this is not true for PRC1 complex components (reviewed by Zheng and Chen, 2011; Holc and Berger, 2012). In barley, exogenous ABA application induced expression of components of the PRC2 complex such as HvE(Z) and HvFIE in seedlings (Kapazoglou et al., 2010). In Arabidopsis, mutations in the two EMBRYONIC FLOWER (EMF) genes display strikingly similar developmental defects (Aubert et al., 2001; Yoshida et al., 2001). EMF2 is a homologue of the Su(z)12 component of the metazoan PRC2 complex. It is currently unclear whether EMF1 is associated with PRC1 or PRC2 function (Beh et al., 2012; Kim SY et al., 2012). The recent identification of EMF1 as a structural homologue of the Drosophila PRC1 complex component PSC, its ability to inhibit remodelling activity of SWI/SNF ATPases (Beh et al., 2012), and its ability to act as a potent repressor of transcription (Calonje et al., 2008) provide support for the idea that EMF1 may be associated with PRC1. Genome-wide expression analysis of the emf mutants revealed that EMF's regulate plant hormone and stress signalling-related genes (Kim et al., 2010b). Both EMF1 and EMF2 bind directly to the promoter of ABI3, and expression of ABI3 and its targets are de-repressed in 7- and 14-d-old emf mutant seedlings (Kim et al., 2010b). More recently, genome-wide binding studies revealed that genes occupied by EMF1 and marked by H3K27me are significantly enriched for Gene Ontology terms such as ‘ABA response’ and ‘abiotic stress response’ (Kim SY et al., 2012). A bypass of the embryo lethality of the single unique PRC2 complex component, FIE, allowed assay of the gene expression defects and post-embryonic phenotypes caused by absence of PRC2 function (Bouyer et al., 2011). This revealed germination defects as well as de-repression of embryonic genes and of positive regulators of ABA responses (Bouyer et al., 2011). Further evidence for a PRC-dependent role in water stress-related responses comes from...
conditional knockdown of EMF1, which led to increased salt tolerance, whilst removal of a factor with opposing (trithorax group-related) activity had the opposite phenotype (Carles and Fletcher, 2009; Pu et al., 2013). It remains to be determined in the latter two studies which of the observed changes in gene expression are direct. Moreover, no evidence is available as yet that the observed changes in gene expression contribute to the altered water stress responses.

Histone arginine methyltransferases

Mutants lacking the Arabidopsis arginine methyltransferase PRMT5/SKBI (henceforth referred to as PRTM5 for simplicity), which catalyses symmetric arginine dimethylation, display salt and ABA hypersensitivity (Wang et al., 2007; Schmitz et al., 2008; Zhang et al., 2011). Low doses of exogenous ABA result in the growth arrest of germinated prmt5 but not wild-type embryos (Zhang et al., 2011). Some of the reported gene expression changes in prmt5 mutants relative to the wild type (Zhang et al., 2011) are inconsistent with the observed hypersensitive phenotype (Merlot et al., 2001; Rubio et al., 2009; Yoshida et al., 2010). Hence, these changes in gene expression may be an indirect consequence of the mutant phenotype. As PRTM5 activity also regulates mRNA splicing (Deng et al., 2010; Zhang et al., 2011) and circadian gene expression (Hong et al., 2010; Sanchez et al., 2010), it will not be trivial to identify the genes, whose misexpression underlies the ABA hypersensitivity of prmt5. Indeed, a genetic screen for Ca²⁺ underaccumulation (cau) mutants identified an allele of prmt5 that displays increased drought tolerance and stomatal closure (Fu et al., 2013). The drought tolerance is at least in part due to de-repression of the direct PRMT5/H4Rsm2 target and calcium accumulation sensor CAS (Fu et al., 2013).

