Chapter 36

Transgenerational Genome Instability in Plants

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Chapter Outline

1. INTRODUCTION

It is commonly accepted that cell division and organism reproduction are characterized by the faithful replication of the genetic material, DNA. The DNA sequence represents a master key that is used to reproduce cells and organisms with the identical genetic makeup. In contrast, the regulation at the epigenetic level, including epigenetic inheritance, represents a more versatile and flexible mechanism controlling gene expression and inheritance of old traits as well as the appearance of new traits. Why would it then be so important to preserve the integrity of DNA if downstream mechanisms are able to alter outcomes in terms of proteins produced, metabolites, and phenotype appearance? Epigenetic mechanisms are frequently reversible because they do not represent permanent chemical changes. In contrast, changes in nucleotide sequences such as base substitutions, deletions, and mutations are irreversible, unless a reversion mutation (mostly a single base modification) occurs. Thus, it is of an outmost importance to preserve the master key code.

Plants maintain genome integrity at all times, whether it is at the stage of active development and cell division or cell growth. An active metabolism, including photosynthesis, cellular respiration, and other physiological activities associated with the function of peroxisomes and lysosomes, poses a continuous challenge for plant genomes. These types of internal

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stresses result in the production of free radicals that are either damaging DNA directly or triggering changes in DNA via a variety of signaling pathways they are involved in. Radicals are also able to oxidase lipids and proteins, thus rendering them incapable of a normal cellular activity.

Being sedentary in nature, plants are at a constant state of war with the environment. Environmental stimuli represent external stresses that include, but are not limited to, changes in light intensity, temperature fluctuations, water and nutrients availability, wind and other mechanical stimuli, and an entire realm of biotic interactions that include physical and chemical influences. A good review describing different types of abiotic and biotic stresses that plants are exposed to is written by Madlung and Comai [1].

To survive these environmental pressures, organisms have to respond using the mechanisms that are already available, but they also have to develop new adaptive changes that provide advantages to them if new conditions persist. Not being able to escape external stresses, plants are limited to mechanisms of tolerance and resistance, the strategies that plants are extremely proficient at Ref. [2]. Adaptive metabolic changes in somatic cells and heritable transgenerational changes are among more sophisticated mechanisms of survival [3]. Through the process of evolution, organisms have developed efficient adaptive mechanisms of survival, and plants seem to be very efficient in doing that [4].

Plants also have the ability to maintain genome stability in the ever-changing growth environment. Many plant species seem to possess additional copies of various DNA-repair genes that often have redundant functions [5]. That is why studying DNA-repair capacity in plants using mutants is so challenging. For example, plants possess four Rad51 paralogs, AtXrcc2, AtXrcc3, AtRad51B, and AtRad51C. A mutation in any of these genes results in hypersensitivity to DNA-damaging agents such as mitomycin C [6]. Moreover, the *atrad51c* and *atxrcc3* mutants show meiotic defects and thus are difficult to propagate.

Genome integrity is maintained through a number of different mechanisms, with the direct repair of DNA damage being perhaps the most important one. There are multiple levels of control of the process of DNA-damage repair, including scanning and the identification of damage, the global or local relaxation of chromatin, the recruitment of the repairsome, actual repair steps, and the reestablishment of a similar or perhaps different status of chromatin, including changes in DNA methylation and histone modifications [7]. Since there is a possibility that chromatin compaction has a buffering ability against various factors that can damage DNA, it is plausible to think that genome stability of a given chromosomal region can be relaxed not only by choosing different DNA-repair pathways but also by introducing or removing various epigenetic modifications [8].

The control over DNA repair and genome stability is thus regulated by a variety of genetic and epigenetic factors [8]. While different DNA-repair pathways are described in great details in the other chapters of this book, the purpose of this chapter is to describe the epigenetic mechanisms that can affect and modify genome stability, with a special emphasis given to transgenerational responses.

2. GENOME STABILITY MAY DEPEND UPON THE CHOICE OF THE DSB DNA-REPAIR PATHWAY

Double-strand break (DSB) is the most dangerous DNA lesion because a single unrepaired DSB may lead to cell-cycle arrest or apoptosis. DSBs are repaired via two major repair pathways: nonhomologous end joining (NHEJ) and homologous recombination (HR) [9]. The repair via NHEJ involves a direct rejoining of break ends and does not require a significant homology between the interacting DNA molecules. In the cases when rejoining via a direct ligation process is not possible, NHEJ proteins Ku70/Ku80 search for microhomology aligning one or several complementary bases to direct repeats, and thus resulting in a removal of DNA between direct repeats. As a result, the repair via NHEJ is relatively inaccurate and is typically associated with small- and large-scale deletions, ranging from a single base pair to large DNA sequences of several thousand nucleotides [9]. Insertions and point mutations are frequent outcomes as well. In contrast, the HR mechanism requires an extensive sequence homology and the presence of a repair template. The repair via HR is quite accurate if perfectly homologous templates such as a sister chromatid or a homologous chromosome are used to prime repair synthesis. However, HR repair using a template with imperfect homology could result in gene conversion events leading to a loss of heterozygosity. In rare cases, if HR repair occurs in DNA regions containing multiple repeats, the process can result in gene translocation and duplication events as well deletions of an entire chromosome. Overall, NHEJ can be characterized as a fast-track error-prone repair mechanism, whereas HR represents a rather slow but relatively error-free process [10]. Detailed information about types of NHEJ and HR repairs in plants can be found in Chapter 12.

The balance between the occurrence of NHEJ and HR is tightly regulated and depends on such factors as the availability of repair templates, the phase of a cell cycle, the rate of cell proliferation, and even the specific function of a given cell type (reviewed in Ref. [11]). NHEJ is a predominant DNA-repair pathway, and cells use it more often while being in the

G1 phase of a cell cycle. In contrast, HR is rather a minor pathway that is more active during S and G2 phases when sister chromatids are formed [12]. Thus, HR plays an important role in the actively dividing cells and during early development of an organism. Moreover, in plants, tissues with a higher ploidy level seem to use HR less frequently. Experimental data show that older plant leaves that tend to have a larger number of genomes per single cell (due to endo-reduplication) have a lower frequency of HR when prorated to a single genome [13]. This is not surprising because an increase in ploidy results in an increase in the number of copies of potential templates available for HR repair, posing a threat to genome stability and possibly leading to large-scale deletions and chromosomal translocations. It is also possible that the genome size has also some effects on the frequency of the use of HR repair [14,15]. It may be more difficult and time-consuming to find homologous sequences in the larger genomes.

Chromatin structure in more complex genomes may also contribute to an additional difficulty for HR to occur more frequently. Since mutation frequency varies in different cell types and during different developmental stages of an organism, there definitely exist some types of chromatin-based regulation of genome stability. Further, we attempted to summarize the accumulated data on epigenetic control over the stability of highly repetitive genomes in plants.

3. EPIGENETIC REGULATION OF PLANT GENOME STABILITY

Plants dramatically differ from most other higher eukaryotes in a specific feature of their life—they are sedentary organisms. The prolonged nature of environmental conditions that has an impact on plant growth continuously poses challenges to plant defense systems, sometimes over many generations. Unlike other organisms that can leave their environment, plants cannot use escape and avoidance tactics to minimize the damaging influence of stress. It is thus logical that plants possess both short-term response systems and long-term defense strategies allowing them to cope with acute and chronic stresses. In fact, stresses typically persist for a long time, and therefore they should not be considered acute to plants. An acute stress is usually defined as a stress that affects an organism in the short term. Such definition is relative in relation to plants and can be applied only if the very same stress persists for a substantially longer period of time. When speaking about stresses that plants face, it would be more appropriate to use a term such as "high and low levels of an acute stress." A rapid alteration in homeostasis, including massive changes in the number and amount of produced metabolites, is one of the mechanisms through which plants respond to stresses. These immediate responses include flexible changes in gene expression, an increase or a decrease in the production of mRNAs, synthesis of proteins, protein and RNA degradation, synthesis and re-compartmentalization of various metabolites, balancing the salt concentration, pH, hormones, and many other events. Although these responses are critical for plant survival, their description is not the focus of this chapter; all the necessary information can be found elsewhere, including reviews by [16,17].

Many of the events described before are controlled by epigenetic mechanisms operating in somatic cells, including small RNA-mediated mRNA degradation, changes in DNA methylation and histone modifications, repositioning of histone and non-histone chromatin-binding proteins in the nuclear matrix. All these mechanisms are important for immediate plant survival. How does the epigenetic machinery protect plants and plant genomes from stress? In the following sections, we present some information on the role of each of the mechanisms mentioned in the preceding paragraphs in genome protection against stresses.

