Review

Transposable Elements as Focal Points for Epigenome Engineering in Plants

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ABSTRACT

Transposable elements are a major component of plant genomes and an important source of structural and regulatory variation. Because uncontrolled activity can be deleterious, plants rely on multilayered epigenetic pathways—DNA methylation, histone modifications, and small-RNA-directed silencing—to restrict transposons. However, transposable element sequences are also exapted as promoters, enhancers, and stress-responsive modules. In this review, we summarize how transposons and their epigenetic states influence gene expression, recombination and genome plasticity, with emphasis on evidence from crop species, and discuss when such states appear stable and heritable enough to be relevant for selection. We then assess emerging approaches for targeted epigenetic manipulation (including CRISPR/dCas-based epigenetic editors and locus-directed RNA-directed DNA methylation) and outline how transposon-rich regions could serve as practical entry points to modulate nearby gene regulation or transposon responsiveness. We highlight design considerations, current limitations, and ethical/biosafety questions, and propose experimental benchmarks needed to evaluate feasibility for crop improvement, with emphasis on evidence from major crops.

KEYWORDS: noncoding DNA; transposable elements; epigenome engineering; crops; stress responses; RNA-directed DNA methylation; CRISPR/dCas9; retrotransposons; ONSEN; MITEs

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ABBREVIATIONS

TE, Transposable element; RdDM, RNA-directed DNA methylation; RDR2, RNA-DEPENDENT RNA POLYMERASE 2; DCL, DICER-LIKE; AGO4, ARGONAUTE 4; siRNAs, small interfering RNAs; DRM2, DNA (cytosine-5)-methyltransferase 2; SUVH5/6, Histone-lysine N-methyltransferase; FWA, FLOWERING WAGENINGEN; GRN, Gene regulatory networks; LTR, long terminal repeats; MITE, Miniature inverted-repeat transposable element; tb1, teosinte branched1; dCas9, catalytically inactive Cas9; gRNA, guide RNA; WGS, Whole Genome Sequencing; ChIP, Chromatin

Immunoprecipitation; ATAC-seq, Assay for Transposase-Accessible Chromatin Sequencing

INTRODUCTION

Most plant genomes are composed of noncoding DNA, and these regions are dominated by transposable elements (TEs). TE content varies widely across crops, from ~40% in Asian rice (*Oryza sativa*) [1], to ~85% in maize (*Zea mays)* [2] and more than 90% in rye (*Secale cereale*) [3]. This abundance, combined with TE mobility diversification across families, rapid propagation, and strategies enabling vertical and horizontal transmission (e.g., pollen enhancer capture, horizontal gene transfer) [4], makes TEs a major source of structural and regulatory variation in plant genomes.

TE annotation relies on a hierarchical classification organized by transposition mechanism and evolutionary relationships (Class–order/superfamily), and this framework is periodically updated as new element types and reference genomes accumulate [5,6]. Within this hierarchy, plant TEs fall broadly into two mechanistic classes. Class I retrotransposons include long terminal repeat (LTR) elements of the Ty1/copia and Ty3/gypsy superfamilies as well as non-LTR retrotransposons. These elements transpose via an RNA intermediate and typically encode enzymes such as reverse transcriptase and integrase/endonuclease activities that mediate copying and insertion of new elements, often contributing strongly to genome size expansion [7,8]. Class II DNA transposons, including miniature inverted-repeat transposable elements (MITEs) and Mutator-like elements (MULEs), mobilize via DNA intermediates and commonly rely on a transposase that catalyzes movement via a cut-and-paste mechanism [5]. This hierarchical structure is also practical for TE-centric epigenome engineering, because candidate selection and guide design are typically performed at the family level [5,6].

Uncontrolled TE activity can be often considered deleterious to genome integrity [9]. As a result, plants deploy targeted epigenetic pathways to restrict TE expression and mobilization, most importantly cytosine DNA methylation, heterochromatic histone marks (e.g., H3K9me2 [10]), and small-RNA-directed targeting via RNA-directed DNA methylation (RdDM) [11]. These pathways lead to TE silencing while preserving the possibility that TE-derived sequences can be exapted, which means repurposed by the host as genes and *cis*-regulatory elements such as promoters, enhancers, insulators, or stress-responsive modules [12]. Exaptation provides a mechanistic bridge between TE silencing and adaptive trait variation, because TE-derived regulatory DNA can be “tuned” through DNA methylation and chromatin state rather than by altering coding sequence [13,14]. For crop improvement, this means that the breeding-relevant unit is often not TE mobility *per se*, but the epigenetic state of TE-adjacent regulatory DNA that shapes expression of nearby genes [15,16].

Once established, repression is maintained by DNA methylation maintenance pathways together with heterochromatic histone modifications (notably H3K9me2 [10]), thereby stabilizing transcriptional silencing across somatic lineages. Because epigenetic reprogramming is comparatively limited in plants, these silencing states are often transmitted and reinforced across generations [17,18].

TEs also display distinct insertion preferences, depending on their structure and class. While many LTR retrotransposons preferentially integrate into heterochromatic regions and remain transcriptionally silent [19], other classes of TEs (e.g., DNA transposons, MITEs) tend to insert in euchromatic regions near genes [20]. In such context, TE activity and epigenetic regulation can directly influence gene expression, chromatin accessibility, and genome plasticity. Therefore, DNA methylation plays a central role not only in TE repression but also in shaping gene regulatory landscapes. Studies in the model plant *Arabidopsis thaliana* have demonstrated the essential contribution of DNA methylation to both TE silencing and the regulation of gene expression [21,22]. Also see Hemenway and Gehring [17] for developmental contexts.