In summary, mounting evidence supports the idea that post-translational modifications of histones are critical for correct water stress responses in plants. One of the biggest remaining challenges is to elucidate the causal defects that underpin the observed water stress-related phenotypes of mutants lacking histone-modifying enzymes. After identification of genes whose expression is altered in a given mutant in a manner consistent with the observed phenotypes, direct association of the histone-modifying enzyme in question with loci of interest should be tested. Coupled with expected changes in the histone modifications at these loci in stress and non-stress conditions in the mutant and wild-type background, this will allow identification of candidate direct targets of the histone-modifying enzyme. Subsequent genetic tests will enable elucidation of the role (if any) of the identified candidate direct targets in the water stress phenotypes observed in mutants lacking activity of a given histone-modifying enzyme. As loss of function of histone-modifying enzymes and other mutants that affect the chromatin organization are pleiotropic, it cannot be ruled out that the altered stress phenotype of constitutive mutants is due to secondary effects of the altered plant morphology (leaf size, stature). Phenotypic and molecular investigations of chromatin regulators should therefore rely as much as possible on inducible loss-of-function mutants. Tissue-specific knockdown of chromatin regulators can minimize pleiotropic defects. Temporally inducible knockdown of a histone-modifying enzyme enables analysis of altered water stress responses shortly after knockdown in wild-type-looking plants, significantly reducing the secondary effects typical of constitutive mutants.

Histone variants

In most organisms including Arabidopsis, there are multiple genes that code for the highly conserved canonical histones (H3, H4, H2A, and H2B), which are mostly expressed during the S phase of the cell cycle (reviewed by Talbert and Henikoff, 2010; Burgess and Zhang, 2013; Skene and Henikoff, 2013). Other less conserved subtypes of histones called histone variants are expressed throughout the cell cycle (reviewed by Talbert and Henikoff, 2010; Skene and Henikoff, 2013). The canonical histones are replaced with histone variants independent of DNA replication. Although they generally do not differ much in sequence from the canonical histones, histone variants can impart distinct characteristics to the nucleosomes, such as stronger or weaker association with the genomic DNA and incompatibility with certain post-translational modifications (reviewed by Talbert and Henikoff, 2010; Skene and Henikoff, 2013). Recent genome-wide studies have revealed the genomic distribution of a subset of the plant histone variants (reviewed by Zilberman et al., 2008; Talbert and Henikoff, 2010; Costas et al., 2011; Wollmann et al., 2012; Skene and Henikoff, 2013).

In plants, linker histone (H1) variants have been linked to the water stress response. The linker histone variant HIS1-3 gene in Arabidopsis is specifically induced by salt, drought, and ABA (Ascenzi and Gantt, 1997; Zhu et al., 2012). Similarly, the tomato linker histone variant H1-S gene is also induced by and accumulates in the chromatin in response to water deficit (Scippa et al., 2000). H1-S also accumulates in a drought-tolerant genotype of tomato (Trivedi et al., 2012). Indeed, knockdown of H1-S levels by antisense RNA in transgenic tomato triggered an altered physiological response to water loss such as altered stomatal conductance, transpiration, and net photosynthetic rate (Scippa et al., 2004). Transgenic plants showed an increased association of the heterochromatin with the nuclear membrane under water stress conditions (Scippa et al., 2004); this may trigger increased silencing of these regions (Hubner et al., 2013). Although upregulation of expression of variants of the linker histone H1 in response to drought is a conserved response in higher plants, detailed mechanistic insight into how this histone variant affects chromatin structure or gene expression during water stress is as yet not available. The H2A variant H2A.Z is largely conserved through evolution (reviewed by Talbert and Henikoff, 2010). Genome-wide studies have revealed that the localization of H2A.Z correlates inversely with DNA methylation in both heterochromatin and in gene bodies of active genes (reviewed by Zilberman et al., 2008). It has been proposed that the anti-correlation between H2A.Z and DNA methylation is primarily due to the exclusion of H2A.Z from methylated DNA (Coleman-Derr and Zilberman, 2012). Moreover, Gene Ontology terms enriched among genes upregulated in h2a.z triple mutants include ‘Response to water deprivation’
and ‘Response to ABA’ (Coleman-Derr and Zilberman, 2012). The authors propose that H2A.Z deposition in gene bodies confers higher variability in the expression of inducible genes including those that respond to water stress. By contrast, gene-body DNA methylation may stabilize constitutive expression of housekeeping genes by antagonizing H2A.Z deposition (Coleman-Derr and Zilberman, 2012). It will be of interest to determine the effect of reduced availability or incorporation of these and additional histone variants on water stress responses in plants. Given their widespread roles in chromatin stability, conditional disruption of histone variant availability or incorporation may allow more precise investigation of such phenotypes.

