Mechanistic and evolutionary questions about epigenetic conflicts between transposable elements and their plant hosts

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Abstract

Transposable elements (TEs) constitute the majority of plant genomes, but most are epigenetically inactivated by their host. Research over the last decade has elucidated many of the molecular components that are required for TE silencing. In contrast, the evolutionary dynamics between TEs and silencing pathways are less clear. Here, we discuss current information about these dynamics from both mechanistic and evolutionary perspectives. We highlight new evidence that palindromic sequences within TEs may act as signals for host recognition and that *cis*-regulatory regions of TEs may be sites of ongoing arms races with host defenses. We also discuss patterns of TE aging after they are silenced; while there is not yet a consensus, it appears that TEs are removed more rapidly near genes, such that older TE insertions tend to be farther from genes. We conclude by discussing the energetic costs for maintaining silencing pathways, which appear to be substantive. The maintenance of silencing pathways across many species suggests that epigenetic emergencies are frequent.

Introduction

Transposable elements (TEs) and their plant hosts engage in a continuous battle, whereby TEs seek to proliferate and hosts strive to control their proliferation. In evolutionary terms, it is difficult to declare a winner. On one hand, TEs have been successful by any measure; the majority of plant genomes are composed of TEs that vary from young intact insertions to old fragmented copies. This composition suggests that TEs often overcome

host defenses, at least on evolutionary timescales. On the other hand, most TEs are epigenetically silenced under normal conditions, so that they do not proliferate. This near-universal suppression implies that plant hosts are largely in control of TE activity. It is the conflicting nature of these two observations that makes the dynamics of host-TE interactions a particularly fascinating topic.

Epigenetic silencing of TEs is mainly conducted via a process known as RNA-directed DNA methylation (RdDM). As its name implies, RdDM uses RNAs, in particular small interfering RNAs (siRNAs), to guide epigenetic modifications that lead to pre-transcriptional silencing of TEs. RdDM has been studied intensively for many years, leading to the elucidation of its mechanisms and pathways. Here, we will not cover the interactions among RdDM components in detail, as these have been presented in several recent reviews [1-3]. Rather, we will first provide an overview of the potential states of a TE within a host genome – and the silencing pathways that influence those states – before highlighting questions of particular interest from mechanistic and evolutionary perspectives.

An overview of the TE life cycle within plant genomes

We begin by considering an active TE, which is transcribed by RNA polymerase II (*Pol II*) to start its life cycle towards producing new copies (Figure 1). At some point, the endogenous *RNAi* pathway recognizes these TE mRNAs and degrades them. This process is aided by key factors such as *RNA-directed RNA polymerase 6 (RDR6)* that converts single-stranded mRNA (ssRNA) into double-stranded RNA (dsRNA), *Dicer-like 2 and 4 (DCL2/DCL4)* that cleave dsRNA into 21-22nt siRNAs, and *Argonaute 1 (AGO1)* that

loads these siRNAs to guide mRNA cleavage (by sequence complementarity between the siRNAs and target mRNAs) [4,5]. This post-transcriptional regulation can degrade large numbers of TE mRNAs, but, most importantly, it may also trigger the “initiation” of pre-transcriptional TE silencing, hence providing an entry-point into RdDM (Figure 1).

The transition from post-transcriptional *RNAi* to pre-transcriptional RdDM was unknown until recently, but there are now two models of how this occurs. The first suggests that some 21-22nt siRNAs are not loaded onto *AGO1*, which directs mRNA cleavage via *RNAi*, but onto a related factor, *AGO6*, that guides chromatin modifications [6]. The second proposes that the overproduction of TE mRNAs eventually overwhelms the processing capacity of *DCL2/DCL4*, thereby triggering the biogenesis of 24nt siRNAs by another Dicer-like protein, *DCL3* [7]; the production of 24nt siRNAs is a hallmark of pre-transcriptional RdDM silencing (Figure 1).

