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Epigenetic regulation of stress responses in plants

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Gene expression driven by developmental and stress cues often depends on nucleosome histone post-translational modifications and sometimes on DNA methylation. A number of studies have shown that these DNA and histone modifications play a key role in gene expression and plant development under stress. Most of these stress-induced modifications are reset to the basal level once the stress is relieved, while some of the modifications may be stable, that is, may be carried forward as 'stress memory' and may be inherited across mitotic or even meiotic cell divisions. Epigenetic stress memory may help plants more effectively cope with subsequent stresses. Comparative studies on stress-responsive epigenomes and transcriptomes will enhance our understanding of stress adaptation of plants.

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Introduction

Information content of the genome (DNA sequence) and its expression in response to stress are crucial for the adaptability of a genotype. Expression of the genome is influenced by chromatin structure, which is governed by processes often associated with epigenetic regulation, namely histone variants, histone post-translational modifications, and DNA methylation. Developmental and environmental signals can induce epigenetic modifications in the genome, and thus, the single genome in a plant cell gives rise to multiple epigenomes in response to developmental and environmental cues [1]. Understanding stress-induced epigenetic processes in stress tolerance of plants requires answers to the following questions: How much of the stress-induced gene expression changes are associated with alterations in DNA methylation and histone modification marks? Are stress-induced DNA and histone modifications during acclimation or during the first experience of stress memorized and inherited mitotically and meiotically? What are the adaptive values of epigenetic stress memory? This review briefly describes epigenetic processes, and then focuses on recent data on the epigenetic regulation of stress responses and its heritability in plants.

Epigenetic regulation of stress responses

Retention of stress memory for short durations is well known in plants, as evident from acclimation responses [2,3]. The stress memory can be retained for only short durations if the memory depends on the half-life of stress-induced proteins, RNAs, and metabolites, while the memory can last longer if it involves reprogramming in phenology and morphology of plants. Epigenetic processes, that is, stable or heritable DNA methylation and histone modifications, can also be a choice of retaining stress memory for longer times. Methods to decipher epigenetic changes are briefly described in Box 1.

Histone modifications

N-terminal regions of nucleosome core complex histones undergo various post-translational modifications. In addition, each histone has variants encoded by different genes. The combinations of histone variants and post-translational modifications can be considered a 'histone code', which plays a key role in chromatin structure and thus determines the transcriptional state and expression level of genes. Some histone modifications, namely acetylation, and certain phosphorylation and ubiquitination [4,5], enhance transcription, while biotinylation and sumoylation repress gene expression [6,7]. Trimethylation of H3K4 activates transcription, while dimethylation of H3K9 and H3K27 represses transcription [5]. Because several of the histone modifications are associated with changes in gene transcription in general, it is not surprising that stress-induced gene regulation is associated with histone modifications in all cases that have been investigated. Changes in histone variants, histone modifications as well as DNA methylation are often referred to as epigenetic regulation. However, such changes may or may not be truly epigenetic in nature because common epigenetics definition requires mitotic or meiotic heritability.

Drought induced the linker histone variant H1-S in tomato. H1-S appears to be involved in the negative regulation of stomatal conductance, because stomatal conductance and transpiration rates were higher in antisense transgenic H1-S tomato plants than in wild type (WT) plants [8].

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Box 1 Deciphering epigenetic changes

Histone modifications:
Chromatin immunoprecipitation (ChiP) — histones bound to the DNA *in vivo* are covalently crosslinked to DNA *in situ* by vacuum infiltration of plant tissue with formaldehyde. Then chromatin is isolated as part of cell extract, fragmented, and protein–DNA complexes are immunoprecipitated with antibodies specific against modified histone, for example, acetylated or dimethylated H3K9. DNA is isolated from the immunoprecipitate and analyzed by PCR [4,9••,15••,16•,18•,51].

ChiP-Seq — this method combines ChiP with next-generation sequencing technology such as Solexa sequencing to analyze genome-wide-specific histone modifications [52].

DNA methylation:
Methylation-sensitive restriction endonucleases — the classical method of cytosine methylation analysis is the restriction analysis of template DNA with methylation-sensitive restriction enzymes. Restricted DNA is then ligated to restriction site specific adaptor and analyzed by PCR or restricted genomic DNA is analyzed by Southern blotting [22,27•,49••,50•].