**DNA methylation**

Methylation on the fifth carbon of cytosine bases is an important epigenetic mark that influences chromatin structure and gene expression (reviewed by Jones, 2012). In plants, cytosine methylation is found in the context of CG, CHG, and CHH (H=A, C, or T). Symmetric CG maintenance methylation is catalysed by DNA methyltransferase I (MET1), a homologue of the mammalian methyltransferase DNMT1 (reviewed by Chan et al., 2005; Goll and Bestor, 2005; Law and Jacobsen, 2010). Symmetric CHG maintenance methylation is catalysed by Chromomethyltransferase 3 (CMT3), a plant-specific methyltransferase. Asymmetric CHH methylation is maintained through de novo methylation by Domains Rearranged Methyltransferase 2 (DRM2), a homologue of the mammalian DNMT3A/b and the RNA-directed DNA methylation pathway (reviewed by Chan et al., 2005; Goll and Bestor, 2005; Law and Jacobsen, 2010). DDM1 is a SWI/SNF superfamily chromatin remodeler required for all DNA methylation (CG, CHG, and CHH) over long transposable elements and in heterochromatin (Vongs et al., 1993). DDM1 was recently shown to cooperate with the CMT2 methyltransferase to mediate CHH DNA methylation in parallel with the RNA-directed DNA methylation pathway (Zemach et al., 2013).

In Arabidopsis, centromeric and pericentromeric regions, repetitive DNA sequences, and transposons are heavily methylated. Many genic regions are also highly methylated, and this is correlated with high gene expression, whereas promoters are mostly depleted of DNA methylation (reviewed by Zhang et al., 2006; Saze et al., 2012). In plants, DNA methylation is associated with diverse biological processes including development and environmental responses (reviewed by Law and Jacobsen, 2010; Saze et al., 2012; Sahu et al., 2013).

Studies from various plant species have shown that abiotic stress may trigger hyper- or hypomethylation in different genomic contexts: hypomethylation of promoters, hyper- or hypomethylation at coding regions, and hypomethylation of transposons (Sahu et al., 2013). For example, genome-wide analysis identified differentially methylated DNA regions in Arabidopsis seedlings treated with simulated drought (treatment with polyethylene glycol). The methylome was widely affected by changes in the water potential, with the most dramatic DNA hypermethylation observed near the transcription start site (±500 bp) of protein-coding genes related to stress responses (Colaneri and Jones, 2013). Moreover, it has been proposed that DNA methylation may contribute to stress adaptation. Mangrove trees grown near a salt marsh had smaller statures than riverside-grown trees and their genomes were globally hypomethylated (Lira-Medeiros et al., 2010). Likewise, in rice, changes in DNA methylation in response to drought were more pronounced in drought-tolerant genotypes (Wang et al., 2011). The altered DNA methylation may contribute to increased differential gene expression upon drought sensing. A subset of the DNA methylation changes induced by drought remained after removal of the stress (Wang et al., 2011). In Arabidopsis, low relative humidity was linked to de novo DNA methylation and stable repression of genes involved in stomata development, resulting in lower stomata frequency (Tricker et al., 2012, 2013). A T-DNA insertion distal to the AtHKT1 gene, which encodes a sodium transporter, has been identified as a suppressor of sos3 (salt overly sensitive 3). The insertion prevents a distal enhancer element and RNA-directed DNA methylation from controlling expression of AtHKT1, which plays an important role in salt tolerance (Baek et al., 2011). met1-3 mutants and met1-3-derived epigenetic recombinant inbred lines show normal germination in non-stress conditions; by contrast, they fail to germinate in the presence of 150 mM NaCl, a concentration that does not impact germination (Reinders et al., 2009). Defects in DNA methylation may thus affect phenotypic plasticity (a topic that has received attention from an evolutionary perspective) in response to adverse environmental conditions (Lira-Medeiros et al., 2010; Wang et al., 2011; Draghi and Whitlock, 2012). It will be critical to identify which of the observed DNA methylation changes contribute to altered water stress response or plasticity.