Once RdDM has begun, the “establishment and spreading” of silencing requires two plant-specific RNA polymerases (*Pol IV* and *Pol V*). *Pol IV* transcribes TEs, and the resulting ssRNAs are processed this time by *RDR2* and *DCL3* to produce 24nt siRNAs (Figure 1). These 24nt siRNAs are then loaded onto *AGO4/AGO6* to direct them to scaffolding, chromatin-bound transcripts of TEs, which are produced by *Pol V*. The association of the *AGO* complex with *Pol V*-derived transcripts mediates cytosine methylation and the deposition of repressive histone marks to the genomic TE locus [1-3]. Crucially, recent work in *Arabidopsis* has shown that *Pol IV* and *Pol V* are recruited to loci that already contain H3K9me and cytosine methylation, respectively [8,9]. These observations indicate that RdDM acts in a self-reinforcing loop that increases the deposition of silencing marks [5], as shown in Figure 1, eventually generating a dense

heterochromatic environment that restricts access to the *Pol II* transcriptional machinery. After sufficient deposition, a TE is effectively in a silenced state (Figure 1).

Once silencing is established, repressive chromatin modifications are faithfully copied during DNA replication, enabling inheritance of the silenced chromatin state in daughter cells; this “maintenance” phase of silencing [5] leads to deeply silenced TEs (Figure 1). Overall, these mechanisms appear to be very effective, because only a few TEs have been observed to actively transpose and proliferate, such as the *Ac/Ds* and *Mutator* families of maize and the *mPing* element in rice [10-12].

Nonetheless, although most TEs are inactive, many may retain the ability to transpose. Genome-wide and biochemical analyses have shown that silenced elements can be activated during epigenetic loss or remodeling (Figure 1), as for example in mutants that interrupt silencing [13,14], in specific cell types like the companion cell of the female gametophyte or the pollen vegetative nucleus [15,16], in developmental transitions [17], and even during stressful conditions [18].

Initiation of silencing

After articulating the states of the TE life cycle, we now address questions about host-TE interactions, starting by again considering the initiation of silencing. In this context, we first discuss the potential for memory in the host response, before considering how hosts might identify TE mRNAs for post-transcriptional *RNAi*. We then argue that *cis*-regulatory regions of TEs may be locations of evolutionary conflict with host defenses. Finally, we note the paradox that TEs appear to be quickly resiled, and yet they are also capable of amplification bursts that produce thousands of genomic copies.

How efficient is the homology-dependent siRNA surveillance system?

If a TE has escaped suppression and is reactivated, host defenses must employ intricate ways to reinitiate silencing. As discussed by Fultz et al. [1], one mechanism may be mediated by *trans*-acting 24nt siRNAs that have sequence homology to the active TE. Presumably, these 24nt siRNAs are produced from *Pol IV*-derived transcripts of silenced relatives of the same family, thereby acting as the host's "immune memory" to identify active elements. In support of this model, there is evidence that reactivated TEs in *Arabidopsis* and maize are silenced in *trans* by the *Pol IV*-RdDM pathway [18-20]. The presence or absence of immune memory may also determine whether a horizontal TE invasion will proliferate rapidly in its new host before it is silenced (Figure 1). If, for instance, an endogenous, silenced TE shares substantial sequence homology with an invading TE, then its 24nt siRNAs may constrain the invader's ability to proliferate.

There are, however, indications that this homology-dependent pathway is not always sufficient to silence active TEs. For example, the *Evade* element of the *ATCOPIA93* family in *Arabidopsis* remains active despite the presence of silenced relatives [21]. Instead, it is suppressed only when enough mRNA transcripts are produced to overwhelm *DCL2/DCL4* processing [7]. Also, recent genome-wide studies of LTR retrotransposons [22,23] have shown that certain families, such as *Ji* and *Opie* in maize, have homology to large numbers of 21-22nt siRNAs, implying that at least some members first undergo post-transcriptional *RNAi* (Figure 1). If the homology-dependent pathway were able to suppress active TEs quickly, then one would not expect large populations of 21-22nt siRNAs.

How are TE mRNA transcripts recognized?