Given their abundance, frequent proximity to genes, and strong epigenetic control, TEs therefore represent compelling targets (and tools) for targeted epigenome engineering. Traditional genetic methods typically rely on permanent DNA sequence changes, for example gene knockouts created by nucleases or insertional mutagenesis using *Agrobacterium tumefaciens* T-DNA, which is the transfer DNA region of the Ti plasmid that integrates into plant chromosomes and is widely used to disrupt genes or introduce transgenes [23]. By contrast, emerging epigenome-engineering approaches aim to reconfigure the epigenetic state (e.g., histone modifications or DNA methylation) at specific loci to tune transcriptional outputs while leaving the DNA sequence intact.

Epigenome engineering holds particular promise for many agricultural applications, as epigenetic modifications can be stably inherited across generations, as demonstrated in multiple organisms such as *A. thaliana* [24] or rice (*Oryza sativa*) [25], while avoiding permanent changes to nucleotide sequences. Consequently, epigenetically modified plants may not align with traditional genetically modified organisms (GMO) definitions, which often focus on the introduction of foreign DNA and/or permanent changes to nucleotide sequence. This creates both technical and regulatory questions about how to classify products where the intended change is an altered chromatin/methylation state that may be heritable yet leaves the DNA sequence unchanged [26].

Because epigenome editing can, in principle, generate plants without foreign DNA and without DNA-sequence changes, it is often discussed separately from transgenic GMOs. However, in practice regulatory oversight remains dependent upon jurisdiction and typically depends on (*i*) whether any transgene DNA is present in the final line, (*ii*) whether the product is considered novel in agronomic behavior or composition, and (*iii*) jurisdiction-specific definitions and evolving interpretations of policies. The brief overview below is intended as a high-level orientation rather than an exhaustive guide, and we recommend consulting the latest jurisdiction guidance for their intended product and market [27–31].

Regulatory treatment of epigenome-edited crops continues to evolve. In the European Union, a provisional agreement has been reached on rules for plants obtained by new genomic techniques, with implementation details still developing [27,28]. In China, the Ministry of Agriculture and Rural Affairs has introduced a dedicated pathway for gene-edited plants, including trial guidelines for safety evaluation and subsequent trial rules in 2023 that further clarify review procedures and classification criteria, but implementation continues to evolve [31]. In Canada, oversight is generally product/novelty-based through the framework for Plants with Novel Traits and related food/feed assessments [29]. In the United States, the USDA-APHIS biotechnology framework emphasizes organism properties and provides exemption/confirmation and review pathways [30].

In this review, we aim to define and highlight a TE-centric epigenome engineering framework and to discuss its potential for advancing crop improvement strategies.

TRANSPOSABLE ELEMENT SILENCING MARKS

Spatial Organization and Maintenance Systems

In many plant genomes, TEs are enriched in pericentromeric, gene-poor regions packaged as heterochromatin, where their repression is maintained by the combined action of cytosine DNA methylation, H3K9me2, and RdDM. While heterochromatic TEs largely rely on maintenance pathways, RdDM is particularly active in euchromatic regions [32] and at TE-gene boundaries, where it establishes *de novo* methylation and contributes to chromatin boundary formation [33,34]. RdDM is a major plant-specific *de novo* methylation pathway, playing a central role in silencing euchromatic TEs, preventing the spread of heterochromatin into adjacent genes, and reinforcing silencing when TEs become transcriptionally active. This pathway relies on specialized RNA polymerases and small RNAs to guide methylation machinery to homologous genomic loci [35–38].

Molecular Mechanisms of TE Silencing

At the molecular level, cytosine methylation occurs in CG, CHG, and CHH contexts (H = A, C or T) and is maintained by partially distinct enzyme systems. In Arabidopsis, CG methylation is maintained primarily by MET1, CHG methylation largely by CMT3 in a reinforcing loop with H3K9me2, and much heterochromatic CHH methylation by CMT2, whereas euchromatic CHH methylation and *de novo* methylation are largely established through RdDM centered on DRM2 [39–41]. Chromatin accessibility is a major constraint on these pathways. The nucleosome remodeler DDM1 promotes methyltransferase access to H1-rich heterochromatin, helping explain why long pericentromeric TEs can remain heavily methylated and relatively resistant to perturbation [42]. Recent structural work has further clarified how DDM1 remodels nucleosomes and couples ATP hydrolysis to chromatin remodeling, thus providing a mechanistic basis for its role in heterochromatin maintenance and TE silencing [42,43].

In the canonical RdDM pathway, RNA Polymerase IV (Pol IV) initiates the process by transcribing TE-derived regions into short RNAs that are converted into dsRNA by RDR2 and processed by DCL3 into predominantly 24-nucleotide (nt) siRNAs, which are loaded onto AGO4/AGO6 [44]. In parallel, Pol V generates scaffold transcripts at target loci. Base-pairing (direct or indirect) between AGO-bound siRNAs and Pol V transcripts enables recruitment of DRM2, leading to cytosine methylation (CG/CHG/CHH) and transcriptional repression. Importantly, recent structural and biochemical studies of Pol IV, Pol V and RDR2 have refined our mechanistic understanding of how these polymerases generate distinct noncoding RNAs and coordinate RdDM targeting [38,45–47].

