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Abscisic Acid-mediated Epigenetic Processes in Plant Development and Stress Responses

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Abstract

Abscisic acid (ABA) regulates diverse plant processes, growth and development under non-stress conditions and plays a pivotal role in abiotic stress tolerance. Although ABA-regulated genetic processes are well known, recent discoveries reveal that epigenetic processes are an integral part of ABA-regulated processes. Epigenetic mechanisms, namely, histone modifications and cytosine DNA methylation-induced modification of genome give rise to epigenomes, which add diversity and complexity to the genome of organisms. Histone monoubiquitination appears to regulate ABA levels in developing seeds through histone H2B monoubiquitination. ABA and H2B ubiquitination dependent chromatin remodeling regulate seed dormancy. Transcription factor networks necessary for seed maturation are repressed by histone deacetylases (HDACs)-dependent and PICKLE chromatin remodeling complexes (CRCs), whereas ABA induces the expression of these genes directly or through repression of HDACs. Abiotic stress-induced ABA regulates stomatal response and stress-responsive gene expression through HDACs and HOS15-dependent histone deacetylation, as well as through the ATP-dependent SWITCH/SUCROSE NONFERMENTING CRC. ABA also probably regulates the abiotic stress response through DNA methylation and short interfering RNA pathways. Further studies on ABA-regulated epigenome will be of immense use to understand the plant development, stress adaptation and stress memory.

Keywords

abiotic stress memory; abscisic acid; chromatin remodeling; DNA methylation; histone deacetylases

Abscisic acid (ABA) regulates plant growth and development such as germination, lateral root development, seedling growth, seed development, seed dormancy, transition from vegetative to reproductive phase and abiotic stress tolerance. Diverse roles of ABA in plants suggest the existence of multiple receptors and signal transduction pathways. Only recently three possible ABA-receptors, namely, FCA (flowering time control protein A) (Razem et al. 2006), ABAR (ABA receptor) similar to H subunit of Mg-chelatase (CHLH) (Shen et al. 2006) and G protein-coupled receptor (GCR2) (Liu et al. 2007a) have been identified. Although ABA signaling pathways are not yet thoroughly understood, it is very well established that most of the ABA responses (barring the quick stomatal closure response) are regulated by ABA-mediated transcriptional regulation, which have been extensively reviewed (Schroeder et al.

2001; Finkelstein et al. 2002; Zhu 2002; Chinnusamy et al. 2004; Nambara and Marion-Poll 2005; Yamaguchi-Shinozaki and Shinozaki 2006; Wasilewska et al. 2008). Recent discoveries reveal that besides genetic regulation, epigenetic regulation plays a key role in ABA-mediated plant processes. This review will briefly introduce the epigenetic processes, namely, histone modifications and cytosine DNA methylation, and further focus on the role of epigenetic processes in ABA-mediated developmental and stress response processes.

Epigenetic Processes

Epigenetics is defined as the study of changes in gene function that are mitotically and/or meiotically heritable, and that do not entail a change in DNA sequence (Wu and Morris 2001). Later, the definition included chromosomal marks that are transient as well as heritable (Bird 2007). Epigenetic modifications play pivotal roles in genomic imprinting (Feil and Berger 2007), paramutation (Alleman et al. 2006), defense against transposon proliferation (Vaucheret and Fagard 2001) and transcriptional gene silencing (Gong et al. 2002). Besides these phenomena, genomes are responsive to, and regulated by developmental and environmental cues through epigenetic processes.

