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Epigenetic contribution to stress adaptation in plants

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Plant epigenetics has recently gained unprecedented interest, not only as a subject of basic research but also as a possible new source of beneficial traits for plant breeding. We discuss here mechanisms of epigenetic regulation that should be considered when undertaking the latter. Since these mechanisms are responsible for the formation of heritable epigenetic gene variants (epialleles) and also regulate transposons mobility, both aspects could be exploited to broaden plant phenotypic and genetic variation, which could improve long-term plant adaptation to environmental challenges and, thus, increase productivity.

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Introduction

Environmental cues are perceived and transmitted by a myriad of plant signal transduction pathways that, by turning on specific transcription factors in the nucleus, lead to the activation of genes encoding effector proteins that enable adaptation to environmental challenges. In recent years, it has become clear that dynamic changes in chromatin properties and the biogenesis of small RNAs also contribute to transcriptional and post-transcriptional regulation of gene expression important for stress responses [1–4]. Although these epigenetic mechanisms are crucial to appropriate plant reactions to stress that result in short-term acclimation, their involvement in the formation of long-lasting, heritable phenotypic traits is less clear. It can be envisaged that plants, given their sessile life style and late differentiation of the germline, could perceive stresses during vegetative growth and 'memorize' them, possibly by epigenetic mechanisms, in cell lineages that later contribute to the germline. Environmentally induced epigenetic states could thus be passed to the progeny. On the other hand, germline progenitor cells in particular need to be protected against such environmental effects to maintain the integrities of their genomes and epigenomes and thus ensure continuity of transgenerational inheritance. Thus, the stability of transgenerational inheritance of the genome and the epigenome under environmental stress conditions and the molecular mechanisms underlying this stability are important areas of study.

It is well documented that alterations in epigenetic marks induced by mutations in genes encoding epigenetic regulators have a durable impact on epigenome organization and function [5•,6–10,11•,12••]. The induction of alternative epigenetic states not only triggers the formation of novel epialleles but also promotes the movement of DNA transposons and retroelements that are very abundant in plant genomes [5•,13•,14,15•]. Therefore, novel 'epigenetically induced' heritable phenotypes can be of true epigenetic or genetic nature and both types can increase the ability of plants to adapt to environmental challenges [5•,11•].

The aim of this review is not to analyse the numerous examples of stress-induced heritable phenotypic alterations in which the underlying molecular mechanisms have remained elusive, but rather to highlight recent advances in the experimental induction of epigenetic and genetic diversity by interference with defined epigenetic mechanisms in combination with environmental stress conditions. These studies have allowed initial assessment of the degree of hidden epigenetic variation and the stability of epigenetic regulation under stress conditions. They have also revealed novel mechanisms controlling the mobility of retrotransposons. We will briefly discuss molecular mechanisms of the formation and maintenance of epialleles. However, considering that the only epigenetic mark for which the mechanism of inheritance has been well established is DNA methylation, we will mainly consider this mark as being the driving force for epiallelic variation.

Modifying stress responses by unlocking the epigenome

In plants, DNA cytosine methylation is found in three sequence contexts: CG, CHG and CHH where H stands for A, C, or T. Because of their symmetrical nature, CG and CHG methylation can simply be copied after DNA replication, but non-symmetrical CHH methylation has to be established *de novo* following each DNA replication cycle. The MET1 methyltransferase maintains methylation at

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CGs and it is thought to act at the DNA replication forks using newly replicated, hemi-methylated DNA as a substrate. CG methylation is considered to be the most stable [16] and its genome-wide distribution seems to provide the blueprint that coordinates transgenerational epigenetic inheritance, implemented further by other epigenetic mechanisms [6,17]. Methylation at CHG is largely maintained by the activity of the plant-specific chromomethyltransferase CMT3 [16], which is directed by histone modifications, especially the dimethylation of histone 3 at lysine 9 (H3K9me2) catalysed by histone methyltransferase KRYPTONITE (KYP). Non-symmetrical methylation is guided by small interfering RNAs (siRNAs) in an RNA-directed DNA methylation (RdDM) process in which target loci are transcribed by a plant-specific RNA polymerase IV (POLIV), producing substrate RNAs for siRNA biogenesis. Transposable elements (TEs), various repeats and transgenes are targeted for silencing by the concerted action of all DNA methylation pathways, including RdDM [18,19].

