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Review

Shaping Plant Adaptability, Genome Structure and Gene Expression through Transposable Element Epigenetic Control: Focus on Methylation

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Abstract: In plants, transposable elements (TEs) represent a large fraction of the genome, with potential to alter gene expression and produce genomic rearrangements. Epigenetic control of TEs is often used to stop unrestricted movement of TEs that would result in detrimental effects due to insertion in essential genes. The current review focuses on the effects of methylation on TEs and their genomic context, and how this type of epigenetic control affects plant adaptability when plants are faced with different stresses and changes. TEs mobilize in response to stress elicitors, including biotic and abiotic cues, but also developmental transitions and 'genome shock' events like polyploidization. These events transitionally lift TE repression, allowing TEs to move to new genomic locations. When TEs fall close to genes, silencing through methylation can spread to nearby genes, resulting in lower gene expression. The presence of TEs in gene promoter regions can also confer stress inducibility modulated through alternative methylation and demethylation of the TE. Bursts of transposition triggered by events of genomic shock can increase genome size and account for differences seen during polyploidization or species divergence. Finally, TEs have evolved several mechanisms to suppress their own repression, including the use of microRNAs to control genes that promote methylation. The interplay between silencing, transient TE activation, and purifying selection allows the genome to use TEs as a reservoir of potential beneficial modifications but also keeps TEs under control to stop uncontrolled detrimental transposition.

Keywords: transposable elements (TEs); epigenetics; RNA-dependent DNA Methylation (RdDM); small interfering RNA (siRNA); microRNA (miRNA)

1. Introduction

1.1. Epigenetic Modifications

The genetic information that modulates the phenotype and that can be inherited without being coded into the DNA sequence is known as epigenetic information. In contrast to canonical genetic mechanisms, epigenetic marks regulate the access to the genetic information more than the alteration of the genetic sequence itself. During the last decade the elucidation of structural elements and mechanisms that explain epigenetic control in a wide range of eukaryotes fostered the advent of a novel genetics perspective.

In eukaryotes, the structure of chromatin, regulates the accessibility of genes to the transcriptional machinery, thereby controlling gene expression. Structurally, DNA is packaged by means of nucleosomes, where histones (H2A, H2B, H3, and H4) are present as octamers, around which 147 bp of DNA are wrapped in almost two turns. The positioning and spacing of nucleosomes as well as post-translational histone modification, together with DNA methylation, affect the overall packaging of DNA and the accessibility of the transcription unit to specific regulatory elements, which results in altering gene expression [1].

In plants three main epigenetic mechanisms have been described: DNA methylation, histone modifications and RNA-interference (RNAi) [2]. DNA methylation occurs specifically over cytosine nucleotides that are followed by a guanine and sometimes by other nucleotides; in many cases, DNA methylation stops interaction with transcription factors and impairs gene activation. Histone modifications are more diverse and include methylation, phosphorylation, acetylation, ribosylation and ubiquitination of mostly histone H3, but post-translational modifications upon histones H4, H1, and H2A have also been described. These protein modifications constitute the "histone code" of chromatin epigenetic marks [3]. Regarding RNA-interference mechanisms, small RNAs, together with factors commonly associated with (RNAi) processes, target complementary DNA sequences and recruit factors that can induce chromatin modifications, specifically, the formation of heterochromatin, to silence targeted genes [4,5].

1.2. Plant Transposable Elements

Transposable elements (TEs) are DNA sequences that move through the genome via a cut and paste mechanism using a DNA intermediate (Class II TEs—DNA transposons), or a copy and paste mechanism with an RNA intermediate (Class I TEs—retrotransposons). In plants, TEs account for an important proportion of genomes, although the proportional representation range varies: Fourteen percent in the genome of *Arabidopsis thaliana* (L.) Heynh [6] to more than 80% in the genome of maize [7]. TEs are usually represented by numerous families corresponding to different superfamilies, orders and classes [8], each of which have a specific set of characteristics including their mode of transposition, presence of promoter sequences, order of genes coding for proteins and mechanisms of replication.

TEs are activated by stress through motifs embedded in their promoters [9–16], which can lead to bursts of transposition that increase their copy number. Since plants commonly experience stress throughout their life cycle, activated TEs can potentially jump to new genomic locations leading to gene-altering effects that can have positive or negative consequences [17–19]. Insertions that fall inside genes typically inactivate gene function [20–22], although many of these insertions are never observed since they can result in lethality if the gene is essential. Insertions inside introns can trigger alternative splicing patterns [23–25], while insertion in adjacent gene regions can generate new regulatory functions that modify gene expression and function [26–28].

Given the risk for lethality or significant modification of gene expression, plant genomes possess mechanisms to stop indiscriminate genome expansion and alterations by TEs. One of these mechanisms is based on epigenetic control, which allows the recognition and silencing of TE sequences.

1.3. TE Epigenetic Regulation Mechanisms

The emphasis on TE epigenetic regulation research stems from the study of DNA methylation marks that often result in TE inactivation. Very early in transposon research, the study of reversible inactivation of TEs through methylation demonstrated that this type of epigenetic control was a traceable fingerprint of TE activity. In one of these early studies, alterations in the methylation of *Mutator* transposons in maize resulted in changes in variegation patterns in maize kernel colors [29]. Also, in a lethal maize line which was unable to produce proper photosynthetic machinery, the phenotype was expressed in the presence of an unmethylated *Mu* TE insertion, but was suppressed when the TE was methylated and unable to mobilize [30]. The behavior of reverting mutants and patchy phenotypes due to TEs, explains some of the observations previously posed by Barbara McClintock

for the movement and mutations produced by the *Activator-Dissociator* (*Ac/Ds*) TEs [31]. But, it was not until a mutant for methylation was found in *Arabidopsis thaliana* [32], that testing for differential activation of TEs linked to methylation patterns could be clearly argued [33,34]. As more evidence accumulated and mechanisms for directed silencing were uncovered, it became clear that methylation of TEs was performed via a self-regulation using TE-derived transcripts for the generation of small interfering RNA (siRNAs) [2,35].

