

Epigenetic Memory for Stress Response and Adaptation in Plants

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In contrast to the majority of animal species, plants are sessile organisms and are, therefore, constantly challenged by environmental perturbations. Over the past few decades, our knowledge of how plants perceive environmental stimuli has increased considerably, e.g. the mechanisms for transducing environmental stress stimuli into cellular signaling cascades and gene transcription networks. In addition, it has recently been shown that plants can remember past environmental events and can use these memories to aid responses when these events recur. In this mini review, we focus on recent progress in determination of the epigenetic mechanisms used by plants under various environmental stresses. Epigenetic mechanisms are now known to play a vital role in the control of gene expression through small RNAs, histone modifications and DNA methylation. These are inherited through mitotic cell divisions and, in some cases, can be transmitted to the next generation. They therefore offer a possible mechanism for stress memories in plants. Recent studies have yielded evidence indicating that epigenetic mechanisms are indeed essential for stress memories and adaptation in plants.

Keywords: DNA methylation • Epigenetics • Histone modification • Small RNA • Stress responses • Transposable elements.

Abbreviations: bHLH, basic helix–loop–helix; DMR, differentially methylated region; HSE, heat shock element; LTR, long terminal repeat; MAPK, mitogen-activated protein kinase; PRC2, Polycomb Repressive Component 2; PTI, pattern-triggered immunity; RdDM, RNA-directed DNA methylation; SI, stomatal index; siRNA, small interfering RNA; ta-siRNA, trans-acting small interfering RNA.

Introduction

Plants are constantly exposed to the vagaries of the environment, such as changes in temperature, light intensity, nutrient and water availability. In addition to such abiotic stresses, plants are also challenged by various pathogens and insects. To survive these difficulties, plants have developed elaborate sensing mechanisms mediated by signaling cascades and gene transcription networks that respond to environmental cues

(Yamaguchi-Shinozaki and Shinozaki 2005, Fu and Dong 2013). As environmental perturbations may occur repeatedly, it is advantageous to plants to be able to remember past incidents and to use this stored knowledge to adapt to new challenges. One of the best known of these 'memory' systems is that termed 'defense priming', which controls the response to a pathogen or herbivore attack (Pastor et al. 2013a). In defense priming, the plant displays a more rapid and robust response to the pathogen or herbivore in a second attack compared with the first one, thereby increasing its chances of survival. Priming can also be found for various abiotic stress responses such as drought stress (Bruce et al. 2007, Ding et al. 2012) (Fig. 1). A range of priming strategies has been identified among plant species: accumulation of intermediate compounds in the cellular compartments; modification of key regulatory proteins, e.g. phosphorylation of mitogen-activated protein kinases (MAPKs); and epigenetic mechanisms (Conrath 2011, Pastor et al. 2013b). In recent years, the epigenetic mechanisms that control gene expression of several model processes have been described, for example genomic imprinting, vernalization response, defense against parasitic elements and stress responses (Chinnusamy and Zhu 2009, Ikeda 2012, Kim et al. 2012b, Kohler et al. 2012, Saze et al. 2012, Song et al. 2013). Although the molecular mechanisms of the response to a stress stimulus vary in the different processes, nevertheless a comprehensive overview of stress memories should be helpful to understand the general role of epigenetic mechanisms for environmental adaptation in plants and, possibly, genome evolution in plants (Mirouze and Paszkowski 2011).

Drought Stress

Water is vital to life and is a fundamental molecule in most cellular processes; therefore, its availability has a direct impact on development and survival, particularly in plants. The importance of available water has been demonstrated in *Arabidopsis thaliana*, in which drought signals are transduced into effects on gene expression (Yamaguchi-Shinozaki and Shinozaki 2005). As changes in gene expression patterns are generally accompanied by changes in the chromatin status (Campos and Reinberg 2009), the modification of histone

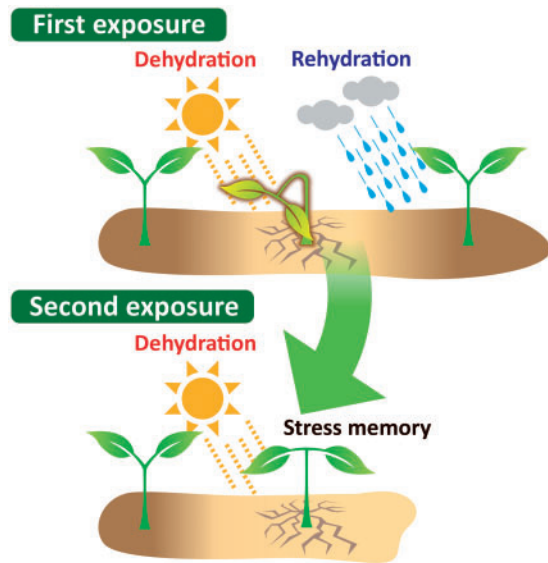


Fig. 1 An example of stress memory in plants. Plants have elaborate mechanisms for stress memory. For example, a plant that experiences a period of drought wilts under the dehydration stress and then recovers after rehydration (upper panel); during a second drought stress, the plant ‘remembers’ the past drought experience, allowing it to achieve better resistance to dehydration and improve its survival prospects (lower panel) (Ding et al. 2012).