Bisulfite method — sodium bisulfite converts cytosines, but not 5′-methylcytosines, into uracil, under denaturing conditions. PCR amplification of bisulfite-treated DNA results in conversion of uracil to thymine. Bisulfite-treated DNA is analyzed by PCR or DNA sequencing [4,23•,33••,49••,50•]

Methylated-DNA immunoprecipitation (MeDIP) — genomic DNA is fragmented and precipitated with 5-methylcytosine-specific antibody. The precipitated DNA is then analyzed by PCR or whole-genome tiling microarrays [53,54].

Shotgun bisulfite-sequencing — this combines bisulfite treatment of genomic DNA with next generation sequencing technology such as Solexa sequencing. The converted sequences are mapped to the reference genome sequence to identify methyl-cytosines [21,55].

In rice seedlings, submergence induced histone H3K4 trimethylation and H3 acetylation in alcohol dehydrogenase 1 (*ADH1*) and pyruvate decarboxylase 1 (*PDC1*) genes. These histone modifications were correlated with enhanced expression of *ADH1* and *PDC1* under stress. The modifications, however, were dynamic and were restored to the basal level after stress was relieved by reaeration [9••].

Environmental and endogenous signals can repress the target genes through reduction in histone acetylation levels. The REDUCED POTASSIUM DEPENDENCY3 (RPD3) family histone deacetylases (HDACs), namely HDA6 and HDA19, mediate histone deacetylation in response to biotic and abiotic stresses in *Arabidopsis*. *HDA6* is induced by jasmonic acid (JA) and ethylene [10]. HDA6 is involved in transcriptional gene silencing (TGS) [11] and RNA-directed DNA methylation (RdDM) in *Arabidopsis* [12]. Wounding, infection by *Alternaria brassicicola*, and plant hormones (JA and ethylene) induced the expression of the *HDA19*/*HD1*/*AtRPD3A* gene. Overexpression of *HDA19* in transgenic plants reduced histone acetylation levels and increased the expression levels of *ETHYLENE RESPONSE FACTOR-1* (*ERF1*) and *PATHOGENESIS-RELATED* (*PR*) genes. In contrast, *RPD3A*-RNAi plants exhibited higher histone acetylation, which was accompanied by downregulation of *ERF1* and *PR* genes in *Arabidopsis* [10]. Enhanced *HDA6* and *HDA19* expression caused by stress and hormonal signals thus might affect chromatin modifications at several loci.

ABA downregulated the expression of *AtHD2C* (a member of plant-specific HD2 family of HDACs). Transgenic *Arabidopsis* plants overexpressing *AtHD2C* exhibited enhanced expression of ABA-responsive genes and greater salt and drought tolerance than the WT plants [13•]. In rice, expression of different members of the HDAC families is also differentially regulated by abiotic factors such as cold, osmotic and salt stress, and hormones such as ABA, JA, and salicylic acid [14].

Besides the HDACs, the WD-40 repeat protein TBL1 (*T*ransducin *B*eta-*L*ike protein-1) is associated with histone deacetylation in humans. The *Arabidopsis hos15* (*high expression of osmotic stress responsive genes15*) mutant was hypersensitive to freezing stress, and was hypersensitive, in terms of germination, to ABA or NaCl. *HOS15* encodes a protein similar to TBL1, which interacts with histone H4. HOS15 is probably involved in H4 deacetylation because acetylated H4 was higher in *hos15* mutants than in WT plants, and thus regulates stress tolerance through chromatin remodeling in *Arabidopsis* [15••].

Drought-induced expression of stress-responsive genes is associated with an increase in H3K4 trimethylation and H3K9 acetylation in *Arabidopsis* [16•]. In *Drosophila*, H3 Ser-10 phosphorylation activates transcription during heat shock responses [17]. In *Arabidopsis* also, high salinity, cold stress, and ABA triggered rapid and transient upregulation of histone H3 Ser-10 phosphorylation, H3 phosphoacetylation, and H4 acetylation followed by stress-type-specific gene expression [18•].

Histone acetyltransferases (HATs) interact with transcription factors and are involved in activating stress-responsive genes. GCN5 is the catalytic subunit of the Spt-Ada-Gcn5 acetyltransferase (SAGA) and transcriptional adaptor (ADA). Like ADA2 and GCN5 in the response of yeast to extreme temperature stress, in *Arabidopsis* as well, GCN5 and ADA regulate cold tolerance by interacting with *C*-repeat *B*inding *F*actor-*1* (CBF1). CBF1 activates transcription of its downstream cold-responsive genes probably through the recruitment of ADA/SAGA-like complexes that may mediate chromatin remodeling in target genes [19].