Analysis of plants affected in DNA methylation has revealed drastic developmental phenotypes, suggesting that phenotypic plasticity is suppressed in wild-type plants by DNA methylation [20]. To assess the extent of the phenotypic plasticity buffered by DNA methylation, two populations of epigenetic recombinant inbred lines (epiRILs) were constructed. The CG methylation-depleted genomes of the two populations originated from the *met1* mutant [5•] or the *ddm1* mutant [11•,12••]. The *DDM1* gene encodes a chromatin remodelling factor required for the maintenance of DNA methylation, and its depletion affects the distribution of methylation in all sequence contexts, including methylation at CGs. The parents of the F1 hybrids initiating both populations of epiRILs (wild type and *met1* or *ddm1* mutants) were isogenic, differing only in the levels and distribution of DNA methylation [5•,11•,21]. The F2 progenies were screened for the homozygote wild-type *MET1* or *DDM1* alleles, to ensure the presence of a functional DNA methylation machinery and, therefore, the maintenance of epigenetically chimeric chromosomes created by recombination during F1 meiosis. Subsequent to the F2, the *met1*-derived lines were propagated by self-pollination and single seed descent for eight generations in order to obtain a high degree of 'epi-homozygosity' (uniparentally derived DNA methylation status at most loci). A similar approach was applied to *ddm1*-derived lines, with the exception of the F1 backcross to the wild type before propagation by single seed descent [11•]. The F1 backcross aimed to reduce the proportion of *ddm1*-derived genomes within this population.

Genome-wide DNA methylation profiling of three *met1*-derived epiRILs at the eighth inbred generation revealed mosaic chromosomes with clearly visible methylated (wild type derived) and hypomethylated (*met1* derived) chromosomal segments that were inherited stably for many generations in a wild-type background [5•,21]. However, the hypomethylated segments also displayed dispersed wild-type DNA methylation levels, suggesting that DNA methylation was inherited in both a Mendelian (parental blocks) and a non-Mendelian fashion (dispersed patterns). Furthermore in *ddm1*-derived epiRILs, analysis of local DNA methylation patterns on chromosome 4 revealed that certain loci producing small RNAs progressively regained methylation through the RdDM pathway upon inbreeding. In contrast, loci not associated with small RNAs were inherited stably in a hypomethylated state [12••]. Interestingly, comparison of methylation inheritance at the same loci in *met1*-derived epiRILs indicated that the epigenetic fate of the loci was not only locus dependent but also differed with respect to the initial mutation used in the construction of the epiRIL populations [21]. Therefore, the two different epiRIL populations are likely to differ in the range of phenotypic variation generated. Acquired morphological and developmental traits, such as altered flowering time, plant height, biomass, leaf morphology and stem fasciation, were documented for both sets of epiRILs [5•,11•]. For *met1*-derived epiRILs, phenotypic diversity in the responses to pathogens and salt-stress conditions was also assessed and novel variation was observed as reflected by increase or decrease in resistance compared with each epiRIL parent [5•]. These phenotypes were stably transmitted to subsequent generations. Importantly, when the range of pathogen sensitivity/resistance within the *met1* epiRIL population was compared to naturally occurring diversity in pathogen responses amongst Arabidopsis accessions, the epiRILs exhibited 60% of the natural genetic variation for this trait. Obviously, such phenotypic plasticity in response to adverse environmental conditions, hidden in wild-type plants by epigenetic buffering, could be of special value for plants with limited genetic diversity and should be considered in future for crop improvement when genetic resources appear to have reached their limits (Figure 1). However, the exploitation of this approach in a controlled and predictable fashion requires deeper knowledge of the range and stability of induced epigenetic diversity as well as broader genetic resources allowing for controlled alteration of crop epigenomes [22].

So far, few experiments have aimed at generating epigenetic diversity in crop species, where mutations in *DDM1* or *MET1* genes are not available. Akimoto and colleagues treated rice seedlings with a chemical affecting DNA methylation (azacytidine, an inhibitor of DNA methyltransferases). The progeny of the treated plants was used to initiate lines for further propagation over several generations. Interestingly, one line showed acquired resistance to a bacterial pathogen (*Xanthomonas oryzae*) that correlated with the hypomethylation of a resistance gene promoter [23] — a truly encouraging result.

