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Epigenetic control of transposon transcription and mobility in *Arabidopsis*

Etienne Bucher[1], Jon Reinders[2] and Marie Mirouze[3]

The mobility of genetic elements called transposable elements (TEs) was discovered half a century ago by Barbara McClintock. Although she had recognized them as chromosomal controlling elements, for much of the consequent time TEs were primarily considered as parasites of the host genome. However the recent explosion of discoveries in the fields of genomics and epigenetics have unambiguously shown the importance of TEs in genome function and evolution. Bursts of endogenous TEs have been reported in plants with epigenetic misregulation, revealing the molecular mechanisms underlying their control. We review here the different steps in TE invasion of the host genome involving epigenetic control and environmental stress responses. As TEs propagate in plant genomes and attract epigenetic marks, their neo-insertions can lead to the formation of new, heritable epigenetic variants (epialleles) of genes in their vicinity and impact on host gene regulatory networks. The epigenetic interplay between TE and genes thus plays a crucial role in the TE-host co-evolution.

Addresses
[1] Botanical Institute, University of Basel, Hebelstrasse 1, 4056 Basel, Switzerland
[2] DuPont Pioneer, DuPont Experimental Station E353, Route 141 and Henry Clay Road, Wilmington, DE 19880-0353, USA
[3] Institut de Recherche pour le Développement (IRD), UMR322 URL5300, Université Montpellier 2, 911 Avenue Agropolis, 34394 Montpellier, France

Corresponding authors: Bucher, Etienne (e.bucher@unibas.ch), Mirouze, Marie (marie.mirouze@ird.fr)

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Introduction

In the last decade remarkable efforts have been made in the sequencing of complete eukaryotic genomes. A surprising result was that even though genome sizes can vary greatly between different species, the total number of protein coding genes stays roughly the same. The main genomic difference between even very closely related species is in the non-protein coding part of the genome, which is mostly composed of transposable elements (TEs). Thus it is very likely that TEs are the major contributors to the expansion that has made genomes more complex. Owing to their successful amplification, comprising up to 85% of some eukaryotic genomes, TEs were perceived only as 'selfish DNA' without conferring any benefit to the host [1]. However this view is now changing. Pioneering studies in maize have revealed not only the existence of TEs but also their epigenetic control [2,3]. Despite the low TE content in the *Arabidopsis thaliana* genome, this model plant has also contributed interesting examples on TE control, notably by using mutants of epigenetic regulators in studies combining genetic and genomic approaches. Here we discuss these recent findings describing the mechanisms that control TEs in *Arabidopsis*. We distinguish the epigenetic control of transcription, affecting a large number of TEs, from the epigenetic control of transposition, which has been so far limited to a few examples of TEs. We also discuss how *Arabidopsis* TEs can influence endogenous gene expression and explore how the potential evolutionary roles of TEs might benefit host genomes.

TEs transcription in epigenetic mutants

Although the *Arabidopsis* genome is TE-poor (17%, [4]) compared to most crops (e.g. 85% in maize [5]), the mechanisms responsible for TE control were not clearly understood until epigenetic mutants were characterized in this species. One of the first proteins found to be involved in DNA methylation, a key hallmark of epigenetics (see Box 1), was DECREASE IN DNA METHYLATION 1 (DDM1) that encodes a putative chromatin remodeling protein. First discovered in a mutant screen for plants that lose DNA methylation at centromeric repeats [6], *ddm1* and other epigenetic mutant plants were subsequently shown to release transcriptional silencing of TEs [7,8] (for a review see [9]). As a result, TEs have even become a useful tool in the study and classification of epigenetic mutants as different epigenetic mutants reactivate specific subsets of TEs [10,11]. Indeed, several layers of different epigenetic marks often converge to repress TE transcription (see [12•] for a detailed review). The features involved in repressing TE transcription include the aforementioned DNA methylation (see Box 1, Figure 1), dimethylation of histone H3 lysine 9 (H3K9) [13] and the presence of heterochromatic 24 nucleotides (nt) small interfering RNAs (siRNAs) that guide the RNA-directed DNA methylation (RdDM) machinery [14,15]. This combination of marks allows the genome to be defined as 'genic' or 'non-genic' chromatin domains [16•]. Interestingly some mutants have upregulated TEs without any detectable

Box 1 DNA methylation in *Arabidopsis*: three different pathways.

