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Roles of transposable elements in the regulation of mammalian transcription

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Abstract

Transposable elements (TEs) comprise about half of the mammalian genome. TEs often contain sequences capable of recruiting the host transcription machinery, which they use to express their own products and promote transposition. However, the regulatory sequences carried by TEs may affect host transcription long after the TEs have lost the ability to transpose. Recent advances in genome analysis and engineering have facilitated systematic interrogation of the regulatory activities of TEs. In this Review, we discuss diverse mechanisms by which TEs contribute to transcription regulation. Notably, TEs can donate enhancer and promoter sequences that influence the expression of host genes, modify 3D chromatin architecture and give rise to novel regulatory genes, including non-coding RNAs and transcription factors. We discuss how TEs spur regulatory evolution and facilitate the emergence of genetic novelties in mammalian physiology and development. By virtue of their repetitive and interspersed nature, TEs offer unique opportunities to dissect the effects of mutation and genomic context on the function and evolution of *cis*-regulatory elements. We argue that TE-centric studies hold the key to unlocking general principles of transcription regulation and evolution.

From protists to mammals, almost all eukaryotic genomes are populated with transposable elements (TEs)[1]. TEs are parasitic mobile DNA elements that are diverse in their genetic structure and transposition mechanisms[1] (FIG. 1). As they propagate in the genome, TEs

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are potent insertional mutagens. When landing within exons, they are usually disruptive, whereas TE insertions in non-coding sequences, within or between genes, can have immediate or latent effects on host gene expression in *cis* or in *trans*. As for any mutational process, the effects of TE insertions on host gene expression may range from deleterious to adaptive. Over evolutionary timescales, mutations in their sequences can render TEs incapable of transposing. However, the regulatory sequences of TEs can be co-opted for host regulatory functions regardless of their transposition competence. Mechanistically, TEs can affect gene expression transcriptionally[2,3], post-transcriptionally[4–6] or in *trans* through their encoded products, which include both non-coding RNAs (ncRNAs) and proteins[5,7].

Co-opted

Co-opted or exapted refers to the process by which transposable element (TE) sequences are repurposed for host function. Classically, this term implies that the process conferred adaptive changes that increased organismal fitness. in the context of gene regulation, the term is also used to describe the process by which TEs give rise to novel *cis*-regulatory elements that cause demonstrable changes in host gene expression.

In this Review, we discuss recent literature illuminating how TEs influence mammalian gene regulation at the transcriptional level through *cis* and *trans* mechanisms. These studies have been enabled by technological advances in genome editing (TABLE 1; BOX 1), which have opened new frontiers for the identification and characterization of TE-derived *cis*-regulatory elements (CREs), and for their functional validation in embryonic development and adult tissues[8–11]. We also discuss challenges in demonstrating and interpreting the adaptive relevance of gene expression changes driven by TEs. We surmise that, although many TEs exert measurable effects on gene expression or cause regulatory divergence between species, only a minority of these effects contribute to phenotypic changes at the organismal level. Finally, we argue that TE-derived CREs provide a unique model system for interrogating the mammalian *cis*-regulatory lexicon owing to their distinctive features compared with non-TE CREs.

How TEs influence gene transcription

In their prescient ‘gene-battery’ model, Roy Britten and Eric Davidson were among the first to realize the potential of interspersed DNA repeats as raw material for the wiring of *cis*-regulatory circuits[12,13] (reviewed in REFS[2,14–16]). We now appreciate that these repeats are mostly derived from the accumulation of diverse TE families that can account for a substantial fraction of the genome. For instance, at least half of the human genome is comprised of TEs and their remnants[17,18] (FIG. 1). A body of literature has now emerged to support the contribution of specific TE families to the dispersion of transcription factor binding sites (TFBSs) and CREs throughout mammalian genomes[14,15,19–24] (reviewed in REF.[25]). In fact, it has been estimated that the majority of CREs newly evolved during primate evolution are directly derived from TEs[26,27].

Gene-battery model

Theory developed by britten and Davidson postulating that repetitive sequences are a driver of the evolution of protein expression control with a limited number of effectors.

Nevertheless, the mechanisms by which TEs influence host gene-regulatory networks are far more diverse than initially envisioned and can broadly be divided into five classes, discussed in this section (FIG. 2): (1) introduction of TFBSs, promoters and enhancers, (2) modification of 3D chromatin architecture, (3) production of regulatory ncRNAs, (4) co-option of TE-derived coding sequences as new transcriptional effector proteins, and (5) collateral effects of TE silencing mechanisms.

TEs function as *cis*-regulatory elements

In order to promote their parasitic existence, many TEs contain regulatory sequences that facilitate their own transcription. Over evolutionary timescales, TEs are typically silenced by the host and accumulate mutations, eventually leading to their immobilization, after which they no longer retain the biochemical activities necessary to promote their own spread. However, remnants of their ancestral regulatory sequences often persist and can be repurposed for the transcriptional control of host genes (FIG. 2a).

An early survey of genome-wide binding patterns of transcription factors in human and mouse revealed that a particular family of TEs is often over-represented in the set of binding sites for a given transcription factor[20]. This correlation suggests that the progenitor of a TE family frequently contains and disperses sequences that can attract host transcription factors. As a result, the process of TE amplification spreads large amounts of TFBSs throughout the genome. A seminal study of 26 transcription factors in human and mouse demonstrated that between 2% and 40% of their binding sites were derived from TEs[21]. In human embryonic stem cells (hESCs) and mouse embryonic stem cells (mESCs), ~19% of various TFBSs of pluripotency factors are located in TEs[22,23]. It is worth noting that TEs frequently harbour modules of multiple TFBSs, which may expand their regulatory potential and transcriptional activity to a wide range of cell types, tissues and developmental stages[19,23,25,28–31].

TEs have remarkable *cis*-regulatory activities in stem cells.—New TE insertions must occur in the germline to be vertically transmitted. Thus, one would expect TEs to possess promoters that support their transcription in the germline or its progenitors to successfully propagate. Indeed, the transcriptional activity of TEs is frequently elevated in totipotent and pluripotent embryonic stem cells, and early germ cells, which share many transcriptional features, rely on a partially overlapping set of transcription factors, and have broadly permissive chromatin landscapes that may further facilitate TE activation[32]. As a result, one might predict that recently active TE families would have a propensity for being co-opted as early embryonic CREs since such sequences would be pre-existing within the TEs and readily available upon their insertion. Consistent with this prediction, multiple examples of young TE families having deposited CREs that are active in early embryonic cells have now been documented in humans and mice (FIG. 3). For example, the long terminal repeat 5 (LTR5HS) of the human endogenous retrovirus type-K (HERV-

K) functions as an enhancer upon activation by the pluripotency transcription factors octamer-binding transcription factor 4 (OCT4), Krüppel-like factor 4 (KLF4) and KLF17 (REFS[33,34]). As these elements were inserted after the split of Old World monkeys from hominoids (apes), they might have contributed to hominoid-specific embryonic gene expression patterns[33–35].