3.1 Chromatin Structure, a Response to Stress and Genome Stability

Responses to stress, including responses to genotoxic stress, involve transcriptional activation and repression of various genomic loci. Changes in chromatin structure play the most active role in this process. Chromatin decondensation involves the action of ATP-dependent remodeling complexes, covalent modifications of histones, deposition of histone variants, and/or changes in cytosine methylation. Moreover, noncoding small RNAs (smRNAs) add another level of complexity to the process as they can alter chromatin structure by directing heterochromatin formation at specific genomic sequences.

Chromatin in cells exists in different states of packaging that involve the wrapping of the DNA around the histone core. These structures, called nucleosomes, prevent the process of transcription, replication, and DNA repair from occurring. Specific histone modifications allow the unpacking of chromatin. Unpacking the damaged DNA is a double-edged sword which allows DNA-repair enzymes to fix the damage but at the same time makes DNA more vulnerable for further assaults. Specific histone modifications, primarily acetylation and methylation, make DNA more or less accessible to potential damaging agents and various rearrangements [18].

There are several experimental evidences which suggest the interdependence of DNA methylation and histone modifications. Effector proteins such as HETEROCHROMATIN PROTEIN1 (HP1) can be recruited to methylated histones: HP1 binds to methylated H3K9 and helps propagate heterochromatin to the adjacent regions of a chromosome [19]. This interaction is apparently important for a response to changes in the environmental conditions. For example, the *Arabidopsis*

homolog of HP1, HETEROCHROMATIN PROTEIN1 (LHP1), is involved in regulating flowering time in response to environmental stimuli.

Methylated DNA also recruits various chromatin modifiers; methylated cytosines serve as substrates for binding of nuclear proteins named methyl-CpG-binding domain proteins (MBDs) [20]. MBDs bound to 5-methyl-cytosines recruit enzymes that modify core histone proteins and change the local chromatin structure. Similarly, HP1 protein binds methylated DNA and recruits histone modifiers [21].

3.1.1 Changes in Chromatin Structure in Response to Stress: Heterochromatin Decondensation

Nucleosome positioning, redistribution of heterochromatin and euchromatin in the nucleus, and the differential binding of chromatin-modifying proteins (excluding histones) and MBDs to DNA represent another level of complexity for an efficient response to developmental cues and environmental factors.

Chromatin condensation is critical for maintaining transcriptional gene silencing at repetitive elements. The removal of nucleosomes from specific genomic locations in response to stress could be both an active and a passive process. The fact that the original nucleosome loading and epigenetic regulation of repeats are restored fairly quickly upon recovery from stress suggests that the removal of nucleosomes can indeed be an active process. Alternatively, nucleosome loss from specific genomic positions can be associated with replication and transcription, thus representing a passive process. A study by Pecinka et al. showed that long-term exposure to heat in *Arabidopsis* resulted in the activation of some repetitive elements [22]. Surprisingly, the activation occurred without loss of DNA methylation and with only minor changes to histone modifications. Repetitive elements were primarily activated by the loss of nucleosomes and heterochromatin decondensation. The recovery from stress was characterized by nucleosome loading and transcriptional silencing. Curiously, in chromatin-assembly factor-1 (CAF-1) mutants impaired in chromatin-assembly functions, the recovery stage and nucleosome loading were considerably delayed [22]. The substantial dissociation of heterochromatin was observed beyond the recovery phase when silencing and nucleosomes had been reinstalled; the loss of heterochromatin was observed in differentiated tissues of plants exposed to heat, and it lasted in the exposed leaves until they started to show signs of senescence. Heat-induced decondensation of chromocenters and a general loss of nucleosomes presumably allowed a better accessibility of DNA to transcription complexes. A similar heterochromatin decondensation was observed in 2-day-old *Arabidopsis* plantlets in response to cell culturing, although regular chromocenters were formed in a stepwise process after a longer period in culture. The loss of heterochromatin also occurred in older plants upon floral transition in development, however, heterochromatin decondensation was not sufficient for repeat activation. Thus, local heterochromatization occurs during normal physiological and developmental processes and (un)specific responses to stress. Indeed, when plants were exposed to low-light stress, heterochromatin decondensation was more permanent and was directed toward areas with repetitive elements [23]. The reversibility of these changes was confirmed by prolonged culturing of plants exposed to low-intensity light; at a higher-light intensity, chromatin decondensation was eliminated.

Hence, is heterochromatin decondensation at genomic repeats a common response to stress? Pecinka et al. argue that it does not seem to be the case as they did not observe this phenotype after freezing (−4°C for 24 h) or UV-C irradiation (3000 J/m2) [22]. In fact, exposure to an abiotic stress may interfere with the plants' capacity to withstand a biotic stress. Indeed, even moderately increased temperatures can reduce biotic stress resistance by pathogens. In plants exposed to long-term heat stress, the activation of some repetitive elements is paralleled by silencing and transcriptional repression of repetitive loci carrying clusters of resistance genes [22].

Heterochromatin decondensation in response to heat stress seems not to occur equally in all tissues; the nuclei of meristematic cells do not undergo heat-induced decondensation. Actually, this does make sense. If one considers that heat stress response is transient in nature and should largely occur in somatic tissues only, the lack of changes in the meristem indicates a safeguarding mechanism for minimizing epigenetic and possibly genetic changes in the germ line. It further supports the hypothesis that decondensation is a controlled process that occurs only either during specific stages of plant development or in response to specific stresses such as heat and high light–intensity stresses. Moreover, exposure to these stresses may result in the transcriptional activation of heterochromatin-embedded genes in differentiated cells but not in dividing cells.

These results demonstrate that environmental conditions can transiently overcome epigenetic regulation and, perhaps, provide a chance for more permanent epigenetic and possibly genetic changes. The transcriptional activation of repeats occurring without DNA methylation resembles the effect of mutations in MOM1, FAS1, FAS2, BRU1, and RPA2; mutants of these plants also exhibit various degrees of activation of repetitive elements that occur without changes in methylation.

3.1.2 The Role of Chromatin-Remodeling Factors

Several chromatin-remodeling factors in plants help control gene expression and genome stability through DNA methylation and histone modifications. One of the best-known proteins, the DECREASED DNA METHYLATION1 (DDM1)

protein, is a member of the SWI2/SNF2 DNA helicase family. Members of this family of proteins are involved in the control of DNA repair, recombination, gene expression, and replication [24]. It is suggested that one of the possible mechanisms of interactions of the SWI2/SNF2 family proteins with chromatin requires the disruption of DNA–histone interactions. DDM1, in particular, is involved in the regulation of DNA methylation status via changes in histone methylation as well as interactions with AtMBDs. It has been shown that the mutant of DDM1, *ddm1*, exhibits a disrupted localization of AtMBDs at chromocenters, suggesting that DDM1 may facilitate the localization of MBDs at specific nuclear domains [25].

The importance of *ddm1* for the control of DNA methylation is reflected by the fact that the *ddm1* mutant shows up to 70% reduction in global genome methylation [26]. As a consequence, this triggers the activation of transposons and retrotransposons, the transcriptional activation of a previously silent disease-resistance gene array, and the profound phenotypic instability amplified with every generation of self-propagation. The fact that *ddm1*-induced hypomethylation of various genes can be stably inherited through mitotic and meiotic cell divisions might be one of the reasons of the phenotypic instability [27]. One of the possible mechanisms of the involvement of DDM1 in the control of DNA methylation is the maintenance of CpG methylation at RNA-direct DNA methylation (RdDM)-targeted sequences after the RNA signal is removed. Although the data on genome instability in *ddm1* is scarce, one can hypothesize that the genome of *ddm1* is unstable since plants have the increased activity of transposons and retrotransposons. *ddm1* plants are more sensitive to a variety of stresses and appear to have a higher frequency of DSBs.

Another potential chromatin-remodeling factor is the nuclear MAINTENANCE OF METHYLATION 1 (MOM1) protein. It is involved in DNA methylation–independent silencing of repetitive sequences in *Arabidopsis* by preventing the transcription of 180-bp satellite repeats of transposons [28]. Curiously, in *mom1* mutants, releasing transgene silencing, the activation of transcription of 180-bp satellite repeats and *106B* dispersed repeats, and derepression of silencing of some 5S repeats occur without reducing/alternating their DNA and histone-methylation patterns. This suggests the existence of two distinct epigenetic-silencing pathways: one that is DNA-methylation dependent and the other one that is DNA-methylation independent. Although MOM1 is involved in chromatin remodeling, the mutant is not hypersensitive to the DNA-damaging agent methyl methane sulfonate (MMS). Other chromatin modifiers, such as BRU1, FAS1, FAS2, and RPA2, are also dispensable for DNA methylation, but all of them are hypersensitive to the MMS-induced DNA damage.