Two conceptual ideas are especially useful for anticipating outcomes of TE-targeted perturbations. First, RdDM is not a single linear pathway but a family of routes converging on DRM2: alongside canonical Pol IV-RDR2-DCL3-AGO4/6-Pol V targeting, noncanonical routes can be initiated by Pol II transcripts (including those from reactivated TEs) and can generate methylation through alternative small-RNA and scaffold-RNA entry points [45,48,49]. For example, a common noncanonical mode is sometimes described as RDR6-RdDM, in which Pol II-derived transcripts (including those produced by reactivated TEs) are converted into dsRNA by RDR6 and processed by DCL2/DCL4 into 21–22 nt siRNAs [50,51]. These siRNAs engage AGO proteins and still converge on DRM2-dependent methylation and in some cases using Pol V scaffold transcripts at the target locus. This matters for epigenome editing because loci that continue to produce TE-derived transcripts and small RNAs can undergo rapid remethylation after demethylation [52], whereas loci lacking sustained small-RNA reinforcement tend to progressive lose induced methylation once the initiating trigger is removed. Second, RdDM frequently concentrates at TE boundaries, especially for TEs near genes, producing local CHH-methylated “islands”, a pattern well described in maize [53,54]. This

spatial patterning is highly relevant for predicting whether epigenome editing at a TE will stay confined or influence nearby promoters and enhancers [53].

Following the establishment of DNA methylation, TE repression is reinforced by a self-reinforcing loop between DNA methylation and H3K9me2. The H3K9 methyltransferases SUVH4/KYP, SUVH5 and SUVH6 can read methylated DNA via their SRA domains and deposit H3K9me2, which in turn promotes recruitment/activation of chromomethylases such as CMT3 (and, in heterochromatin, CMT2), thereby stabilizing silencing [32,55].

Functional Consequences and Variability

When TEs are located near genes or regulatory regions, TE-associated methylation can spread (or be excluded), influencing gene expression. A classic example is FLOWERING WAGENINGEN (FWA) in *A. thaliana*, where loss of methylation over SINE-like TE-derived repeats in the 5' regulatory region causes ectopic FWA expression and a stable late-flowering epiallele [56]. Crop studies likewise show that TE-associated methylation can shape local regulatory outputs; for example, RdDM at TE-derived sequences (including MITEs) can modulate expression of nearby genes and small-RNA loci with developmental consequences in rice.

Many loci also reflect a dynamic equilibrium between methylation pathways and active DNA demethylation, which is crucial for preventing inappropriate methylation spreading into gene regulatory regions. Active demethylation is initiated by DNA glycosylases/lyases of the ROS1/DME family, where recent structural work has clarified how ROS1 excises 5mC and couples base removal to repair [48,57]. Recent reviews summarize how demethylation contributes to boundary formation and stress/developmental regulation [48].

TE insertions can also generate novel *cis*-regulatory modules whose activity is environment and tissue-dependent. In blood orange (*Citrus sinensis*), insertion of a copia-like LTR retrotransposon upstream of the MYB factor Ruby created a fruit-specific, cold-responsive module that activates anthocyanin biosynthesis [58] (Table 1), with subsequent work supporting a key contribution of the retrotransposon promoter/solo LTR to cold-inducible Ruby expression [59].

In plants, TE-associated methylation, particularly non-CG methylation (mainly CHH) that often peaks at TE boundaries near genes, can be more dynamic than CG methylation, which contributes to epigenetic variability and the potential emergence of TE-linked epialleles [39,60]. Population and evolutionary studies show that variation in methylation near TEs can be associated with stable, and sometimes heritable, differences in gene expression and phenotype without underlying DNA sequence change. This suggests that TE-linked epialleles may contribute to domestication and diversification in crops [61,62].

Table 1. Emblematic TE-linked epigenetic variants relevant to crops and experimental design[1].

Name	Organism	Origin TE	Genomic impact	Reference
Karma	Oil palm (*Elaeis guineensis*)	LTR retrotransposon	'Mantled' somaclonal variation is associated with hypomethylation of the Karma retrotransposon embedded in a floral regulator	[63]
ONSEN	*Arabidopsis thaliana*	LTR retrotransposon	Transcriptionally activated upon heat stress and can massively transpose when small-RNA pathways are compromised	[64,65]
mPing	Rice (*Oryza sativa*)	MITE	High copy number in four cultivated rice accessions and alters expression of nearby genes	[20,66]
Tcs1	Blood orange (*Citrus x sinensis*)	LTR retrotransposon	3'LTR acts as a cold-responsive *cis*-regulatory element that promotes *Ruby* gene expression	[58,59]

[1] Selected example of TEs whose insertion, activation, or epigenetic state modulates the expression of nearby genes or regulatory pathways, illustrating how specific TE copies or families can contribute to phenotypic variation and adaptive traits.

TRANSPOSABLE ELEMENT FAMILIES AS REUSABLE REGULATORY MODULES

TEs intrinsically carry *cis*-regulatory sequences within their structure, including promoters, terminators, and sometimes enhancer-like modules that are required for expression of TE-encoded proteins and for mobilization within the genome [67,68]. Consequently, TEs can participate in the rewiring of gene regulatory networks by introducing regulatory sequences into new genomic contexts, and when amplified, by dispersing similar motifs across several loci [68,69].

This capacity to harbor and disseminate regulatory motifs has been widely documented and positions TEs as potent sources of regulatory innovation. A well-characterized example is ONSEN (ATCOPIA78), a Ty1/copia LTR retrotransposon in *A. thaliana* (Table 1). Multiple studies show that ONSEN is robustly induced by prolonged heat stress, and that its LTR contains a heat-responsive element bound by the plant heat shock response machinery (including HSFA2), effectively coupling TE transcription to the host's heat-stress program [64,65,70]. Interestingly, ONSEN illustrates that DNA hypomethylation alone is not necessarily sufficient to activate a stress-responsive TE. Indeed, ONSEN activation depends strongly on the appropriate environmental cue, and the element can remain inactive in unstressed conditions even when epigenetic repression is weakened [65]. In addition, while heat can trigger transcription and the accumulation of extrachromosomal copies, transposition into the next generation is normally restricted by small-RNA pathways, linking environmental activation to host surveillance mechanisms [64].