**Non-covalent changes in chromatin state**

ATP-dependent chromatin remodelling ATPases alter histone–DNA interactions non-covalently by utilizing the energy derived from ATP hydrolysis to promote changes in nucleosome occupancy, nucleosome positioning, or nucleosome composition (reviewed by Clapier and Cairns, 2009; Hargreaves and Crabtree, 2011; Narlikar et al., 2013). Chromatin remodelling can either increase or decrease the accessibility of a given piece of genomic DNA to trans-acting factors and hence facilitate or obstruct transcription, respectively (reviewed by Clapier and Cairns, 2009; Hargreaves and Crabtree, 2011; Narlikar et al., 2013). Four well-studied subfamilies of ATP-dependent chromatin remodelers are the SWI/SNF, ISWI, CHD, and INO80/SWR1 families. Each subfamily has unique domains, which endow it with specialized functions for particular nuclear processes (reviewed by Clapier and Cairns, 2009; Hargreaves and Crabtree, 2011; Narlikar et al., 2013). Among these ATP-dependent chromatin remodelers, only the SWI/SNF and CHD subgroups have been implicated in water stress responses in plants.
SWI/SNF ATPases are conserved from yeasts to humans and plants (reviewed by Flaus et al., 2006; Kwon and Wagner, 2007; Hu et al., 2013; Narlikar et al., 2013). Plant genomes contain three types of SWI/SNF subfamily chromatin remodelling ATPases called BRAHMA (BRM), SPLEYED (SYD), and MINUSCULE (MINU) (reviewed by Jerzmanowski, 2007; Kwon and Wagner, 2007; Sang et al., 2012). The catalytic ATPase subunit forms a core complex together with SWIRM- and SANT- domain proteins (SWI3) and SNF5-domain proteins. Additional accessory proteins, which are frequently tissue- and developmental-stage specific, control targeting and activity of the complex (reviewed by Clapier and Cairns, 2009; Hargreaves and Crabtree, 2011; Kwon and Wagner, 2007). In vitro remodelling activity has not yet been demonstrated for members of this subfamily in plants. In Arabidopsis, the BRM complex containing SWI3C and SNF5 (BSH) has been linked to ABA and drought response (Han et al., 2012). Germinating brm mutants display ABA hypersensitivity and enhanced growth arrest relative to the wild type. Consistent with the mutant phenotype, de-repression of the positive ABA response regulator ABI5 (Lopez-Molina et al., 2001) was observed (Han et al., 2012). ABI5 is a direct BRM target and, based on genetic epistasis tests, the brm mutant growth arrest is due to the ABI5 de-repression (Han et al., 2012). BRM repressed ABI5 expression in the absence of stress by promoting high occupancy of the +1 nucleosome close to the ABI5 transcription start site (Han et al., 2012). In addition, brm mutants displayed increased drought tolerance at multiple stages of development. The molecular underpinnings of this response remain to be elucidated (Han et al., 2012). The MINU1/AtCHR12 ATPase (henceforth referred to as MINU1 for simplicity) has been implicated as a negative regulator of a temporary growth arrest caused by drought and heat stress in adult Arabidopsis plants (Mlynarova et al., 2007). Overexpression of MINU1 induces temporary growth arrest under drought as well as salt and heat stress (Mlynarova et al., 2007). Intriguingly, the expression of several stress-inducible dormancy-related genes was reduced in the inflorescence and 4-week-old rosette leaves of MINU1-knockout and increased in MINU1-overexpressing plant. Whilst it is not yet known whether these genes are directly regulated by MINU1 or responsible for the observed phenotypic defects, MINU1 may play a role in the induction of stress response genes upon perception of the stimulus.