Another remarkable example of this phenomenon is the primate-specific LTR7 of HERV-H. As an endogenous retrovirus (ERV), HERV-H copies are flanked by two identical-upon-insertion long terminal repeats (LTRs) that contain promoter sequences that drive transcription of the ERV (FIG. 1a). Members of this family are bound by the pluripotency transcription factors NANOG, OCT4, KLF4 and transcription factor CP2-like 1 (TFCP2L1), and they have been shown to function as enhancers in hESCs[34,36,37] and as promoters of naive stem cell-specific `chimeric and alternative transcripts`[38] (FIG. 3a). Interestingly, different subfamilies of HERV-H LTRs (LTR7Y, LTR7B, etc.) are transcriptionally active at distinct stages of zygote-to-blastocyst development, indicating that differences in the LTR DNA sequence promote precise stage-specific activation[39]. Activation of HERV-H has also been observed in human primordial germ cells, although this activation involves a different subset of HERV-H insertions than those observed in hESCs[40]. As reported in a recent preprint, dissection of the LTR7 family revealed that there are eight distinct subfamilies of LTR7. These subfamilies arose as the result of a complex evolutionary pattern of recombination and duplication events. A particular subfamily that is highly active in hESCs (LTR7up) has a binding site for SRY-box transcription factor 2 (SOX2) and SOX3, which is essential for promoter activity in vitro[41]. Thus, even within a single TE family, one can observe an extensive diversification of *cis*-regulatory activities. This diversification may be driven by the need to colonize new embryonic cell types in order to evade the emergence of stage-specific host repressors[42–44].

Chimeric and alternative transcripts

Transcripts that result from joining by splicing of RNAs that are not part of the same region of origin. They may comprise exons of different genes or combinations of sequences of TE and non-TE origin.

In mice, two families of LTR elements, RLTR9 and RLTR13, are bound by SOX2, OCT4, NANOG, oestrogen-related receptor-β (ESRRB), and KLF4 and have enhancer activity in mESCs[23] (FIG. 3b). Importantly, the sequence motifs recognized by each of these transcription factors were present in the TE ancestral sequences, which may explain how these elements were able to successfully colonize the mouse genome[23]. The developing placenta also appears to be a niche repeatedly targeted by some TEs. In mouse trophoblast stem cells, RLTR13B and RLTR13D5 elements are bound by caudal type homeobox 2 (CDX2), E74-like factor 5 (ELF5), and eomesodermin (EOMES) and make a substantial contribution to the enhancer repertoire of mouse trophoblast stem cells[45,46].

Some TEs also recruit transcription factors to directly infiltrate germ cells. As a result, these elements can deposit CREs that may be co-opted to control sex-specific gene expression. For example, studies in mice spermatogenesis demonstrated that RLTR10 elements act

as enhancers of male germline genes upon binding of A-MYB, a crucial regulator of male meiosis[47,48] (FIG. 3b). In female mice, a mouse transcript family type C (MTC) retrotransposon drives the oocyte-specific expression of a Dicer isoform that is essential for fertility[49].

Altogether, these examples illustrate that young TE families — often LTR elements with embryonic TFBSs in their ancestral sequence — exhibit exquisitely specific patterns of transcription during early development. In developmental biology, the hourglass model posits that the embryonic morphology (and its underlying gene expression) of species belonging to the same phylum (for example, vertebrates) is divergent at the earlier and later steps of embryogenesis whereas, in the mid-embryonic stages, the different embryos have similar morphology, pointing to a common anterior–posterior body plan[50,51]. We speculate that the species-specific features that characterize early embryogenesis reflect the accelerated transcriptional divergence driven by recent waves of TE colonization, which have introduced large numbers of CREs that are active in different subpopulations of totipotent and pluripotent embryonic cells[3,23,45].

TEs contribute to *cis*-regulatory gene networks in somatic cells.—In contrast to early embryos and germ cells, there is no intuitive reason for TE families to possess *cis*-regulatory activity in somatic tissues. From an evolutionary perspective, somatic activity should be selected against since it seemingly offers no advantage for TE propagation but creates an additional process by which new insertions can cause detrimental changes to their host[52]. Despite these theoretical predictions, many reports have now confirmed that certain TEs do in fact readily transpose in somatic cells[53] and some have been co-opted as CREs in somatic tissues[19,31,34,46,54–60] (FIG. 3). We posit that this process can occur through one or more of the following mechanisms: (1) overlap between *cis*-regulatory programmes of stem cells and somatic cells, (2) retroviral hijacking of transcription factors expressed in immune cell types, or (3) gain of somatic regulatory activity through mutations in the TE sequence that occur after genomic insertion.

As outlined above, TE-derived *cis*-regulatory activity in somatic tissues may be explained by the presence of ancestral binding sites for transcription activators that are also expressed in germ cells or embryonic stem cells. This overlap between embryonic and somatic *cis*-regulatory programmes may be explained by the promiscuity of expression of pluripotency transcription factors. Indeed, it is known that pluripotency transcription factors, such as SOX2, OCT4, NANOG, ESRRB and KLF4, are also individually expressed in various differentiated tissues[61]. Thus, binding of these transcription factors to TE families containing their cognate motifs could trigger regulatory activity of these young TEs in somatic cells. For example, upon binding of SOX2, a HERV-K element acts as an enhancer of *PRODH*, a schizophrenia-linked gene, in human neuronal cell lines[62]. Similarly, SINE-VNTR-Alu (SVA) elements, which are active in the early human embryo, are also reactivated in the fetal and adult brain and liver[34,63] (FIG. 3a).

In a mechanism restricted to ERVs, somatic CRE activity may reflect the hijacking of innate immunity transcription factors by their exogenous retroviral ancestors. This is exemplified by MER41, a family of primate-specific LTR elements that are frequently bound by signal

transducer and activator of transcription 1 (STAT1) and act as interferon-inducible enhancers for several innate immunity genes[64–66] (FIG. 3a). Remarkably, MER41 enhancers are often located distal to the genes they activate but are in contact with their target promoters prior to interferon induction[65], a finding consistent with the function of stress-responsive enhancers observed in other systems[65]. Because MER41 is of retroviral origin, it is tempting to speculate that its hijacking of immunity transcription factors is a relic of strategies evolved prior to its endogenization to promote viral replication in immune cells, similar to a strategy adopted by contemporary exogenous viruses such as HIV1 (REFS[64,67]).