In all plants analyzed to date, DNA cytosine methylation has been found in three different sequence contexts: CG, CHG and CHH (H standing for A, C, or T) (for a review see [67]). Symmetrical methylation refers to CG and CHG sites where the same sequence can be found on the reverse strand. This type of DNA methylation can be copied after DNA replication, whereas non-symmetrical CHH methylation has to be established *de novo* after DNA replication. The MET1 methyltransferase maintains methylation at CGs and it is thought to act at the DNA replication forks using newly replicated, hemi-methylated DNA as a substrate. CG methylation is stable across generations [68••,69••]. CHG methylation is maintained by the activity of the chromomethyltransferase CMT3, which is directed by histone modifications, especially the dimethylation of histone 3 at lysine 9 (H3K9me2) catalyzed by histone methyltransferase KRYPTONITE (KYP). A plant-specific RNA polymerase IV (POL IV) produces transcripts of certain loci that are then a substrate for small interfering RNA (siRNA) biosynthesis. These siRNAs then guide DNA methylation in an RNA-directed DNA methylation (RdDM) process [70–74]. RdDM can lead to *de novo* methylation in all sequence contexts although CHH methylation stands as a hallmark of RdDM since it is not heritable. Finally the chromatin remodeler DDM1 is required to ensure the proper methylation in all sequence contexts although its detailed mode of action is not yet characterized.

DNA corresponding to TEs and repeats is methylated owing to the concerted action of all DNA methylation pathways (for a review see [12•,75•]). Intriguingly 'symmetrical cytosines' (CG and CHG) have high levels of methylation whereas CHH methylation levels are quite low [76,77] and could result from varying levels of CHH methylation in different tissue or cell types.

change in the classical epigenetic marks [17,18], suggesting that additional mechanisms may exist.

TEs transcription under stress conditions

Expression of TEs in stressed plants has been described in many plant species [19]. In *Arabidopsis*, the first studies that were done to transcriptionally profile stressed plants used microarrays containing only a very small subset of TEs, thus they did not reveal the importance of stress-induced TE transcription. Recent reports using tiling arrays showed that TEs with strong repressive epigenetic marks could be transcribed under heat shock [20–22] (see [23] for a review). The mechanisms underlying this reactivation are not yet understood as no change in epigenetic mark was described except for the deposition of a positive mark for transcription (histone acetylation) at upregulated TEs. Interestingly DNA methylation changes in plants challenged with pathogens [24] indicate that DNA methylation could be more dynamic than previously anticipated. The study of plant methylomes under abiotic stresses is likely to deepen our understanding on stress-induced TE transcription.

Consequences of TE transcription

The transcription of some TEs in epigenetic mutants or in cell culture is accompanied by the production of 21nt siRNAs [25••,26••,27••,28••,29]. These 21nt-siRNAs are considered a hallmark of posttranscriptional gene silencing (PTGS), a pathway that can be activated when transcriptional gene silencing (TGS, associated with 24nt-siRNAs) is alleviated (see also [30]). Interestingly PTGS is also involved in anti-viral defense [31] suggesting that this mechanism could have been co-opted to control TEs, as endogenous parasites. In the battle between the host and its TEs it is expected that TEs would have developed ways to counteract the RNA silencing machinery, much like viruses do by encoding suppressors of RNA silencing [32]. Indeed, this has been suggested in experiments that have shown that RNAs produced from TE derivatives could affect the plant small RNA production [33•]. Further experiments are needed to address this question.

Other roles are emerging for the 21nt class of siRNAs. These include a role in directing epigenetic modifications in pollen from the vegetative nucleus to the sperm cell (easiRNAs for epigenetically activated siRNAs, [28••,34]) and a recently described role in DNA repair (diRNAs for double-strand breaks-induced small RNAs [35•]). Finally a fascinating example came from the role of TE-derived 21nt-siRNAs in a 'microRNA-like' function in targeting the degradation of an mRNA from an endogenous gene [26••].