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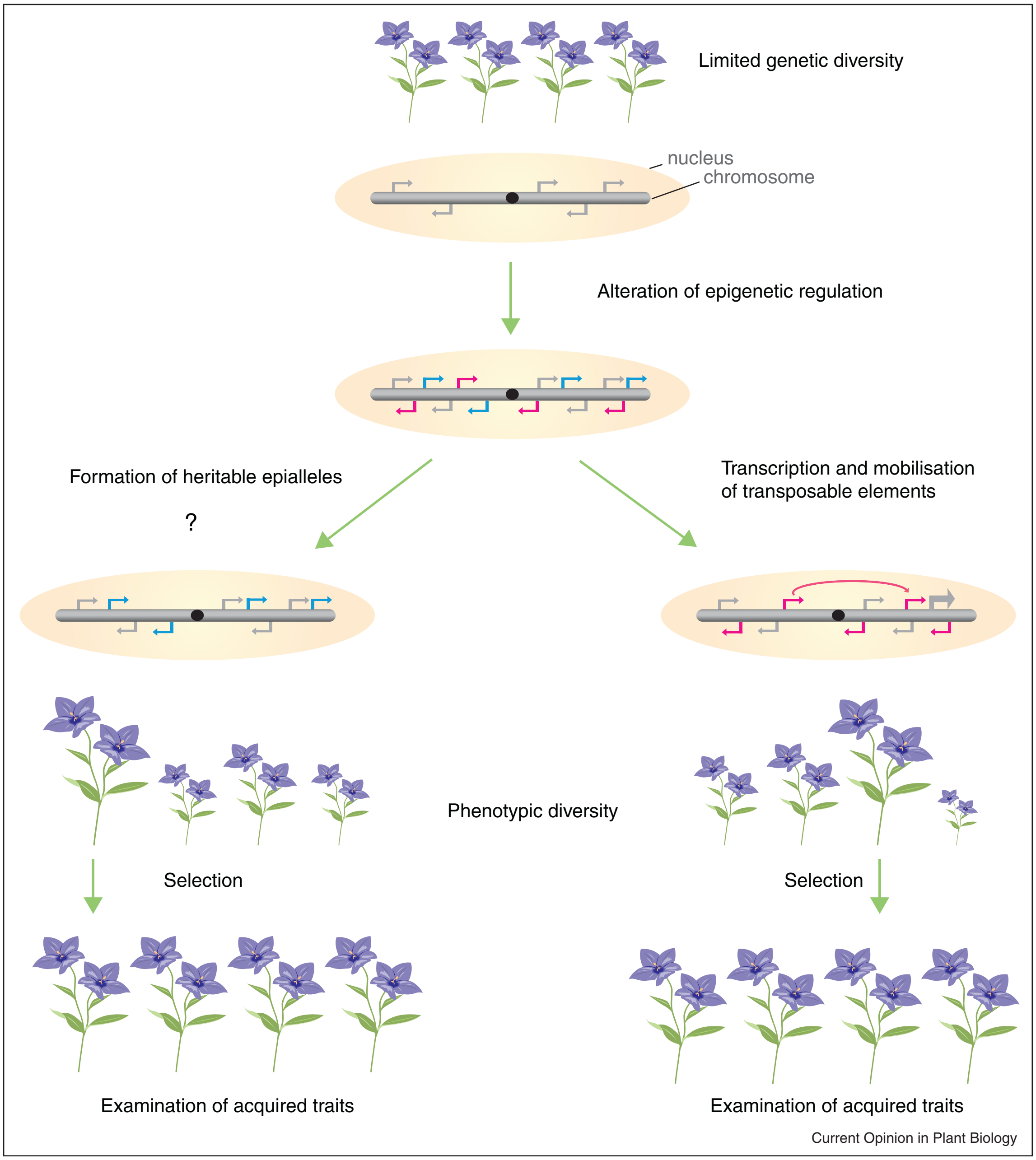
Figure 1