Beyond individual cases, we see growing evidence indicating that TE families can shape transcription at scale by contributing regulatory DNA that is then reused across many insertions [71,72]. In maize and other large-genome crops, many regions of accessible chromatin and candidate *cis*-regulatory elements can occur within TE sequences, and TE polymorphisms can alter local regulatory landscapes between genotypes [69,73]. In particular, genome-wide analyses have shown that a limited number of TE families can be associated with stress-responsive activation of nearby genes, which is consistent with TE insertions providing potential stress-responsive enhancer-like functions [71,74]. We see recent integrative work that continues to refine this view and provides a clearer framework for quantifying the contributions of TEs to regulatory DNA and transcriptional variation in various crop genomes [69].

Conceptually, TE families enriched for motifs under specific conditions can be viewed as reusable regulatory scaffolds. What we mean is that TE amplification can disperse related *cis*-elements throughout the genome, while epigenetic repression and environmental responsiveness can determine when and where these modules are active. This perspective at the "family level" could help to explain how TE landscapes can contribute to coordinated transcriptional responses under stress. Furthermore, it highlights why TE sequence modules are attractive templates for epigenome-engineering strategies if one aims at tuning stress-responsive traits. Moving from this conceptual framework to experimental implementation, it is important to separate regulatory effects from transpositional activity. Therefore, TE-centric epigenome-editing strategies should be evaluated in a manner that is independent of any increase in TE mobilization. This requires parallel identification of TE transcription and small RNA production, evidence of new insertions, and expression of nearby target genes.

A CONCEPTUAL FRAMEWORK FOR TE-CENTRIC EPIGENOME ENGINEERING

In this section, we outline a practical framework for shifting epigenome engineering from single-locus interventions to more TE-centered designs, where related families act as distributed regulatory substrates. Because TE copies are repeated, epigenome editors could be deployed either as a "multi-copy model" (to get family-level perturbation) or as a "driver-copy model" (selected insertions with strong regulatory influence). Both of these models would aim to tune regulatory programs while explicitly monitoring TE activation.

A Novel Angle: Starting from TE Families Rather than Individual Genes

Certain TE families show insertion preferences that are not random along plant chromosomes, with some families integrating preferentially within gene-rich and regulatory regions. For example, in Arabidopsis, TE-accumulation experiments revealed that different TE families have distinct chromatin "preferences", and that Ty1/copia LTR

retrotransposons can be preferentially guided toward H2A.Z-enriched chromatin at environmentally responsive genes. This is consistent with an informed logic to target specific families for coordinated, network-level epigenome engineering [75].

We also find that a number of families preferentially integrate into distal regulatory regions, such as the Hopscotch retrotransposon insertion about 70 kb upstream of the maize teosinte branched1 (tb1) gene, where it acts as an enhancer and contributes to architectural traits selected during domestication [76]. Also, work dissecting the broader tb1 domestication module further highlights how selection can act on regulatory variation embedded in such modules [77]. Other TE families tend to insert in proximal regulatory regions, often very close to host genes. A classic example is the MITE family mPing (Table 1), which has undergone dramatic copy-number amplification in some domesticated rice accessions and shows a strong bias for insertions within a few kilobases of coding regions [20,66].

MITEs are also appealing from an engineering perspective because they are small and non-autonomous, which could reduce the probability of direct gene disruption while still allowing widespread deposition of regulatory DNA. Recent crop examples illustrate that individual MITE insertions in promoters can measurably tune valuable traits. For example, a MITE insertion in the OsTCP4 promoter is associated with increased tiller number and reduced grain size in rice [78,79]. More broadly, MITEs are increasingly recognized as major contributors to regulatory innovation in plant genomes [80,81].

Now, rather than targeting individual genes, a TE-centric strategy would prioritize TE families with characteristics like advantageous chromatin states, defined insertion preferences, and conserved regulatory motifs, such as the stress-responsive elements observed in ONSEN [65], and importantly leverage the fact that a single TE family can be associated with many genes across the genome. Modulating the epigenetic state of such families could shift the expression of an entire gene set participating in a common response or developmental program [71,82–84].

This framework shifts epigenome engineering from a gene-by-gene paradigm toward a network-level approach, in which TE families serve as programmable regulatory nodes. It aligns with the reality that many agronomic traits like stress tolerance, flowering time, and yield are polygenic and often controlled by distributed *cis*-regulatory variation [85] (Table 2).

Table 2. Decision framework for choosing TE-centric versus gene-centric strategies design[1].

Situation	Gene-Centric CRISPR	Conventional Breeding	TE-centric Epigenome Editing	Why TE-Centric Helps
Single gene, large effect	✓✓✓	✓✓	✗	Simple and most predictable intervention
Polygenic/quantitative trait	✗/✓	✓	✓✓✓	Coordinates multi-gene shifts via shared modules
Redundant regulatory elements	✗	✗	✓✓✓	Exploits repeated motifs and redundancy
Distributed enhancer/ network regulation	✗	✗	✓✓✓	Network-level tuning via shared TE-derived modules
Stability requirement is strict	✓✓✓ (DNA mutation)	✓✓✓	✓ (context-dependent)	Requires empirical validation
High TE polymorphism across genotypes	✗	✓	✓✓	Requires TE atlases + pangenome-aware guide design

[1]✓ indicates relative suitability.

Identifying and Targeting "Driver" TE Copies

A TE-centric epigenome engineering strategy therefore requires the identification of "driver" TE copies, which would be individual insertions within a family that have disproportionate regulatory impact on nearby genes and/or on higher chromatin domains. Driver copies are expected to be located where regulatory logic is encoded and read (promoters, enhancers, boundary elements) and/or in chromatin states permissive for regulatory factor binding. This concept builds on growing evidence that TE insertions can donate functional *cis*-regulatory DNA and become integrated into host gene regulatory networks [8,13,68,75,82].