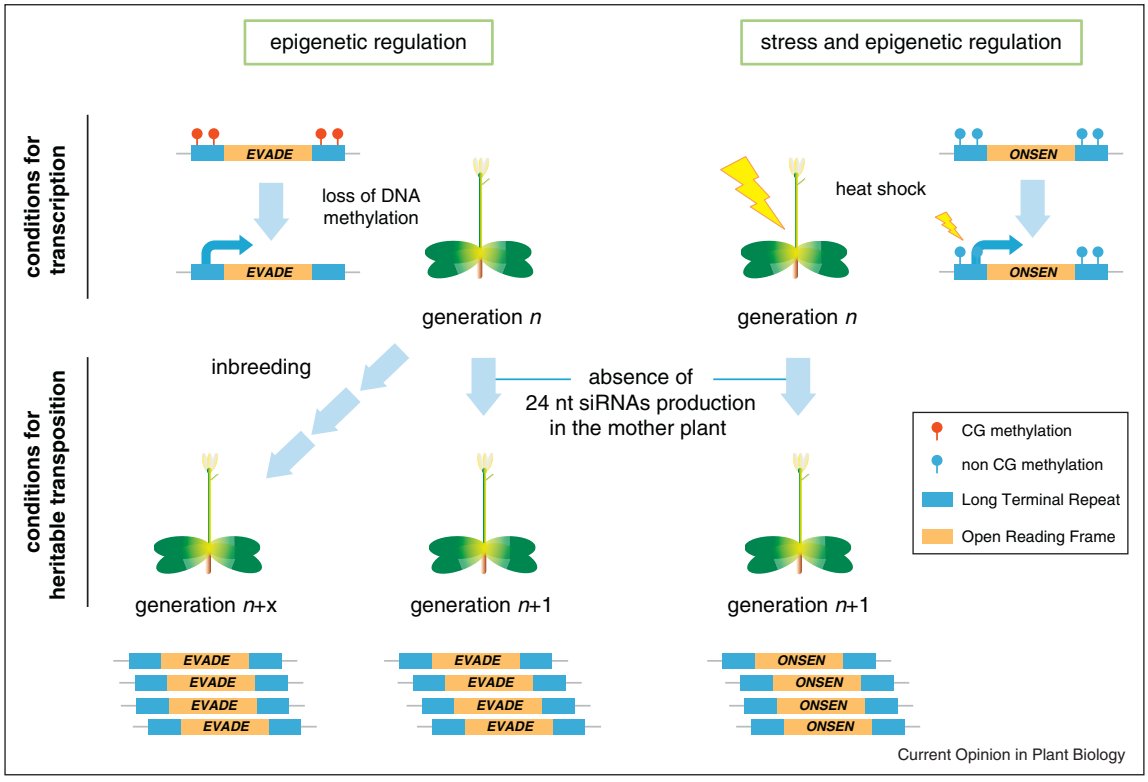
Given the complex roles of TE-derived 21nt-siRNAs, more precisely integrating small RNA sequencing and epigenetic maps with transcriptional regulatory effects may be necessary to better understand their impact.

TE transposition in inbred epigenetic mutants

As stated above TEs are transcriptionally reactivated in a massive way in epigenetic mutants or under stress conditions but transcription is only the first step in the TE lifecycle. Ultimately the TE will transpose and generate neo-insertions defined as insertions of additional copies in a different location in the host genome. However, while TE transcription has been intensively studied, their transposition is poorly documented despite early interesting observations [36,37]. There are two main explanations as to why this has been overlooked so far: (1) most transcriptionally reactivated TEs are unable to generate neo-insertions owing to accumulated mutations in their coding sequence; (2) mobile TEs were typically identified only when their neo-insertions caused an observable phenotype [27••,38••,39••] or by analyzing TE families already known to be active in other plant species [40••]. Now genome resequencing and comparative genomic hybridizations facilitates detecting TE neo-insertions and copy number variation, respectively, at the genome-wide level [27••,38••].