Histone code

The basic unit of eukaryotic chromosome is the nucleosome, which consists of a histone-core complex (H2A, H2B, H3 and H4) wrapped around by 146 bp DNA and the linker histone H1 associated with the linker DNA (8 to 114 bp). Nucleosome structure depends on the histone variants and post-translational modifications in the N-terminal tails of core histones. The *Arabidopsis* genome encodes about 50 genes for histones (5 H1, 13 H2A, 11 H2B, 13 H3 and 8 H4). The N-tails of the core histones offer about 240 sites for post-translational modifications such as acetylation (Peterson and Laniel 2005), methylation (Shilatifard 2006), phosphorylation (Houben et al. 2007), biotinylation (Camporeale et al. 2007), ubiquitination (Sridhar et al. 2007), sumoylation (Nathan et al. 2006) and ADP-ribosylation (Hassa et al. 2006). Histone acetylation, phosphorylation, and ubiquitination activate transcription (Peterson and Laniel 2005; Houben et al. 2007; Sridhar et al. 2007), whereas biotinylation and sumoylation repress gene expression (Nathan et al. 2006; Camporeale et al. 2007). Dimethylation of H3 (K9 and K36) represses transcription, whereas trimethylation of H3K4 activates transcription (Zhang et al. 2007). Specific combinations of histone variants and core histones N-tail post-translational modifications called “histone code” offer enormous combinatorial possibilities for nucleosome assemblies. Histone code plays a key role in chromatin structure and affinity for chromatin-associated proteins, and thus regulates the expression of genetic code (Jenuwein and Allis 2001; Fuchs et al. 2006). Chromatin modification is induced by histone modification-dependent chromatin remodeling complexes (CRCs) and ATP-dependent CRCs (SWITCH/SUCROSE NON-FERMENTING, SWI/SNF complex) that non-covalently modify histones in the nucleosome (Fransz and de Jong 2002).

DNA methylation

Cytosine (5') methylation of DNA is the ubiquitous mechanism of heritable epigenetic modification. DNA methylation maintains the methylated loci in a repressed state. In plants, asymmetric methylation (^mCpHpH) is re-established after each mitosis cell division, whereas symmetric methylation (^mCpG and ^mCpNpG) can be maintained across mitosis and meiosis divisions (Martienssen and Colot 2001). The *de novo* DNA methyltransferases catalyze new cytosine methylation, while maintenance DNA methyltransferases propagate the symmetric methylation marks on the parental DNA (Chan et al. 2005). The *Arabidopsis* bifunctional DNA glycosylase/lyase REPRESSOR OF SILENCING-1 (ROS1) actively removes DNA methylation by a base-excision repair mechanism (Gong et al. 2002; Agius et al. 2006), and thus has a key role in the plasticity of the epigenome (Zhu et al. 2007b). *Arabidopsis*

DEMETER (DME), a protein similar to ROS1, is necessary for endosperm gene imprinting and seed viability in *Arabidopsis* (Choi et al. 2002). Post-translational modifications of histones and DNA methylation determine chromatin modeling and remodeling. These modifications can transmit epigenetic memory within one generation and across generations (Khorasanizadeh 2004; Vaillant and Paszkowski 2007).

ABA-mediated Epigenetic Processes in Seeds and Seedlings

Genetic analyses of ABA-deficient and ABA response mutants showed that ABA plays pivotal roles in storage reserve accumulation, desiccation tolerance, and dormancy of seeds and germination arrest. During seed development, two peaks of ABA accumulation were observed. Maternally-derived early peak of ABA is required for embryo maturation and prevention of vivipary. The embryo synthesizes the second peak of ABA accumulation in seed and this ABA appears to regulate dormancy, seed reserve accumulation and desiccation tolerance. Gene expression during seed development and germination inhibition are regulated by ABA through networks of transcription factors belonging to the B3 domain (ABI3, ABA INSENSITIVE3/VP1, VIVIPAROUS1; LEC2, LEAFY-COTYLEDON2; FUS3, FUSCA3), APETELA2 (ABI4), bZIP (ABI5) and HAP3 subunit of CCAAT binding factor (LEC1) (Finkelstein et al. 2002).