Small RNAs are used for RNA-directed DNA methylation (RdDM) through an RNA-induced silencing complex (RISC) to induce transcriptional gene silencing (TGS). TE-derived transcripts are processed by RNA-Dependent RNA polymerases (RDRs) that produce double-stranded RNA (dsRNA). Double-stranded RNA undergoes processing into siRNAs using specific DICER-like (DCL3, DCL4) proteins, and the siRNAs are then recruited by Argonaute family proteins (AGO4, AGO6). AGO-siRNA complexes interact with DRM1 and DRM2 methyltransferases and are directed to target sites where siRNA binds to transcripts generated by RNA polymerase V (PolV). This process allows to methylate cytosines at CG, CHG, CHH sites (H representing any other nucleotide) and initiate TGS [2]. However, small RNAs can also become part of a larger protein complex that includes members of the Argonaute family (AGO1) and target the source RNA (in this case TEs), to cleave it, resulting in post-transcriptional gene silencing (PTGS), reviewed in [35,36]. Yet a third mechanism comprises post-translational histone modification mainly on heterochromatic genomic regions [35,36].

1.4. Linking Plant Responses to Transposable Element-Derived Epigenetic Changes

Plants rapidly adapt to new conditions (e.g., stress) via processes traditionally termed as phenotypic plasticity, acclimation or hardening [37]. Such changes usually affect the plant's growth, physiology and/or development. Once the initial stress is detected, appropriate signaling cascades are triggered and relayed into alterations of gene expression through transcription factors or modification of epigenetic signals. Epigenetic changes produced by the stress elicitors can be transient or inherited through generations [38,39], and can help the plant to respond faster and more efficiently in case of continuing or recurrent stresses, providing a mechanism for plant adaptation. Since modifications in the transcriptional signals and epigenetic marks in plants can be modulated by stress and development, elicitors to which TEs are responsive as well, both transient and heritable epigenetic modifications can influence TE activation and regulation.

Research on the epigenetic control of TEs, their activation due to developmental and stress changes and the widespread distribution of these elements in plants [40], has opened an interesting field of study which examines how TEs can modulate gene expression and reshape plant genomes. Because of their abundance in plant genomes [40], once epigenetic repression is lifted there is potential for at least some elements to jump to new locations (Figure 1A,B). These novel insertions can undergo: Purifying selection (especially when TEs are inserted close to genes and methylated) [41–43], events of exaptation (adoption of the TE or parts of it for normal gene function) [44], or accumulate with either detrimental or alternatively no apparent effect on the genome. Either way, TEs will remain in their new locations for some time and can be targeted for epigenetic repression (Figure 1C). When TEs fall inside or close to genes, they do not only have a disruptive potential, but can also epigenetically modulate adjacent regions [43,45,46] (Figure 1C,D). Additionally, if TEs are modulated epigenetically and generate hotspots where they cohabit with other genes, they have the potential of transducing and recombining these genes to generate new variants which can evolve into new functions [47]. The synchronization of TE and host stress responses can be viewed as an escape mechanism that benefits TEs but can also provide rapid genomic change with novel functionalities that might improve the capacity of the host to overcome stress. In a scenario where stress controls TE movement and TE insertions can modulate gene expression through their stress response elements and epigenetic marks, TEs can be catalogued as mediators of plant adaptation. We will center our following discussion on methylation-related control of TEs since this mechanism has been widely studied and seems to have a large influence on TE control changes in the genome. Also, we examine how this regulation

mechanism influences changes in the genomic landscape of plants, towards potential adaptation and response to different elicitors.