If the homology-dependent pathway fails, silencing of active TEs must be initiated by *RNAi*. The first step of this process likely entails the recognition of *Pol II*-derived TE mRNAs. Importantly, *RNAi* needs to safely distinguish between TE and genic mRNAs, but this must be difficult given high sequence and structural diversity among TE classes and, hence, the lack of a universal TE ‘barcode’. The recognition of TE mRNAs is mediated by *RDR6*, which converts ssRNA into dsRNA [3]. It has been hypothesized that *RDR6* is assisted by ‘primary’ small RNAs that act either directly as primers or indirectly by their association with *AGO* proteins that cleave TE mRNAs, thereby providing a starting point for dsRNA synthesis [24,25]. Indeed, some miRNAs play this role in Arabidopsis [26]. However, miRNAs also participate heavily in genic mRNA cleavage, and so the mechanism of recognizing TE over genic mRNAs remains uncertain. Furthermore, only a subset of Arabidopsis TE families are silenced via miRNAs [26], implying that more mechanisms must be in place.

Intriguingly, one such mechanism could involve non-miRNA hairpin-derived small RNAs (hpRNAs) that are generated from secondary structures within the TE transcripts themselves. The first evidence for the importance of hpRNAs came from the *MuDR* family in maize. In this example, a spontaneous inverted repeat rearrangement within a single element (termed *Mu killer*) triggered *RNAi* and subsequent silencing of other *MuDR* members [19,27]. In fact, the discovery of *Mu killer* prompted Lisch and Slotkin to suggest that the low fidelity of TE replication may increase the chances of accidental hairpins to form, which in turn would be a critical feature for enhancing *RNAi*

[28]. Axtell further discussed the potential importance of plant hpRNAs and noted that the numerous short hairpins in genomes (which also occur within TEs) may produce hpRNAs [29]. Although hpRNAs have not yet been identified systematically [30], they appear to be abundant in maize [31].

The potential role of hairpins in TE recognition is supported by a new study of maize Sirevirus LTR retrotransposons, which contain multiple palindrome motifs in their LTRs [23]. The palindromes are predicted to form stable stem-loop structures with minimum free energy comparable to those of known miRNA precursors (Figure 2A), raising the possibility that they act as loci for hpRNA biogenesis (Figure 2B). Consistent with this hypothesis, the palindromes are hotspots for siRNA mapping, especially of 21-22nt siRNAs [23]. These siRNAs may be directly produced by the hairpins (i.e. without prior dsRNA synthesis by *RDR6*), based on previous research in Arabidopsis showing that *DCL* proteins can directly process hairpins into hpRNAs [32]. These observations suggest a model in which the palindromes form hairpins in the Sirevirus mRNA, these hairpins are then processed by *DCL* proteins to produce hpRNAs, and the hpRNAs mediate *RDR6* synthesis of dsRNA either as primers or through their loading onto an *AGO* protein, hence initiating the *RNAi* cycle. Alternatively, the hairpins could directly prime *RDR6* synthesis without the need of hpRNAs, i.e. in a ‘primary’ small RNA-independent manner [33]. If these models are correct, palindromic regions may be crucial signals for the initiation of TE silencing.

An arms-race between TE *cis*-regulatory regions and host defenses?

The Sirevirus palindromes raise an interesting evolutionary question. From the selfish view of a TE, it is not entirely desirable to contain a motif that facilitates host recognition and subsequent silencing. Why, then, might Sireviruses contain such motifs? The answer is simple: because they may be required for the element to proliferate. Indeed, the Sirevirus palindromes are found in the *cis*-regulatory region in the upstream half of the LTR of retrotransposons [34,35] and retroviruses [36]. Intriguingly, this region in Sireviruses is also an area of rapid sequence evolution, because the palindromes differ along the sequence of an element, among family members, and among families [23]. As a consequence of this extensive sequence variation, each palindrome is targeted by different siRNAs. This example raises the possibility that *cis*-elements are foci for an evolutionary arms race between TEs and host defenses. Jacobs et al. beautifully describe this interplay for the *L1* and *Alu* TE families in primates [37]. In this case, sequence and structural changes within *L1* and *Alu* regulatory regions led to multiple amplification bursts during primate evolution. These changes, however, were soon followed by changes in the silencing components of the host, leading to cycles of TE proliferation followed by host repression.