Seed maturation

Absciscic acid plays a central role in the accumulation of storage reserve and acquisition of desiccation tolerance during seed maturation. Seed storage protein accumulation requires a B3 transcription factor such as ALF (ABI3-like factor, *Phaseolus vulgaris*), ABI3 (in *Arabidopsis*), or VP1 (in *Zea mays*). The *phaseolin* (*phas*, encoding a major seed storage protein) gene is repressed in vegetative tissues and chromatin remodeling activates transcription of *phas* in seeds. PvALF1 transcription factor activates the ABA-dependent seed-specific expression of *phas*. Repression of *phas* expression is related to the deacetylated chromatin structure on *phas* promoter in leaves. DNase I foot printing assay revealed that the TATA boxes of *phas* are protected in the presence of both PvALF and ABA. ABA-induced expression of *PvALF* and the remodeling of chromatin architecture over the TATA box region of the *phas* promoter are required for *phas* expression (Li et al. 1999). Chromatin immunoprecipitation (ChIP) assay revealed that PvALF potentiates chromatin for transcription by mediating acetylation of H3-K9 and H4-K12 through histone acetyltransferases (HATs), whereas ABA has been shown to induce acetylation of H3-K14 and methylation of H3-K4 (Ng et al. 2006). Thus, chromatin remodeling in a euchromatin region plays a crucial role in ABA-induced gene expression.

Arabidopsis B3 domain transcription factors such as LEC2, FUS3, and ABI3 activate genes involved in the accumulation of storage proteins and lipids in the embryo during seed maturation. After completion of seed maturation, these genes are repressed in matured seeds and vegetative tissues. *Arabidopsis* *pkl* (*pickle*) and *val* (*vp1/abi3-like*) mutants exhibited derepression of embryonic genes in germinating seedlings (Ogas et al. 1999; Suzuki et al. 2007). The *PICKLE* (*PKL*) gene encodes a putative SWI/SNF-class chromatin-remodeling factor of the CHD3 type (harboring a Chromodomain, a SNF2-related helicase/ATPase domain, a DNA binding domain and a PHD zinc finger). *VALs* encode B3 proteins associated with chromatin factors. *val1 val2* double mutant seedlings showed embryonic seedling phenotype, and upregulation of *LEC1*, *ABI3*, and *FUS3* genes. It is suggested that VAL proteins repress the target genes by recruiting a chromatin-remodeling complex that includes PKL and related CHD3 proteins (Suzuki et al. 2007). Histone acetylation dependent chromatin remodeling also appears to regulate seed development as plant-specific histone deacetylases (HDACs) such as HD2A, HD2B, and HD2C have been shown to express in ovules, embryos,

shoot apical meristems, and primary leaves and is strongly induced during somatic embryogenesis in *Arabidopsis* (Zhou et al. 2004).

Seed dormancy

In natural ecosystems, plants complete their life cycle in favorable seasons and the seeds remain dormant during the unfavorable seasons. Thus, seed dormancy is crucial to avoid vivipary (pre-harvest germination of seeds on the mother plant) and germination of seeds under unfavorable conditions (Bewley 1997). Besides, successful agriculture also requires seed dormancy, as seeds harvested in the previous season need to be stored until the next season. Mutations that impair ABA biosynthesis (*aba*) and mode of action (*abi1* to *abi5*, *era1*, *sad1*, *abh1*) also impair seed dormancy and germination under stress conditions. Molecular genetic analyses established the pivotal role of ABA, the original “dormin”, in inducing seed dormancy and prevention of germination. ABA is necessary for seed dormancy in many plants (Finkelstein et al. 2002) and tuber dormancy in potato (Suttle and Hultstrand 1994). However the mechanisms of dormancy and germination arrest are poorly understood. The ABA-mediated chromatin remodeling processes regulate some of the processes involved in seed dormancy and inhibition of germination under unfavorable conditions.

Treatment of potato tubers with bromoethane (BE, artificial breaker of dormancy) resulted in a decline of meristem ABA content due to reduction in the expression of *StNCED* (9-*cis*-epoxycarotenoid dioxygenase) and enhanced expression of *StCYP707A1* and *StCYP707A2* (ABA 8'-hydroxylases that catalyze inactivation of ABA) genes (Destefano-Beltrán et al. 2006). Expression pattern of these genes also showed a similar pattern during the ripening process in *Arabidopsis* seeds. Genetic analysis of *Arabidopsis* mutant, *reduced dormancy4* (*rdo4*), which exhibits reduced seed dormancy, revealed a possible role of chromatin remodeling in seed dormancy through regulation ABA metabolism and response genes (Liu et al. 2007b).