Other reports also indicated the link between chromatin maintenance and stress response. Mutants of a nuclear protein BRU1 involved in the maintenance of chromatin structure were highly sensitive to genotoxic stress and were characterized by an increased frequency of intrachromosomal HR [29]. Similarly, the expression of the *MIM1* gene involved in the maintenance of chromosome structure and required for the efficient HR was significantly increased by DNA-damaging agents.

Another SWI/SNF-like protein, DRD1, represents a novel plant-specific chromatin-remodeling protein that is required for RNA-directed de novo methylation of target promoters [30]. It is also necessary for the total loss of de novo DNA methylation after the RNA-silencing trigger is withdrawn. DRD1 interacts with two other factors, NRPD1b and NRPD2a, which represent subunits of a novel, plant-specific RNA polymerase, pol IVb. Together, DRD1 and the pol IVb complex act downstream of the small RNA (smRNA) biogenesis pathway (see later). Thus, they direct reversible silencing of euchromatic promoters in response to RNA signals possibly through the recruitment of DNA methyltransferases for methylation of homologous DNA sequences. It is noteworthy that among putative DRD1 targets, there are DNA glycosylases, ROS1 and DME, which are involved in active DNA demethylation. The downregulation of *ROS1* in *drd1* and *pol IVb* mutants confirms the importance of the DRD1/pol IVb pathway for the active loss of induced de novo DNA methylation [31].

3.2 The Role of DNA Methylation in the Maintenance of Plant Genome Stability and Response to Stress

DNA methylation is the most versatile mechanism involved in the regulation of gene expression, including the inheritance of specific gene expression patterns through somatic or meiotic cell divisions. Since DNA methylation is typically associated with a more restrictive chromatin state, it is highly likely that regions with higher methylation would be more stable—that is, they will have fewer mutations associated with them. Is that actually true?

There are not many reports indicating a negative correlation between DNA methylation and rearrangements. An earlier work demonstrated that DNA methylation suppresses the occurrence of HR between dispersed sequences, restricting recombination events to the gene-rich regions with a lower level of methylation [32,33]. In *Hevea brasiliensis*, the inverted correlation between DNA-methylation levels and gene rearrangements was observed [34]. In contrast, a study by Mirouze et al. could not find any significant correlation between the level of methylation and HR frequency in the *Arabidopsis* genome [35]. Also, the authors showed that the progeny of crosses between wild-type and *met1* mutant *Arabidopsis* plants impaired in the maintenance of CpG methylation showed the increased meiotic recombination frequency in the hypomethylated chromosome arms but not in the hypomethylated heterochromatic pericentromeric regions. It remains to be shown

what other factors regulate the recombination frequency in the plant genome. Further studies analyzing changes in histone modifications and the binding of nonhistone chromatin proteins may allow to establish a better correlation between genome rearrangements and chromatin structure.

Stress may result in both hypo- and hypermethylation at specific genomic loci, and these changes may represent either a short-term change or a long-term strategy of response to stress [34]. Promoters of stress-responsive genes are often found to be hypomethylated [36,37], whereas methylation at other genomic loci may not be altered, and sometimes may even be increased.

Changes in DNA methylation in response to stress may occur due to many different mechanisms, including the activity of DNA methyltransferases, DNA demethylases such as ROS1, DME1, DML2, and DML3, a passive loss of methylation via the exclusion of DNA methyltransferases from the nucleus, changes in the activity of chromatin-remodeling factors and effector proteins, and many other changes in proteins regulating the chromatin structure. The regulation of methylation is a complex process, and the absence of one or several DNA methyltransferases does not necessarily result in a total loss of DNA methylation. In the *met1* plants that lack the maintenance methyltransferase, global hypomethylation is accompanied by hypermethylation at multiple transposons and repetitive element loci. The expression of DNA demethylases, *DME* and *ROS1*, is repressed in the mutant, likely as an overcompensation mechanism that prevents more extensive losses in methylation. As a result, both de novo non-CG methylation at nonrepetitive loci and RdDM-directed hypermethylation of repetitive elements are increased [38].

3.2.1 Correlation Between DNA Methylation Levels and Genome Stability

Does methylation directly influence genome stability? Unfortunately, there is no clear answer to this question. However, there exists a degree of correlation between methylation of specific cytosine nucleotides and the frequency of point mutations at these sites. Methylated cytosines are prone to frequent spontaneous deaminations as a result of which they are converted into thymines, which leads to C/G to T/A point mutations. This may explain why CG pairs occur much more rarely as compared to other nucleotide pairs. Ossowski et al. analyzed the rate of mutations in *Arabidopsis* plants self-propagated for 30 generations; it was found that a great majority of all mutations were C/G to T/A base substitutions [39]. Such bias can only be explained by a high frequency of spontaneous deamination of methylated cytosines. In contrast, the deamination of nonmethylated cytosines results in the formation of uracils that are easily recognized by the DNA-repair machinery. DNA methylation also seems to play a critical role in the control of the activity of transposable elements and in the protection of plant cells against the expression of integrated foreign DNA elements. Considering that cytosine methylation protects DNA from cleavage by an endonuclease, it can also protect it against multicopy transposable elements and aberrant gene duplications. Thus, a higher level of DNA methylation at certain loci may function as a defense mechanism against foreign invasive DNA molecules and as a protection against the cell's own transposable elements.

There also exists an inverted correlation between the level of methylation at certain genomic loci and the frequency of large chromosomal rearrangements at these loci. Although it is a common wisdom that hypomethylated loci are more prone to genomic rearrangements, there is not much data on plants that can confirm this. A higher frequency of deletions/insertions of transposable elements at long terminal repeats associated with hypomethylation was observed in the first two generations after allopolyploidization of wheat [40]. The progeny of tobacco plants exposed to tobacco mosaic virus (TMV) exhibited a higher frequency of rearrangements at *R* gene–like loci, and these changes were paralleled by hypomethylation [41].

Many stresses may directly influence the level of methylation in the genome. According to literature, salts of Cd, Ni, and Cr cause oxidative damage that induces DNA hypomethylation [42]. The mechanisms by which ROS generate hypomethylation are the activation of DNA damage–specific endonucleases, such as those associated with the formation of single-stranded breaks, makes DNA a poor acceptor of methyl groups; exposure to heavy metals results in the formation of premutagenic 8-oxo-2'-deoxyguanosine adducts which strongly inhibit methylation of adjacent cytosines; oxidative stress induces an increase in nicotinamide levels, and through its metabolite trigonelline, NIC can trigger hypomethylation in the genome [43]. Hypomethylation in response to oxidative stress triggered by heavy metal exposure can be caused by either indirect effects of heavy metals or a specific defensive mechanism by which cells regulate gene expression.

The importance of DNA methylation for the maintenance of gene-expression patterns and genome stability is reflected by the fact that DNA-repair mechanisms evolve a specific enzyme to excise methylated cytosines from DNA. ROS1 is a methylated cytosine-specific glycosylase that excises methylated cytosines through the process of base excision repair [44]. This enzyme is rather unique in plants since it combines the function of a DNA-repair enzyme with that of an active demethylating process. Curiously, in the *ros1* mutant, the expression of several transposons was found to be decreased due to an increase in methylation levels at CpNpG and CpNpN sites [45]. Active DNA demethylation is thus important in

pruning methylation patterns of the genome, and even previously silent transposons need the dynamic control by methylation and demethylation. Such control is required for the plant epigenome to efficiently respond to developmental and environmental cues. For more detailed information on types of active demethylation processes, see the review by Zhu [44].

Methylation seems to be one of the most versatile epigenetic mechanisms of stress response in plants. The immediate stress response of plant somatic tissues results in changes in methylation of various areas of the genome, with genes involved in stress response being primarily hypomethylated. Exposure to cold causes demethylation and transcriptional activation of a *ZmMI1* gene in maize seedlings; the *ZmMI1* gene contains a retrotransposon-like sequence, and its activation mirrors cold-induced root-specific demethylation in the *Ac/Ds* transposon regions followed by their activation [46]. Hypomethylation in tobacco plants with *NtMET1* antisense results in the upregulation of 31 genes, with most of them being related to stress response [36]. One of the pathogen-responsive genes, *NtAlix1*, undergoes demethylation and activation in response to viral infection, thus confirming that the induction of this gene under natural stress conditions requires sequence demethylation.

The relationship between gene expression and DNA methylation was studied in hypomethylated transgenic tobacco plants expressing an anti-DNA methyltransferase sequence [47]. One of the identified genes coding for a glycerophosphodiesterase-like protein (NtGPDL) was earlier reported to be responsive to aluminum stress. Indeed, when detached leaves from wild-type tobacco plants were treated with aluminum, NtGPDL transcripts were induced within 6 h, and the corresponding genomic loci were demethylated at CCGG sites within 1 h. Exposure to salt and low temperature, but not to pathogen, induced similar demethylation patterns [47].