TE transposition was reported in epigenetic mutants, for instance in mutants impaired in DNA methylation: *met1*, *met1 cmt3*, *ddm1* (see Box 1, Table 1). Noticeably neo-insertions could only be detected after inbreeding, in

Figure 1



Example of distinct regulation of two endogenous LTR retrotransposons in *Arabidopsis thaliana*. We illustrate the differences in epigenetic control of transcription and transposition using the two LTR retrotransposons *EVADE* (*left side*) and *ONSEN* (*right side*). Loss of CG methylation triggers transcriptional activity of *EVADE* whereas heat shock activates *ONSEN* transcription. Transposition could not be detected in both cases in the first generation of plants but heritable neo-insertions were detected upon inbreeding (*EVADE*) or in a mutant background unable to synthesize 24nt-siRNAs (*EVADE* and *ONSEN*). Legend as follows: *red lollipops*, CG methylation; *blue lollipops*, non-CG methylation; *blue boxes*, LTRs; *orange boxes*, ORFs.

Table 1

Examples of transpositionally active TEs in *Arabidopsis thaliana*. The conditions in which these elements can transcribe and transpose are given. Retrotransposons are TEs that transpose through a 'copy and paste' mechanism using an mRNA intermediate while DNA transposons transpose through a 'cut and paste' mechanism [66].

Name	Origin	Type	Condition for transcription	Condition for transposition	Ref.
AtCOPIA13	Endogenous	Retrotransposon (*copia* type)	Hypomethylation	Inbred *ddm1*	[38••]
AtCOPIA21	Endogenous	Retrotransposon (*copia* type)	Hypomethylation	Inbred *ddm1*	[38••]
AtGP3	Endogenous	Retrotransposon (*gypsy* type)	Hypomethylation	Inbred *ddm1*	[38••]
EVADE (*AtCOPIA93*)	Endogenous	Retrotransposon (*copia* type)	Hypomethylation	*met1*-epiRILs and inbred *met1*, inbred *ddm1*	[27••] [38••]
ONSEN	Endogenous	Retrotransposon (*copia* type)	Heat stress	Inbred *nrpd1*	[25••]
Tnt1	Transgenic (Tobacco)	Retrotransposon (*copia* type)	Wounding	Inbred *ddm1*	[78•]
Tto1	Transgenic (Tobacco)	Retrotransposon (*copia* type)	Tissue culture	Tissue culture	[79,80]
AtMu1	Endogenous	DNA transposon	Hypomethylation	Pollen vegetative nucleus, *ddm1* mutant	[28••] [38••,40••]
CACTA	Endogenous	DNA transposon	Hypomethylation	Inbred *ddm1*, inbred *met1 cmt3*, *met1*-epiRILs and *ddm1*-epiRILs	[39••] [82] [41•,81•]
VANDAL21	Endogenous	DNA transposon	Hypomethylation	Inbred *ddm1*	[38••]

some cases after several generations. *EVADE* is an example of a long-terminal repeat (LTR) retrotransposon mobilized in both *met1* [27••] and *ddm1* [38••] inbred mutants (Figure 1). Interestingly, this retrotransposon was also mobilized in *met1*-derived epigenetic inbred lines (*met1* epiRILs). The *met1* epiRILs are genetically wild-type plants but were obtained from an initial cross between a wild-type plant and a *met1* mutant to create an F2 generation where only wild-type *MET1* individuals were inbred [41•]. *EVADE* was clearly mobilized from the 4th selfed generation of the epiRILs [27••], indicating that inbreeding was required for its movement. By combining the loss of CG methylation and the loss of the siRNA pathway (using a *met1 nrpd2a* double mutant) the necessity for several generations of inbreeding was not required and immediate, dramatic bursts of *EVADE* transposition were detected [27••], suggesting that multiple epigenetic pathways were required to control *EVADE* mobility.

Transposition of TEs is restricted by epigenetic regulation even when transcription is released by stress. For example neo-insertions of an *Arabidopsis* endogenous retroelement named *ONSEN* were detected after heat-stress [25••]. However, for *ONSEN* to transpose the stressed plants had to be deficient in the siRNA pathway (for example mutated in the PolIV subunit NRPD1, see Box 1). Interestingly, *ONSEN* transposition could only be observed in the next generation of heat-stressed plants (Figure 1) in the progeny of stressed homozygous, but not heterozygous *nrpd1* plants. This suggests that 24nt-siRNAs generated from the mother plant are important in restricting TE transposition. Further experiments are needed to reveal whether additional TEs are under the same epigenetic control for their transposition and how these 24nt-siRNAs exert their 'anti-transposition' function.