The CHD subgroup chromatin remodeler PKL has also been implicated in the ABA response. CHD chromatin remodelers have two tandem chromodomains known to bind methylated lysines, and these domains were shown recently to couple ATP hydrolysis to remodelling (Hauk et al., 2010). Like SWI/SNF ATPases, CHD remodelers can both promote and repress transcription. The vertebrate Mi2-NuRD complex contains histone deacetylase and methyl CpG-binding domain (MBD) proteins in addition to a CHD domain chromatin remodeler (reviewed by Clapier and Cairns, 2009). PICKLE (PKL) is the best-characterized CHD remodeler in Arabidopsis and most closely resembles CHD3. Recently, in vitro chromatin remodelling activity was demonstrated for PKL (Ho et al., 2013). PKL is required for repression of embryonic genes during seedling development and promotes the developmental transition to vegetative growth (Henderson et al., 2004). pkl mutants display exaggerated ABA responses during germination, and fail to germinate in conditions where the wild type germinates properly (Perruc et al., 2007). The ABA-dependent growth arrest of germinating pkl plants is mediated mainly by failure to developmentally repress genes strongly expressed during embryogenesis, including ABI3 and ABI5. Increased expression of ABI3 and ABI5 in pkl mutants relative to the wild type in the presence of ABA treatment is correlated with a reduced level of two repressive histone modifications, H3K9me2 and H3K27me2, at the promoters of these genes (Perruc et al., 2007). Epistasis tests revealed almost abi5-like germination and growth responses in pkl abi5 double mutants, suggesting that the majority of the phenotypic defects can be explained by failure to repress ABI5. It is not known whether ABI5 is directly regulated by PKL. Elucidation of the direct PKL targets is critical, as there is currently evidence for PKL acting both as a trithorax group protein (to counteract polycomb repression) and as a promoter of polycomb repression (Aichinger et al., 2009; Zhang et al., 2012; Jing et al., 2013).

Trade-offs between growth and water stress responses?

Although the underlying mechanisms are largely unknown, growth arrest in adverse environments is thought to be advantageous for plant survival (Lopez-Molina et al., 2001; Achartd et al., 2006; Skirycz and Inze, 2010). One hypothesis is that limited resources available to monocarpic (annual) plants in particular can be allocated either to stress response or to continued growth (Bennett et al., 2012). In support of this idea, ABA and drought stress not only induce expression of stress response genes but also repress expression of genes linked to growth and metabolism (reviewed by Shinozaki and Yamaguchi-Shinozaki, 2007; Chaves et al., 2009; Sreenivasulu et al., 2012). In addition, when major drought-responsive transcription factors are overexpressed, transgenic plants display growth retardation in non-drought conditions (reviewed by Shinozaki and Yamaguchi-Shinozaki, 2007). In conditions when the stress does not threaten survival, growth inhibition may lead to an unnecessary reduction in plant growth and hence productivity and yield (Tardieu, 2003; Bennett et al., 2012).