DNA methylation

DNA cytosine methylation, both asymmetric (^{m}CpHpH)-methylation and symmetric (^{m}CpG and ^{m}CpHpG)-methylation, is associated with repressive chromatin in gene promoters and with repression of gene transcription. *De*

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novo methyltransferases DRM1 (DOMAINS REARRANGED METHYLASE 1) and DRM2 catalyze new cytosine methylation, while the maintenance of symmetric CG and CHG methylation is mediated by the DNMT1-like enzyme MET1 and the plant-specific enzyme Chromomethylase 3 (CMT3), respectively [20]. Recent studies suggested that MET1 and CMT3 may also catalyze *de novo* methylation, while DRM1 and DRM2 are also important for the maintenance of symmetric methylation [1,21].

Stresses can induce changes in gene expression through hypomethylation or hypermethylation of DNA. In maize roots, cold stress-induced expression of *ZmMI1* was correlated with a reduction in methylation in the DNA of the nucleosome core. Even after seven days of recovery, cold-induced hypomethylation was not restored to the basal level [22]. In tobacco, aluminum, paraquat, salt, and cold stresses induced-DNA demethylation in the coding sequence of the *NtGPDL* (a glycerophosphodiesterase-like protein) gene correlated with *NtGDPL* gene expression [23•].

Osmotic stresses induced transient DNA hypermethylation in two heterochromatic loci in tobacco cell-suspension culture [24]. DNA hypermethylation was also induced by drought stress in pea [25]. In the facultative halophyte *Mesembryanthemum crystallinum* L., drought and salt stresses-induced a switch in photosynthesis mode from C_3 to CAM. This metabolic change was associated with stress-induced-specific CpHpG-hypermethylation of satellite DNA [26].

Transposons constitute a significant portion of plant genomes and are maintained in a repressed state by DNA methylation. Environmental factors may activate transposons through DNA demethylation. In *Antirrhinum majus*, cold stress induced hypomethylation, and transposition of the *Tam-3* transposon [27•].

Stress-induced histone modifications can also influence DNA methylation. Knockout mutants and RNAi lines of stress-inducible *HDA6* of *Arabidopsis* and *HDA101* of maize showed an increase in histone acetylation accompanied by changes in histone methylation pattern and derepression of silenced genes [28,29]. Specific histone modification-dependent pathways appear to mediate methylation of about two-thirds of the methylated loci in the *Arabidopsis* genome [1]. Thus, dynamic histone modification marks could be converted into DNA methylation marks, which are often more stable.

RNA-directed DNA methylation

Genetic analysis using *Arabidopsis* mutants impaired in genes for siRNA biogenesis or action revealed the involvement of small interfering RNAs (siRNAs) in RdDM [20,30]. Integration of the *Arabidopsis* floral epigenome with the floral transcriptome and small RNA profiles revealed a direct correlation between the ability of genomic sequences to produce small RNAs and DNA methylation [21]. In fact, siRNAs are involved in the methylation of at least one-third of methylated loci [21]. Studies on the *repressor of silencing 1* (*ros1*) mutant of *Arabidopsis* revealed that the DNA glycosylase ROS1 actively demethylates DNA by a base excision repair mechanism and can counteract RdDM [31,32]. ROS3, a RNA recognition motif-containing protein, binds to small RNAs and may direct sequence-specific demethylation by ROS1 and related DNA demethylases [33••].

Gene silencing processes can be sensitive to temperature. Temperature and other abiotic stresses can also regulate specific small RNAs. Low temperature promoted virus-induced gene silencing, while high temperature delayed it [34]. Endogenous siRNAs that are regulated by abiotic stress have been identified in *Arabidopsis* [35]. In *Arabidopsis*, 24-nt SRO5-P5CDH nat-siRNA downregulates the expression of *P5CDH* mRNAs through mRNA cleavage, leading to decreased proline degradation, and enhanced proline accumulation and salt stress tolerance [36]. This and other stress-regulated siRNAs conceivably could also lead to changes in histone modifications and DNA methylation. Microarray data showed that abiotic stresses and ABA influence the expression of many of the genes implicated in RdDM pathways in *Arabidopsis* (our unpublished data). Further studies are clearly needed to unravel the roles of RdDM pathway under stress.