Germ-cell specific epigenetic control of TEs

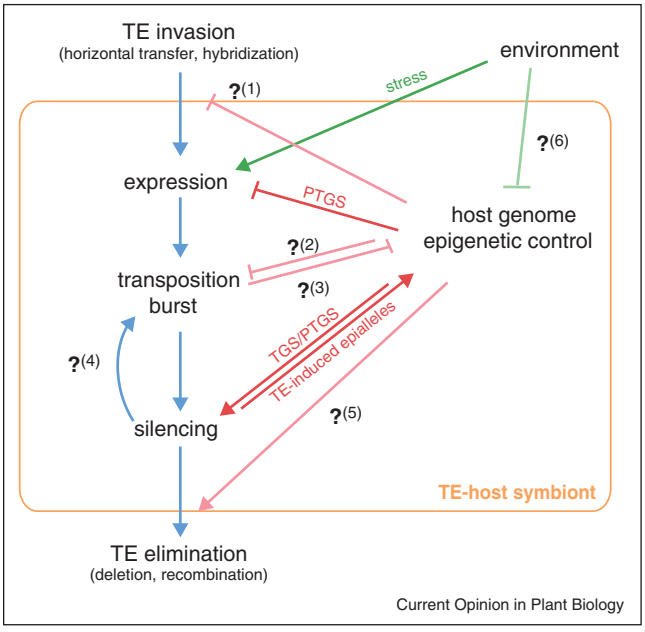
For a TE to stably increase its copy number in the host genome, there are two possible ways: to generate neo-insertions in germ cells or in meristematic cells. In the former case, specialized TE control machinery has been described. When studying TE transcription in pollen, Slotkin *et al.* have shown that the sperm-cell-accompanying nuclei undergo DNA demethylation that allows transcriptional activation of some TEs [28••]. Furthermore this release of transcription leads to the transposition of at least one family of TE (MULE) as neo-insertions were detected in the vegetative nucleus [28••]. Nevertheless, these neo-insertions are not transmitted and thus have no impact on the next generation. This study showed that TEs are 'revealed' by generating 21nt-siRNAs that would migrate from the vegetative nucleus to the gametes to re-enforce silencing and establish TE immunity, thereby protecting genome integrity in the next generation (see the review by K. Slotkin in the same issue). On the female side, a loss of DNA methylation has also been described in the endosperm (the embryo nourishing tissue) although in this case TE transposition was not analyzed [42••,43••]. The endosperm is also the source of PolIV-dependent 24nt-siRNAs that are required to silence TEs [44,45]. The sporophytic maternal tissues also play a role in TE silencing and could be a source of mobile siRNAs targeting TEs in the female gametes [46]. Surprisingly, *MET1* expression is repressed in ovules [47] and *DDM1* expression was not detected in the male gametes [28••] suggesting that still unknown mechanisms could play a role in maintaining TE silencing at these specific developmental stages. Additionally, whether a PTGS-based mechanism is activated in response to transcriptionally active TEs and how all of these diverse small RNA signals are transmitted and perceived in the germ cell requires more understanding. Despite these caveats a model has emerged in which developmentally controlled hypomethylation is essential to establish TE immunity [34].

Consequences of TE transposition on host gene evolution

TE transposition leads to neo-insertions and in some cases these insertions reveal target site preferences [48]. An interesting case to discuss is the insertion of TEs within or close to promoters. TE neo-insertions can be targeted for TGS and thus accumulate repressive epigenetic marks, and these marks may also affect downstream gene expression. Hence, genes previously under classical transcriptional control may additionally become epigenetically controlled. It has consequently been proposed that TEs are at the origin of epigenetic gene regulation [49] and that imprinting could be seen as a consequence of epigenetic control of TEs [42••]. Indeed, it appears that genes reported as being under epigenetic control are often associated with TEs. *Arabidopsis* examples include the flowering repressor gene *FLOWERING WAGENINGEN* (*FWA*) [50,51] and *BONSAI* (*BNS*) [52,53], which are associated with SINE-related repeats and a LINE retrotransposon, respectively. In these two examples remnants of the TEs attract DNA methylation resulting in transcriptional repression of the host gene.