Finally, an alternative explanation for TE *cis*-regulatory activity in somatic cells is that it emerged following genomic insertion through mutation of the TE sequence, which led to gain of new TFBSs. Consistent with this model, TE-derived enhancers active in somatic tissues are predominantly observed in members of older TE families, which also tend to be present across more species[68] than the young ERV families that participate in early embryonic regulatory programmes. For instance, in human fetal brain, several members of the ultraconserved element 29 family of DNA transposons (UCON29) are hypomethylated, marked as enhancers by mono-methylation of histone H3 Lys4 (H3K4me1), and located near genes that show fetal brain-specific activation[54]. LTR77-derived CREs are preferentially active in blood, CRE activity of the MER121 family is enriched in mesodermal tissues, and CRE activity of several old short interspersed elements (SINE) families is enriched in fetal and epithelial tissues[54]. Neural development in mammals is partially regulated by a set of lineage-specific enhancers that are enriched in MER130 DNA transposons (an old repeat family that contains key binding sites for neurodevelopmental transcription factors, including neural differentiation factors, neurogenins and neurofibromin 1) and in deeply conserved insertions of the Amniotes-clade SINE1 (AmnSINE1) family of retrotransposons[57,58]. Importantly, all these TE families have been inactive for at least 100 million years and their ancestral (consensus) sequences did not originally contain the lineage-specific TFBSs responsible for the *cis*-regulatory activity observed today. These observations suggest that these older TEs acquired CRE activity by recurrent post-insertional mutations of individual copies rather than by dispersion of built-in TFBS modules such as those observed in younger LTR elements. It seems that the ancestral sequence of these TE families contained ‘proto-motifs’ for specific transcription factors that matured into bona fide binding sites upon mutation. Thus, the proto-motif content and abundance of certain TE families may predispose them to serve as a substrate for evolving some but not other TFBSs[69,70]. Additionally, their nucleotide composition and location in the genome may influence their mutational trajectory, notably due to the presence of hypermutable `methyl-Cpg sites`.

Methyl-CpG sites

The cytosine in 5′-Cpg-3′ dinucleotides (C-phosphate-g) can be methylated to form 5-methylcytosine, which frequently triggers a C-to-T mutation through deamination.

TE-derived promoters drive transcription of chimeric RNAs and protein isoforms.—Besides acting as enhancers that modulate gene expression, TEs can also

introduce functional promoters in new genomic locations, thereby creating new host gene transcripts[71,72] (FIG. 2a). These TE-driven transcripts may encode protein isoforms (for example, truncated) with novel biochemical properties and functions[73] or new patterns of expression[49]. One of the most well-characterized examples of this process is the antisense promoter activity of long interspersed nuclear elements (LINE1), which generates chimeric transcripts with human genes[74–76]. The LTRs of ERVs are also a common source of alternative promoters that drive the production of chimeric transcripts in a tissue-specific or developmental-specific fashion[71,77–79]. For example, a recent study shows that the mouse MT2B2 LTR promoter generates an embryonic-specific RNA isoform of cyclin-dependent kinase 2-associated protein 1, which encodes a truncated protein essential for pre-implantation development[80]. Although the vast majority of LINE1 and LTR promoters are repressed in normal cells, they may be reactivated in disease states — notably in tumours and transformed cells[81]. This reactivation may result not only in expression of their own proteins, which can drive cancer-specific transposition[82], but also in aberrant host gene transcription. Both mechanisms can directly contribute to oncogenesis[83] (reviewed in REF.[84]). Widespread de-repression of latent TE promoters can be experimentally induced with epigenetic drugs such as histone deacetylase or DNA-methyltransferase inhibitors, which may be harnessed for cancer treatment by driving the expression of tumour-specific antigens, or as nucleic acid adjuvants that potentiate immunotherapies[85–88]. Thus, the activity and proper control of TE-derived CREs are integral to cellular homeostasis.

TEs influence 3D chromatin architecture

The relationship between 3D organization of the genome and gene expression has been a subject of intense investigation (reviewed in REFS[89–95]). The development of chromatin conformation capture methods for measuring the contact frequency between distant regions of DNA revealed that, in the interphase nucleus, the genome partitions into `topologically associating domains` (TADs) in the megabase scale[89,96–98]. TADs are proposed to have a key role in specifying CRE function by restricting enhancers to only activate promoters within the same TAD[91,99]. However, the precise role of TADs in gene regulation remains controversial. Deletions of individual TAD boundary elements can result in dysregulation of the expression of adjacent genes, in some cases with clear phenotypic consequences[100–104]. Paradoxically, acute global perturbations of TAD-organizing proteins, such as the cohesin complex and CCCTC-binding factor (CTCF), apparently have a relatively minor effect on transcription despite global loss of `chromatin loop domains`[104].

Topologically associating domain

(TAD). large architectural domains of chromatin demarcated by insulator proteins that generally restrict the space in which three-dimensional DNA contacts and enhancer–promoter interactions are favoured.

Chromatin loop domain

Folding of chromatin that causes two regions of the genome that are separated by (a large) linear space along the DNA to come into close three-dimensional proximity.

The contribution of TEs to 3D genome architecture has just begun to be explored. Several TE families are specifically enriched at TAD boundaries or harbour insulator activity[105,106], and manipulative experiments suggest that some TEs have a direct influence on the folding of chromosomes (FIG. 2b). Notably, HERV-H elements are enriched at TAD boundaries that are transiently established in hESCs prior to their in vitro differentiation, and CRISPR–Cas-mediated deletion of two individual HERV-H elements leads to loss of their cognate boundaries[107]. Interestingly, this boundary function appears to be dependent on the transcription of the elements, which may suggest that they drive the formation of transient TAD boundaries during stages of embryonic development in which HERV-H is specifically expressed[107]. Similarly, in mice, widespread hypomethylation and transcriptional activation of the murine endogenous retrovirus type-L (MERVL) elements at the two-cell stage of embryonic development[77,108] results in the establishment of stage-specific TAD boundaries at these elements (reported in a preprint[109]). It remains to be seen whether TE-derived TAD boundaries established at these early embryonic stages and the spatial genomic remodelling they engender have functional consequences for development.