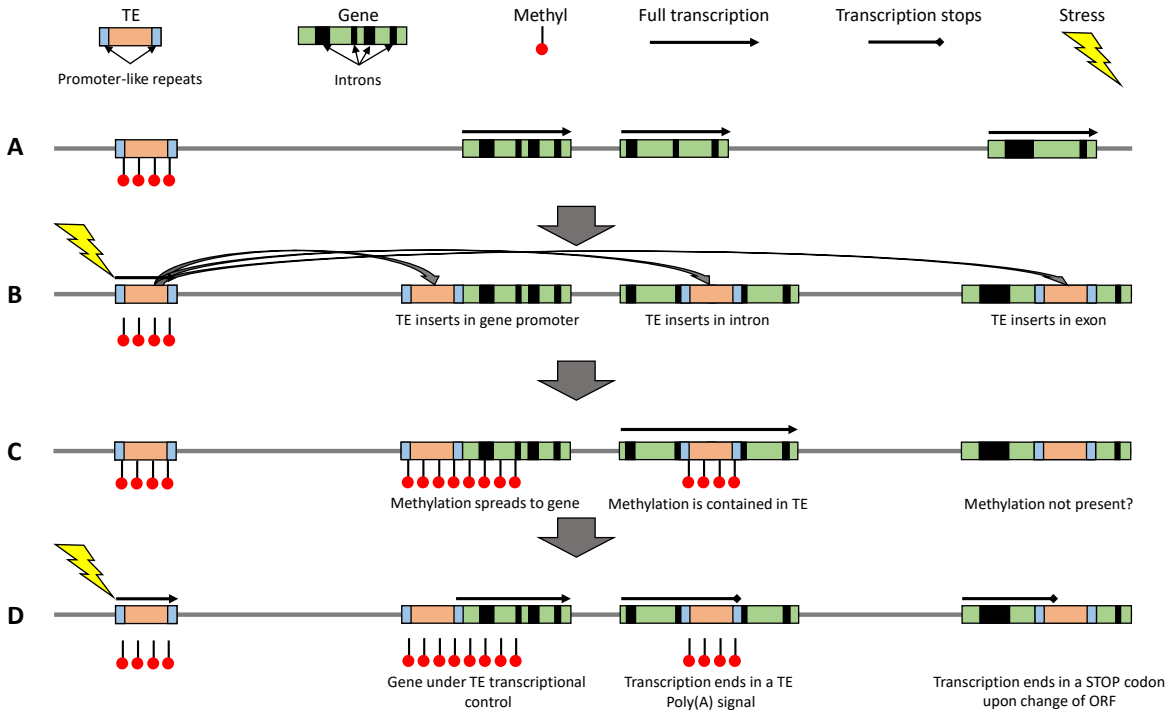

Figure 1. Epigenetic control of transposable elements TEs through methylation. (**A**) Under absence of elicitors a methylated TE remains silent and genes which are far from the TE can be transcribed normally; (**B**) Upon stress, the TE becomes hypomethylated and can be transcribed and transposed to new locations: A promoter region of a gene, an intron or an exon; (**C**) When normal conditions resume the original TE and the TEs located in the promoter and intronic regions of genes are silenced again through DNA methylation (RdDM.) Methylation from the TE inserted in the promoter of the gene spreads to the adjacent gene and either lowers or suppresses expression. Methylation in the intronic TE is usually contained and helps with correct splicing of the transcript. In the case of an insertion into an exon the result is commonly a disruption of the reading frame which renders a non-functional gene; methylation is rarer in TEs falling in exons [48]; (**D**) Upon a second round of stress silencing is lifted again. The first gene carrying a TE in its promoter can fall under the transcriptional control of the TE's stress responsive motifs. Demethylation of the TE inserted in the intron of the second gene can result in a read-through transcript that ends prematurely on a cryptic polyA signal inside the TE. The TE insertion disrupting an exon will likely end transcription prematurely when the open reading frame (ORF) changes from the gene into the TE generating a stop codon.

2. Stress, Development and Genome Size: How TE Epigenetic Changes Alter the Genomic Landscape and Influence Plant Adaptability

Abiotic and biotic stresses [9,49,50], developmental changes [51], and events of genomic shock such as polyploidization [52,53], are elicitors of plant adaptation. The reprogramming of the genomic landscape during these events, can lift repression of silenced TEs, and constitutes one mechanism that allows TE movement (Figure 1) (an alternative mechanism uses stress-induced *cis*-elements in TE promoter regions). TE transposition events due to these elicitors can result in both detrimental and/or favorable changes that can decrease or favor plant's adaptability.

2.1. Prominent Examples of Abiotic Driven Changes

One of the most studied plant TEs in relation to activation by an abiotic stress is the *copia*-type element *ONSEN*. The activation of retrotransposon *ONSEN* in *Arabidopsis* is elicited by heat stress [49],

through heat shock transcription factors that bind to a *cis* regulatory sequence in the transposon's promoter region. *ONSEN* expression increases in mutants that are deficient in siRNA generation [9,49]. Experiments on this TE show that siRNA regulation influences transcription, but hypomethylation does not necessarily increase retrotransposon expression [9,49], showing that RdDM might not be the controlling mechanism in this case. The generation of an *Arabidopsis* line carrying a GFP (green fluorescent protein) gene controlled by an *ONSEN* promoter in a siRNA mutant background, results in increased signal detection of GFP, confirming that siRNA is involved in transcription control of the TE [54]. Furthermore, the mutants displayed transposition of the elements compared to no transposition in wild-type plants, and these new insertions could be inherited to the progeny [54]. More research may be required to test if siRNA mechanisms and methylation status are independent in the case of this retrotransposon, but a more recent experiment shows that impairing transcription of TEs through mutations in RNA polymerase II (Pol II) results in DNA methylation decrease, and increased *ONSEN* mobilization [55]. At some point *ONSEN* retrotransposons acquired heat response elements in their promoter sequences allowing them to transpose upon heat stress [9,56]. The acquisition of such motifs in different Brassicaceae family members represents an alternative mechanism used by these TEs to escape epigenetic regulation via methylation. Such strategy is not rare among TEs, with many of them incorporating stress response elements in their promoters and linking their potential amplification to their host stress response. Furthermore, insertion of these TEs upstream from genes confers heat responsiveness [56]; if the ability to respond to heat is beneficial for the gene, it is possible than the insertion is positively selected.

The *NAC* gene from maize provides another example of how TE-derived epigenetic modifications can impact abiotic stress responses. NAC transcription factors regulate many processes in plants including responses to stress. A Genome-Wide Association Study (GWAS) found drought sensitivity polymorphisms associated to a miniature inverted-repeat transposable element (MITE) insertion in the gene promoter of a NAC transcription factor. Samples carrying the TE insertion in the gene promoter displayed high levels of DNA methylation through activation of the RdDM pathway, resulting in lower expression of the gene and an overall lower tolerance to drought [57] (Figure 1C). This insertion event spread among temperate maize after domestication but not among the maize ancestor (teosinte) and tropical or sub-tropical maize. The phenomenon is a clear example of how selection for yield can result in other random mutations which become detrimental under a regime of low water status.