Practically, the identification of candidate driver copies can be framed as an integrative prioritization approach that would consider the following: (*i*) TE annotations and subfamily assignments; (*ii*) proximity to genes and to experimentally defined regulatory elements (ATAC-seq peaks, enhancer-associated histone marks); (*iii*) evidence of local epigenetic constraint or plasticity (such as siRNA production, edge-localized CHH, methylation variability across genotypes); and (*iv*) evidence of genotype–phenotype links, including TE insertion polymorphisms, methylation quantitative trait loci (QTL) and expression QTLs that implicate variation due to TE proximity (Box 1).

Once identified, driver TE copies could become prime candidates for targeted epigenetic manipulation. Tools based on catalytically inactive Cas9 (dCas9) fused to epigenetic modifiers can install or erase marks at chosen loci without introducing double-strand breaks. For example, locus-specific DNA demethylation has been achieved in Arabidopsis by recruiting the human TET1 catalytic domain [86]. Conversely, a SunTag system that brings multiple copies of a plant DRM catalytic domain to a chosen genomic site can trigger strong, targeted DNA methylation, which remains stable at loci like FWA, even after the transgene has been removed [87].

Recent work continues to broaden the plant epigenome-editing toolkit beyond DNA methylation, including locus-specific manipulation of histone marks. For example, in Arabidopsis, targeted removal of H3K27me3 using a dCas9–JMJ13 fusion was recently used to de-repress the Arabidopsis CUC3 locus [88]. Taken together, these studies emphasize that locus choice, chromatin context, and effector design can strongly influence both the magnitude and the durability of engineered epialleles [85,89]. A key advantage of the driver-copy strategy lies in leverage: because individual driver TEs can influence multiple genes through shared motifs, local chromatin effects, or long-range interactions, the editing of a small number of strategically positioned TE insertions could yield coordinated transcriptional changes across a pathway (Figure 1, Table 2).

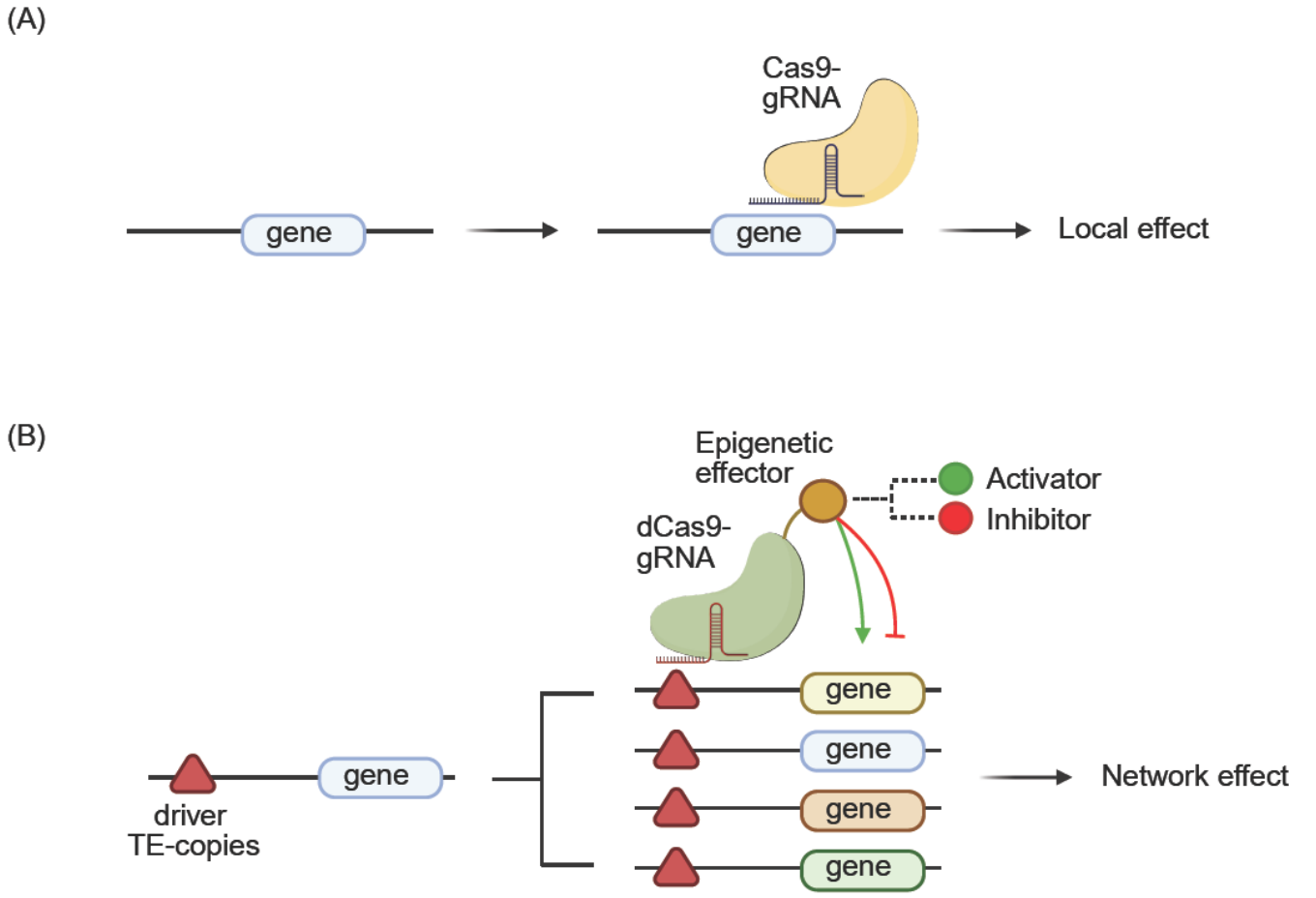

Figure 1. Summary figure illustrating approaches from gene-centric to TE-centric epigenome engineering. (**A**) Classical CRISPR/Cas9-based approaches target individual genes and produce localized effects on gene expression. In contrast, (**B**) TE-centric epigenome engineering uses dCas9, a catalytically inactive Cas9, fused to epigenetic effectors (CRISPRa/i); no double-strand breaks are introduced. They are directed toward conserved sequence motifs shared by multiple driver TE copies. Targeting these strategically positioned TE copies enables coordinated epigenetic modulation of multiple genes distributed across the genome, resulting in network-level regulatory effects.