The *RDO4*, which was named later as *HISTONE MONOUBIQUITINATION1* (*HUB1*), encodes a C3HC4 RING finger E3 ligase that catalyzes histone H2B monoubiquitination. Mutation in *HUB2*, a homolog of *HUB1*, also reduced seed dormancy in *Arabidopsis*. *hub1* and *hub2* mutants are deficient in ubiquitinated H2B. Dormancy-related genes that are involved in ABA metabolism and response such as *NCED9* and *ABI4* showed reduced expression, whereas *CYP707A2* showed enhanced expression in the freshly harvested seeds of *hub1* mutant as compared with wild-type. Besides these genes, expression of *DOG1* (*DELAY OF GERMINATION 1*), *ATS2* (a caleosin-like protein) and *PER1* (similar to the per-oxiredoxin family of antioxidants) were reduced in freshly harvested seeds of *hub1* mutant. These expression patterns were similar to that of wild type after-ripened seeds (Liu et al. 2007b). Histone H2B monoubiquitination is associated with activation of gene transcription and deubiquitination is linked to repression (Sridhar et al. 2007). Hence, chromatin remodeling through HUB1 and HUB2 mediated H2B monoubiquitination plays a key role in seed dormancy (Liu et al. 2007b) probably by regulating ABA level in seeds, ABA sensitivity and other mechanisms.

Germination inhibition

Osmotic stress and salt concentration will be high during the non-rainy season and will decrease during the rainy season in salt-affected soils. In nature, scanty rainfall frequently occurs in non-rainy seasons with ensuing longer drought spells. If seeds germinate under these unfavorable conditions, then the chance for their survival is less. Hence, plants have impregnated adaptive mechanisms in seeds to prevent germination if osmotic stress occurs during the initial processes of germination. Exogenous application of ABA or osmotic stress within 48 h of imbibition can arrest or delay germination of *Arabidopsis* seeds. Germination arrest is mediated by induction

and maintenance of *ABI3* and *ABI5* transcription factors expression. Chromatin remodeling is crucial for this germination arrest mediated by ABA and osmotic stresses. In *Arabidopsis*, The PKL (SWI/SNF type chromatin remodeling factor) expression is induced by imbibition and it mediates repression of embryonic traits during germination (Henderson et al. 2004; Li et al. 2005). During germination *LEAFY COTYLEDON1*, (*LEC1*, a transcriptional regulator that promotes embryonic identity) and *FUSCA3* (*FUS3*) are repressed in wild type, whereas *pk1* mutants showed expression of *LEC1* and *FUS3* upon seed imbibition (Ogas et al. 1999). ABA has been shown to have a modest effect on the penetrance of *pickle* root phenotype (Henderson et al. 2004). Further, *pk1* mutants exhibit hypersensitive germination responses to ABA, and persistent high expression of *ABI3* and *ABI5* on ABA induction. ChIP studies comparing wild-type and *pk1* mutant seeds treated with or without ABA revealed ABA-treated *pk1* mutant seeds with 2.0–2.5-fold lower H3-K9 and H3-K27 methylation levels at *ABI3* and *ABI5* promoters than wild-type plants. Thus, chromatin remodeling mediated by PKL represses *ABI3*, *ABI5* and some late embryogenesis abundant genes expression after imbibition to promote germination (Perruc et al. 2007).