Generation of heritable phenotypic variation through alteration of epigenetic regulation. Epigenetic regulation is destabilized in plants derived from a homogenous plant population with a limited genetic diversity. This can be achieved through mutations affecting epigenetic regulation, by environmental stress treatment or by a combination thereof. Alteration of epigenetic regulation can lead to the formation of heritable epialleles (left) and to transcriptional activation of transposable elements (TEs) also resulting in their mobilisation (right). The question mark on the left reflects a shortage of examples illustrating well-documented, transgenerationally stable epialleles, especially stress-induced ones. Epiallelic and transposon-driven variation in gene expression generate phenotypic diversity that can be subjected to a desired selection (here illustrated as selection for large individuals). In subsequent generations the newly selected traits can be examined for their stability, mode of inheritance, chromosomal location and the molecular mechanisms involved in their formation. For simplicity, a nucleus with a single chromosome is shown (as grey bar, with centromere marked as black dot, and nuclear membrane as brown circle). Arrows represent chromosomal transcripts (grey: genes not subjected to epigenetic regulation, blue: genes silenced by epigenetic mechanisms, red: transposons). New TE insertion can affect expression of neighbouring genes (now thick grey arrow).

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Transient destabilization of the epigenome under stress conditions

For the environment to impact on long-term, transgenerational adaptation, the perceived environmental cues must be memorized in a form that is propagated through mitotic and meiotic divisions, even when the initial stimulus is removed. In this issue, Igor Kovalchuk discusses environmentally induced heritable phenotypes and possible molecular mechanisms that may be involved [24]. Moreover, one of the current authors (JP) together with Ueli Grossniklaus recently reviewed selected aspects of transgenerational epigenetic inheritance, including stress induction of heritable traits [25]. Therefore, we will restrict discussion of this subject here to just a few critical points.

In 2006, Molinier *et al.* demonstrated that ultraviolet-C radiation and the bacterial elicitor flagellin induced a high frequency of intra-chromosomal homologous recombination. This trait was inherited in the absence of the stress trigger and was transmitted in reciprocal crosses [26•]. Transmission of the hyper-recombination phenotype was documented for several consecutive generations, which was a critical aspect of this study, since the authors could then largely exclude parental effects of the stressed plants. Since the acquired hyper-recombination phenotypes were observed throughout the population, the authors suggested that the mechanisms responsible for the stress-induced phenotypes were likely to be epigenetic. However, the true mechanisms of the acquisition and inheritance of this trait remain unknown and, in fact, the experimental characterization of the mechanisms may be very difficult, since stress-triggered induction of hyper-recombination phenotypes are not as widespread as initially anticipated. A more recent publication describing an analogous experimental setup reported conflicting results [27]. Importantly, although the authors observed effects on stressed plants manifested by increased levels of intra-chromosomal homologous recombination similar to those reported by Molinier *et al.*, transgenerational transmission of this trait was not detected [27]. Thus, further investigations are needed to assess the robustness of this and other similar experimental systems before studies of the underlying molecular mechanisms can start. At the moment, it is largely unknown whether indeed epigenetic control is involved in the generation and propagation of stress-induced heritable traits [24].

An alternative approach to link epigenetic regulation and response to environmental stress has been tried recently by testing the influence of stress on the stability of previously well-characterized epialleles. Three independent research groups investigated transgenic and endogenous Arabidopsis loci that were already known to be subject to epigenetic regulation (suppressed by transcriptional gene silencing, TGS), using them as markers to readout the stability of epigenetic states under various stress conditions applied to plants. They examined whether subjecting Arabidopsis plants to various abiotic stress treatments, such as elevated salinity, water deprivation, UV radiation or temperature shifts would destabilize TGS at the marker loci. All three groups found that a transient shift to a higher temperature (37 or 42 °C) destabilized TGS [28•,29•,30•]. Stress-induced TGS release was observed at various transgenic and endogenous loci and was also confirmed at the genome-wide level by transcriptomic analyses [28•,29•]. Therefore, this system seems to be sufficiently robust and, thus, experimentally approachable for the investigation of molecular mechanisms involved in stress-induced destabilization of epigenetic transcriptional suppression. Noticeably, TGS destabilization was only transient, with silencing being re-established within 2 days after the stress ceased [28•,29•]. Initial results have indicated that transient silencing release and its rapid re-establishment is related to temporary changes in nucleosome occupancy [29•].