Box 1. Criteria for Selecting "Driver" TE copies for TE-Centric Epigenome Editing. This box summarizes the genomic, epigenetic, and functional criteria used to identify TE families or subfamilies suited as regulatory targets.

Not all transposable elements (TEs) are equally suitable for TE-centric epigenome-engineering strategies. "Driver" TEs can be defined as TE families or subfamilies whose genomic, epigenetic, and functional properties make them particularly effective regulatory targets.

Key selection criteria include:

(i) Defined TE family and subfamily identity: prioritize well-annotated family and subfamilies to leverage shared sequence features and evolutionary context.
(ii) Accessible chromatin context: prioritize insertions in open chromatin and/or near experimentally defined regulatory elements, such as ATAC-seq peaks or enhancer marks.
(ii) Evidence of epigenetic constraint or plasticity: prioritize TEs with signatures of active regulation (siRNA production, edge-localized CHH methylation, variable DNA methylation across genotypes or conditions.
(iv) Evidence for genotype-phenotype associations: prioritize TE families/copies supported by functional or association evidence, including TE insertion polymorphisms linked to phenotypic variation, methylation quantitative trait loci (meQTLs), or expression QTLs (eQTLs).

TE-Aware Guide Design and Off-Target Logic

Once a driver TE family has been selected, a critical next step would be integrating TE annotations into guide RNA (gRNA) design strategies. This departs from most conventional gene-centric CRISPR applications, which typically prioritize unique targets. In a TE-centric framework, repetition can be an intended feature, where guides can be designed in "multi-copy mode" to recognize conserved motifs shared across many family members, or alternatively in "copy-specific mode" to target a small set of prioritized driver copies using subfamily-specific variants or unique flanking sequence.

Guide selection can be used in two complementary modes. In a family-level (multi-copy) design, guides are chosen from conserved 20-nt windows (adjacent to the relevant PAM) identified on a TE family consensus or multiple-sequence alignment. Sites to be prioritized would be present across a high fraction of copies to maximize on-family coverage. In a driver-copy (copy-specific) design, guides are anchored to a single-nucleotide polymorphism (SNP) or insertion-deletion variant (indel), a subfamily-specific variant, or a TE flanking junction unique to the candidate insertion, and then screened across the pangenome to ensure near-null matches elsewhere. In both design modes, off-target assessment should focus on cross-family and genic risks, and not only repeated hits within the intended TE family.

This then reframes the notion of off-target effects. In multi-copy mode, hits across intended family members are on-target outcomes aligned with experimental design. In this context, off-target risks primarily arise from unintended targeting of closely related TE families, as well as unique genic sites that share short sequence homologies. Accordingly, a TE-aware

design pipeline should maximize coverage within the intended family while minimizing cross-family interactions and, importantly, should incorporate empirical validation of both chromatin changes (e.g., genome-wide DNA methylation profiles, whole genome bisulfite sequencing (WGBS)) and transcriptional outcomes (RNA-seq, TE expression profiling). Guide design should consider pangenome diversity within TE copies to preserve intended on-family coverage across cultivars and inbred lines.

TE-Centric Epigenomic Screens

Following the targeted epigenetic modifications of selected TE families or driver copies, a very important step would be to perform epigenomic screening to assess molecular and phenotypic outcomes. We envision TE-centric screens as pooled (or semi-pooled) libraries of epigenome-editing constructs designed around TE families (multi-copy guides) or prioritized driver copies (copy-specific guides), extending emerging CRISPR screening frameworks in plants to epigenetic effectors [90,91].

Such screens should be coupled with multi-omics readouts to comprehensively characterize effects: (*i*) local on-target editing (targeted bisulfite or ChIP/CUT&Tag), (*ii*) genome-wide chromatin remodeling (WGBS, ATAC-seq), (*iii*) transcriptomic impact (RNA-seq), and (*iv*) quantitative phenotyping under relevant environments (Table 3). Ideally, recent advances in plant screening toolkits and high-throughput platforms, including protoplast and single-cell approaches, now provide additional routes to scale discovery [91,92].

Table 3. Minimum monitoring assays.

Risk Mode	What to Expect	Minimum Monitoring Examples
TE transcriptional reactivation	TE RNA increases; potential stress-responsive bursts	TE-qPCR, RNA-seq, condition/stress tests
TE mobilization	New insertions in progeny	Insertion-site mapping, TE-capture or whole genome sequencing (if possible)
Pleiotropic network effects	Broad shifts in expression; impact on growth	RNA-seq and targeted trait assays, basic fitness readouts
Off-target chromatin remodelling	Global changes detected in methylome, accessible chromatin, histone marks	Whole genome bisulfite sequencing or targeted bisulfite, ATAC/ChIP-seq/CUT&Tag (subset targets)

A practical implementation is staged scaling rather than immediate large libraries. A pilot phase can begin with for example ~10–50 guides, which could correspond to 2–5 TE families with 2–5 guides each, or ~10–20 driver-copy guides to first validate delivery and molecular readouts in transient systems. This is followed by expansion to ~100–500 guides once targeting and assays are robust, coupled with early molecular triage (RNA-seq and targeted bisulfite assays) before committing to stable lines and multi-generation validation. This staging aligns throughput with decision points, thus making screens feasible while keeping downstream validation focused on a manageable set of prioritized TE families or copies. This

staged approach makes screens tractable while keeping interpretability central, which is essential when perturbing repetitive genomic substrates at scale.