Several other reports showed changes in DNA methylation in response to stress. The nuclear genome of *Mesembryanthemum crystallinum* plants underwent a twofold increase in the level of CpNpG methylation in response to high salinity [48]. An increase in methylation was noticed in response of *M. crystallinum* plants to drought and temperature stresses upon switching from C3- to C4-type photosynthesis. A correlation between an age-dependent increase in methylation and resistance to the blight pathogen *Xanthomonas oryzae* in rice was also proposed [49]. Virus infection of tomato plants triggered changes in DNA methylation at several marker loci where the majority of polymorphisms detected were associated with genomic regions involved in defense and stress responses [50]. Exposure to heavy metal stress resulted in hypomethylation at several marker loci in hemp and clover [51]. Verhoeven et al. showed that exposure of an apomictic dandelion population to salicylic acid led to genome-wide and possibly stress-specific changes in DNA methylation in exposed plants which can be faithfully transmitted to the immediate progeny [52].

Intriguingly, secondary effects of changes in DNA methylation may include the altered frequency of genome rearrangements. Reports since 2000 have indicated that a decrease in DNA methylation at given genomic loci could attract genome rearrangements [41,53]. If these observations are accurate, then changes in DNA methylation in response to stress may have a significant impact on the rate of genetic changes in genomic loci targeted by DNA methylation. Thus, changes in DNA methylation in response to stress are not just mandatory, but in fact they could be one of the critical components of transgenerational response and ultimately of the process of directing and accelerating plant genome evolution.

3.2.2 Changes in Transposon Activity Associated With Changes in DNA Methylation and Response to Stress

A methylation-dependent activation of transposons in response to stress is a common phenomenon affecting genome stability. Exposure to cold temperatures decreases DNA methylation and as a consequence increases the rate of excision of the *Tam3* transposon [54]. The mechanism of this event is quite fascinating because the *Tam3* transposase binds the GCHCG (H = not G) sequence immediately after DNA replication and thus prevents de novo sequence methylation. A variety of abiotic and biotic stresses were shown to activate the *Tos17* (rice) [55], *Tto1* (tobacco) [56], *Tnt1* (tobacco) [57], and *BARE-1* (barley) [58] retrotransposons. Three different subfamilies of *Tnt1* retrotransposons showed different tissue-specific activation patterns and demonstrated a different inducibility by pathogen elicitors [57]. The stress-mediated activation of *Tnt1* and *Tto1* retrotransposons presumably occurs through the binding of host transcription factors to their promoter sequences that carry a similarity to sequences found in plant defense gene promoters. Thus, it can be hypothesized that adaptive processes in plants and plant genome evolution may occur through the simultaneous activation of stress-responsive genes and retrotransposons. This hypothesis was supported by the finding that in rice plants exposed to cold, the *mPing* element transposed into a rice homolog of the flowering time gene *CONSTANS* [59]. This event resulted in the alteration of flowering time in the progeny of stressed plants.

Another group of genes altered by transposable elements is the cluster of resistance genes (*R*-genes). These genes are involved in pathogen recognition and resistance due to a specific gene-for-gene interaction. As pathogens try to avoid recognition through mutations of avirulence (*Avr*) genes, plants are forced to use the same procedure with *R*-genes. Thus, there

is a constant arm race between pathogens and plants. There exist many mechanisms of *R*-gene evolution, including HR and transposition. It is suggested that a number of transposable elements and their derivatives that are present at the *R*-gene loci play a significant role in a rapid diversification of this gene family [60]. It would be curious to know whether *R*-genes enjoyed a higher frequency of diversification because of the presence of transposons in their sequences, or *R*-genes could be diversified because transposons nonrandomly integrated into the *R*-gene-coding areas. Thus, the reports mentioned earlier support the long-standing hypothesis proposed by Barbara McClintock that all kinds of stresses can potentially reshape plant genomes via transposon activation [61].

3.3 The Role of Histone Modifications in the Maintenance of Genome Stability

Proteins that are associated with histone modifications can be broadly classified into writers, readers, and erasers (Fig. 36.1) [62]. Writers include enzymes involved in modifications such as acetylation, methylation, phosphorylation, and others, and their activity results in local changes in chromatin relaxation or compaction. Readers represent a set of proteins containing bromodomain, chromodomain, or Tudor domains. Most of these proteins cannot directly influence the chromatin structure, they are rather involved in recruiting other chromatin modifiers or erasers of the established chromatin marks. Finally,

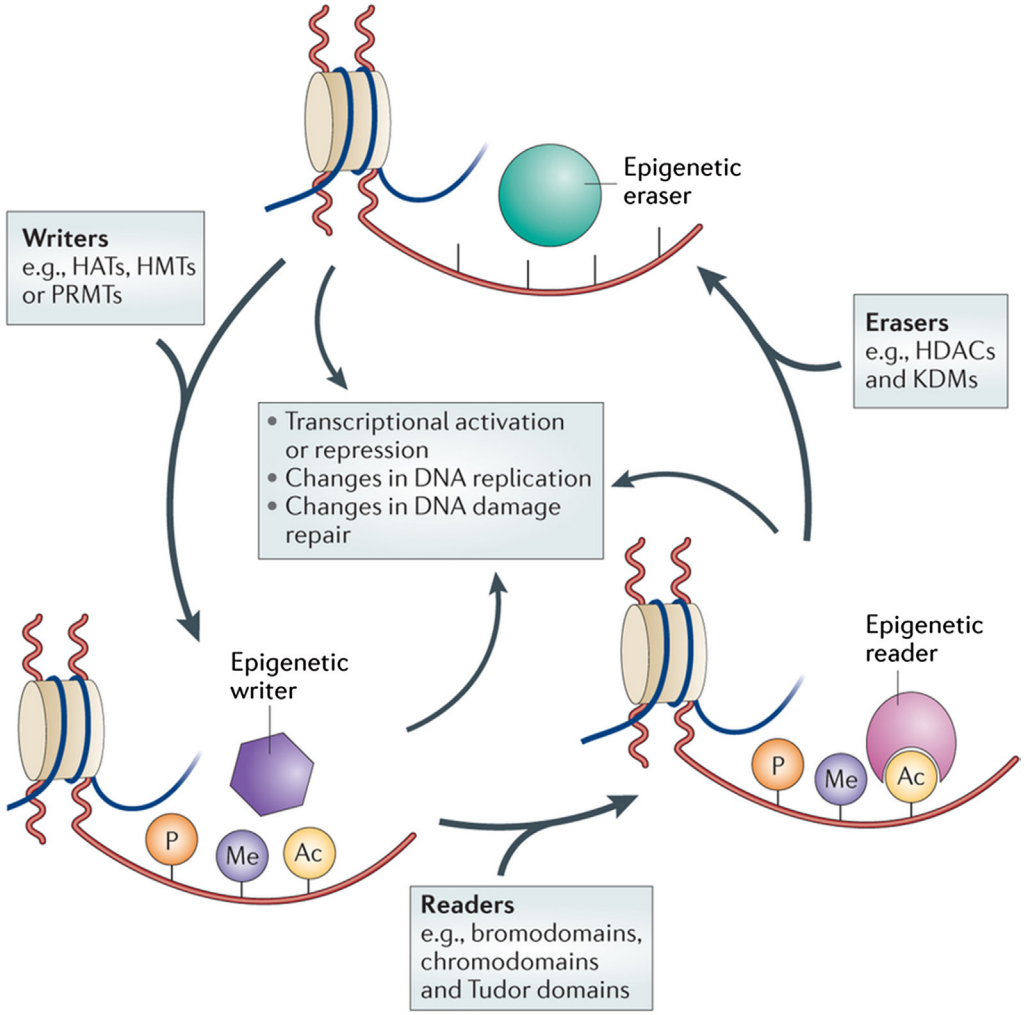

FIGURE 36.1 Epigenetic writers, readers, and erasers. The epigenetic regulation is a dynamic process. Epigenetic writers such as histone acetyltransferases (HATs), histone methyltransferases (HMTs), protein arginine methyltransferases (PRMTs), and kinases lay down epigenetic marks on amino acid residues on histone tails. Epigenetic readers such as proteins containing bromodomains, chromodomains, and Tudor domains bind to these epigenetic marks. Epigenetic erasers such as histone deacetylases (HDACs), lysine demethylases (KDMs), and phosphatases catalyze the removal of epigenetic marks. The addition and removal of these posttranslational modifications of histone tails lead to the addition and/or removal of other marks in a highly complicated histone code. Together, histone modifications regulate various DNA-dependent processes, including transcription, DNA replication, and DNA repair. *Reproduced from Falkenberg KJ, Johnstone RW. Histone deacetylases and their inhibitors in cancer, neurological diseases and immune disorders. Nat Rev Drug Discov 2014;13(9):673–91 with permission.*

erasers are proteins that reverse the chromatin marks and represent proteins such as histone deacetylases (HDACs) and histone demethylases (KDMs).