Detailed analyses of the epigenetic importance of the *cis*-regulatory regions of plant TEs are limited, and their potential role in host recognition remains virtually unexplored. However, studies across TE families in diverse organisms (mice, *Drosophila*, tomato, hominoids) have revealed that *cis*-regulatory regions are often arranged as arrays of repeats, with some also exhibiting symmetrical properties like those of maize Sireviruses [38-41]. Therefore, the complex, sometimes palindromic organization of regulatory motifs may be common across TE classes. In fact, for the *Tnt1* family of

tomato and other *Solanaceae* species, the *cis*-regulatory region encompasses the highest sequence variability among its family members, and this variability associates with different stress-related expression patterns [42,43]. These observations suggest that the evolution of this region equipped *Tnt1* subgroups with diverse regulatory capacity to respond to different stimuli and to colonize new hosts [43,44]. Similar patterns of diversity within the *cis*-regulatory region was also recently reported among *Tcs* elements in citrus [45].

Collectively, these observations highlight the need for in-depth research on the evolutionary interplay between *cis*-regulatory TE motifs, secondary structures and epigenetic silencing. It is possible that these regions evolve quickly to evade the host, but have underlying structural restrictions that continue to facilitate host recognition. For example, at least three kinds of information could support the role of *cis*-regulatory palindromes in host recognition. First, it must be shown experimentally that these regions are sufficient to generate hpRNAs. Second, their existence needs to be surveyed systematically across TE families and hosts. It is already known that most Sireviruses contain palindromic structures across plant species [46], but it is unclear if other TEs typically have similar structures. Finally, observations about siRNA targeting need to be generalized. Is it common that palindromic regions are hotspots for siRNA mapping? To our knowledge, only the maize Sirevirus study has been performed at a sufficient level of detail to investigate this question.

The paradox of the time to regain control

There is increasing evidence that epigenetic defenses respond quickly to TE activation, suppressing their activity within only a few host generations. For example, the *ONSEN* LTR retrotransposon was activated in heat-stressed *Arabidopsis* seedlings. However, *ONSEN* only managed to insert new chromosomal copies in the progeny of an siRNA-compromised genetic background and not in the progeny of wild-type plants or in vegetative tissues [18]. Another study in *Arabidopsis* showed that, after severe loss of methylation, many TEs were remethylated in only a few host generations [20]. Finally, when the *Evade* element was awakened in recombinant inbred lines engineered to erase most of their methylation, it was transcriptionally silenced after ~15 host generations, or after ~40 new copies had been integrated in the genome [7].

Based on these observations, we are faced with a paradox: if TEs are rapidly resilenced, then how do the bursts of amplification occur that ultimately produce thousands of TE copies? Unfortunately, we do not yet have a compelling answer to this question, even though myriad studies have identified evidence for such bursts. For instance, comparative analysis of species from the *Oryza* genus [47] – as well as work on the 1Gb sequence of chromosome 3B in wheat [48,49] and the fully sequenced 2.3Gb maize genome [50,51] – have documented the accumulation of thousands of copies for several TE families, typically within an estimated timescale of thousands of years. Given that experimental data have demonstrated only small increases in TE copy number, how do bursts of thousands of copies occur? Do TE families escape silencing for longer lengths of time than have been observed experimentally? Or, do they undergo repeated cycles of short bursts that last only a few host generations, producing a few new copies

before they are resilenced, and then somehow burst again? Thus far, we have very few insights into the timescale and conditions of bursts.

Establishment, Spreading and Maintenance of silencing

In contrast to the various mysteries associated with the initiation of silencing, the next two phases of host response, that is the RdDM-mediated establishment & spreading of silencing and the maintenance of repressive chromatin modifications (Figure 1), are much better understood. Yet, it is during these phases that TEs begin to age and gradually degrade. These quiescent TEs shape host genomes substantially, because *i*) they are the primary determinants of genome size [52], *ii*) they may be exapted for regulatory and other purposes [53], and *iii*) their methylation perturbs gene expression [54]. Given these effects, it is surprising that little is known about the epigenetic profiles of TEs as a function of their age, and how this relationship influences genome structure and function. We address these issues below.

The intriguing epigenetic dynamics of TE ageing

Only a handful studies have investigated the evolutionary relationship between the age of TEs and either siRNA targeting [23,54,55] or methylation [23,54,56]. These analyses have shown that older elements are typically targeted at lower levels by siRNAs compared to younger elements. This implies that older TEs are likely to be deeply silenced, a state that can be maintained independently of siRNAs (Figure 1). If true, one also expects methylation to increase with age, as elements reach their maximum methylation levels. This expectation holds for LTR retrotransposons in maize [23] and

even mice [57,58], but not rice [56]. It is not yet clear if the differences between rice and maize are species-specific or specific to the TE families examined.