tails in response to dehydration has been analyzed in the chromatin surrounding drought stress-responsive genes (Kim et al. 2008, Kim et al. 2012a). Under drought stress conditions, *RD29A*, *RD20* and *AtGOLS2* transcripts accumulate; during rehydration, the amounts of these transcripts fall to the basal level. In agreement with the changing levels of transcripts, acetylation of lysine 9 of histone H3 (H3K9ac), which is correlated with the active state of gene expression, and the presence of RNA polymerase II in these genic regions transiently increase under drought stress conditions and then fall during the rehydration recovery period. Alterations to trimethylated histone H3 lysine 4 (H3K4me3), which is correlated with active transcription, suggest that this chromatin mark may play a role in transcription memory for these genes since it is enriched by drought stress and maintained at some levels during the rehydration process (Kim et al. 2012a).

Ding et al. (2012) reported that multiple exposures to drought stress conditions enable plants to respond to a new stress by more rapid adaptive changes to gene expression patterns compared with plants not previously exposed to a drought stress. While the levels of induction of *RD29A* and *COR15A* transcripts are the same in each stress treatment, the *RD29B* and *RAB18* transcripts accumulate in a progressive manner, i.e. the accumulated levels of transcripts are higher than in the previous stress treatment. The progressive change in gene expression and transcript accumulation may be the result of a progressive increase in H3K4me3 and phosphorylation of serine 5 (Ser5P) of RNA polymerase II during the process of recovery from dehydration (Ding et al. 2012). Although gene transcription falls to the basal level present in unstressed plants, the relatively high levels of H3K4me3 and the RNA

polymerase II (Ser5P) occupancy persisted, and may, therefore, function as a stress memory.

The Polycomb Repressive Component 2 (PRC2) and Trithorax Group (TrxG) proteins are known to repress and to activate gene transcription in an epigenetic manner; their interaction with the chromatin of the *FLOWERING LOCUS C (FLC)* gene plays a central role in the memory of the duration of low winter temperatures which is crucial for vernalization (Buzas et al. 2012, Yun et al. 2012, Song et al. 2013). However, the actions of *CURLY LEAF (CLF)*, a SET-domain component of PRC2, and *ARABIDOPSIS TRITHORAX-LIKE PROTEIN 1 (ATX1)*, a component necessary for gene activation (Avramova 2009), do not fully explain transcriptional memory (Ding et al. 2012, Liu et al. 2014). Genome-wide DNA methylation differences have also been investigated with regard to a possible role in memory of drought stress; to date, however, no correlation has been identified between gene expression patterns and DNA methylation levels in *A. thaliana* (Colaneri and Jones 2013).

Low Humidity

Water loss through the stomata is a crucial aspect of the processes for obtaining and absorbing nutrition from the soil, and for respiration and photosynthesis. Low humidity conditions therefore also pose a stress to plants (Roelfsema and Hedrich 2005). The development of the stomata is controlled by an elaborate genetic network (Lau and Bergmann 2012, Pillitteri and Torii 2012) in combination with environmental variables such as light intensity and carbon dioxide levels (Casson and Hetherington 2010). Basic helix–loop–helix (bHLH) transcription factors are involved in the fate determination pathway that transforms leaf epidermis cells into guard cells; one of these transcription factors, *FAMA*, has been shown to be controlled by PRC2 (Lee et al. 2014). Recent studies in *A. thaliana* reported that the stomatal index (SI), the ratio of stomata to other epidermal cells, is developmentally controlled by the levels of relative humidity (Tricker et al. 2012). Low relative humidity triggers the accumulation of small interfering RNAs (siRNAs) that interact with *FAMA* and *SPEECHLESS (SPCH)* loci that encode bHLH transcription factors required for stomatal development. Under low humidity conditions, wild-type plants and plants with the *chromomethyltransferase 3 (cmt3)* mutation show reduced dry weight; in contrast, plants with double mutation of *domain rearranged methyltransferase 1 and 2 (drm1/2)* or with the *methyltransferase1 (met1)* mutation are resistant to the effects of low humidity. Plants with the *dicer-like 3 (dcl3)* or *RNA dependent RNA polymerase 6 (rdr6)* mutation have a higher SI than wild-type plants in conditions of low humidity, suggesting that the RNA-directed DNA methylation (RdDM) pathway contributes to this phenomenon (Tricker et al. 2012). Note that the mechanisms of RdDM and epigenetics have been described in many excellent reviews (e.g. Matzke and Mosher 2014).