The anticipation that stress-induced destabilization of epigenetic regulation would be heritable is possibly too naïve given the well-documented cooperation of multiple epigenetic mechanisms in stabilizing and buffering epigenetic states of transcriptional activity [6,31]. Furthermore, transient destabilization of TGS occurred in differentiated tissues, whilst meristematic cells appeared to be protected [29•]. This suggests that acquisition of environmentally induced epigenetic changes may also be influenced by differing competences of plant tissues, further hindering passage of epigenetic alterations to the progeny. More controlled and precisely designed experiments are now needed to clarify these unknowns and discrepancies.

Epigenetic regulation of genome stability and its impact on stress adaptation

One important effect of epigenetic regulation is to restrict the transcription of TEs and thus prevent their movement. Right from the time transposons were discovered, McClintock linked regulation of TE transcription and transposition to genetic and environmental triggers [32] and a plethora of examples of environmentally induced transposon activities was subsequently reported (reviewed in Refs. [33–35]). Moreover, many examples of transposon-mediated transcriptional control of neighbouring genes added to the complexity of the regulatory networks that can be initiated by stress-triggered transposon activities influencing host genes [36,37••,38–40]. In recent years, progress in understanding epigenetic and developmental mechanisms involved in the control of transposon transcription and movement has shed new light on these dynamic interactions. A recent series of excellent reviews has addressed various aspects of transposon biology and their influence on the evolution and regulation of host genomes [35,41–44]. Therefore, here we will comment

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only on a very narrow set of the latest experiments describing epigenetic, developmental and environmental control of retrotransposons movement in Arabidopsis.

Retrotransposons are TEs propagating through a 'copy-paste' mechanism using an RNA template for reverse transcription. They represent the most abundant class of TEs, constituting the major part of many plant genomes, and although bursts of their transposition are thought to have shaped the architecture of plant chromosomes during evolution [45–48], their uncontrolled proliferation would be undesirable. Recently, it has become clear that transcriptional and post-transcriptional epigenetic control are of crucial importance to the restriction of retrotransposition, which may also be triggered by environmental stress.

Early experiments on the activation of retrotransposition of transgenic retroelements or the mobilisation of their endogenous copies in tissue culture cells suggested that control of their mobility is highly complex, involving poorly defined molecular mechanisms [49–52]. This is in contrast to the relatively detailed studies of epigenetic mechanisms affecting transcriptional silencing of retrotransposons, which have documented the involvement of multiple TGS pathways in the silencing of diverse families of retrotransposons. Notably, although retroelements were transcribed in mutants impaired in various TGS pathways, their movement was not observed. Only recently has proliferation of retroelements in Arabidopsis strains with altered epigenomes been reported. The group of Tetsuji Kakutani, when comparing the DNA of inbred strains of *ddm1* mutants and the wild type by genomic hybridization, discovered significant increases in copy number of several different retroelements, indicating their replicative movement [13•]. At the same time, retrotransposition of one such element, named *Evadé*, was observed in several *met1*-derived epiRILs. Since *Evadé* remained immobile in the *met1* mutant, where alleviated TGS permitted the synthesis of its full-length transcript, which could have been used for extrachromosomal DNA (ecDNA) synthesis preceding retrotransposition, it was hypothesised that ecDNA production is prevented post-transcriptionally by unknown mechanisms [15•]. Indeed, when the *met1* mutant was combined with a mutant deficient in siRNA biogenesis or histone methyltransferase KYP, ecDNA appeared and an uncontrolled burst of *Evadé* transposition was observed [15•]. Interestingly, genome re-sequencing showed the burst of retrotransposition to be limited to *Evadé*; other potentially active elements [13•] remained immobile, suggesting very selective epigenetic control that distinguishes different retroelement families. These results provided the first step towards the selective control of retrotransposition for native retrotransposons using the inactivation of particular epigenetic pathways. Moreover, it became immediately obvious that such experimentally controlled retrotransposition would be much easier to handle if it could be combined with conditional regulation by either chemical or physical switches.