The zinc finger protein CTCF has been demonstrated to demarcate many TAD boundaries, to mediate chromatin loop formation, and to be generally crucial for proper 3D genome organization[110]. Early studies reported that a large fraction of CTCF binding sites in various mammalian genomes derive from several classes of TEs[20,21]. For example, in humans, CTCF binding sites often derive from primate-specific ERVs whereas, in the mouse genome, B2 SINEs are a major source of murine-specific CTCF binding sites[20,111]. The observations that TEs have dispersed so many lineage-specific CTCF sites in mammalian genomes have posed a conundrum because TAD boundaries are highly conserved between human and mouse and they often correspond to regions that are deeply syntenic across metazoan genomes[97,112]. How are genomes protected from the formation of new TADs introduced by TE-driven CTCF sites and from a widespread remodelling of genome organization? Recent work has provided two solutions to this conundrum. First, TAD boundaries have been identified where one CTCF site introduced by a lineage-specific TE has functionally replaced an adjacent ancestral CTCF site, resulting in CTCF anchor turnover without a major change in 3D genome organization[113]. Another mechanism mitigates the widespread remodelling of TAD boundaries through CTCF sites dispersed by B2 SINEs, which are preferentially bound by the ChAHP complex (abbreviation for CHD4, ADNP and HP1 complex) that recognizes a motif nearly identical to that bound by CTCF but with higher affinity[114]. Thus, ChAHP binding tends to outcompete CTCF binding at B2 SINEs and prevent the establishment of ectopic TAD boundaries at these sites[114].

TEs can also influence host chromatin structure in a CTCF-independent way by acting as insulator elements. In the developing mouse embryo, a B2 SINE ensures proper spatiotemporal activation of the growth hormone locus by blocking the spread of repressive

chromatin. This insulator activity is dependent on the transcription of the B2 element by both RNA polymerase II (Pol II) and Pol III, which is surprising because such tRNA-derived SINEs are not naturally transcribed by Pol II[115]. By leveraging the polymorphic nature of B2 SINEs between lab mouse strains, a recent study identified a polymorphic B2 insertion that constrains the spread of histone H3 Lys9 acetylation (H3K9ac) and DNA hypomethylation in the strain where it is present[116]. This particular element was bound by CTCF; however, ChIP-seq data revealed that B2 SINEs are typically enriched at the boundaries of active-chromatin marks, and that the majority of these elements are not bound by CTCF[116]. The RNA expression levels of B2 SINEs are relatively low in the tissues where these boundary elements were observed, which argues against the model that the expression of these elements is universally required for their boundary function. A parallel situation exists in humans, where mammalian-wide interspersed repeats (MIR) are enriched at CTCF-independent boundaries between active and repressive chromatin features. These SINEs are also bound by Pol III[105], suggesting that, in mammalian cells, one or more components of the Pol III machinery may facilitate insulator function as has been proposed for TFIIIC in yeast[117].

Gene regulation by TE-derived ncRNA

Long non-coding RNAs (lncRNAs) contain a much higher density of TE-derived sequences than protein-coding genes, with TEs comprising ~30% of the total lncRNA sequence in human and mouse[118,119]. As they do for protein-coding genes, TEs provide crucial signals for the biogenesis of lncRNAs. For example, the expression of ~10% of human lncRNAs is driven by LTR-derived promoters[118]. In addition, TE sequences transcribed as part of lncRNA species can affect gene expression through a variety of mechanisms[120,121] (FIG. 2c).

Several TE-derived lncRNAs have been implicated in embryonic development. Notably, some are highly expressed in pluripotent stem cells, where the transcriptome has been reported to be 30% more complex than those of differentiated cells, presumably due to the pronounced transcriptional activation of TEs[122]. Depletions of lncRNA transcripts derived from HERV-H in hESCs and from ERV-K and mammalian apparent LTR retrotransposon (MaLR) elements in mESCs suggest that they are essential for the maintenance of pluripotency in vitro[36,38,122,123]. In another example, the MER41-containing lncRNA BRAF-activated non-protein coding RNA (*BANCR*) stimulates the migration of ESC-derived human and non-human primate cardiomyocytes and cardiac enlargement in a mouse model[124]. The molecular mechanisms by which these ERV-derived lncRNAs modulate development remain murky but may involve a combination of transcriptional[36,38,107] and post-transcriptional[123,125,126] processes.

LINE1-derived RNAs have been reported to have a role in mouse embryonic pre-implantation development[127] and in mESC self-renewal[128]. LINE1 RNA is highly expressed in the early mouse embryo, peaking at the two-cell stage, where it accumulates predominantly in the nucleus. Perturbation of LINE1 transcription at those early stages using designer transcription activator-like effectors (TALEs) fused to either activating or repressive transcription factor domains revealed that the precise temporal pattern of LINE1

transcription is required to avoid developmental arrest[127]. Interestingly, this phenotype cannot be rescued by the addition of exogenous LINE1 RNA in *trans*, which might indicate that it is the nascent LINE1 RNA or the process of LINE1 transcription that is required for development. Despite the dramatic phenotypic consequences of LINE1 perturbation to embryonic development, only modest changes in host gene expression were observed in these experiments[127]. Furthermore, another study reported that LINE1 nuclear RNA is essential to safeguard mESC identity by repressing the two-cell stage gene expression programme[128]. This conclusion was drawn by depleting LINE1-derived nuclear RNA using antisense oligonucleotides, suggesting that stable expression of LINE1 RNA is required for this regulatory activity. Further experimentation is required to explore the potential role of LINE1 expression in development as it remains unclear which LINE1 insertions or RNA species these different perturbation techniques were able to target.

Another example of regulatory activities of a TE-derived ncRNA is provided by the mouse B2 SINE RNAs upon heat shock. The heat shock transcriptional response is characterized by robust induction of a small set of heat-responsive genes alongside modest repression of many other genes[129]. B2 SINE RNAs bind to the stress-responsive genes and repress their transcription in basal conditions but, upon heat-shock, the Polycomb protein enhancer of zeste homologue 2 (EZH2) is recruited and accelerates the degradation of B2 RNA and de-repression of the stress-responsive genes[130]. B2 RNA degradation occurs through the activity of a self-cleaving ribozyme, which is greatly stimulated by EZH2 (REF.[131]). B2 SINE RNA has also been implicated in repressing the transcription of non-induced genes after heat shock[132,133]. Additionally, a recent report revealed that B2 SINE RNA cleavage can also be stimulated by heat shock transcription factor 1 (HSF1; a transcription factor that has a major role in the heat-shock response) in a mouse hippocampal neuronal cell line[134]. These results further strengthen the evidence of a *trans*-regulatory role for B2 SINE RNAs in this type of transcriptional stress response. It is possible that these regulatory functions may extend to other SINE RNAs because Alu transcripts also accumulate during and modulate the heat-shock response in human cells[135] and Alu RNA possesses EZH2-stimulated self-cleaving RNA activity[131].