Changes to the DNA methylation status in some loci in plants can be heritable over multiple generations (Kakutani 2002). It will be of interest to address the role of DNA

methylation in this phenomenon. Interestingly, a transgenerational effect of low humidity stress has been identified for the *Sl* phenotype; this effect is manifested for at least one generation by changes in DNA methylation status at various sites of the *SPCH* and *FAMA* bHLH transcription factor loci (Tricker et al. 2013a, Tricker et al. 2013b). Since all the sites examined overlapped with siRNA targets of the locus, the next question is how these DNA methylation states are protected from the genome-wide reprogramming during reproductive development (Calarco et al. 2012, Gutierrez-Marcos and Dickinson 2012, Ibarra et al. 2012, Jullien et al. 2012). More studies are required to understand fully the transgenerational effect response to low humidity conditions.

Heat Stress

As climate change will have an impact on plant growth and crop yield, the mechanisms of stress response to elevated temperature levels have gained much attention (Hedhly et al. 2009). Recently, transposition of the retrotransposon *ONSEN* in *A. thaliana* has been reported to involve an epigenetic mechanism (Ito et al. 2011, Matsunaga et al. 2012). *ONSEN* is a long terminal repeat (LTR)-copia type retrotransposon that is transcriptionally activated under heat stress. Transcripts from *ONSEN* loci are reverse transcribed as extrachromosomal DNA and then integrated into the host genome. Transposition of *ONSEN* occurs more frequently in the progeny of the RdDM machinery mutants that have been exposed to a 24 h heat pulse of 37°C, suggesting that the RdDM machinery has a role in the prevention of transgenerational propagation of retrotransposons in this organism (Ito et al. 2011). The *ONSEN* retrotransposon has a heat shock element (HSE) within its promoter region that can bind host heat shock transcription factors and enable the retrotransposon to be transcribed under heat stress conditions (Cavrak et al. 2014).

Recently, one of the epigenetic pathway responses to heat stress was clarified (Li et al. 2014). In this pathway, a non-coding RNA, *Trans-acting siRNA precursor 1 (TAS1)* is converted to a double-stranded RNA by *RDR6* using an miR173 guide; in turn, this double-stranded RNA is processed into a phased array of 21 nucleotide *trans-acting* small interfering RNAs (ta-siRNAs) by the DICER-LIKE 4 (*DCL4*) RNase III enzyme (Allen et al. 2005). *TAS1*-derived siRNAs are heat inducible and bind to the *HEAT-INDUCED TAS1 TARGET (HTT)* genes that confer thermotolerance in the plant through the action of the HSP70 protein complex. HsfA1 transcription factors bind directly to the HSE in the *HTT* promoter and thereby induce thermotolerance (Li et al. 2014). Interestingly, the small RNA machinery antagonistically represses the Hsf1A targets, *HTT* genes and *ONSEN* retrotransposons, under ambient temperatures. Small RNAs and Hsf1A transcription factors may orchestrate fine-tuning of the heat stress responses by controlling the transcription of target genes. Alternatively, the small RNA pathway may serve as a stress memory, as in the case of repeated drought stress (Ding et al. 2012). H3K4me accumulation and RNA polymerase II occupancy of the *RD29B* and *RAB18* genes are observed

during the rehydration process during recovery from drought. In this scenario, the biogenesis or accumulation of small RNAs that target *HTT* or *ONSEN* might be delayed during the recovery process after heat stress.