There are also interesting dynamics among the epigenetic state of a TE, its age, and its proximity to genes. Generally speaking, TEs near genes are thought to be younger than TEs far from genes. There are at least two reasons to expect such a relationship. First, elements near genes may be removed by natural selection more efficiently, because of their deleterious effect on gene expression, which is in turn stronger when a TE is in a silenced state [54,56,59]. (We note, however, that the strength of deleterious effects varies among TE families [60]). Second, genes tend to reside in high recombination regions, where natural selection is more efficacious [61] and rates of TE removal are higher [62]. These two factors combine to suggest a model in which TEs are more prone for deletion near genes, such that older elements have a better chance to survive far from genes. To test this model, we retrieved the age for ~6,500 maize Sireviruses from [63] and examined the age distribution in relation to gene proximity (Figure 3). Insertion age exhibits a positive (but weak) correlation with distance to the nearest gene (Pearson $r = 0.1$; $P < 10^{-20}$), suggesting that Sireviruses are removed from the genome quicker when they reside near genes.

However, there is no firm agreement on these purported relationships. Recently, Maumus and Quesneville developed a method to estimate the age of elements based on their divergence from a consensus sequence [64]. In contrast, most studies have so far relied on either dating retrotransposons by the divergence between their LTRs, as with the Sirevirus example (Figure 3), or by inferring new vs. old insertions by detecting orthologous loci in other species. Using their age estimates in *Arabidopsis*, Maumus and

Quesneville found the exact opposite of previous studies, i.e. that older TEs are both closer to genes and more epigenetically active (i.e. less methylated). It is unclear why these results are so markedly different. One option for resolving this ambiguity may be to survey LTR retrotransposons across several species, because the LTR dating method is generally well accepted. Altogether, these results portray the need for more synthesis of the age, genomic distribution and epigenetic profiles of TEs.

Do zombie TEs exist?

Careful examination of Figure 3 reveals an anomaly: as Sireviruses age, they tend to be farther from genes, with the exception of the small number of elements in last two categories – i.e., elements estimated to be more than 2.5 million years old. Intriguingly, these very old elements are distinct for even more reasons [23]. First, they have epigenetic properties similar to those of their youngest counterparts (<0.5 million years), that is somewhat lower cytosine methylation but high levels of siRNA targeting. Furthermore, they tend to *i*) have an unexpectedly large number of intact palindromes, *ii*) often contain complete *gag* and *pol* genes (suggesting potential competence) and *iii*) retain extensive homology with siRNAs that cross-map to much younger elements.

What could explain these observations? Lisch and coauthors have hypothesized that plant genomes contain ‘zombie’ TEs that are co-opted by the host to trigger both their own silencing and the *trans*-silencing of active relatives [28,65]. By being beneficial to the host, zombies are predicted to be both evolutionary conserved and epigenetically active, hence resembling loci that produce piwi-interacting RNAs in *Drosophila* [66]. Also, they would be expected to reside in regions near genes where RdDM is ongoing

and the chromatin environment remains more permissive [67-70]. Thus, one potential explanation for the discontinuity in Figure 3 is that the very old elements have been coopted by the host to produce siRNAs capable of silencing younger, potentially more dangerous, elements. We note that the existence of zombies is as yet only hypothetical, but some properties of very old Sireviruses may be consistent with this hypothesis.

Escape from silencing

Finally, despite the post-transcriptional *RNAi* and pre-transcriptional RdDM silencing pathways, TEs do escape suppression (Figure 1). Nonetheless, although TEs can reactivate during epigenetic loss or remodeling, the question remains as to whether they can themselves avoid host defenses.

Have TEs evolved escape strategies?

Several mechanisms have been proposed to facilitate escape from silencing. These include recombination between different elements, which generates new variants unrecognizable to host defenses [71,72]. Another way to escape silencing is to switch hosts via horizontal transfer. Invading a naïve genome where prior “immune memory” is absent may provide TEs with a chance to freely proliferate. A growing body of data suggests that these events occur more often than initially thought between vertebrates [73] and between invertebrates [74], but research on plants remains underrepresented [75]. Nonetheless, a recent study investigated the genomes of 40 angiosperms and found evidence for extensive horizontal transfer of LTR retrotransposons [76]. Some of these elements underwent subsequent amplification bursts, suggesting both that they remained

active after initial colonization and that horizontal transfer may be an important factor in plant genome evolution.