These examples illustrate how ncRNAs derived from TEs act as regulators of transcription through a variety of mechanisms. In recent years, the number of molecular techniques available for the study of TE-derived ncRNAs has increased exponentially, so we foresee that many new and exciting roles of TE-derived ncRNAs are yet to be discovered.

Transposase-derived transcription factors

While it has long been speculated that the repeat sequences that TEs spread throughout genomes provide a fodder for the dispersion of TFBSs that facilitate the emergence of new gene regulatory networks[12,13], it has more recently come to be appreciated that the proteins encoded by TEs themselves provide complementary pathways to achieve this outcome. Transposase proteins encoded by DNA transposons contain DNA-binding domains that recognize and bind their cognate TE sequences dispersed in the genome. Thus, it is easy to envision how the fusion of a transposase DNA-binding domain to a transcription-regulation domain could form a new transcription factor instantaneously capable of recognizing a

ready-made network of binding sites distributed across the genome by its parent TE family (FIG. 2d). The fact that well-characterized transcription factors, such as paired box (PAX) proteins, possess DNA-binding domains that appear to have originated from transposases provided a hint that this process of transposase capture may be a recurrent theme in the birth of transcription factors (reviewed in REF.[16]). However, the ancient origin of most transposase-derived transcription factors has made it difficult to trace the steps by which these proteins and their *cis*-regulatory network were assembled.

A recent study has shed new light on the prevalence and modalities by which transposases supply protein domains for the emergence of new transcription factors in vertebrate evolution. A survey of ~600 tetrapod (limbed vertebrates) genomes suggests that fusion of transposase domains with host regulatory domains is a recurrent mechanism for the assembly of novel transcription factors during evolution[136]. This study identified 94 independent events across the phylogeny of transposase domains fused to a variety of host domains, with an apparent proclivity for `KRAB domains`. A *mariner*-transposase-*KRAB* fusion gene dubbed *KRABINER*, which evolved in the vespertilionid bat lineage, was further characterized. Reporter assays, loss-of-function and rescue experiments in bat cells show that KRABINER behaves as a canonical sequence-dependent transcription factor that binds thousands of genomic sites, including hundreds of cognate *mariner* transposons, and modulates the transcription of a large set of genes and CREs[136]. Together with previously characterized host-transposase fusion proteins[137–141], these new findings demonstrate that the fusion of host and transposase domains can give rise to a wide variety of proteins with novel architectures and functions, including transcription factors. Because the emergence of new transcription factors is often pivotal to the emergence of major evolutionary novelties (for example, PAX6 in the evolution of eyes[141]), it is tempting to speculate that transposase capture has had a profound impact on phenotypic diversification.

KRAB domains

Repressive transcription factor domain, characterizing KRAB containing zinc-finger repressor proteins.

Collateral effects of TE silencing

Organisms have evolved strategies to tame the activity of TEs[142,143], and there are also a few examples that describe how TEs have evolved mechanisms to temper host defenses[144]. One of the major silencing mechanisms of TEs in mammals is DNA methylation[145,146], which can lead to long-term, heritable transcription silencing (reviewed in REF.[147]). The need for DNA methylation to suppress TEs is underscored by the observation that, when some TEs are demethylated, their enhancer potential is unmasked, leading to aberrant activation of nearby genes[54,148].

A classic case of the interplay between DNA methylation and the activity of TE-derived CREs are the *Agouti* `metastable epialleles`. These elements confer a range of yellow coloured coating to the brown-furred C57BL/6J mice in a manner dependent on the DNA methylation levels of the cryptic promoter that controls the transcription of intracisternal

A-particle (IAP) retrotransposons[149–154]. In mice, IAP retrotransposons with disparate levels of CpG methylation across individuals (that is, with metastable epiallele features) mostly belong to the youngest and most polymorphic IAP subtypes[155,156]. Current data suggest that, at the implantation–gastrulation transition, the developing embryo may be exposed to environmental cues that modulate the activity of methylation maintenance complexes, giving rise to these differential methylation patterns[157]. Following their establishment in early development, these DNA methylation patterns are maintained throughout the organism[157]. Recent work demonstrates that DNA methylation at metastable epialleles is highly dependent on genetic background[158]. Additionally, studies in humans have revealed that metastable epialleles overlap with ERVs and LINE1s, suggesting that these elements contribute to human epigenetic variation[159].

Metastable epialleles

Alleles that are differentially epigenetically regulated and maintained throughout the organism's lifetime and, in some cases, also across generations. They typically arise due to the variable levels of DNA methylation established during early development and can respond to environmental stressors and potentially drive phenotypic variation.

Another process through which TEs are silenced is the targeted deposition of repressive histone modifications. For example, in mESCs, TEs are silenced through tri-methylation of histone H3 Lys9 (H3K9me3) by SET domain bifurcated histone lysine methyltransferase 1 (SETDB1), which is recruited to TEs by KRAB zinc-finger proteins (KZFPs) through interaction with tripartite motif containing 28 (TRIM28; also known as KAP1)[43]. SETDB1 knockout leads to widespread de-repression of class I and class II ERV elements and transcription of chimeric RNAs, suggesting that repression of these elements not only prevents mutagenic transposition but also deleterious *cis*-regulatory effects[160]. An elegant experiment using `trans-chromosomic mESCs` demonstrated that a subset of SVA and LINE1 retrotransposons on the transferred human chromosome were activated in the non-primate nuclear environment because mice lack the transcription repressors ZNF91 and ZNF93 (REF.[42]). ZNF91 and ZNF93 are two KZFPs that have rapidly evolved in the primate lineage to adapt their DNA binding domains to specifically repress these retrotransposons, but the youngest LINE1HS elements have escaped repression through a deletion of the ZNF93 binding site[42]. Interestingly, interplay between TEs and KZFPs has also been described in naive hESCs, human primordial germ cells and, recently, in cancer[34,40,161]. In naive hESCs, accessible chromatin regions marked by H3K27ac and KLF4 binding are enriched in young TEs such as LTR5HS of HERV-K, LTR7 of HERV-H, and SVA elements. Curiously, these TEs, which are upregulated during pre-implantation development, function as enhancers of young KZFPs that, in return, target them for repression[34]. Thus, TEs and KZFPs appear to form complex regulatory loops that modulate the expression of developmental genes in a cell type-specific fashion. Interestingly, many KZFPs are expressed in somatic tissues, where they may mitigate the deleterious *cis*-regulatory effects of the TEs they target. By comparison, lack of KZFP expression in the germline may facilitate the spread of TEs and their fixation in the population. Thus, rather than a defence system against transposition, the KZFP system may actually enable the

genomic accumulation of TEs with strong CREs (such as LTR elements), which increases the likelihood of these elements being subsequently co-opted for host functions.