Plant development under stress

Reprogramming of cell differentiation in response to environmental stress leads to phenological and developmental plasticity, which are important mechanisms of stress resistance. Phenotypic plasticity helps adjust the durations of various phenological phases in plants, and thus allows plants to avoid exposure of critical growth phases, and especially reproductive development, to stress. Further, adjustment of growth and development is critical for effective use of resources under stress.

Germination and vegetative growth

Osmotic stress reduces the uniformity of seed germination and seedling establishment. Several HDACs are induced by ABA in *Arabidopsis* [13•] and rice [14]. *Arabidopsis* HDA19/HD1 interacts with a global corepressor of transcription, AtSIN3, which in turn interacts with AtERF7 (APETALA2/EREBP-type transcription factor). Suppression of *AtERF7* and *AtSIN3* in plants caused hypersensitivity to ABA during germination and seedling growth [37]. *Arabidopsis HDA6/HDA19* double repression lines showed growth arrest after germination and formation of embryo-like structures on true leaves [38]. These results suggest that ABA accumulation leads to change in expression or activity of HDACs, which in turn regulate growth under stress.

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Transgenic *Arabidopsis* overexpressing a SNF2/BRAHMA-type chromatin remodeling gene *AtCHR12* exhibited growth arrest of primary buds and growth reduction of the primary stem. These responses were more pronounced under drought and heat stress than under nonstress conditions. Conversely, the growth arrest response under stress was less in the *AtCHR12*-knockout mutant than in the WT plants [39••].

Reproductive development

Flowering and seed development are crucial for plant reproduction. Hence, plants have evolved mechanisms to flower when environmental conditions are appropriate. In *Arabidopsis*, low temperatures during vernalization induce epigenetic mechanisms which repress the *FLOWERING LOCUS C* (*FLC*, a MADS-box protein) gene, and the repressed *FLC* chromatin is maintained till transition to flowering. The mechanisms of mitotic inheritance of the repressed epigenetic state of *FLC* chromatin and resetting during reproduction are not fully understood [40]. Because the low temperatures that induce vernalization also induce cold acclimation, some of the gene expression programs could be under common epigenetic control.

Mutations in some of the genes involved in stress-related epigenetic processes cause changes in flowering time. The *hos15*, a freezing sensitive mutant of *Arabidopsis*, was late flowering owing to downregulation of flowering-regulatory genes *SOC* and *FT* [15••]. Plant hormone and stress-regulated *HDA6* and *HDA19* may act as a link between stress and developmental cues that control flowering and plant development. Reduction in *HDA19* expression in antisense transgenic plants/T-DNA mutants resulted in developmental abnormalities including delayed flowering [41,42]. HDA6-RNAi lines and *axe1-5*/*hda6* mutants showed hyperacetylation of histone H3 globally, downregulation of JA-responsive genes, upregulation of *FLC*, and delayed flowering [43•].

In *Arabidopsis*, FCA and FPA proteins form an autonomous flowering pathway by downregulating flowering repressor *FLC*. Both FCA and FPA are RNA-binding proteins that can regulate DNA methylation [44]. ABA and drought stress induced the expression of chromatin remodeling gene *PsSNF5* (*Pisum sativum SNF5*). PsSNF5 interacts with *Arabidopsis* SWI3-like proteins (SWI3A and SWI3B), which in turn interact with FCA [45,46]. ABA-induced SNF5 and FCA may regulate flowering time and stress responses through chromatin remodeling.

Because stresses reduce crop yield and quality, and ABA regulates seed development partly through epigenetic processes [47], effects of stress on ABA accumulation or epigenetic processes therefore may affect seed/fruit development under stress.

Senescence

Abiotic stresses induce premature leaf senescence, which leads to reduced photosynthesis and thus less biomass accumulation. JA – and ethylene-responsive-HDACs, HDA6 and HAD19, appear to modulate leaf senescence. *Arabidopsis* HDA6-RNAi lines and *axe1-5* (*hda6*) mutants exhibited downregulation of JA-responsive genes and senescence-associated genes, and delayed senescence as indicated by higher chlorophyll content and PSII activity as compared to WT plants [43•]. In contrast, *HDA19* antisense transgenic plants/T-DNA mutants showed early senescence [41].