In plants, transcriptionally active chromatin exhibits an enhancement of H3 and H4 acetylation, trimethylation of lysine 4 from histone H3 (H3K4me3), whereas silent chromatin contains hypoacetylated H3 and H4, methylated lysine 27 (H3K27) and lysine 9 of histone H3 (H3K9) [63]. Histone acetyltransferases (HATs) and histone deacetylases modulate the expression of developmental and stress-sensitive genes.

Modifications of histones play many essential roles in the maintenance of genome stability. First, since the chromatin structure is directly correlated with the association of DNA with certain modified histones, it can be predicted that repressive histone marks, such as H3K9me and H3K27me, contribute to genome stability, whereas permissive chromatin marks, such as H3K4me, H3K36me, and H3K9ac, may contribute to genome instability. This notion may not necessarily be true because open chromatin is also associated with a higher DNA-repair capacity. A more detailed analysis of mutation rates associated with open and closed chromatin may be necessary to prove or disprove this hypothesis.

Second, high rates of exchange (removal or addition) of various histone modifications is required for a proper response to stress and DNA damage to allow either a more efficient access to damaged DNA or a more efficient transcription of loci encoding DNA-repair factors. One of the reports of 2014 demonstrated the production of DSB-induced ncRNAs (diRNAs) from genomic regions with strand breaks [64]. It demonstrated an important role of various epigenetic factors in the production of diRNAs and showed that loci with higher transcription rates have a higher frequency of diRNA production and higher repair rates.

Finally, various histone variants are playing a very critical role in DNA repair and genome stability. Phosphorylation of a specific histone variant H2AX resulting in the formation of γH2AX foci is one of the critical steps in the recognition of a strand break and the assembly of DSB-repair factors around the strand break [65].

Writers, readers, and erasers are critical for a proper response to both stress and DNA damage. HDACs, for example, have been implicated in defense against pathogens. HC toxin from *Cochliobolus carbonum* specifically targets the HDAC activity causing histone hyperacetylation in susceptible corn cultivars [66]. It should be noted, however, that among all classes of plant HDACs, proteins from the reduced potassium dependency protein 3/histone deacetylase 1 (RPD3/HDA1) and HD2 classes are the only proteins that become inhibited by the toxin [67]. In *Arabidopsis*, the *AtHDAC19* gene is induced in a similar manner by the fungus *Alternaria brassicicola* and an exogenous application of JA [68]. The overexpression of the *AtHDAC19* gene enhances fungal resistance through the apparent activation of the ethylene-responsive factor 1 (ERF1), whereas silencing of the gene increases fungal susceptibility. The expression of another HDAC, AtHDAC6, was also shown to be induced by JA application [68]. This enzyme was also shown to affect transgene silencing and DNA methylation. *AtHDAC19* is possibly involved in *Arabidopsis* resistance to a bacterial pathogen *Pseudomonas syringae*. The proposed mechanism may involve a decrease in histone acetylation through interactions of HDAC19 with WRKY38 and WRKY62, two transcription factors that repress the SA pathway [69]. The locus-specific suppression of transcription of these two WRKY genes results in the activation of the SA-dependent pathway, and thus in the resistance to bacterial pathogens.

The histone-mediated transcriptional regulation plays an important role in plant protection against stress. Besides various modifications mentioned earlier, histone variants such as H2A.Z were shown to be a part of the response to temperature stress in *Arabidopsis* [69a]. At moderately high temperatures, tight wrapping of H2A.Z and the amount of H2A.Z are reduced at the promoter of heat-responsive genes such as HSP70. A similar effect was observed in *Drosophila* where exposure to temperature stress resulted in nucleosome depletion at HSP70 loci.

3.4 ncRNAs Are Likely Involved in the Regulation of Genome Stability and DNA Repair

Many types of non-coding RNAs (ncRNAs) have been implemented in response to stress and are associated with direct or indirect regulation of genome stability. A more detailed role of ncRNAs in the regulation of DNA repair and genome stability is covered in Chapter 25.

4. TRANSGENERATIONAL RESPONSES

4.1 Types of Transgenerational Effects and Possible Mechanisms of Their Appearance

Transgenerational response is a phenomenon in which plants exhibit changes in the progeny in response to the adverse environment experienced by their parents [70]. Transgenerational changes may include alterations at many levels: DNA methylation and histone modifications, changes in transcriptome, including mRNA and ncRNA transcripts, changes in

metabolome and proteome, and in stress tolerance and genome stability (reviewed in [2,71,72]. Such changes may be heritable; although it is still unclear what changes are considered to be heritable, we suggest those alterations that persist for two consecutive generations, S1 and S2, where S1 is the first progeny of plants exposed to stress, and S2 is the second generation of stressed plants. Nonheritable transgenerational changes are typically those that last for a single generation after stress exposure and disappear in the next generation if stressful conditions are not maintained. Most commonly, such changes occur due to differential seed viability/quality caused by the accumulation of metabolites/nutrients that give a certain advantage to plants grown under specific environmental conditions.

Heritability of transgenerational changes depends on the epigenetic regulation. Similarly to animals, in plants, early development reprograms epigenetic modifications such as DNA methylation and histone modifications accumulated during sporophyte development. In contrast to animals, however, reprogramming is less dramatic in plants. While in animals the majority (70–90%) of DNA methylation marks are erased, in plants, most of the marks may be faithfully retained and passed on to progeny [73]. Therefore, heritable epigenetic marks may be responsible for passing the memory of stress exposure across generations.

For a long time, heritable transgenerational changes, often referred to as "soft inheritance," were believed to be impossible or extremely rare. Hard inheritance (or Mendelian inheritance) requires mutations to occur in order to introduce a new trait. Such mutations would have to be beneficial to have a chance of becoming fixed in a population. Since mutations are extremely rare, new traits/species emerge rarely and may require many generations to become common in a certain population [2,74,75]. In contrast, soft inheritance allows an immediate response to the environment, and it is flexible (reversible) allowing the population to respond to the environment frequently and efficiently.

Moreover, it is possible that epigenetic modifications triggered by environmental stimuli are converted to genetic changes (Fig. 36.2). For example, cytosine hypomethylation or the establishment of permissive chromatin marks can lead to the increased frequency of genomic rearrangements, whereas cytosine hypermethylation may result in the increased