The two examples above demonstrate how TEs are key players in plant potential for adaptation to stress conditions. How epigenetic marks regulate TEs in promoter regions may be a process of fine tuning over time. Recently inserted elements might be more prone to tight regulation and spread of silencing on adjacent regions [46], however TEs that progressively increase their copy number are perhaps more difficult to silence since resources to silence them become limited [45]. The acquisition of TEs in regulatory genes can be beneficial if it provides the possibility of selective activation of the gene only under conditions when the gene is needed, thus providing an efficient mechanism of resource utilization.

2.2. TEs and Plant Defense Responses

TEs can become part of genes through processes of exaptation [44,58], where complete or partial TE sections are acquired for normal gene function. When TEs become part of promoters of normal plant genes, epigenetic silencing provides a tight mechanism to control expression of the host gene under specific elicitors (Figure 1C,D). In *Arabidopsis*, the use of a flagellin bacterial peptide, a trigger of plant defense responses, results in demethylation and transcription of several transposable elements along with demethylation of promoter regions of defense genes that carry TE-like repeats/sequences [50]. Likewise, a triple mutant for three of four demethylases in *Arabidopsis thaliana*, displayed numerous downregulated stress response genes that carried transposable element-derived sequences in their promoters [59]. Such promoter sequences with increased methylation in the mutant background impaired gene function and consequently resistance to the fungal pathogen *Fusarium oxysporum*

Schltdl. decreased. Another example, providing support to the co-activation of defense genes and TEs showed that in *A. thaliana* plants that were challenged with *Pseudomonas syringae* pv. *tomato* (Okabe) Young, Dye & Wilke, methylation levels decreased in both TEs and some defense genes [60]. Hyperactivation of plant defenses using salycilic acid (SA) also results in TE activation and regulation of some genes in the vicinity of TEs, suggesting that TE epigenetic marks may affect nearby genes. These examples indicate that biotic response genes can also use regulation through TE incorporation and corroborates that both, abiotic and biotic responses, can use common promoter signals. This may also account for the common crosstalk observed between genes traditionally observed in biotic or abiotic interactions.

Finally, since TEs not only transpose but actively recombine internally and with other similar TEs, suppressing methylation in response to a stress event can result in TE-mediated genome restructuring. For example, TEs clustered with specific gene families (e.g., *R*-genes) can result in TE-directed gene shuffling/recombination, accelerating the evolution of these genes and providing new mechanisms of defense against pathogens [47]. In addition to promoting recombination on these gene clusters, TEs can also exert their influence on these hotspots through extension of methylation, transduction of downstream genes, mutation through gene truncation, and indel events [61]. This demonstrates that changes in the TE epigenetic landscape on these dynamically active regions can contribute to plant diversification of defense components.

Overall, TEs provide alternative mechanisms for control of gene expression upon biotic stress but are also important in accelerating genome restructuring and gene evolution. Both factors embody important mechanisms of plant adaptability and can be retained through processes of natural selection when changes are acquired in germline cells.

2.3. Benefits of Activating/Deactivating TEs during Development

Silencing of TEs is lifted during certain developmental stages. The change in the methylation status of TEs could be a by-product of the necessary reactivation of other silenced genes when plants go through shifts in development [51]. An interesting case of transposable element regulation occurs in the endosperm where active imprinting-dependent demethylation takes place to allow allele-specific gene expression. In both, *Arabidopsis* and rice, demethylation in the endosperm during gene imprinting results on TE activation and siRNA generation, accompanied by hypermethylation of transposable elements in the embryo [62–64]. The increased activity of TEs in reproductive tissues is also observed in the pollen vegetative nucleus of *Arabidopsis* plants, where specific TEs are reactivated through demethylation [65]. It is possible that the process of endosperm imprinting that determines tissue specific expression of genes could have been derived from targeted methylation of TEs inserted close to genes. The methylation on TEs could have been extended to nearby genes and the alternative gene expression (through methylation-demethylation) during certain stages of development was potentially selected as a favorable strategy for certain genes [66]. Interestingly, the activation of TEs and production of the TE-derived small interfering RNAs on the endosperm, can result in the selective control of TEs in nearby cells directly involved in reproduction (through TE-derived siRNAs that act as mobile signals between cells). In such reproductive cells, drastic changes due to TE movement could result in detrimental changes for the progeny, and therefore siRNA-mediated silencing of TEs without TE activation seems like a suitable strategy to preserve genome integrity.

Another example of TE development-driven changes occurs with flowering time genes. The *FWA* (Flowering Wageningen) gene, which controls flowering, is silenced in wild type *Arabidopsis thaliana* plants and is only expressed during specific stages of development in the endosperm. Mutants for DNA methylation processing, express *FWA* ectopically, demonstrating that silencing depends on cytosine methylation [67]. DNA methylation analysis of the gene indicates that the direct repeats responsible for silencing originated from a Short Interspersed Nuclear Element (SINE) TE that maps close to *FWA's* transcriptional start site. Finally, in maize a major allele for flowering repression shows an insertion of a MITE associated with heavy RdDM within and outside the boundaries of the TE.

This pattern results in an early flowering phenotype indicating that epigenetic associated changes of an inserted TE can alter a neighboring gene [68]. These examples can be catalogued as mechanisms by which methylation marks provided by a TE determine an exaptation event which is selected for novel gene functions.