Because TE-centered interventions can, in principle, tune gene regulation or increase mobilization frequency, TE-centric screens should include explicit monitoring of mobilization risk. This could include TE transcript levels, extrachromosomal DNA, and insertion-site mapping. When feasible, whole-genome or TE-capture sequencing of edited lines and progeny should be included to detect new insertions. This requires that regulatory reprogramming be clearly distinguished from mobilization risk and monitored with appropriate molecular readouts.

Taken together, these design principles could serve as the basis to position TE families as reusable regulatory substrates for precision epigenome engineering and for dissecting how epigenetic states at repeats shape complex traits like environmental responses.

Risks, Constraints, and Design Safeguards

A central challenge of TE-centric epigenome engineering lies in the inherent connectivity and copy number of many TE families. Because multi-copy targets are distributed across chromosomes and often lie in distinct chromatin environments, simultaneous perturbation of many insertions can generate broad, context-dependent transcriptional changes that propagate through regulatory networks [24,93–95]. This network-level leverage is a strength of the approach, but it also necessitates careful target selection, comprehensive mapping of on-target and collateral chromatin changes, as well as rigorous phenotypic characterization to distinguish beneficial rewiring from pleiotropy or fitness costs [85,88,96,97].

A first design safeguard is therefore to favor partial and context-dependent perturbations over constitutive reprogramming. In practice, this can mean prioritizing copy-specific or subfamily-specific targeting of a small number of “driver” insertions before moving to whole-family approaches, and/or inducible [97], tissue-specific, or stress-responsive expression of epigenome editors to restrict when and where chromatin is altered [71,98]. These designs mirror natural TE regulation, where activation is often conditional, and enable causal testing in defined environments while minimizing unintended effects during normal development [65].

A second key safeguard is to separate modulation of local regulatory outputs from unintended TE transcriptional reactivation and mobilization. Because TE silencing is closely coupled to genome stability, broad loss of DNA methylation or heterochromatic histone marks can increase TE expression and, in some cases, enable mobilization [15,47]. This is particularly relevant for stress-responsive elements such as ONSEN, whose transcription is strongly induced by heat stress and can produce extrachromosomal copies, while heritable transposition is normally

constrained by small-RNA pathways [64,65]. Therefore, epigenome-editing strategies should be calibrated to tune *cis*-regulatory effects without crossing thresholds that promote TE replication. Practical approaches include targeting TE-derived enhancer-like sequences rather than the TE promoter, editing flanking regulatory regions, and reinforcing partial silencing states (instead of fully erasing them).

The stability of engineered epigenetic states across generations represents an additional constraint. In plants, transgenerational inheritance of DNA methylation variants is well documented, but many cases reflect an imperfect reinforcement rather than complete resetting, and also durability can be very locus and context-dependent [18,99]. To provide a quantitative anchor from plant epigenetics, studies in hybrid maize show that although hybridization can induce thousands of *trans*-acting methylation changes, only a small fraction (~3%) of these epialleles are transmitted through multiple backcross and selfing generations, suggesting that many induced methylation states tend to be removed in the absence of reinforcement mechanisms [100]. In contrasts, targeted CRISPR/dCas9-TET1cd-mediated demethylation of the Arabidopsis NMR19-4 region yielded heritable demethylation and associated phenotypic effects detectable up to four generations, which illustrates that some engineered epialleles can segregate stably over several generation [101]. These contrasting outcomes likely reflect locus- and pathway-specific differences in epigenetic maintenance (Table 4). In plants, reproduction involves partial, cell-type specific reprogramming rather than global methylation erasure as seen in animals, and the local chromatin environment can determine whether an induced state is reinforced (including via RdDM) [45] or gradually erodes across generations. Therefore, TE-centric approaches may be more likely to yield durable outcomes at some TE-associated targets than at others, depending on genomic context. For agricultural purposes, stability should therefore be treated as an empirical property of each target, where engineered states may persist for some loci, yet progressively revert at others in the absence of continued selection or reinforcement [98,102–104]. Therefore, claims of stable TE-centered engineering should ideally be supported by multi-generation segregation tests (including transgene-free lines), methylome profiling at and around the target, and phenotyping across diverse environments. Because selection regimes can shape which epigenetic states persist in populations, long-term field assessments would be particularly important when engineered configurations are intended to contribute to stress adaptation or yield stability in crops [105,106].

Taken together, these considerations highlight the need for cautious, TE-aware epigenome-engineering strategies that balance new avenues with genome integrity, phenotypic stability, and agronomic considerations.

Table 4. Examples of plant epigenome-editing systems with reported inheritance.