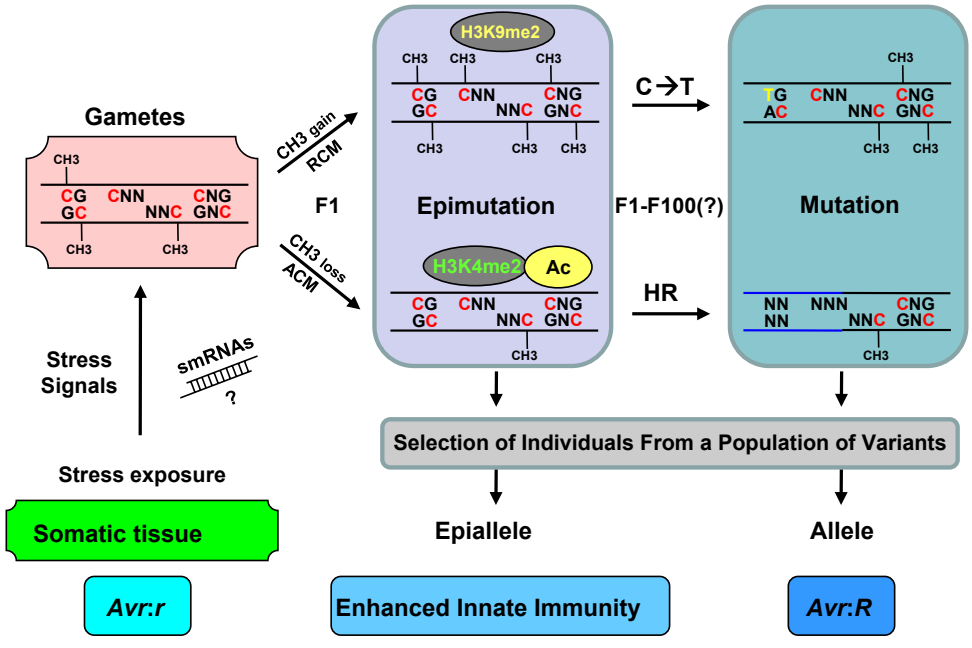

FIGURE 36.2 **Stress-induced epigenetic and genetic changes—an evolutionary perspective.** In the proposed scenario, stress generates mobile signals, for example, smRNAs, that can reach the gametes and influence DNA methylation patterns. The loss or gain of DNA methylation accompanied by repressive chromatin marks (RCMs) or active chromatin marks (ACMs) represent epimutation events. The diagram shows three types of cytosine methylation, CpG, CpNpG, and CNN. H3K9me2 exemplifies the repressive chromatin mark, whereas H3K4me2 and "Ac" (acetylation) exemplify active chromatin marks [50]. The hypermethylated regions are prone to a higher frequency of C to T mutations, whereas the hypomethylated regions have a higher frequency of homologous recombination. It is not clear how many generations are required to translate epigenetic mutations into stable genetic ones. Individuals with (epi)mutations that are beneficial for the growth in the specific environment have better chances to survive and reproduce. Thus, new epialleles and alleles are established in the population. The lower panel applies our scenario to plant–pathogen interactions. It can be hypothesized that compatible pathogen interactions in which plants do not have a functional *R*-gene (*Avr*:*r*) result in the cascade of the earlier described events. In the short term, epimutations/epialleles allow plants to withstand pathogen encounters through enhanced innate immunity. A long-term strategy requiring exposure to the same pathogen over multiple generations leads to the production of new resistance genes (*Avr*:*R*) as well as resistance to pathogens due to incompatible interactions. *Reproduced from Boyko A, Kovalchuk I. Genome instability and epigenetic modification–heritable responses to environmental stress? Curr Opin Plant Biol 2011;14(3):260–6.*

frequency of C to T point mutations due to the frequent deamination of methylated cytosine [76,77]. It is therefore prudent to suggest that in many cases, the appearance of new traits and new species in response to adverse conditions is largely driven by epigenetic mechanisms, and genetic mechanisms come second (Fig. 36.2).

4.2 Transgenerational Changes in Response to Abiotic Stress

Abiotic stress can be broadly classified as stress of nonbiological origin, such as temperature changes, water availability, exposure to toxic chemicals or radiation (UV, gamma and so on). Plants respond to stress at many levels, with the main emphasis given to mechanisms of stress survival and setting seeds. Some plants are able to tolerate abiotic stress if it is repeated in the processes known as adaptation and acclimation [78,79]. These processes operate at the somatic level, but they are also known to occur across generations. Abiotic stress is known to destabilize genomes of somatic cells. The number of stresses increases the frequency of HR in a direct or an indirect manner [37,80–82].

Work since 2000 have also demonstrated that a stress-induced increase in the frequency of somatic HR can be inherited [37,41,81,83–85]. It is important to stress out, however, that transgenerational changes in HR frequency were not always found to occur, and they likely depend on many parameters such as tests/measurements utilized, stress conditions, and plant species used. For example, one of the earlier works by Molinier et al. demonstrated that a single exposure of *Arabidopsis thaliana* plants to stress of UV radiation (UVC, specifically) results in the increased frequency of somatic HR in four consecutive nonstressed generations [86], thus representing the truly heritable epigenetic inheritance. In contrast, works by Boyko et al. [85], Kathiria et al. [87] and Rahavi et al. [88] and others provided experimental evidences that the increased frequency of somatic HR is mostly restricted to the immediate progeny of stressed plants, and if stress is not maintained, the frequency of HR drops to the endogenous level observed in unstressed plants. It is possible that the persistence of changes in the frequency of HR observed by Molinier et al. is a unique feature of a particular transgenic line used [86]. For example, the transgenic *A. thaliana* line in which an increase was observed was found to be very unstable without any stress exposure, and a simple propagation of these plants for several generations under normal conditions resulted in a dramatic increase in recombination frequency. Moreover, a work by Pecinka et al. [89] actually shows that a transgenerational increase in HR frequency occurs only in specific transgenic *Arabidopsis* lines tested and only in response to few stresses. The analysis of HR frequency in response to 10 different stresses showed that transgenerational changes occurred in response to two to three stresses, and changes were low and stochastic.

One possible explanation for such discrepancy observed in transgenerational changes in HR frequency could be the intensity of stress used for the analysis. It is possible that only a mild stress may lead to the inheritance of changes in the recombination frequency because a severe stress may have a significant negative effect on plant physiology, somatic cell death, and the negation of epigenetic factors that otherwise would lead to changes in the recombination frequency in progeny. This hypothesis was confirmed by a study that analyzed changes in the HR frequency in response to NaCl; whereas a transgenerational increase in the recombination frequency was most prominent in response to 25 mM NaCl, it was milder in response to 75 mM, and it did not exist in response to 100 mM [90]. This observation was actually consistent with the results published by Pecinka et al. [89]. The existence of response to mild rather than harsh environmental conditions is reminiscent of the long-known phenomenon of hardening in plants. Hardening in plants describes the increased tolerance to a severe stress when plants experienced a mild stress prior to exposure to a severe stress [91]. In case of a transgenerational response, this phenomenon may be referred to as transgenerational hardening.

Our work demonstrated that in most cases transgenerational changes in the recombination frequency occurred only in the immediate progeny; only two stresses tested (25 mM of salt and UVC) increased the recombination frequency in two consecutive generations, and changes in the second generation were smaller than those in the first one [85].

Would the recombination frequency increase more if plants were propagated in the presence of stresses for more than one generation? Would such changes last longer? The answers to these questions were in part obtained from the work of Rahavi et al. [88]. The authors studied changes in the recombination frequency in response to heavy metal salts such as Ni^{2+}, Cd^{2+}, and Cu^{2+}. They propagated plants on heavy metal salts for up to five generations, then stress was removed from them in each generation, starting after generation one. In most cases, an increase in the number of generations exposed to stress resulted in a higher increase in the recombination frequency, although in many cases, this increase reached the plateau already after the first or second generation of exposure. Propagating the progeny of stressed plants under normal conditions resulted in the decreasing recombination frequency, and in those cases where plants were propagated under stress conditions for more generations, a decrease in the HR frequency was less noticeable. In several cases, propagating plants in the presence of stress for three to four generations resulted in an interesting phenomenon—the removal of stress did not decrease the recombination frequency even after two generations of growth under normal conditions. These results indicate that stress memory is inherited, and the more generations are exposed to stress, the stronger and the longer lasting the memory of stress is [88].

Transgenerational responses may also depend on the timing of stress application. Since plants establish the germline relatively late during the development, exposure to stress early during development may allow to pass on the memory of stress application more efficiently (while cells are transitioning to gametes). In contrast, it is likely that stress exposure later during development when gametes are formed may not lead to the efficient generation of stress memory. This is exactly what we have observed in the experiment where we exposed *Arabidopsis* plants to heat, cold, and UVC at different time points during development: 7, 14, 21, and 28 days post germination (dpg). The analysis of HR frequency showed that the highest increase was observed in the progeny of plants exposed at 7 dpg. Similarly, the analysis of plant phenotype in the progeny showed that the progeny of plants exposed at 7 dpg had the largest seeds and the largest leaves when grown under normal conditions and when exposed to stress [92].

What type of genome instability is the most common in the progeny of stressed plants? To address this question, we have used three different transgenic lines, which allowed us to analyze the point mutation frequency, the HR frequency, and microsatellite instability [37]. Exposure to various stresses revealed that changes in the HR frequency were the most prominent among three types of genome instability that we tested. Changes in the microsatellite instability occurred in response to UVC, heat, and cold but were less prominent than changes in the recombination frequency. Finally, changes in the frequency of point mutations in the progeny were only observed in response to UVC, but not in response to any other stress. It would be interesting to analyze why changes in the HR frequency are affected the most in the progeny. HR is a mechanism of crossing over involved in a physical exchange between sister chromatids during meiosis. Such events result in gross chromosomal rearrangements and are likely the most effective in generating novel alleles [93]. If we hypothesize that transgenerational changes in genome stability in response to stress are directed at the diversification of the genome, HR should be a mechanism that is affected the most.

4.2.1 Changes in DNA Methylation in the Progeny

In the preceding paragraphs we have discussed on various epigenetic mechanisms that regulate genome stability and are responsive to stress. Transgenerational changes in DNA methylation in response to stress has been observed in many reports. One of the earliest reports by our laboratory showed that the progeny of plants exposed to ionizing radiation exhibit global genome hypermethylation [94]. Moreover, hypermethylation appeared to be dose dependent; a higher dose of radiation experienced by parental pine tree plants in Chernobyl increased the level of methylation to a higher extent in the progeny [94].

Similarly to the effect of ionizing radiation, exposure to stresses such as salt, flood, heat, cold, and UVC also resulted in hypermethylation in the progeny [85]. When plants were propagated for two generations, DNA methylation did not increase further in the second generation and stayed at the same level as in the first progeny of stressed plants. Curiously, when the progeny of salt-stressed plants were propagated under normal conditions, they maintained higher levels of methylation, but at the same time, they showed the decreased recombination frequency and the lower stress tolerance [85]. This is an interesting phenomenon. We can assume that transgenerational changes are triggered by differential expression of ncRNAs that target various genomic loci to establish differential methylation and differential gene expression, leading to changes in stress tolerance [2]. DNA methylation is maintained at a higher level no matter whether plants are or are not exposed to stress for the second time, while the recombination frequency and stress tolerance depend on the second stress exposure, which suggests that changes in DNA methylation are more robust, they are maintained in the absence of stress, and they are likely in part disconnected from the capacity to tolerate stress.