2.4. Can Polyploidy Transiently Affect TE Activity?

Polyploidy represents another event that has been characterized as a type of genomic shock where transposon-mediated changes can take place and affect gene expression and genome structure. For example, *Brassica napus* L. has experienced polyploidization due to hybridization of parental species (*Brassica rapa* L. and *Brassica oleracea* L.). In a study where *Brassica napus* was resynthesized from its parental species, non-additive TE insertions appeared in *Brassica napus*, which led to hypothesize that these changes were influenced by methylation changes that occurred after the polyploidy event [52] (Figure 2). Likewise, newly synthesized *Arabidopsis* polyploids showed increased TE transcriptional activity which was directly related to decreases in methylation of their respective loci. This activity possibly led to transposon involvement in chromosomal rearrangements, showing the impact TEs can have on genome restructuring [69]. A study of polyploidization in wheat evidenced a decreased number of siRNAs matching TEs with concomitant higher transcript levels of *Wis-2* and *Veju* retrotransposons, and decrease in methylation of the latter TE [70]. Also, in wheat, an event of polyploidization resulted in larger hypomethylation vs. hypermethylation in the first generation after the polyploidy event, and increased hypermethylation in later generations in loci corresponding to the retrotransposon *Veju*. These events were associated with TE deletions and insertions, showing that methylation changes promote TE rearrangements [71]. During polyploidization when silencing is lifted, at least some TE families might actively transpose, with potential disruptive effects on gene function (Figure 2). Although this would seem counterproductive for the host genome, the same polyploidization event has now generated multiple copies of each gene, and while some of them will keep fulfilling their basic function, others are free to evolve through novel mutations (Figure 2C).

While in the examples above stress commonly produces transient demethylation, hypermethylation is also possible. A rice autopolyploid with a whole genome duplication (WGD) showed most TE families were hypermethylated [72]. The methylation was accompanied by transcript downregulation of genes located nearby the TEs, and with an abundance of siRNAs mapping to the TEs, supporting RdDM as the main mechanism for TE silencing [72]. In this case the authors argued that silencing of TEs and subsequent influence upon adjacent genes allowed the newly synthesized genome to compensate for genome dosage effects. Evidently, since most genes are duplicated, the silencing of duplicated copies allows similar level of expression of genes between the 2× and 4× genomes, which prevents a waste of cell energy and resources [72].

It could be assumed that polyploidization would always drive increases in TE copy number and TE-mediated genomic changes, but many events of polyploidization in plants do not show TE transposition [73]. Additionally, when TE-mediated rearrangements take place, they do not necessarily have to involve whole-genome scale changes [52], and are usually restricted to a few specific TEs [73]. Furthermore, processes of purifying selection start taking place at least for some TEs falling in vicinity of genes [41,42], and transposon decay and recombination are used to stop uncontrolled genome size increase [74].

The examples above provide some evidence that even upon polyploidization, a controlled burst of transposition can have some positive effects in terms of rearrangement and genome energy utilization. However, as we will see in the next section, the differential activity of TEs among closely related species can account for large genome size differences.