Besides the histone methylation, histone deacetylation is also involved in ABA sensitivity during germination. Some of the histone deacetylases (HDACs) are induced by ABA in *Arabidopsis* (Sridha and Wu 2006) and rice (Fu et al. 2007). Impairment of expression of *AtERF7* (APETALA2/EREBP-type transcription factor) showed a role for HDA19 in ABA sensitivity during seed germination. *Arabidopsis* ERF7 interacts with a global corepressor of transcription, AtSin3, which in turn interacts with HDA19. HDA19 and AtSin3 enhance the AtERF7 mediated transcriptional repression. RNA interference (RNAi) mediated suppression of *AtERF7* and *AtSin3* in transgenic plants and resulted in increased sensitivity to ABA during germination and seedling growth. ABA signaling appears to repress the genes through histone acetylation (Song et al. 2005). A transgenic *HDA6-RNAi* repression line treated with the HDAC inhibitor (trichostatin A) showed growth arrest and elevated expression of *LEC1*, *FUS3*, and *ABI3* during germination. *lec1*, *fus3*, and *abi3* mutants suppressed the growth-arrest phenotype of the *HDA6-RNAi* repression plants. The double repression line of *HDA6/HDA19* displayed arrested growth after germination and the formation of embryo-like structures. These results suggest that HDA6 and HDA19 redundantly regulate the repression of embryonic properties and growth arrest during germination (Tanaka et al. 2008). In *Arabidopsis*, HDA6 has also been implicated in transgene silencing and the regulation of ribosomal RNA transcription (Probst et al. 2004; Earley et al. 2006). These lines of evidence suggest that histone deacetylation plays important roles in induction of embryonic genes and germination inhibition under unfavorable environments.

Epigenetic Processes in ABA-mediated Abiotic Stress Responses

The prominent role of ABA in abiotic stress adaptation of plants is well known. Stomatal regulation by ABA is crucial for regulation of transpiration and avoiding water-deficit stress in plants. ABA-mediated regulation of ion channels in guard cells is crucial for stomatal closure under unfavorable conditions. Calcium, protein kinases and phosphatases, and membrane trafficking components have been implicated in ABA signaling in guard cells (Schroeder et al. 2001). Similarly, calcium, protein kinases, protein phosphatases and transcription factor networks regulate expression of ABA-responsive genes and stress adaptation (Finkelstein et al. 2002; Zhu 2002; Chinnusamy et al. 2004; Yamaguchi-Shinozaki and Shinozaki 2006; Wasilewska et al. 2008).

Histone code regulates stomatal response

Histone variants offer a means to alter histone code. In tomato, the linker histone H1 variant, H1-S, was induced by drought through an ABA-dependent pathway (Bray et al. 1999; Scippa et al. 2000). This H1-S might be positively involved in drought tolerance by controlling

transpiration as H1-S antisense tomato transgenic plants showed about 37% higher stomatal conductance, transpiration and photosynthetic rate than the wild type plants (Scippa et al. 2004). ABA might mediate stomatal response by chromatin remodeling through change in histone acetylation status at certain loci. Histone deacetylation also appears to regulate guard cells. *Arabidopsis* ERF7 interacts with AtSin3 and HDA19 to repress transcription of some genes. Transgenic *Arabidopsis* plants overexpressing *AtERF7* show reduced sensitivity of guard cells to ABA, and increased transpirational water loss (Song et al. 2005). Further, ABA represses the expression of *AtHD2C*, and overexpression of *AtHD2C* resulted in a lower transpiration rate than that of the wild type plants (Sridha and Wu 2006). These results show that ABA-induced stomatal response mediated by chromatin remodeling plays a crucial role in stress tolerance.

ABA-mediated stress-responsive gene expression

Histone deacetylation results in a non-permissive chromatin conformation that represses transcription. *Arabidopsis* histone deacetylases HD2A, HD2B and HD2C have been implicated in transcriptional repression of genes (Wu et al. 2003). Expression analysis of rice *HDAC* genes revealed that the expression of several *HDACs* is influenced by plant hormones as well as abiotic stresses. ABA represses the expression *HDT701*, *HDT702*, *SRT701* and *SRT702* in rice. Abiotic stresses such as cold, mannitol and salt also repress *SRT701* and *SRT702* (Fu et al. 2007). Abiotic stresses induce ABA accumulation, which in turn might repress the *HDACs*. In *Arabidopsis*, ABA represses the expression of *AtHD2C*. Transgenic *Arabidopsis* plants overexpressing *AtHD2C* exhibited ABA-insensitive phenotype as well as greater tolerance to salt and drought stresses than the wild type plants. The enhanced tolerance of transgenic plants constitutively overexpressing *AtHD2C* was partly due to increased expression of ABA-responsive LEA-like genes (*RD29B* and *RAB18*), and decreased expression of *ABI2*, *ADH1* (alcohol dehydrogenase 1), *SKOR* (K^+ outward rectifier), *KAT1* and *KAT2* (K^+ inward rectifier) (Sridha and Wu 2006). These results show that ABA mediates abiotic stress responses through dynamic regulation of histone acetylation levels.