Very recently, during microarray-based transcript analyses in experiments with heat-induced destabilization of TGS (discussed above), it was observed that the transcription of a particular family of *copia* retrotransposons, named *Onsen*, was activated [28•,29•]. Moreover, full-length transcripts of these retroelements persisted longer than transcripts of other transiently activated chromosomal targets following destabilization of TGS by heat stress [28•]. Importantly, in addition to the full-length transcripts, *Onsen* DNA was also detected and persisted in an extrachromosomal form for several days after heat stress [53•]. *Onsen* RNA and ecDNA accumulation was higher in mutants deficient in siRNA biogenesis. Although levels of both RNA and ecDNA diminished during subsequent plant growth and no new chromosomal insertions were detected, a surprisingly high frequency of retrotransposition was observed in the progeny of heat-stressed plants. However, this occurred only in mutants deficient in siRNA biogenesis (not in the wild type, even when subjected to heat stress) [53•]. Therefore activation of *Onsen* retrotransposition requires an environmental trigger (heat stress) and is under epigenetic (siRNA pathway) and developmental control (transgenerational transposition). The documentation of such elaborate regulation of retrotransposition provides an excellent handle on its control ('stop' and 'go') and, therefore, also for the detailed dissection of the underlying molecular mechanisms.

Retrotransposons influence the regulation of neighbouring genes and may carry regulatory sequences co-opted from the host genes that respond to specific environmental stimuli (e.g. the sequence of *Onsen* LTRs (Long Terminal Repeats) includes a common heat response motif) [53•]. Remarkably, genes in the vicinity of *Onsen* neo-insertions, found at random positions in gene-rich chromosomal regions, acquired transcriptional responsiveness to elevated temperature. Therefore, regulatory elements of retrotransposons when relocated by epigenetic activation of their transposition may generate further retrotransposon-derived regulatory networks able to respond to environmental cues. The example of *Onsen* resembles natural processes of plant genome evolution during which identified bursts of retrotransposon mobility may have contributed to the acquisition of new adaptive traits [54]. Although controlled induction of retrotransposition bursts by altering specific pathways of epigenetic regulation in combination with environmental triggers is still at the pioneering stage, this could well become an interesting source of novel traits or a tool to assemble new regulatory gene networks that can be subjected to subsequent selection (Figure 1) [22].

Aside from their mobility, there are further ways in which TEs can modify gene expression and play a role in stress

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adaptation. For example, TEs generate a vast repertoire of small RNAs that can regulate subsets of stress-related genes [55•] or act as general transcriptional repressors upon stress [56•]. These small RNAs, being mobile [57•,58•], could influence gene expression in distant organs, which would be especially important in the stress adaptation of perennial plants. Finally, as attractors of the epigenetic silencing machinery, TEs can also spread TGS to nearby genes [59]. Therefore, the recent identification of molecular mechanisms controlling the regulation of retrotransposon transcription and retrotransposition represents an essential step towards uncovering retrotransposons as a so far hidden source of genetic variation that could contribute to future plant breeding strategies.

Conclusion and outlook

Examples of stable transgenerationally inherited epialleles are still scarce and studies of plant populations displaying epigenetic variation are rather preliminary. So far these have not provided firm evidence of novel, well-documented epialleles contributing to heritable changes in stress responses. On the other hand, there are many examples of acquired traits related to the activities of transposons, and especially retroelements, which are an abundant component of plant genomes. Thus, we propose that the most attractive way by which epigenetic regulation could contribute to enrichment of novel traits related to plant stress adaptation is, directly or indirectly, the controlled generation and exploitation of retrotransposon-induced genetic diversity. We could even envisage that plant populations with a variety of new retroelement insertions would be able, under selective stress conditions, to 'recruit' activated retrotransposons as fast drivers of evolution, as has been shown for yeast [60]. Next-generation sequencing technologies, the availability of methylomes from plants responding to stress, and access to tissue-specific or single cell-specific genome and epigenome information [61•] may provide us with sufficient resolution power and, thereby, a more dynamic and thus more complete appreciation of the mobile part of the genome, 'the mobilome'. This will shed new light on its role in adaptive plant responses and their evolution.

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