It may seem obvious that such epigenetic repression would be deleterious to the host in most cases [54]. However reversibility is an intrinsic property of epigenetic modifications and has been observed in natural epialleles [55,56]. Therefore, if a TE-induced epigenetic repression does not cause an immediate loss of fitness, whether the repression remains or is reversed creates additional variation. This gives the host the opportunity to evolve new epigenetically controlled gene networks that can be advantageous. Considering that in *Arabidopsis* thousands of protein-coding genes are associated with

Figure 2



Proposed model for the roles of epigenetic control in TE-host co-evolution. The main steps occurring during TE invasion of the host genome (*blue arrows*), the interplay between the host's epigenetic machinery and the TE (*red*, experimentally supported, *pink*, proposed regulation) and the influence of the environment (*green arrows*) are represented. TEs enter the genome via an unknown mechanism possibly through horizontal transfer or genomic hybridization. During co-evolution within a host genome, TEs can be transcriptionally active, some even exhibiting transposition bursts within a species, and become silenced again. Eventually some TEs can be eliminated from the genome through deletion and/or recombination. While host regulation can exert epigenetic control through TGS and PTGS mechanisms, TE regulation can be co-opted to affect host gene expression by attracting epigenetic marks to nearby genes. Some of these regulatory steps are incompletely understood and specific questions are highlighted as follows: (1) How is a new TE recognized by the epigenetic machinery upon its entry into a naive genome? (2) How are siRNAs involved in controlling the TE burst? (3) Did TEs evolve anti-silencing functions? (4) Is TE silencing reversible? (5) Is TE deletion epigenetically controlled? (6) Does stress directly have an impact on the efficiency of the host epigenetic machinery?.

nearby TEs, the evolutionary potential within its genomes implies that the full extent of epigenetic control of gene expression has only begun to be revealed.

Conclusion and outlook

Ever since their discovery TEs have intrigued researchers. The discovery of mobile endogenous TEs in *Arabidopsis* has now opened up many new possibilities to study the lifecycle of TEs as it was done in yeast [57]. Interestingly, the studies of these mobile elements that the 24-nt siRNA pathway played a central role in not only limiting TE transcription, but also transposition. How the 21 and 24-nt siRNA pathways potentially converge to regulate actively replicating TEs remains an interesting question to pursue. Additionally, other steps during TE invasion in the host genome might also be under epigenetic control (Figure 2) as suggested recently for the integration step [58•], and further studies are needed to better understand the epigenetic crosstalk between TEs and their host.

The epigenetic control of TEs in meristematic cells also deserves attention. Contrary to animals, plants do not produce germ cells early in development, but rather differentiate germ cells from somatic meristematic cells. Very interestingly in maize the role of PTGS in meristems is crucial for the silencing of a DNA transposon [59] (see the review by D. Lisch in the same issue). However, the meristem-specific TE control is not clear yet in *Arabidopsis*. Recently the finding that small RNAs are mobile in plants and can transport epigenetic information (for example, what sequence to be methylated) from one organ to another [60••,61••,62] make it likely that TE silencing in meristematic cells could be influenced by the somatic activity of TEs experienced during the plant lifetime. Currently, the meristem epigenome is unknown but newly developed techniques [63] should allow characterizing cell-specific epigenomes in the near future. Whether epigenetic regulation plays a role in diplontic selection for instance (counter selection of meristematic cells that have accumulated somatic mutations leading to a loss of fitness [64]) is definitely an interesting question, especially for long living organisms in which meristematic cells undergo quiescent stages with epigenetic changes [65].

Additionally, to what degree the epigenome integrates environmental stimuli to mediate host-genome responses remains an important question. For *ONSEN*, heat stress not only lead to transposition, but also provided heat-responsiveness to genes neighboring *ONSEN* new integration sites [25••]. In specific cases transposition might thus be beneficial to the host by more rapidly facilitating the evolution of new pathways necessary for responding to environmental change. Whether this is a recurring theme in evolution requires further investigation but it certainly opens fascinating perspectives for plant adaptation and crop improvement.

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