Finally, in a fabulous review, Lisch suggested that TEs might evade suppression by capturing gene fragments as a form of camouflage [65]. In theory, if these TEs are targeted by siRNAs, the same siRNAs could target the ‘parent’ genes from which the fragments were captured and likely alter their expression. Lisch’s hypothesis was based primarily on the observation that *Pack-MULE* and *Helitron* TEs systematically acquire exons during transposition [77,78]. However, most research on gene capture by TEs has focused on the evolutionary patterns of selection and possible expression of these captured fragments [78-80], and not on the epigenetic consequences on either the TE or the parent gene. Furthermore, the extent of gene capture by LTR retrotransposons, which make up the majority of plant genomes, has not been examined extensively. Studies on the potential effect of gene capture for TE evasion are urgently needed.

Energetic costs of TE silencing and their evolutionary consequences

Thus far, we have considered the epigenetic states of TEs and addressed several mechanistic and evolutionary questions that suitably fit under these states (Figure 1). If we now step away from the intricacies of the TE life-cycle, we can point out another evolutionary implication: the host response to TEs is not only complex but also likely to be energetically costly. The energetic costs include the production and activation of multiple polymerases, methylases, protein complexes and siRNAs. In evolutionary terms, it does not pay to maintain an energetically expensive process unless it contributes to

fitness. But how costly are these host mechanisms and under what conditions would they be maintained?

Let us first consider the maintenance of TE repressive chromatin modifications as a *de facto* necessity for cell integrity; we assume, therefore, that hosts cannot afford to diminish these processes regardless of energetic costs. Our focus then turns to siRNAs, which appear to be produced abundantly from both pericentromeric heterochromatin and euchromatic chromosomal arms based on sequencing and mapping of *Pol IV*-derived transcripts and their associated siRNAs in Arabidopsis [81]; in fact, 65% of *Pol IV* transcripts mapped to TE loci, compared to 9% to genes [81]. Surprisingly, few papers have directly calculated the proportion of siRNA libraries that map to TEs, despite abundant information from several plant species (for example, <http://mpss.udel.edu/>). However, limited evidence in Arabidopsis suggests that siRNAs that map to TEs constitute a substantial proportion of small RNAs [30], and up to 11.3% of siRNAs map to a set of ~6,500 maize Sireviruses that constitute only 2.8% of the genome [23]. Given that Sireviruses totally occupy ~20% of the maize genome [51], it is likely that a much higher proportion of siRNAs corresponds to them. More detailed calculations across plant species and tissues would be valuable, but the overarching impression is that most siRNAs map to TEs.

This raises the question: why incur the energetic costs to produce siRNAs when most TEs are highly methylated [82-84] and, therefore, presumably deeply silenced? There are at least two potential answers. First, as discussed above, siRNAs – and particularly 24nt siRNAs – may function as immune memory. Under this scenario, siRNAs act as a buffer against the possibility of TE activity, even though most

methylation is maintained independently of RdDM in heterochromatic regions [70]. The retention of siRNA surveillance would then be similar to the evolution and retention of acquired immune memory in vertebrates, which is costly but maintained under frequent cycles of reinfection [85]. Consistent with this scenario, some TEs are expressed in mutant backgrounds with impeded 24nt siRNA biogenesis [13,70], indicating that the TEs may be released from “establishment and spreading” rather than a “deeply silenced” state (Figure 1). A second possibility is that siRNA production is necessary for a particular step of the host lifecycle, so that it cannot be lost. The fact that Arabidopsis TEs are epigenetically released in companion cells of the male and female gametophyte and then apparently reprogrammed in the sperm and egg cells is consistent with this assertion [15,16]. However, if this were the only necessary function for siRNAs, evolution would be expected to dampen production in non-reproductive tissues, thereby conserving costs.