Stress memory

UV-C radiation or flagellin (an elicitor of plant defense) induced a high frequency of somatic homologous recombination, and the hyper-recombination state was transmitted as a dominant trait to untreated progenies of stress-treated parents [48••]. Similarly, tobacco mosaic virus (TMV) infection resulted in a high frequency of somatic and meiotic recombination rates in tobacco. The progeny of TMV-infected plants exhibited hypomethylation in several leucine-rich repeat (LRR)-containing loci and a higher frequency of recombination in hypomethylated LRR-containing TMV (*N*-gene) resistant gene [49••].

The adaptive value of stress-induced epigenetic plasticity was studied in hypomethylation progenies of 5-aza-deoxycytidine (inhibitor of DNA cytosine methylation)-treated rice seeds. In one of the progenies, methylation was completely erased in *Xa21G*, a Xa21-like protein gene. The erasure of promoter methylation and inheritance of this epigenetic state resulted in constitutive expression of *Xa21G* in the progeny line and enhanced resistance to the pathogen *Xanthomonas oryzae* pv. *oryzae*, race PR2 [50•].

Conclusions

Stress-induced changes in histone variants, histone N-tail modifications, and DNA methylation have been shown to regulate stress-responsive gene expression and plant development under stress. Transient chromatin modifications mediate acclimation response. Heritable, epigenetic modifications may provide within-generation and transgenerational stress memory (Figure 1). It is unclear how much of the stress-induced histone and DNA modification changes that have been observed to date may be epigenetic in nature because little is known about their mitotic or meiotic heritability. Abiotic stress-induced epigenetic changes might have an adaptive advantage. However, stress memory could have a negative impact on crop yield by preventing the plant from growing to its full potential. Thus, stress memory has implications for the use of seeds from stressed crop to raise ensuing crops by the farmers, breeding for stress environments and *in situ* conservation of plant species. Recent progress in understanding DNA methylation and

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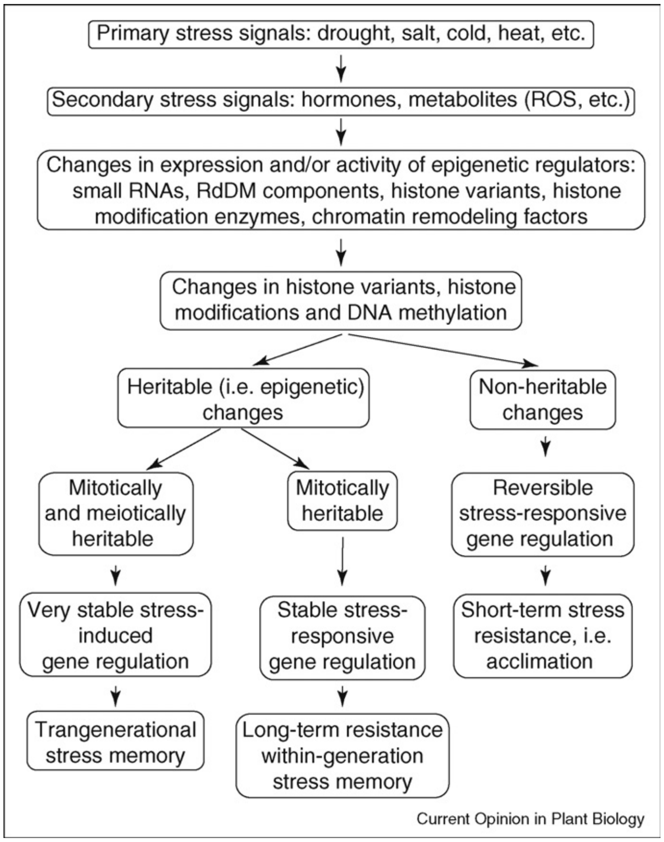
Figure 1



Epigenetic regulation of stress tolerance. Primary and secondary stress signals induce changes in the expression and/or activity of epigenetic regulators namely, small RNAs, RdDM components, histone variants, histone modification enzymes, and chromatin remodeling factors. These epigenetic regulators modify histone variants, histone modifications, and DNA methylation. Some of these are heritable epigenetic modifications, while others are transient changes. Transient chromatin modifications mediate acclimation response. Heritable epigenetic modifications provide within-generation and transgenerational stress memory.

demethylation, histone modifications, small RNAs and in developing powerful and versatile tools to study these epigenetic processes makes it possible to critically analyze epigenetic stress memory and harness it for crop management and improvement.

Conflict of interest

There is no conflict of interest relating to this article.

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