Trans-chromosomic mESCs

Mouse embryonic stem cell (mesC) clones to which a human chromosome has been transferred, resulting in aneuploid cells in which the effects of a non-primate cellular context on a primate chromosome can be assayed.

An additional H3K9me3-related repression mechanism is the silencing of retrotransposons by the human silencing hub (HUSH) complex, which was first identified in humans as a mediator of `position-effect variegation`[162]. A genome-wide screen of proteins affecting LINE1 retrotransposition in human cells revealed that the ATPase MORC2 and HUSH interfere with the mobilization and mutagenic potential of these elements by promoting the deposition of H3K9me3 on evolutionarily young LINE1 elements that reside in euchromatin[163]. In naive mESCs, TRIM28 and HUSH also co-repress young LINE1s and a subset of ERVs[164]. As many of these LINE1s are located within the introns of active host genes, their silencing by HUSH has a collateral repressive effect on the genes that harbour them, even when the silenced LINE1s are located a long distance from the gene promoters[163,164]. Host-gene silencing is likely mediated by a decrease in Pol II elongation rates caused by the presence of intragenic islands of H3K9me3 heterochromatin[165]. Recently, it has also been reported that HUSH repression of LINE1s safeguards the cells from inflammation triggered by the sensing of LINE1 and ERV RNAs in the cytoplasm by the double-stranded RNA sensors melanoma differentiation-associated protein 5 (MDA5) and retinoic acid-inducible gene I (RIG-1)[166]. These examples reveal that TE silencing not only affects TE activity but can also have collateral effects on the regulation of host-gene transcription (FIG.2e).

Position-effect variegation

Phenomenon that defines the distinct expression levels that a gene or transgene exhibits when located at different positions in the genome, usually in correlation with the activated or repressed status of the neighbouring chromatin.

Phenotypic and evolutionary effects

The complex relationship between genotype and phenotype is challenging to decipher, yet it forms the foundation of our understanding of how genomic variation underlies the diversity of living organisms. In this section, we discuss the phenotypic consequences of gene regulatory changes triggered by TEs in mammals. TE insertions are thought to be generally deleterious in nature, yet TEs have colonized mammalian genomes to an impressive extent. This accumulation is likely driven by the small effective population size of mammalian species, which promotes the fixation of mildly deleterious TE insertions through genetic drift[167]. Although the majority of TEs in mammalian genomes have long

lost the ability to transpose, their accumulation constitutes a vast amount of DNA with pre-existing functionalities, including CREs, which can provide fodder for phenotypic evolution.

TE-driven phenotypic change

Mammalian TE sequences can be co-opted to assemble new protein-coding genes (reviewed in REF.[7]) or contribute in non-coding capacity to the rewiring of gene regulatory networks. Some studies estimate that about 20% of CREs in the human genome may have been co-opted from TEs[168]. The extent to which exaptation of TEs drives phenotypic changes during evolution remains largely unknown, but there are some documented cases of TE-derived regulatory innovations with clear adaptive consequences.

A paradigmatic example is the evolution of the salivary-specific expression of the digestive enzyme amylase, which emerged during hominoid evolution through the insertion of an ERV-derived CRE upstream of the gene *AMY1C* (encoding α-amylase 1C)[169,170]. Another adaptation of mammals driven by a TE-derived CRE is the expression of prolactin in the endometrium, which is required for embryonic implantation[171]. In a striking case of convergent evolution, different mammal species utilize different TEs as endometrial promoters of prolactin: MER39 and MER20 in primates, MER77 in mice, and the LINE L1–2_LA in elephants[172]. Multiple members of the MER20 and MER121 families might also have had important roles in the evolution of pregnancy by dispersing numerous TFBSs for the transcription factors progesterone receptor (PGR) or Fork head box O1 (FOXO1A), thus potentially contributing to endometrial decidualization[173,174]. The placenta is another evolutionary novelty of mammals where TEs appear to have made repeated contributions through *cis*-regulatory innovations (FIG. 3a). Notably, insertion of a primate-specific transposon-like human element 1B (THE1B) controls the expression of corticotropin-releasing hormone (CRH), which modu lates gestational length[175], and another TE controls the expression of placensin, a hormone that promotes placental cell invasiveness[176].

TE-derived CREs that spur phenotypic novelties are not restricted to physiological or reproductive processes but may have also affected anatomical innovations. Enrichment in specific families of ERVs, such as LTR9, has been detected in neural crest enhancers with divergent activity between human and chimp[177]. These evolutionary gains in enhancer activity in neural crest cells have been linked to changes in the TFBS content of the enhancers, notably within a 17-bp sequence motif termed Coordinator. This motif was apparently not present in the progenitor sequence of LTR9 elements; thus, repeated mutations in a Coordinator-like proto-motif might have contributed to the adoption of some of these LTRs for neural crest enhancer function during recent human evolution[177]. Consistent with progressive gain of TEs and cooperative action of transcription factors[91], a Wnt family member 5A (*WNT5A*)-dependent enhancer that regulates mammalian secondary palate development originated from concerted co-option of three distinct TEs (AmnSINE1, X6b_DNA and MER117)[178].

As examples continue to aggregate in the literature, it is becoming increasingly apparent that TE-derived sequences have provided a fertile ground for the emergence of CREs that drove adaptive changes in gene expression during mammalian evolution. Nonetheless, we

lack systematic studies that delete large numbers of TE-derived CREs to test their regulatory activity in animal models or even in cells. The scarcity of loss-of-function experiments in mammalian model organisms such as mice (but see REF.[80] for a recent example), often precludes the demonstration of the direct involvement of TEs in driving organism-level phenotypes. In humans, these experiments introduce additional challenges. For example, many human enhancer regions do not have an orthologous sequence in the mouse[179], and even if the mouse orthologous sequence exists, its spatiotemporal pattern of activity or the strength of it may not be conserved; thus, deletion of the mouse sequence may lead to much smaller gene expression changes than those seen for the human enhancer[180].