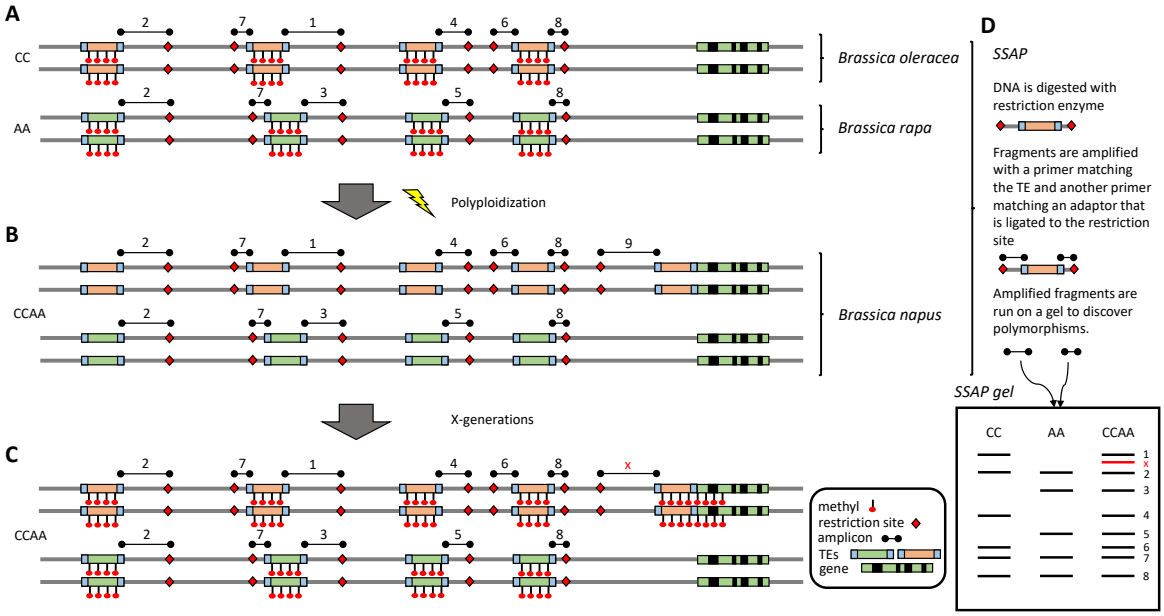

Figure 2. Allopolyploidization and its effects on TE epigenetic regulation. The diagram shows the hypothetical outcome of a cross between the diploid genomes of *Brassica oleracea* (CC genome) and *Brassica rapa* (AA genome) to produce an allotetraploid (*Brassica napus*—CCAA genome). (**A**) Homologous DNA regions of the parent genomes contain four TEs and one gene; the regions present some divergence due to speciation; (**B**) After polyploidization a tetraploid is formed (*B. napus*) and methylation is transiently suppressed, allowing TEs to move to new genomic locations. In this example just one TE moves to the promoter region of the depicted gene of the CC subgenome; (**C**) After a variable number of generations methylation is reinstated and the novel TE insertion silences the gene in the CC subgenome, but the homeologous gene in the AA subgenome expresses normally; (**D**) In the original experiment [52], SSAPs (Sequence-Specific Amplification Polymorphisms) [75] were used to detect sequence polymorphisms from transposable element insertions in the two parents and the synthesized allotetraploid. In SSAP DNA is digested with a restriction enzyme, and amplicons are generated from one primer binding the TE and a second primer binding an adapter ligated to the restriction site, resulting in a pattern of bands when run on an electrophoresis gel. The gel diagram shows the putative pattern of bands from the hypothetical parental genome sections and the resulting allopolyploid. The different amplicons corresponding to these bands can be followed in sections A to C of the figure (common bands with the same size have the same number). The novel insertion of a TE in the CC subgenome of *B. napus* generates band x, which constitutes a non-additive band supporting an event of transposition during polyploidization.

2.5. TEs Can Boost Genome Size Divergence among Related Species

Transposable elements have been characterized as having a large influence on plant genome size variation, because of their capacity to amplify their copy numbers when bursts of transposition occur [17]. These effects can be viewed even among closely related species. For example, while the genomes of *Arabidopsis thaliana* and *Arabidopsis lyrate* (L.) O'Kane & Al-Shehbaz are mostly syntenic [76], the genome of the latter is 1.5 times larger. The two species diverged only 10 million years ago, but besides other differences in chromosome number, and gene structure, transposable elements account for most of the size difference (with *A. lyrata* having three times as many TE insertions as *A. thaliana*), and for the disruption of syntenic collinearity [45,76]. As with most plants, epigenetic control of TEs in these two species depends largely on siRNA-guided DNA methylation. However, while the number of siRNAs that map to TEs is proportionally similar for both species, the ratio of siRNAs that map to unique TEs differs, with *A. thaliana* bearing a larger proportion of siRNAs targeting unique sites [45]. Since the number of siRNAs reaching multiple copies of the same element is less, and enzymes for processing silencing are also limited, a larger quantity of TE copies in *A. lyrata* would

be harder to silence. This potentially results in further mobility of TEs in *A. lyrata*, and concomitant genome size increase (an example of this process is given in Figure 3). Consistent with this view, higher expression of TEs was observed in *A. lyrata* when compared with *A. thaliana* under normal vegetative conditions [77]; although this latter study did not find a difference in the methylation load between species, the methylation analysis only concentrated in a few genic regions and did not explore genomic regions corresponding to TEs. Similarly, a 1.5-fold genome size difference between the genomes of *Zea mays* L. and its close relative *Zea luxurians* (Durieu & Asch.) R.M. Bird, is largely due to a higher content of TEs in the latter species, and it was suggested (but not tested) that epigenetic mechanisms could account for difference in proliferation of TEs between these two species [78]. A genome size duplication in a wild relative of rice, *Oryza australiensis* Domin., is due to retrotransposition of three LTR-retrotransposon families in the last three million years [79]; although there is no experimental proof of epigenetic changes during these events, it would be logical to infer that TE silencing was lifted during genome expansion. In the same way, TE lineage specific bursts are linked to genome size differences among *Gossypium* species [80]. As illustrated by these examples, TE-dependent genome size difference among closely related species can be a consequence of very specific evolutionary stories, where some species undergo one or more events of genomic shock that transiently modify activity of some TEs, which incrementally become more difficult to control solely by epigenetic mechanisms (Figure 3).