Consistent with the hypothesized trade-off between growth and drought response, several chromatin regulators have been implicated in stress-mediated temporal growth arrest at different stages of plant development. A highly dehydration-sensitive developmental phase in the life of a plant is immediately after germination (Lopez-Molina et al., 2001). Several chromatin regulators act at this stage to trigger water stress-dependent growth arrest, which resembles the growth arrest during late-embryogenesis in seed development. In several cases, the hyperactive stress response is due to a delay or failure to repress the embryonic developmental programme (which is geared towards desiccation tolerance and growth arrest) upon germination.
One example of this type of regulator is PKL. The hypersensitive germination response to ABA of pkl mutants is due to failure to developmentally repress ABI3 and ABI5 accumulation and is restored by removing ABI5 function (Perruc et al., 2007). Other embryonic genes such as LEC1, LEC2, and FUS3 are constitutively de-repressed and cause formation of embryonic structures on adult pkl mutant plants (Dean Rider et al., 2003; Henderson et al., 2004; Aichinger et al., 2009). Likewise, a delay in the developmental repression of the embryonic programme is observed under conditions of reduced histone deacetylase activity (Tanaka et al., 2009). Likewise, a delay in the developmental repression of the embryonic programme is observed under conditions of reduced histone deacetylase activity (Tanaka et al., 2009). Double mutants between pkl and histone deacetylase hda6 enhanced persistence of embryonic traits and embryonic gene expression (Tanaka et al., 2008). Polycomb group protein and RETINOBLASTOMA-RELATED protein (RBR) are also required for persistent silencing of late embryonic genes including AB13 by increasing their histone H3K27 trimethylation (Bouyer et al., 2011; Gutzat et al., 2012; Kim SY et al., 2012; Yang et al., 2013). Although the role of RBR in the abiotic stress response has not been investigated, seedlings with reduced RBR function arrest their growth after germination in non-stress conditions; this is accompanied by de-repression of embryonic genes linked to ABA responses including AB13 and ABI5 (Gutzat et al., 2012).

The SWI/SNF ATPase BRM, by contrast, displayed normal developmental downregulation of embryonic genes (AB13, ABI5) at the onset of autotrophic growth and was instead required for repressing expression of positive regulators of water stress responses in the absence of the stimulus (Han et al., 2012). Moreover, the overall reduced vegetative growth of brm mutants under non-stress conditions is partly restored by removing ABI5 function or by disturbing ABA signalling pathway. However, a role for BRM in repression of the embryonic programme cannot be entirely ruled out. Several embryonic genes were expressed in mutants lacking BRM and its close homologue SPLAYED based on transcriptome studies (Bezhani et al., 2007). Of note, the expression of key embryogenesis regulators such as AB13, LEC1, and LEC2 was either not changed or only marginally upregulated (FUS3) in adult brm hypomorph mutants (Tang et al., 2008). The SWI/SNF ATPase MINU1 is thought to be required for induction of stress-inducible genes that mediate growth arrest under abiotic stress, although direct targets of MINU1 remain to be identified (Mlynarova et al., 2007).

Taken together, these studies highlight a role for chromatin modifying and remodelling enzymes at the nexus of growth versus stress response pathways, both by modulation of developmental programmes and by enabling proper stimulus-dependent changes in gene expression.

### Links between stress signalling pathways and chromatin modifying or remodelling enzymes

As outlined above, many chromatin changes including a change in histone variant incorporation, histone modifications, nucleosome occupancy or positioning, or DNA methylation accompany stress-induced changes in gene expression. A critical question is how chromatin regulator activity is controlled to allow precise stimulus-dependent changes in the accessibility of the genome. One way to achieve this may be a direct communication between components of the stress signal transduction pathway and chromatin modifying or remodelling activities.

The question of whether histones in the context of chromatin can directly receive and deliver signals from cellular signal transduction cascades to facilitate specific cellular responses has recently received much attention (reviewed by Badeaux and Shi, 2013; Johnson and Dent, 2013; Suganuma and Workman, 2013). Another intersection between cellular signal transduction and chromatin is indirectly through post-translational modifications of chromatin modifying or remodelling enzymes (reviewed by Badeaux and Shi, 2013). Studies in mammals revealed that histone and DNA methyltransferases are directly phosphorylated by a downstream component of phosphoinositide signalling, the AKT kinase (Cha et al., 2005; Esteve et al., 2011). Likewise, SWI/SNF chromatin remodellers have been shown to be phosphorylated by p38 (Simone et al., 2004), as well as acetylated (Bourachot et al., 2003) and SUMOylated upon signal perception (Galisson et al., 2011).