Impact of TE insertion polymorphisms

Thus far, all known examples of human TEs with adaptive CRE activity come from insertions that have long been fixed in the genome. However, could `TE insertion polymorphisms` contribute to human phenotypic variation, including to disease susceptibility? In humans, only LINE1, Alu and SVA elements retain the ability to transpose and, as such, they represent the vast majority of TE insertion polymorphisms between and within populations[181,182]. De novo insertions of these elements can disrupt proper gene function leading to highly penetrant phenotypes and monogenic diseases[181,183,184]. There is also growing evidence that TE insertion polymorphisms that segregate in non-coding regions of the human genome can have subtler regulatory effects that may contribute to Mendelian or polygenic diseases or to the emergence of complex traits (FIG. 4). Recent studies suggest that these TE insertion variants can be mapped as *cis*-`expression quantitative trait loci` with substantial effects on gene expression, especially at loci involved in immune response and cognitive function[185–188]. Decrease in chromatin accessibility at or near TE insertion sites seems to underlie gene expression differences associated with most polymorphic TE insertions[188]. For instance, insertion of an SVA into a cell type-specific enhancer leads to a decrease in expression of the *B4GALT1* gene (encoding β−1,4-galactosyltransferase 1), which has been associated by `genome-wide association studies` (GWAS) with susceptibility to autoimmune disease[187]. Another study identified 44 polymorphic Alu insertions in non-coding regions that are in linkage disequilibrium with GWAS single-nucleotide polymorphisms associated with various complex diseases, suggesting that these elements may contribute to the disease phenotypes through *cis*-regulatory effects[189].

TE insertion polymorphism

A TE insertion allele that is not yet fixed but segregating in the population; emerges from transpositions that occur in the germline and may be transmitted to the offspring, thereby increasing TE frequency in the population.

Expression quantitative trait loci

Loci that explain expression variation among individuals in a population.

Genome-wide association studies

(GWAS). a population genetics method of linking genetic variants present within the population with various traits and diseases.

Although no ERV is known to actively transpose in humans, the HERV-K family was recently active (~250,000 years ago) and seeded several insertions that remain unfixed in the human population[190,191]. Analysis of data from the 1000 Genomes and Human Genome Diversity projects revealed associations between HERV-K polymorphisms and human traits and diseases, and notably with neurological and immune afflictions[192]. For example, a polymorphic HERV-K insertion increases the expression of the gene encoding the C4A and C4B complement factors and has been correlated with higher schizophrenia risk[193] (FIG. 4a). Another polymorphic HERV-K insertion has been associated with increased drug abuse due to an upregulation of the addiction-related gene *RASGRF2* (encoding Ras-specific guanine nucleotide releasing factor 2)[194] (FIG. 4b). These findings are concordant with the enhancer function of HERV-K in several cellular contexts[34,35] and beg for further functional experiments to characterize the effects of HERV-K insertion polymorphisms on gene expression variation.

Evolutionary sequence conservation, or purifying selection, is a hallmark of functional constraint. Thus, it can be used as a proxy to quantify the fraction of TEs that have undergone co-option for organismal function. It has been estimated that ~6% of TEs annotated in the human genome harbour non-exonic sequences that have evolved under functional constraint throughout mammalian or primate evolution, suggesting they have assumed some regulatory functions[168]. However, this benchmark is not always appropriate in defining TE co-option or, more broadly, the functionality of non-coding regions. Enhancers are sometimes poorly conserved at the sequence level, and TEs that have given rise to enhancers are often clade specific and, as such, have not had sufficient time to exhibit measurable sequence conservation when closely related species are compared.

Very few cases have been described of human TEs co-opted as CREs that give rise to gene expression changes that lead to adaptive phenotypic changes at the organism level. It is likely that many TE-driven regulatory changes have resulted in subtler but still adaptive effects that are difficult to capture pheno-typically. Notably, redundancy is a common feature of gene-regulatory networks (at the level of both regulatory elements[195] and protein-coding genes[196]). This phenomenon has been noted previously by a study that found that many enhancers have ‘shadow enhancers’ or redundant regulatory elements that may provide regulatory buffering and protect the enhancers against harmful changes[195–197]. TE-driven regulatory changes likely contribute to this adaptive redundancy and, in this case, their deletion would not lead to obvious phenotypic changes or such changes would only reveal themselves in certain, suboptimal environmental conditions[198].

Using TEs to decipher transcription

TEs have several characteristics that enable unique lines of investigation into broader mechanistic aspects of transcription regulation. First, TE sequences from the same family

are essentially identical upon their insertion. The very high genomic copy number (hundreds to thousands) of some TEs provides considerable advantages when investigating their regulatory activity. This repetitive nature has been a boon for the design of large-scale perturbative experiments utilizing sequence-guided catalytic mutant Cas9 (dCas9)-based synthetic transcriptional effectors (TABLE 1) because a large copy-number of elements can be targeted simultaneously using relatively few guide RNAs[34,35,45]. This efficiency allows the interrogation and comparison of the activity of hundreds to thousands of CREs in a single experiment (FIG. 5a). Second, although the different insertions of a specific TE family are identical or highly similar in sequence, they are integrated into distinct chromosomal environments, allowing for probing of the effect of the local chromatin context on CRE function. Furthermore, TE families are not evenly distributed in the genome but show distinct biases for insertion and retention in different genomic locales[1]. For example, in the human genome, Alu elements are enriched in gene-dense regions, whereas LINE1s are depleted from these regions[18]. These patterns enable contrasting the effects of distinct genomic environments on *cis*-regulatory activity. Third, just as the current sequences of the different TE families have permitted the reconstruction of their ancestral sequences[10,18], it is possible to finely assess how mutations in TE-derived regulatory elements that occurred after insertion have modulated their activity (FIG. 5b). This property also enables the examination of how the unique genomic context of otherwise identical regulatory elements has influenced their mutational trajectory towards the gain or loss of CRE activity during evolution (FIG. 5c). Finally, TEs often have exquisitely specific patterns of expression, which enable inferences to be made regarding the role of sequence motifs — and by extension of transcription factors that recognize them — in a given cell type or tissue. Due to these special characteristics, TEs can be thought of as a model system within the genome that can bolster our general understanding of mechanisms governing the control of gene expression.

Consequences of TFBS mutation

TEs often harbour clusters of TFBSs, and their ancestral sequence can be approximated by generating phylogenetic trees or consensus sequences[10,18] (taking into account that a consensus does not necessarily represent the actual ancestral sequence as selective forces can introduce bias in mutational patterns). Accordingly, a particular TE family can be used to measure the effects of mutations in TFBSs on their function as CREs. For example, if an LTR element containing a Pol II promoter and a specific combination of TFBSs is dispersed in many sites throughout the genome, one could investigate, in a highly granular manner, the effect of point mutations in each of these different TFBSs on the transcriptional output of the associated promoter. For example, HERV-K LTR5HS is the youngest ERV in the human genome and, consequently, the sequences of its 697 LTRs are >90% identical[18,35]. Despite this sequence homogeneity, LTR5HS copies display heterogeneous levels of the enhancer-associated modification H3K27ac in NCCIT embryonic carcinoma cells, with approximately 30% of the copies exhibiting high levels of H3K27ac while the remaining fraction shows low or negligible levels of the modification[35]. Likewise, copies of the RLTR13D6 and RTLR9 families in the mouse genome are also highly similar in sequence but exhibit varying levels of H3K27ac in mESCs and have disparate effects on gene expression[45]. These examples highlight the power of using TEs to study gene regulation despite having highly

similar sequences; members of the same family may possess different activities, offering the opportunity to evaluate whether variation in regulatory activity is caused by mutations that led to gain or loss of TFBSs (FIG. 5b) or by local genomic features unique to each TE insertion site. Currently, the presence of suboptimal TFBSs or the vari able spacing between them are being uncovered as key determinants of enhancer function[199]. We speculate that the multi-copy nature of TEs and their variable regu lation will boost the exploration of the *cis*-regulatory grammar in the coming years.