Study	Organism	Tool/ effector	Target/ context	Transgene-free tested?	Generations followed	Outcome
[87]	*Arabidopsis thaliana*	dCas9-SunTag-NtDRMcd (targeted methylation)	FWA locus (DNA methylation gain)	Yes	Multiple (reported)	Stable, meiotically heritable methylation after segregation
[86]	*Arabidopsis thaliana*	dCAs9/TET1cd (targeted demethylation)	FWA/CACTA1 (DNA methylation loss)	Partly	Reported	Efficient on-target demethylation; heritable in at least on case
[101]	*Arabidopsis thaliana*	dCas9-TET1cd	NMR19-4 (spontaneous epiallele)	Reported	Up to 4	Transgenerational demethylation and phenotypic effects
[100]	Maize (*Zea mays*)	Natural/induced trans-acting epialleles (small-RNA mediated)	Genome-wide	N/A	Multiple	~3% transmitted across backcross and selfing generations

CONCLUSIONS AND PERSPECTIVES

Realizing the full potential of TE-centric epigenome engineering will require dedicated conceptual, computational, and experimental resources that treat TE families as key engineering targets. A first priority is the creation of TE-family atlases for major crop species that integrate (*i*) high-confidence family annotations and hierarchical classification, (*ii*) insertion preferences and pangenome diversity, (*iii*) chromatin context (such as DNA methylation, histone marks, accessibility), and i*v*) predicted regulatory motif content and links to nearby gene regulatory networks. Because TE-derived regulatory logic is often family-specific and context-dependent, such atlases would provide the practical baseline for identifying families, and a small subset of candidate “driver” insertions, with high regulatory leverage and potential agronomic relevance [82,83,107]. Robust TE annotation pipelines such as EDTA provide an important foundation; coupling these catalogs to epigenomic and regulatory-network components will enable identification of families and driver insertions with high regulatory leverage [108] (Figure 2, Box 2).

At the experimental level, we anticipate the value of standardized perturbation frameworks built around TE-family–specific panels of programmable editors (e.g., DNA methylation writers and erasers, histone-mark editors, and CRISPRa/i modules) that can be used as modular toolkits. In addition, inducible or tissue-restricted expression systems could be used to implement partial, context-dependent perturbations. Recent advances in plant editing vectors and screening-compatible toolboxes increasingly support pooled or semi-pooled designs, including protoplast-based prescreens to triage guide activity before whole-plant validation [91]. Looking forward, emerging high-throughput phenotyping and single-cell screening approaches may further accelerate the discovery of TE-linked regulatory nodes and their downstream programs [92].

For translation to crops, TE-centric epigenome engineering is attractive because it can harness pre-existing TE-encoded regulatory circuitry to tune complex traits without altering DNA sequence, which is an important concern around genetically modified organisms, where regulatory

treatment of these outputs is jurisdiction-dependent and mechanism specific. A credible path to application in this context would combine target selection grounded in mechanistic and population evidence, rigorous molecular validation of the edited state and its specificity, and breeding-compatible workflows that allow segregation of editor transgenes or use transgene-minimized delivery where possible [109]. Because engineered epialleles can show locus-dependent durability and environment-dependent expression, multi-generation and multi-environment testing should be treated as a core design criterion.

Ultimately, reframing TEs from genomic obstacles or junk DNA to designable regulatory scaffolds represents a shift in plant systems engineering. By embracing TE family structure, chromatin context, and network connectivity, as well as by building tools that explicitly manage the associated risks, TE-centric approaches could expand the practical design space of plant biotechnology toward coordinated modulation of complex traits in an increasingly variable environment.

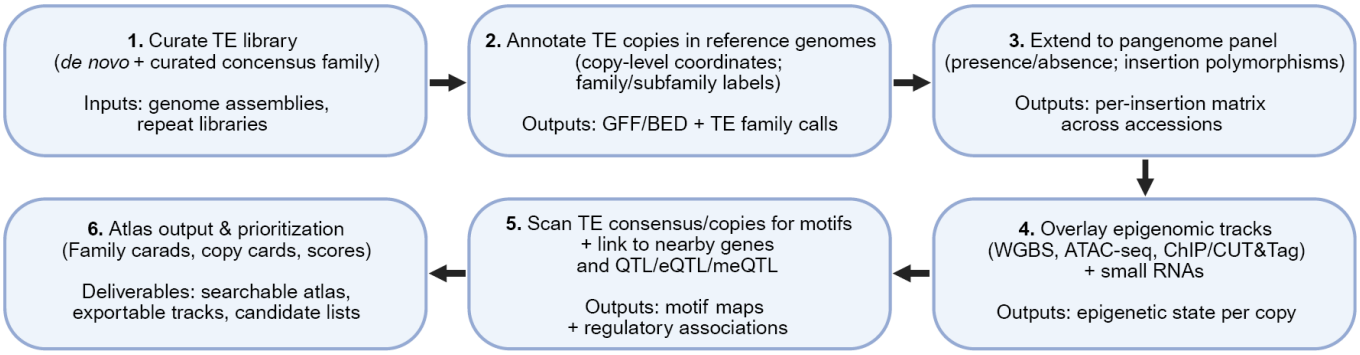

Figure 2. Workflow for constructing a transposable element (TE) family atlas.

Box 2. This box summarizes the essential components needed to construct a streamlined TE-family atlas. Minimum viable TE-family atlas (fields to include).

A minimum viable TE-family atlas should include:

- Family + subfamily consensus sequences (classitication + versioning)
- Copy-level annotation per genome coordinates, orientation, truncation/age proxies)
- Pangenome presence absence per insertion and per family
- Epigenomic state per copy (methylation profiles, accessibility, key histone marks) siRNA mapping + evidence of RdDM engagement
- Motif maps (stress-response elements, TF motifs)
- Links to nearby genes, regulatory elements + QTL overlays
- Exportable tracks (BED/GFF/bigWig) + summary “family cards” and “driver-copy cards”.

Suggested outputs for readers:
“Family cards”: copy counts, chromatin states, motif enrichment, genotype distribution
“Copy cards”: top-ranked candidates with coordinates, local epigenome, nearby genes, evidence links

DATA AVAILABILITY

No data were generated for this review.

AUTHOR CONTRIBUTIONS

Conceptualization, AJ and ZJL; writing—original draft preparation, AJ; writing—review and editing, AJ and ZJL; visualization, AJ; supervision, ZJL; funding acquisition, ZJL. All authors have read and agreed to the published version of the manuscript.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest in relation to this work.

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