Plant Immunity

The mechanisms of defense priming in plants have been studied for many decades. Tobacco plants, for example, accumulate a nicotine pool in their vacuoles after attack by insects or following treatment with jasmonic acid (Baldwin and Schmelz 1996). The toxic nicotine pool protects the plants from subsequent insect attacks and, therefore, serves as a stress memory. In many cases, priming induces a rapid and robust defense capacity in plants by enhancement of molecular mechanisms related to plant immunity (Conrath 2011). Pre-treatment of a plant with salicylic acid or a synthetic analog enhances the expression of several *WRKY* transcription factors in response to a subsequent stress. This 'gene priming' is associated with altered patterns of histone modifications, histone H3 and H4 acetylation and H3K4me3 (Jaskiewicz et al. 2011). Various research groups predicted an important role for epigenetic mechanisms in plant immunity (Bruce et al. 2007, Conrath 2011), and the reports showing increased immunity in *ago4* or the RdDM mutants support this idea (Agorio and Vera 2007, Lopez et al. 2011). An important observation is that plants defective in *METHYLTRANSFERASE 1 (MET1)* or that carry the triple methyltransferase mutations, *drm1*, *drm2* and *cmt3 (ddc)*, show increased resistance to the pathogenic bacterium *Pseudomonas syringae* pv. *tomato* DC3000 (Downen et al. 2012). Genome-wide analyses have also shown changes in DNA methylation patterns, termed differentially methylated regions (DMRs), in response to pathogen attack. The identified DMRs significantly influence gene expression patterns. Thus, increased resistance might be explained by the release from the silent state of pathogenesis-related genes that are induced after recognition of effector proteins derived from avirulent bacteria or the plant hormone salicylic acid. Indeed, genes associated with DMRs that occur under stress conditions are also up-regulated in the *met1* and *ddc* mutants. A genome-wide analysis also showed that the population of siRNAs changes after salicylic acid treatment; in particular, transposon-associated 21 nucleotide siRNAs but not 24 nucleotide siRNAs are increased (Downen et al. 2012).

Using a reporter system in which an LTR region of the *AtGP1* gypsy-type retrotransposon is fused with β -glucuronidase (*GUS*), Yu and colleagues (2013) showed that application of the bacterial flagellin-derived peptides *flg22* causes increased expression of *AtGP1-GUS* in the vascular tissue. Recognition of *flg22* by the pattern recognition receptor FLAGELLIN SENSING 2 activates pathogen-associated molecular patterns that trigger immunity [pattern-triggered immunity (PTI)] (Boller and Felix 2009). The PTI response induces activation of MAPK cascades, production of reactive oxygen species (ROS) and expression of immune response genes (Jones and Dangl 2006). In addition to these PTI responses, *flg22* induces the suppression of genes in

the RdDM machinery and enables expression of previously silenced genomic sequences, such as *ONSEN*, *EVADE*, *AtSN1* and 5S rDNA repeats. These hypermethylated elements are transiently demethylated at CHH (H = A, T or C) sites. Interestingly, the activity of the DNA demethylase of *ROS1* restricts pathogenic bacterial growth, and this is probably mediated by *ROS1*-mediated activation of the *RESISTANCE METHYLATED GENE 1 (RMG1)*, which is a nucleotide-binding and leucine-rich protein (Yu et al. 2013). These analyses and others clearly show the role of the RdDM machinery and the need for an active DNA demethylase in plant immune responses.

Conclusions and Perspectives

There is growing evidence that epigenetic mechanisms contribute to stress responses and memory in plants. Changes in DNA methylation in some genomic regions can be heritable over many generations, for example that affecting the imprinted *FWA* gene of *A. thaliana* (Kinoshita et al. 2007). Therefore, it is tempting to speculate that many of the observed transgenerational responses to stress conditions could be attributable to epigenetic mechanisms. On the other hand, it has also been shown recently that plants have an elaborate reprogramming system for epigenetic marks during sexual reproduction (Calarco et al. 2012, Ibarra et al. 2012, Jullien et al. 2012). Several excellent reviews have pointed out that more careful analyses are still needed to provide the critical proof required to accept the reality of transgenerational epigenetic adaptation in plants and animals (Calarco et al. 2012, Pecinka and Mittelsten Scheid 2012, Grossniklaus et al. 2013). Although we have focused on studies of *A. thaliana* in this review, it is well known that this species carries a relatively small population of transposable elements that are mostly restricted to the pericentromeric genomic regions (Fedoroff 2012). The RdDM machinery, together with other mechanisms to maintain DNA methylation, mostly target repetitive elements and contribute to the silencing of these elements. It is possible that epigenetic mechanisms play more dynamic roles in crop plants, which have relatively higher populations of repetitive elements even in gene-rich euchromatic regions. Indeed, DNA methyltransferases and DNA demethylase mutations in rice show more severe defects compared with the corresponding mutants in *A. thaliana* (Moritoh et al. 2012, Ono et al. 2012, Hu et al. 2014, Wei et al. 2014, Yamauchi et al. 2014). Future studies using crop plants will undoubtedly provide further insights into epigenetic stress memory and adaptation in plants.

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Disclosures

The authors have no conflicts of interest to declare.

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