Further evidence for roles of histone acetylation in ABA and abiotic stress response was emerged from genetic analysis of *hos15-1* (for high expression of osmotically responsive genes) mutant of *Arabidopsis*. An *RD29A::LUC* reporter-facilitated genetic screen led to the identification of the mutant *hos15-1* mutant, which showed super-induction of *RD29A::LUC* in response to exogenous ABA, cold and salt stresses. *hos15-1* mutants exhibit hypersensitivity to inhibition of germination by ABA and NaCl stress and also hyper-sensitive to freezing stress. *HOS15* encodes a protein similar to the human WD-40 repeat protein TBL1 (transducin beta-like protein-1), a component of the chromatin repressor complex involved in histone deacetylation. ABA and abiotic stresses (cold, NaCl) enhanced the expression levels of *HOS15*. In *Arabidopsis*, *HOS15* interacts with histone H4 and the level of acetylated histone H4 is higher in *hos15* mutants as compared with that of the wild type. Under cold stress conditions, substantially higher levels of acetylated histone H4 are associated with higher expression levels of *RD29A* in the *hos15* mutant than in wild type. Consistent with this, the induction of the *RD29A* gene is higher in the *hos15* mutant than in the wild type under cold stress. Thus, ABA induces expression of *HOS15* which in turn regulates ABA and abiotic stress responses through H4 deacetylation-dependent chromatin remodeling in *Arabidopsis* (Zhu et al. 2007a).

ATP-dependent CRCs also play a role in ABA-mediated stress responses. ABA and drought stress induce the expression of *PsSNF5* (*Pisum sativum* *SNF5*), a CRC protein, in germinating embryos as well as vegetative tissues. *PsSNF5* interacts with *Arabidopsis* SWI3-like proteins (*SWI3A* and *SWI3B*) in heterologous expression systems (Ríos et al. 2007). In yeast two-hybrid assay, *AtSWI3A* and *AtSWI3B* also interact with FCA, one possible ABA receptor

(Sarnowski et al. 2005). Thus, ABA regulates the expression levels of *PsSNF5*, which in turn can mediate chromatin remodeling to regulate ABA-responsive gene expression (Ríos et al. 2007).

Regulation of DNA methylation by ABA

DNA methylation is associated with repression of gene expression, whereas demethylation leads to enhanced expression of genes (Zilberman et al. 2007). Cold stress induced DNA demethylation in maize (Steward et al. 2002). In tobacco, Aluminum, salt and cold stresses induced demethylation of the glycerophosphodiesterase-like protein (*NtGPD*) gene, followed by upregulation of *NtGPD* in leaves (Choi and Sano 2007). In tobacco cell-suspension culture, osmotic and salt stresses induced DNA hypermethylation in two heterochromatic loci, which was reversible when cells were returned to non-stress media (Kovarík et al. 1997). Water-deficit stress also induced specific cytosine hypermethylation (CCGG) in the pea genome (Labra et al. 2002). Although, ABA accumulation and the role of ABA in gene expression under abiotic stress are well known, the role of ABA in the DNA methylation/demethylation dependent gene expression is not understood.

H2B monoubiquitination (K143) regulates the methylation of histones H3 and deubiquitination of H2B directs CpNpG and CpNpN methylation. H2B deubiquitination by the nuclear ubiquitin protease SUP32/UBP26 is required for transcriptional gene silencing (TGS) (Sridhar et al. 2007). The *HUB1* gene has been shown to monoubiquitinate histone H2B, which positively mediates ABA-induced seed dormancy, as *bub1* mutation reduced the expression of *NCED9* and *ABI4*, but enhanced the expression of *CYP707A2* (Liu et al. 2007b). These results suggest a potential role of ABA-mediated processes such as stress response in addition to seed development.