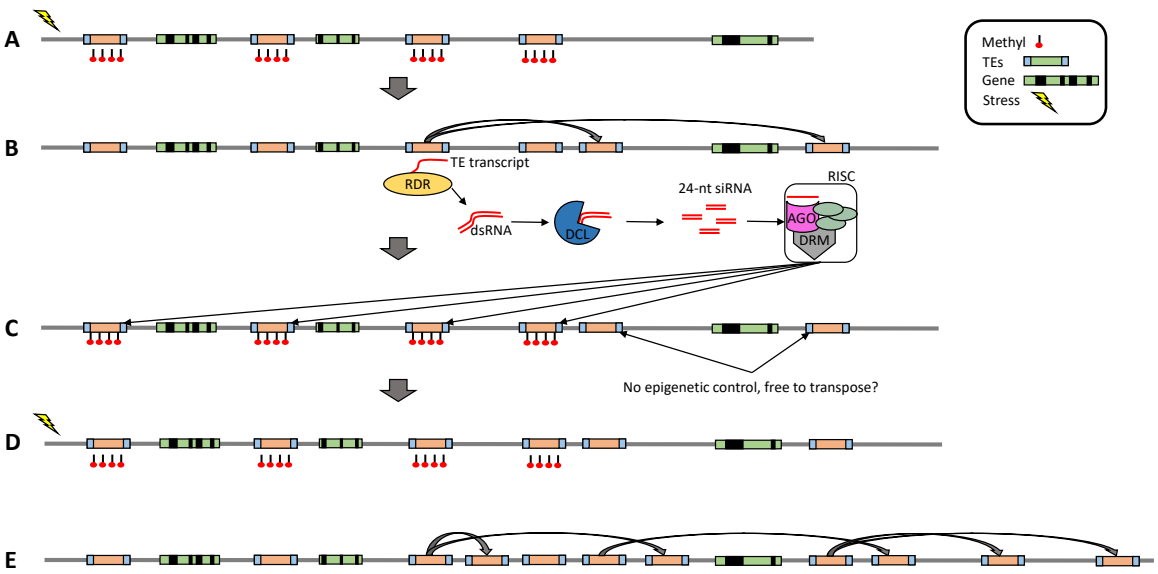

Figure 3. Potential mechanism of genome size increase due to TEs. (**A**) Multiple copies of a TE are demethylated upon stress; (**B**) TEs jump to new locations increasing genome size. At the same time, TE transcripts are recruited by an RNA-dependent RNA polymerase (RDR) to generate a double stranded RNA (dsRNA) that is broken down into double stranded 24-nt siRNAs by a DICER (DCL) protein. siRNAs are loaded to Argonaute proteins (AGO) which form a RISC complex (RNA-induced silencing complex) with methyltransferases (DRM) and other proteins. The siRNA binds to complementary target RNAs being produced in the multiple TE copies bringing the complex into place to promote methylation; (**C**) The RISC complex promotes methylation of multiple TEs but as more copies of TEs transpose to novel locations, resource limitation makes methylation less effective in some of the TEs, leaving some partially or completely unmethylated. Such TEs could now transpose more freely without epigenetic control; (**D**,**E**) A new stress triggers the process again further increasing genome size. As more copies of a TE are dispersed through the genome, epigenetic mechanisms for silencing all copies become increasingly limited.

While genomic obesity can be controlled by mechanisms like epigenetic silencing and TE recombination, the regulation of TEs works better on smaller genomes with a lower number of TEs [45,74]. This pattern can promote further TE activity in genomes which already had several events of TE-related expansion. Larger genomes with high amounts of TEs would have to rely on alternative mechanisms to halt uncontrolled TE expansion. The fact that larger genomes with high number of TEs persist indicates that host plants can benefit or tolerate such expansion. Obese genomes can host more mutations, and these mutations are usually outside of genes and map to regulatory regions [81], providing for a faster decay of TEs, but also for potential adaptive regulatory changes. At the same time a larger amount of TEs generates a higher likelihood for structural variants through processes of recombination, transduction, indels and inversion, which can be important for plant adaptation. Overall an increase in genome size due to TE expansion creates variation upon which natural selection can take place.

3. Is Extension of TE Methylation into Surrounding Regions detrimental?

Patterns of insertion of TEs into plant genomes can influence genome restructuring, as well as modify gene expression when TE-directed methylation spreads to adjacent regions and causes decreases in gene expression [43,45,46,48,82,83] (Figure 1C). This TE-mediated repression seems unfavorable for genes and therefore, one would expect a mechanism to diminish this action. In fact, while methylated TEs close to genes decrease gene expression in *Arabidopsis thaliana*, TEs close to genes have lower levels of methylation than TEs found farther from genes [43,45,46]. More heavily methylated TEs are usually localized in heterochromatic regions where methylation does not spread from TEs but from surrounding regions into TEs [48]. Partial methylation of TEs close to genes works as a necessary trade-off, where some gene expression is sacrificed in order to stop further transposition or read-trough transcripts from TEs close to genes [43].

But why would gene-associated TEs have lower methylation than TEs that are far from genes? Some authors suggest that TEs that fall close to genes undergo purifying selection, which results in quicker removal of these elements than the ones located in heterochromatic regions [41–43]. If this is true, younger insertions are more common in regions nearby genes. Younger insertions that have not undergone removal yet, can yield several copies as products of recent transposition bursts. If we assume limitations in enzyme availability and siRNAs, targeting multiple young transposon copies becomes increasingly difficult, and epigenetic modification would not be performed in every TE (Figure 3). In the meantime, siRNAs uniquely mapping to single or low copy TEs would probably tag their targets successfully for methylation. This argument is supported by studies in *Arabidopsis* showing that siRNAs mapping to a unique TE, produce a higher level of methylation [45,46]. Interestingly, the genome seems to further purify methylated TEs (but not unmethylated TEs) nearby genes [43], decreasing even more the potential negative effects of methylation spreading into genes. Although detrimental effects on gene expression are seen when TEs insert close to genes, plants can partially control the insertional epigenetic effects and occasionally benefit from some insertions when they provide alternative or novel promoter-like regulatory functions.

Methylation spread from TEs is found commonly for elements inserting up or downstream from genes, but insertion within genes can have alternative outcomes. TEs that interrupt genes can either directly disrupt gene expression when they fall inside exons and alter the reading frame (Figure 1), or can modify the splicing of the host gene (Figure 1D). For example, a resistance gene in *Arabidopsis* that responds to infections of *Peronospora parasitica* (Persoon) Constantinescu, contains a *Copia* retrotransposon in its first intron, which affects gene splicing [24]. Histone methylation in the retrotransposon region correlates with correct splicing of the element and production of normal gene transcripts, while poor methylation generated by a mutation in a controller of methylation results in an alternative splice site inside the retrotransposon. Heterochromatization due to histone modification and DNA CHG methylation is abundant in transposons inserted in introns of genes in the maize genome and does not prevent correct transcription of host genes [84]. Therefore, methylation

corresponding to TEs inside introns does not extend into exons and instead helps delimiting boundaries that result in correct gene expression (Figure 1C). Evidently, the mechanisms of methylation spread from TEs are dominated by genomic context and have been selected during evolution to execute different functions.