Conclusions and Future directions

Our primary interest in this review has been to convey questions about the evolutionary dynamics between TEs and hosts. Some questions were more broad and evolutionary, while others specific and mechanistic. All questions, however, shared a common feature in that their answers are likely to be multifaceted. For example, the evolutionary pressures that maintain the host response system may reflect the importance of 24nt siRNAs in immune memory, the frequency of ongoing emergencies, and perhaps even essentiality for host reproduction. Similarly, signals for host recognition are likely to vary across TEs classes and also among species.

This raises an important point: most studies have so far focused on *Arabidopsis*, with a few on rice and maize as well. Nonetheless, these species are not “normal”, because they all have smaller genomes than the angiosperm average [52]. Moreover, *Arabidopsis* is particularly bizarre because its elements tend to be substantially older than those of its sister species *Arabidopsis lyrata* [86], suggesting a dearth of transpositions in recent past. It is possible, then, that these model plants are atypical with respect to some features of the host response. We therefore endorse continued epigenetic studies across a wide variety of plant species. A helpful first-step will be to study the evolution of genes known to contribute to epigenetic mechanisms. For example, one recent paper has found that lower plants lack important RdDM-related genes [87], suggesting that host-TE interactions differ substantially across the plant kingdom. Similarly, patterns of DNA methylation vary substantially across plant species [88].

There is also an urgent need for more work on the host response as a function of development, even in model plants. One of the most exciting discoveries of the last decade was the finding that TEs were epigenetically released and then reprogrammed within the male and female gametophyte [15,16]. Is this reprogramming a necessary step in the *Arabidopsis* lifecycle? Is it shared with other plants? If so, how broadly? Such studies are technically difficult, but they will ultimately help illuminate critical features of host-TE interactions.

Finally, we know very little about the focal regions of TE-siRNA interactions, because most studies have focused on mapping siRNAs to exemplar TEs [6,26,31,67,70,82,89,90] instead of intact elements. Unfortunately, the process of building a consensus is likely to omit the regions of interest, i.e. those that evolve rapidly

due to an ongoing arms race between TEs and hosts. The accurate identification and classification of large numbers of individual TEs remains, however, a challenging task [91,92]. Nonetheless, a recent review has stressed the need for fine-scale characterization of plant TEs to properly assess epigenetic dynamics [93], and here we amplify the sentiment for continued bioinformatic and evolutionary analyses of TEs .

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Figure captions

Figure 1. States of TEs and their interactions with the host's epigenetic silencing pathways. Active TEs may derive from either horizontal transfer or the escape of endogenous TEs from epigenetic suppression. The initiation of suppression may depend on homology-dependent silencing or *RNAi*, which is facilitated by known or yet-to-be-identified triggers. *RNAi* cleaves TE mRNAs post-transcriptionally (PTS), but also places the first heterochromatic marks on TE insertions, hence initiating pre-transcriptional silencing (TS). After initiation, RdDM strengthens suppression in a self-reinforcing loop for the establishment and spreading of silencing. These silenced TEs may slowly reach their maximum methylation levels while ageing, a process that may be largely maintained independent of RdDM. See text for additional details.

Figure 2. The epigenetic and sequence patterns of the maize Sirevirus LTRs. (A) Minimum free energy (MFE) of siRNAs from a previously published lead library [89] that mapped along the LTRs of two abundant maize Sirevirus families, *Ji* and *Opie*. Mapping of siRNAs and calculation of their MFE was conducted as in [23]. The dotted lines represent the -40 MFE cutoff of known miRNA precursors [31]. A schematic shows the location of palindromes, which correspond to the hairpin-prone regions of the LTRs. (B) Predicted secondary structure of an *Opie* LTR. Arrows point to two hairpins formed by palindromes and potential locations for processing by *DCL* proteins into hpRNAs. Note that longer hairpins may form by the folding of two (or more) pairs of palindromes.

Figure 3. The proximity of Sirevirus elements to maize genes as a function of their insertion age. We retrieved information for 6,456 Sireviruses from MASiVEDb (<http://databases.bat.infospire.org/masivedb/>) and allocated them into age groups as in [23]. The number at the top of each boxplot indicates the number of elements within each group. Gene information was retrieved from the Filtered Gene Set of the maize B73 RefGen_V2 genome. my, million years.

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horizontal transfer

: TE
 : cytosine methylation
 : repressive histone mark
 : questions addressed in the text