Small RNAs

Non-coding regulatory small RNAs (~20–24 nt long) namely microRNAs (miRNAs) and small-interfering RNAs (siRNAs) have emerged as important players in plant development and stress responses (Jones-Rhoades et al. 2006; Sunkar et al. 2007). RNA-dependent RNA polymerases (RDR) play a crucial role in siRNA and miRNA biogenesis. *AtRDR6/SGS2/SDE1* (*RNA-dependent RNA polymerase6/SUPPRESSOR OF GENE SILENCING2/SILENCING DEFECTIVE1*) catalyzes the synthesis of dsRNA from various RNAs derived from transgene, viral replication, natural antisense transcripts or miRNA-cleaved mRNA (Vazquez et al. 2004; Borsani et al. 2005; Wassenegger and Krczal 2006). *OsRDR6* promoter contains an ABA-responsive DNA *cis*-element (ACGTG) at the –680 region and exogenous ABA application enhanced the *OsRDR6* transcript level in rice cells grown on either acetate or sucrose. Further, ABA silences the *isocitrate lyase* (*ICL*) gene (a glyoxylate cycle enzyme) through the RDR6-generated siRNA(*ICL*) mediated post transcriptional gene silencing mechanism (Yang et al. 2008). *Arabidopsis* RDR2 is involved in transcriptional gene silencing (TGS) (Chan et al. 2004). Non-coding RNAs can also induce epigenetic changes through chromatin or DNA methylation (Bond and Finnegan 2007). Some ABA-induced small RNAs and RDRs can play a role in epigenetic regulation of abiotic stress response.

An endogenous siRNA identified from stress-treated *Arabidopsis* plants is complementary to At2g27152 (AAO3, a drought stress-inducible ABA biosynthesis enzyme). ABA and abiotic stresses have been shown to upregulate miR402. The predicted target of ABA-upregulated miR402 is At4g34060, which encodes a DNA glycosylase domain protein, DML3 (DEMETER-LIKE PROTEIN 3) similar to ROS1 (Sunkar and Zhu 2004). Two of the homologs of DML3 in *Arabidopsis* are involved in epigenetic regulation. The DME/DEMETER (*DME*) DNA glycosylase is required for endosperm gene imprinting and seed viability (Choi et al. 2002). The ROS1 DNA demethylase has a key role in the plasticity of the

epigenome (Gong et al. 2002; Agius et al. 2006; Zhu et al. 2007b). It is probably that ABA-induced RDR6 might synthesize siRNA that target AAO3 and thus regulate the cellular levels of ABA under stress conditions. ABA-induced miR402 might reduce the transcript levels of DML3, which in turn can result in increases in methylation levels at certain loci and thus, repression of gene expression.

Abiotic Stress Memory

Abiotic stress-induced changes in the genome are necessary for stress memory. UV-C radiation or flagellin (an elicitor of plant defense) stress-induced changes in the genome have been shown to be a dominant trait and transmitted through both the maternal and the paternal crossing partner in *Arabidopsis* (Molinier et al. 2006). Progeny of tobacco mosaic virus (TMV) infected plants showed enhanced frequency of the somatic and meiotic recombination rates at certain loci due to DNA hypomethylation. Thus, stress memory appears to be inherited through epigenetic changes (Boyko et al. 2007). ABA accumulation in vegetative tissues is induced by abiotic stresses. ABA also enhances the somatic recombination frequency (Z Gong et al. unpubl. data, 2008). As discussed in this review, ABA induces chromatin remodeling. Chromatin remodeling regulates transcription, recombination, replication and genome organization (Meyer 2001). It is probably that ABA might also regulate DNA methylation through DML3 and siRNAs. Under abiotic stresses ABA mediates reduction in plant growth. One of the mechanisms of abiotic stress-induced growth arrest under abiotic stress is regulated by chromatin remodeling (Mlynárová et al. 2007). Abiotic stress-induced inheritable epigenetic state might have an adaptive advantage but also a negative effect on the yield of crops, as stress memory can inhibit the expression of full potential of crops. Hence it is imperative to understand the role of ABA in abiotic stress memory to use them for agricultural advantage.