Signalling transduction by SnRK2 kinases and PP2C phosphatases plays an important role in coordinating whole-plant water stress responses. Calcium-dependent protein kinases (CDPKs) are also critical for proper water stress response, ABA signalling and reduction of reactive oxygen species accumulation (Asano et al., 2012), whilst the inositol polyphosphate 1-phosphatase FIERY1 acts a negative regulator of ABA and stress signalling (Xiong et al., 2001). Thus far, there is no report that links these signalling components directly to the chromatin. However, links between other signal transducers and chromatin regulators have been identified. The clade A PP2C phosphatase HYPERSENSITIVE TO ABA 1 (HAB1) interacts physically with SWI3B, a core subunit of the putative Arabidopsis SWI/SNF complex. HAB1 is recruited to ABA response genes, and this recruitment is eliminated upon ABA treatment (Saez et al., 2008). HAB1 may perhaps directly dephosphorylate SWI/SNF complexes containing SWI3B in an ABA-dependent manner. In agreement with this idea, recent phosphoproteomics analyses performed by the Zhu and Shinozaki laboratories revealed that several chromatin regulators, including the BRM SWI/SNF ATPase, are substrates of SnRK2 type kinases in the ABA response pathway (Umezawa et al., 2013; Wang et al., 2013). Whether the observed phosphorylation/dephosphorylation of SW13B or BRM by SnRK2 kinases/PP2C phosphatases modulates SWI/SNF complex activity remains unknown. The Arabidopsis trithorax-like protein and histone H3 lysine 4 methyltransferase ATX1 (Alvarez-Venegas et al., 2003) is involved in dehydration response in both ABA-dependent and -independent pathways (Ding et al., 2011). Intriguingly, ATX1 also interacts directly with phosphatidylinositol (PtdIns5P), and this negatively influences the ATX1 activity (Ndumukong et al., 2010). Dehydration stress increases accumulation of phosphatidylinositol, a precursor of secondary
messengers in stress signalling (Ndamukong et al., 2010). An increase in the cellular levels of PtdIns5P keeps ATX1 in the cytoplasm, thereby diminishing ATX1 binding to target genes linked to proper water stress responses (Ndamukong et al., 2010). The phosphoproteomics studies mentioned above identified additional chromatin regulators as phosphorylated upon dehydration or ABA treatment in a SnRK2 kinase-dependent manner (Umezawa et al., 2013; Wang et al., 2013). Although there was little overlap between the phosphorylated peptides identified in the two studies, chromatin-associated proteins identified include putative components of HDAC complexes (e.g. SIN3-like 2, HD2B), histone acetyltransferase complexes (e.g. SNS1, Eal7 superfamily), histone methyltransferases (e.g. ATXR2, SDG2), chromatin remodelling ATPases (e.g. CHR2/BRM, CHR5/CHD1) and NUCLEOLIN LIKE 1, a nucleolar protein linked to rRNA gene methylation and expression (Umezawa et al., 2013; Wang et al., 2013). In addition, the Arabidopsis histone acetyltransferase GCN5 was shown to specifically interact with PP2C6.6, a clade E PP2C with no visible mutant phenotype. GCN5 is dephosphorylated by PP2C6.6 in vitro, and loss of PP2C6.6 activity induces GCN5-mediated histone acetylation (Servet et al., 2008). A possible link to water stress responses is supported by the reported expression of PP2C6.6 in guard cells (Galbiati et al., 2008).

The possibility that chromatin regulator activity is modulated upon stress sensing is intriguing with regard to the question of how these factors can execute specific roles in the organism. It is furthermore of practical significance. As chromatin regulators broadly alter the stress-inducible transcriptome, they may be able to direct tolerance not only to a unique stress but to combinations of stresses that are frequently encountered in the field (Mittler and Blumwald, 2010; Yang et al., 2010). The ability to precisely modulate the activity of chromatin regulators—via targeted post-translational modifications for example—should allow utilization of their broad reprogramming capacity whilst minimizing detrimental effects on growth or yield.