4. Can TEs Escape Epigenetic Control?

Besides stress activation, strategies used by TEs to avoid silencing include [85]: (i) Inserting in regions close to genes which decreases but not fully eliminates silencing [43]; (ii) capturing gene fragments to resemble normal genes—a strategy used by pack-MULEs (*MUtator*-Like Elements) [86]; (iii) non-autonomous replication (e.g., MITES) to increase copy number [87]; and (iv) the generation of micro RNAs to suppress genes involved in epigenetic control. It could be argued that when TEs become part of the genome as controllers of transcription or when they donate partial or full reading frames to normal plant genes during exaptation events [44,58], they also escape host control. These mechanisms could be better characterized as part of whole genome evolution, where TEs can be viewed as a reservoir of sequences to diversify genome function and structure in short evolutionary times. Nevertheless, incorporation of TEs into the genome and TE-mediated gene capture depend on the restrictions imposed by epigenetic silencing. For example, pack-MULEs, which can reach high copy numbers in the genome [86], tend to fall in regions with higher recombination rates and low methylation level, increasing their chances for gene capture. However, as the pack-MULEs get older, their internal sequences tend to be more methylated [88], probably as a result of silencing directed by the genome.

TEs are not only generators of transcripts which can be processed into siRNAs to produce a feedback loop of transcriptional control through RdDM; TEs can also act as sources of microRNAs (miRNAs) that mitigate the expression of host genes through translation repression or mRNA degradation [89,90]. Since miRNAs can be produced from processing stem-loop RNA structures, MITEs are ideal TE candidates for miRNA production. Most of these elements have lost their transposase and have inverted repeats that can form the desired hairpins with a double RNA stretch that can be processed into a miRNA [87]. However, similar TEs inserted in tandem and opposite direction are also suitable for the generation of TE-derived miRNAs in plants, animals and fungi [89].

The evolution of siRNAs from TEs into miRNAs and their co-option to regulate host gene expression was suggested a decade ago [91], and a clear-cut distinction of siRNAs evolving to control TEs and miRNAs evolving to control genes has now blurred [92]. A study in *A. thaliana* found that at least 20 miRNAs produced by TEs control genes in trans [93]. Under this scenario, miRNAs would be generated from TEs that acquire part of the host or adjacent sequences to be processed into miRNAs. Alternatively, TEs can pick up and copy a fragment of a gene through transduplication [94], and then process it into miRNA. These characteristics can be used by TEs to suppress silencing. For example, if a TE inserts into a gene involved in methylation and an alternative splice form is produced, a miRNA can now start evolving which will target such gene. Such mechanism of evolution has been proposed before by studying miRNAs matching TEs and target genes that have incorporated such miRNA sequences in their reading frames, allegedly derived from TE insertions [90]. Members of *CACTA* DNA transposon family in rice contain microRNA sequences which target a methyltransferase. Two alternate miRNAs species of 24 and 22 nucleotides target the methyltransferase for methylation and mRNA degradation respectively; the decrease in the activity of this methyltransferase results in reduced methylation and increased TE activity [95]. Also, one miRNA derived from an *Athila* retrotransposon in *A. thaliana*, has been shown to target a protein (UPB1b) which under normal circumstances, inhibits TEs translationally. The TE-derived miRNA is therefore able to suppress its inhibition in *trans* by producing a miRNA that targets this gene [93,96]. Yet, another example of TEs escaping epigenetic silencing comes from a *MULE* in *A. thaliana*, which contains an anti-silencing factor. The incorporation of a complete *MULE* transgene results in the mobilization of endogenous *MULE* copies and decreased methylation of the TEs [97]. While the protein responsible for mobilization

is similar to a transposase, methylation changes were linked to a second reading frame designated as *vanC*, acting as an anti-silencing factor. VANC proteins bind a specific tandem repeat which is widespread among several transposons, and are able to use this interaction to erase methylation control [98].

The mechanisms utilized by plant TEs to incorporate themselves as part of a functional genome, and their ability to suppress epigenetic silencing favor permanence of TEs as part of the evolving genome. If most miRNAs are of TE origin, then TEs have provided yet another mechanism of control that benefits regulation of gene expression and processing in their host genomes.

5. Conclusions

Besides TE potential for read-through transcription, gene disruption, generation of intronic sequences and control of gene expression, the epigenetic control of TEs through methylation adds another layer of TE-dependent regulation in the genome. The tight control of TEs through methylation can be both beneficial and detrimental to genomic stability. One could think that most mechanisms of silencing established in the genome would point to mitigation of TE spread if these elements are viewed as mere parasites. However, if RdDM is performed through the generation of siRNAs and miRNAs that are produced by the same element, the process of regulation seems more like a feedback control loop. Lifting silencing transiently during events of stress, development or genomic shock, would in fact give some flexibility to TEs to generate certain amount of change without totally scrambling the genome. This allows the genome to have some room for restructuring and adaptation in response to different elicitors. Additionally, the incorporation of TEs in promoter regions provides a mechanism to modulate stress response in certain genes. Such insertions can be positively selected if these genes become effective in responding to stress and if their modulation provides better energy balance for the cell. Otherwise, these insertions may decay through purifying selection or if their effects are lethal, then individuals carrying them will not be able to survive.

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