Concluding Remarks and Perspectives

Absciscic acid regulates plant development and abiotic stress tolerance through genetic and epigenetic mechanisms. The role of ABA in epigenetic processes is just beginning to emerge (Figure 1). Seed development and germination are vital processes not only for plant survival but also for other organisms including humans, as successful agriculture depends on these processes. ABA plays a pivotal role in seed development and dormancy by direct induction of gene expression or histone ubiquitination, acetylation and methylation-dependent chromatin remodeling. In turn, these epigenetic processes might regulate ABA levels through change in the expression of genes involved in ABA metabolism. Transpiration, stomatal opening and plant growth are regulated under abiotic stresses through repression of histone deacetylation and chromatin modeling. ABA-regulated histone acetylation levels either through ABA-repression of histone deacetylases or repression of HOS15 (a protein similar to the human WD-40 repeat protein TBL1) plays a key role in chromatin remodeling, abiotic stress responsive gene expression and stress tolerance of plants (Figure 2). ABA also probably regulates siRNA pathways and DNA methylation under abiotic stresses. It is not known whether ABA-induced epigenetic changes in response to stresses are heritable through mitosis as well as meiosis and their adaptive advantage. Further studies on ABA-regulated epigenome will be necessary for better understanding of seed development, storage reserve accumulation in seeds, seed dormancy, plant development, abiotic resistance and engineering of these processes for agricultural use.

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Figure 1. Abscisic acid (ABA) mediated epigenetic processes regulate seed maturation, dormancy and germination inhibition

HISTONE MONOUBIQUITINATION1 (HUB1) monoubiquitinates H2B, which in turn regulates ABA-levels by inducing *NCED* and repressing *CYP707A2*. Enhanced ABA level induces *ABI4* expression and H2B monoubiquitination induces the expression of *DOG1*, *ATS2* and *PER1*. These proteins in turn induce seed dormancy. VP1/ABI3-like (VAL proteins recruits SWI/SNF class chromatin remodeling complex, PIKLE (PKL). PKL and histone deacetylases (HDACs) dependent chromatin remodeling repress the expression of genes involved in seed maturation and stress-induced germination arrest. ABA probably downregulates HDACs (HDA6, HDA19) and also induces the expression of these genes

involved in seed maturation and stress-induced germination arrest (ABA2, short chain dehydrogenase; ABI, ABA insensitive; AAO3, abscisic aldehyde oxidase; ATS2, a caleosin-like protein; CYP707A, ABA 8' hydroxylase; DOG1, DELAY OF GERMINATION1; FUS3, FUSCA3; H2B, histone 2B; LEC1, LEAFY COTYLEDON1; NCED, 9 *cis*-epoxycarotenoid dioxygenase; PER1, peroxiredoxin family of antioxidants; UQ, ubiquitin; ZEP, zeaxanthin epoxidase).

Figure 2. Abscisic acid (ABA)-mediated chromatin remodeling regulates stress responsive gene expression and stress tolerance

Abiotic stresses induce accumulation of ABA. ABA enhances the expression levels of *HOS15*, which encodes a protein similar to the human WD-40 repeat protein TBL1 (Transducin Beta-Like protein-1), a component of the chromatin repression complex involved in histone deacetylation. Conversely, ABA can also inhibit histone deacetylation through repression of histone deacetylases (HDACs). ABA and drought induce an ATP-dependent chromatin remodeling complex protein, SNF5. Histone deacetylation dependent and ATP-dependent chromatin remodeling complexes regulate gene expression and stress response (Ac, acetyl group; H4, histone4; HOS15, high expression of osmotically responsive genes 15).