An overall increase in global genome methylation in the progeny of stressed plants does not reflect the situation at the specific loci in the entire genome because it is a mere reflection of all methylated cytosines present in the genome. A more detailed analysis on the level of individual loci in the progeny of salt-stressed plants revealed that many loci essential for stress tolerance and epigenetic regulation were either hypomethylated or hypermethylated. For example, the promoters of *SUVH2*, *SUVH5*, and *SUVH8* genes that were involved in the regulation of the chromatin structure, and the promoter of *ROS1*, a gene that helps demethylate DNA were hypermethylated, whereas the promoters of stress-responsive genes UVH3, ERF1, TUBG1, RAP2.7, and several others were hypomethylated [84]. The essential role of DNA methylation for the establishment of transgenerational stress tolerance was demonstrated by the fact that soaking seeds of the progeny of salt-stressed plants in 5-azaC, a chemical compound that modifies cytosines by preventing methylation, does not allow plants to tolerate a higher level of MMS chemical and eliminates hypermethylation [85].

4.3 Transgenerational Changes in Genome Stability, Methylation, and Stress Tolerance in Response to Biotic Stress

Biotic stress includes various plant pathogens such as bacteria, fungi, viruses, nematodes, insects, and others. Pathogen infection frequently results in changes in plant physiology, the loss of biomass, early flowering, the decreased seed set, the accumulation

of protective metabolites, and many other changes. One of the first evidences that pathogens may destabilize the plant genome comes from the work of Lucht et al. [95]. *Arabidopsis* infection with *Peronospora parasitica* or treatment with chemicals such as 2,6-dichloroisonicotinic acid (INA) or benzothiadiazole (BTH) resulted in an increase in the HR frequency in stressed plants [95]. Later on, the work in our laboratory showed that infection of tobacco plants with TMV also results in an increase in the somatic recombination frequency [96]). Resistance to TMV in tobacco is conferred by the presence of the resistance gene *N* that allows cytoplasmic recognition of the virus. Lines such as Big Havana cultivars that contain the *N*-gene produce a local hypersensitive response and a systemic acquired resistance response that allow plants to localize the virus. Lines that lack the *N*-gene such as SR1 plants succumb to infection. It is important to note that resistance is temperature sensitive; at temperatures exceeding 28°C, lines that contain the *N*-gene also become sensitive. Our work showed that only infection of sensitive plants, either SR1 or Big Havana plants grown at temperatures higher than 28°C, results in the increased somatic recombination frequency. Importantly, the increase was observed in tissues that were not infected with the virus. Moreover, grafting virus-free leaves of infected plants onto naïve tobacco plants also led to an increase in the recombination frequency [96].

Next, we analyzed transgenerational changes in response to TMV. We found that the progeny of infected plants had more plants with a fully recombined luciferase transgene, which indicated that the meiotic recombination frequency also increased. In addition, we found that the somatic recombination frequency in the progeny of infected plants had also an increased rate. Similar effect was observed in response to another virus, oilseed rape mosaic virus (ORMV) [97].

We were curious why the recombination frequency increased only in plants sensitive to TMV. We hypothesized that a boost of HR may be one of the mechanisms for increasing the diversity of resistance genes that potentially leads to the generation of resistance genes that would confer the resistance to TMV. Our analysis of the SR1 genome revealed that despite the fact that SR1 plants do not have an active *N*-gene, they contain many [30–50] loci that carry a substantial (up to 65%) homology to the *N*-gene [41]. The analysis of the rearrangement frequency at these loci in the progeny of infected SR1 plants revealed an over eightfold increase as compared to the progeny of control plants. The same analysis at actin loci did not show any difference, suggesting that an increase in the rearrangement frequency is locus specific [41].

The analysis of DNA methylation in the progeny of infected plants showed global genome hypermethylation. At the same time, a decrease of methylation level was observed at resistance gene-like loci but an increased one at the actin loci [41]. It is highly likely that rearrangements at certain loci are controlled by the level of methylation; hypermethylation may prevent loci that are "irrelevant" to the response to TMV from rearrangement, whereas hypomethylation allows for the recombination and may enable a genetic diversity where it is most needed (Fig. 36.2).

Another important change observed in the progeny of infected plants was a higher tolerance to TMV infection as well as a higher tolerance to *P. syringae* and *Phytophthora nicotianae*. Thus, the progeny of TMV-infected plant have a certain degree of cross-tolerance to bacterial and fungal pathogens. The ability to delay the viral progression is likely triggered by many factors, but our research showed that these plants had a higher endogenous expression of the *PR1* gene and a higher level of callose deposition [87]. These plants were also more tolerant to chemical MMS.

Several studies confirmed our findings that infection with a pathogen leads to changes in the progeny, mainly in the form of a higher tolerance to this pathogen. Luna et al. showed that the progeny of plants that were repeatedly infected with *P. syringae* exhibited a higher tolerance to the same pathogen in the form of a reduced bacterial colonization compared to the progeny of noninfected plants [98]. These plants were also more tolerant to fungal pathogen *Hyaloperonospora arabidopsidis*. A higher pathogen tolerance was also observed in the next generation even when plants were propagated under normal conditions. Slaughter et al. analyzed the impact of a single inoculation with *P. syringae* and found that the immediate progeny had a stronger and quicker response to *Pseudomonas* infection [99]. In contrast to the study by Luna et al., Slaughter et al. found that pathogen tolerance did not persist into the next generation when the progeny of infected plants was propagated without stress [99].

A response to insects also has a transgenerational nature. Wild radish plants exposed to herbivores produce the progeny that are more resistant to herbivory [100]. Also, yellow monkeyflower plants respond to herbivory with an increased trichome density in the progeny; trichome density positively correlates with tolerance to herbivores [101,102]. The analysis of the progeny of *Arabidopsis* and tomato plants that were exposed to caterpillar herbivory showed an enhanced resistance to two out of three herbivores tested [103]. A higher tolerance to herbivores was also observed in the second generation when plants were propagated under normal conditions, but no such tolerance was observed in the third generation [103].

5. POSSIBLE MECHANISMS INVOLVED IN THE REGULATION OF TRANSGENERATIONAL INHERITANCE OF STRESS MEMORY

Which mechanisms control transgenerational changes and in particular changes in genome stability? Several mechanisms may be involved, and our experiments indicate a possible role of DNA-repair proteins and epigenetic regulators such as ncRNAs and DNA cytosine methylation.

5.1 The Potential Role of DNA-Repair Factors

As far as genome stability is concerned, a differential level of metabolites is unlikely to play any role, but it is possible that some differential transcripts accumulated in seeds developed from stressed plants may influence genome stability. For example, an increase in the level of transcript of DNA-repair genes may play a positive role. Our previous analysis indeed showed higher transcript levels of several DNA-repair genes in the progeny [84,85].

Also, it is possible that differential expression of repair genes in plants exposed to stress may also influence the recombination frequency in the progeny. It is not clear, however, whether the presence of all repair factors is essential to observe transgenerational changes in genome rearrangements. For example, an increase in the recombination frequency was observed in wild-type plants *atm* and *rad51b* but not in *ku80* plants in response to several abiotic stressors [83]. In the progeny of stressed plants, an increase in the recombination frequency was observed in wild-type plants and *ku80* mutants, whereas the *atm* mutant was partially impaired. The main changes were observed in *rad51b* mutants; the progeny of these plants completely lacked transgenerational changes in the recombination frequency. It is therefore likely that functional ATM proteins that recognize DSBs and AtRAD51B involved in the HR pathway to repair such breaks are needed for the initiation of a transgenerational signal or its transmission through gametes. Other proteins, such as KU80 and UVH3, appeared to be dispensable for transgenerational changes [83]. It is curious to draw a parallel with recent reports describing the production of diRNAs (see Chapter 25 for details). It was shown that strand breaks trigger the production of diRNA originating from the site of a strand break [64,104]. It was shown that these diRNAs are depleted in *atm* and *atr* mutants as well as in various mutants impaired in epigenetic regulation, namely DCL3, AGO2, and several others. Moreover, it was demonstrated that the NHEJ process (in which KU80 is known to participate) was not impaired when diRNAs were depleted [64,104].