Stress-induced transient or long-term epigenetic memory

In higher plants, stress memory phenomena known as ‘priming’ or ‘acclimation’ have been described (reviewed by Bruce et al., 2007; Conrath, 2011). Pre-exposure to mild stimuli can make plants more stress resistant and boost responses to recurring stress exposure. Well-known examples of priming are seed priming to enhance germination efficiency and crop yield, temperature acclimation, and systemic acquired resistance (reviewed by Bruce et al., 2007; Conrath, 2011; Gutzat and Mittelsten Scheid, 2012). One mechanism proposed for long-term ‘storage’ of the stress memory is a mitotically heritable, or epigenetic, change in the chromatin organization. Another could conceivably rely instead on post-translational modification of chromatin regulators. Epigenetic ‘stress memory’ could be maintained during subsequent development within the life span of an organism that experienced the priming stress in ‘somatic memory’ or might perhaps even be transmitted to the progeny across generations in ‘transgenerational inheritance’, a meiotically heritable change in the chromatin organization.

Unlike the mitotically heritable response to prolonged cold (reviewed by Song et al., 2012; Zografos and Sung, 2012), the mechanisms underlying long-term somatic stress memory are not well understood. Previous studies have shown that histone tail modifications such as H3 acetylation or H3K4 methylation occur at drought-responsive genes upon drought sensing, and correlate with active transcription of dehydration response genes (Kim et al., 2008). However, drought-induced H3K9Ac marks and RNA polymerase II occupancy rapidly declined upon rehydration (Kim JM et al., 2012). By contrast, H3K4me3 decreased much more gradually during a 5 h rehydration period (Kim JM et al., 2012), suggesting that H3K4me3 could be a mitotically heritable epigenetic mark for water stress memory. In accordance with this study, another group proposed that H3K4me3 and stalled RNA polymerase II (PolII Ser5P) could function in mitotic stress memory (Ding et al., 2011). Recurrent dehydration induces a higher rate of expression of dehydration response genes such as RD29B and RAB18 than primary dehydration. This is accompanied by higher H3K4me3 and Ser5P PolII accumulation at these loci (Ding et al., 2011). During rehydration, the RD29B and RAB18 transcript levels revert to basal expression, but H3K4me3 and Ser5P PolII association with both loci remain elevated. The observed stress memory endured until 5 d after recovery (Ding et al., 2011). Likewise, H3K4 hypermethylation mediated by the Set1 histone methyltransferase in Saccharomyces cerevisiae was proposed to provide molecular memory of recent transcriptional events (Ng et al., 2003). It was suggested that elevated H3K4 trimethylation is important for genes to be rapidly switched on and off by environmental stimuli and that it acts to prevent the associated genes from being silenced (Ng et al., 2003). The combined data suggest the presence of a conserved mechanism for stress memory in metazoans.

One of the main difficulties in monitoring epigenetic profiles for long-term stress memory are confounding epigenetic changes caused by altered plant growth and development in stress-challenged plants. Another challenge is determining the period for which plants can ‘remember’ the priming event. An enhanced response to the second treatment shortly after the primary treatment could result from ‘leftover’ proteins and metabolites that were induced by the first stress treatment. Recently, Sani et al. (2013) developed an experimental protocol to monitor epigenetic profiles, which aims to avoid these problems. They showed that a mild transient salt treatment of young Arabidopsis seedlings establishes long-term somatic memory. This was accompanied by specific changes in the H3K27me3 profile, which remained after 10 d of subsequent growth, and resulted in drought/high-salt tolerance priming in the pre-treated plants without morphological differences between primed and non-primed adult plants (Sani et al., 2013).

Interestingly, H3K4me3 is generated by a methyltransferase that belongs to the trithorax group of proteins
alterations may be instructive for altered stress responses, rather than a consequence thereof; (2) to understand which chromatin alterations lead to stress tolerance that is mitotically (or meiotically) heritable; and (3) to devise ways to modulate the activity of ‘instructive’ chromatin regulators in ways that allow enhanced primary or heritable stress tolerance without causing growth or yield trade-offs.

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