5.2 The Role of Epigenetic Regulators

Several experiments in our laboratory and work of others strongly suggest the involvement of epigenetic regulators in transgenerational changes in genome instability. We have suggested earlier (see Fig. 36.2) that locus-specific changes in DNA methylation in the progeny of stressed plants are likely directed to control the expression of these loci and prevent rearrangements from occurring.

In plants, DNA methylation occurs at various sequence contexts, including symmetrical methylation at CG and CNG sites and asymmetrical methylation at CNN sites. In the latter case, there are no maintenance DNA methylation and de novo methylation events that are established through the function of ncRNAs in a sequence-specific manner. *Arabidopsis* contains four Dicer-like (DCL) proteins, among them, DCL1 that primarily functions in miRNA biogenesis and the other three, DCL2, DCL3, and DCL4, involved in biogenesis of small interfering RNAs (siRNAs) [105]. RdDM occurs through a concerted function of sequence-specific siRNAs, PolIV, DCL3, RDR6, DRM2, and several other proteins involved in two major RdDM pathways, PolIV-RdDM and RDR6-RdDM. More details on de novo RdDM silencing and self-reinforcing loops can be found in Bond and Baulcombe [106]. We hypothesized that the RdDM pathways may be responsible for transgenerational changes in methylation and genome stability.

Several experiments partially confirm our hypothesis. Boyko et al. showed that *dcl2* and *dcl3* mutants were partially impaired in a transgenerational increase in the recombination frequency in response to flood, heat, cold, and UVC as well as in a transgenerational increase in methylation [85]. Also, *dcl2* was partially impaired in transgenerational stress tolerance to MMS [85]. Similar data were reported by Rasmann et al.; the *Arabidopsis dcl2 dcl3 dcl4* triple mutant did not inherit resistance to insects in response to parental herbivory [103]. These reports support the essential role of DCLs and siRNAs in changes in DNA methylation, genome rearrangements, and the transmission of stress memory to progeny. Unlike the two aforementioned studies, report by Ito et al. showed that DCL3 may not be necessary for transgenerational transposition of *ONSEN* [107]. The heat-induced expression of *ONSEN* was higher in *dcl3* plants compared to wild-type plants. The authors suggested that DCL3 may be partially restricting the accumulation of *ONSEN* in response to heat stress in somatic tissues. In contrast, they did not find any new insertions of *ONSEN* in the progeny of heat-stressed *dcl3* plants, which was similar to the finding in wild-type plants.

The mechanism of transgenerational changes may involve several steps. First, stress response includes differential expression of mRNAs, ncRNAs, changes in DNA methylation, and histone modifications in somatic tissues. If stress occurs early during development and influences the whole plant, these changes may occur in meristem cells that will give rise to gametes. If stress occurs when gametes are established, they may also be altered in response to stress. Even if meristem cells or gametes are not altered directly, these cells may acquire the information about stress from all other somatic cells through active functions of plasmodesmata and phloem that circulate in a variety of molecules, including ncRNAs. It is

possible that changes in DNA methylation and histone modifications caused by the RdDM mechanism may already occur in meristem cells or early gametes. Second, changes that occur in meristem cells or in the developing gametes have to survive reprogramming, a mechanism that erases the epigenetic marks, such as changes in DNA methylation, histone modifications, and degradation of mRNA in pollen. Epigenetic changes caused by stress also need to survive the second level of reprogramming that occurs after the fertilization event. It is possible that changes in DNA methylation occur in mature gametes or early embryos and are caused by differential expression of ncRNAs produced in gametes or embryos, or even in the endosperm. Third, it is possible that some of the differentially expressed ncRNAs may survive all reprogramming steps and trigger changes directly in the progeny. Our 2015 work in *Brassica rapa* showed that heat stress induces changes in ncRNA and mRNA expression in meristem tissues and gametes; some of these changes were propagated into the developing embryo and even into the progeny [108]. Changes observed in the somatic recombination frequency may be triggered by changes in the chromatin structure (due to either changes in DNA methylation or histone marks) or/and changes in the expression of DNA-repair genes (see earlier). Changes in stress tolerance could be the result of all factors combined, including the differential accumulation of metabolites, changes in DNA methylation, the differential expression of stress-responsive genes, the primed chromatin structure, etc. Fourth, the propagation of stress memory and the maintenance of a high frequency of HR in the next generations may require continuous stress exposure (generation after generation). This is not surprising because if changes in DNA methylation and ncRNAs that trigger it play an essential role, they need to be generated constantly to reinforce transgenerational memory and replenish the molecules depleted during reprogramming. Future research will show whether this theory has merit.

6. CONCLUDING REMARKS

Plants as any other species need to balance between genome stability and genome instability. Whereas the preservation of the genome integrity is important for passing the genetic information on to the progeny, inducing genomic variability in a random fashion or directing it at the defined genomic loci is a prerequisite for the survival of species in adverse environments.

In this chapter, we summarized various mechanisms regulating genome stability, discussed how the choice of DNA-repair pathway influences genome stability, and described the role of epigenetic factors such as DNA methylation, histone modifications, and ncRNAs in controlling genome stability. We described several experimental evidences indicating that the progeny of stressed plants exhibit a variety of changes, including changes in stress tolerance, DNA methylation, and genome stability. We further demonstrated that transgenerational changes are regulated epigenetically. We hypothesized that transgenerational changes are caused by the differential expression of ncRNAs and RdDM mechanisms causing differential changes in DNA methylation and histone modifications. Direct links between a certain type of differentially expressed siRNAs, changes in DNA methylation at specific loci targeted by these siRNAs, and genome stability remain to be established. It is unclear whether such siRNAs are passed from the progeny via gametes, or their expression is induced in the early developing embryo or in the germinated plants. It is also not clear whether the chromatin structure does have a direct effect on genome rearrangements because such links are not evidently established. It is also possible that such siRNAs are propagated in the cytoplasm without the additional transcription. Finally, it remains to be shown whether these siRNAs and rearrangements in the genome are stress specific and are indeed directed towards specific loci in the genome and promoter-specific changes at epigenetic and perhaps genetic levels.

GLOSSARY

Active chromatin marks Posttranslational histone modifications associated with high levels of gene expression and open chromatin

Epigenetic changes Heritable but reversible changes in gene expression that do not involve changes in the DNA sequence. Epigenetic changes are typically associated with reversible modifications of DNA (cytosine methylation) or histones (methylation, acetylation, etc.and so on). The differential expression of non-coding RNAs and sometimes the differential binding of non-histone chromatin modifiers may also be referred to as an epigenetic modification.

Hard or Mendelian inheritance The inheritance of traits based on the DNA sequence; according to Mendelian inheritance, new traits can only appear as a result of a mutation – —changes in the DNA sequence.

Hardening The increased tolerance to severe stress in plants after exposure to mild stress; also referred to as priming, acclimation, conditioning, etc.

Heritable transgenerational effects Transgenerational effects persisting for more than one generation, even when the stimulus causing these changes is removed; these effects are typically associated with changes in the epigenome.

Memory Genetic, epigenetic, or physiological changes that outlast stressful conditions and modify the response to subsequent stress treatments in the same or the next generation (transgenerational memory).

Non-heritable transgenerational effects (responses) Transgenerational effects that do not persist beyond the immediate generation after stress; these changes are not passed on to the subsequent generations if the stressor is removed. Such effects are typically caused by changes in seed quality due to the accumulation of metabolites, nutrients, etc.and so on, giving an advantage to the growing seedling under certain environmental conditions.

Repressive chromatin marks Posttranslational histone modifications associated with low levels of gene expression and condensed chromatin.

Soft inheritance The inheritance of traits that does not include changes in the DNA sequence but rather involves changes in gene expression, typically caused by changes in the epigenetic regulation.

Transgenerational effects (responses) Typically refers to changes in phenotype (associated with epigenetic or physiological changes) that are apparent in the progeny of an organism grown under normal conditions or in response to stress.

Transgenerational hardening A higher stress tolerance in the progeny of plants exposed to mild stress; occurs mostly due to the accumulation of nutrients and metabolites in seeds as well as due to changes in the epigenetic regulation.

LIST OF ABBREVIATIONS

CAF-1 Chromatin-assembly factor-1
DCL Dicer-like
DDM1 DECREASED DNA METHYLATION1
diRNAs DSB-induced ncRNAs
DSB Double-strand break
HATs Histone acetyltransferases
HDACs Histone deacetylases
HP1 HETEROCHROMATIN PROTEIN 1
HR Homologous recombination
KDMs Histone demethylases
MBDs Methyl-CpG-binding domain proteins
MMS Methyl methane sulfonate
MOM1 MAINTENANCE OF METHYLATION 1
ncRNAs Noncoding RNAs
NHEJ Nonhomologous end joining
ORMV Oilseed rape mosaic virus
RdDM RNA-direct DNA methylation
smRNA Small RNA
TMV Tobacco mosaic virus

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