Effects of genomic environment

Another interesting question to ask is whether the genomic context of a TE insertion can influence the element's propensity to evolve regulatory activity. Although most examples of TE-derived TFBSs involve an intact transcription-factor binding motif pre-existing in their ancestral sequence, there are also instances where the TE gained a new TFBS through a mutation introduced after its genomic insertion. For example, a recent study revealed that RSINE1 elements in mouse spread proto-motifs for circadian transcription factors throughout the genome that, in many distinct cases, matured into bona fide TFBSs through CpG deamination[70], which is a common mutation in methylated TEs (reviewed in REF.[200]). A similar mutational process has been observed previously within human Alu elements, which contain proto-motifs for p53, PAX6, and MYC and can generate different combinations of TFBSs through differential CpG deamination[201]. Interestingly, RSINE1 elements inserted nearby pre-existing circadian TFBSs were more likely to acquire circadian TFBSs through CpG deamination in proto-motifs. One possible explanat ion for this pattern is that it is driven by natural selection for mutations that introduce *cis*-regulatory redundancy, an idea that has been explored previously[197]. Epistatic interactions with other TFBSs located outside the TE but near its insertion site may also facilitate the acquisition of new TFBSs within the TE, a mechanism dubbed 'epistatic capture'[69]. Using TEs to measure the effects of genomic context on the evolutionary trajectory of regulatory elements has the potential to unlock many of the secrets of the non-coding genome (FIG. 5c).

How do distinct local epigenomic features or the 3D structure of chromatin shape the regulatory activity of TEs? Several reports modelling the features that predict regulatory potential of enhancer–promoter pairs have recently been published[202,203]. The strength of the CRE, the distance to the cognate promoters, the underlying TFBSs and the 3D topology of chromatin have emerged as the main features influencing a CRE's regulatory potential. Additionally, 4Tran[65] (a technique akin to capture Hi-C[204] but centred around transposons) has revealed that TEs interact with genomic regions that are located in the same compartment and the same TAD, which is consistent with the general behaviour of enhancer–promoter connections[65]. Further study of TE-derived CREs leveraging the experimental and analytical advantages described above may solidify our understanding of how 3D chromatin structure influences transcription regulation.

Tissue-specific transcription factors

Because TEs contain various TFBSs and often have highly specific patterns of expression across different cell types[31], knowing which TEs are expressed in a given cell type might provide a way to infer which transcription factors may be important for transcription

activation in that environment. Rapid diversification of the *cis*-regulatory activity of a TE family — driven by the need to colonize new cell lineages — can cause a group of highly related TE subfamilies to exhibit highly specific activation profiles across different cell types[39] and developmental cell lineages[41]. Careful examination of the sequence motif content of these highly similar subfamilies may facilitate the identification of previously unrecognized transcription factors governing cell type identity[41]. This analysis could even be performed with more granularity by cross-referencing the TFBS content of all expressed TEs for common occurrence of TFBSs and might reveal different transcription factor specificity in distinct yet related cell types. By using TEs as a proxy for transcription factor affinity across cell lineages, we believe that the degree of overlap between developmental programmes in terms of the combination of transcription factors required to generate a particular cell type could also be examined in detail. The advent of single-cell methodologies is likely to greatly enhance this type of analyses. Indeed, recent forays into single-cell RNA sequencing analysis of TE expression have revealed that certain TE families behave as precise markers of cell lineages during vertebrate development[41,205–207].

Conclusions and future perspective

Technological advances in genomics and systems biology over the past few years are ushering in a golden era of TE research. Methods such as CRISPR–Cas9 have empowered the systematic dissection of TE-driven *cis*-regulatory evolution in mammalian cells. These experiments have revealed that, although many TEs bear the biochemical hallmarks of CREs, only a subset of these elements significantly contribute to gene regulation in their native chromosomal context. As the number of functionally validated TE-derived CREs continues to grow, it will soon become possible to explain their potential redundancies, synergies and interactions with each other and with non-TE regulatory elements. Empirical demonstration of TE co-option for adaptive *cis*-regulatory changes remains challenging as regulatory changes driven by TEs may result in subtle phenotypes. However, methodological improvements in TE-centric analyses and the ease and precision with which genomes can now be functionally profiled and modified have elevated TEs from components that were once recalcitrant to genomic analyses to an emerging platform for understanding general principles of gene regulation. Unique features of TEs offer powerful tools to decipher the lexicon of mammalian transcription regulation and probe its evolutionary dynamics with unprecedented depth. These advances open exciting avenues for improving our understanding of gene regulation in health, disease and evolution.

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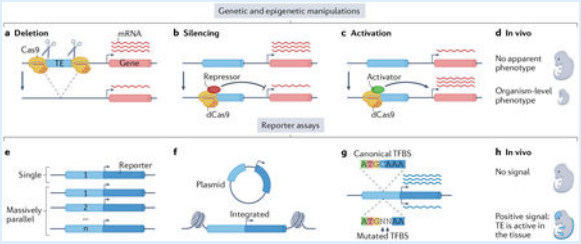
Box 1 |

Functional assessment of transposon-derived *cis*-regulatory elements

Genetic manipulation methods (see the figure, parts **a**–**d**) and modalities of reporter assays (see the figure, parts **e**–**h**) permit the functional validation of *cis*-regulatory elements (CREs). the transposable element (TE)-derived CRE of interest can be removed from the genome using CRISPR–Cas9 by designing pairs of guide RNAs flanking its sequence. Loss of enhancer activity is evaluated by measuring RNA expression of the putative CRE target gene (see the figure, part **a**). the TE-derived CRE or TE family of interest can be epigenetically repressed by a repressor protein domain (for example, KRAB) fused to a catalytic mutant Cas9 (dCas9). A single guide RNA or multiple guide RNAs can be designed to target the TE-derived CREs. Loss of enhancer activity is evaluated by measuring RNA expression of the putative CRE target genes (see the figure, part **b**). The TE-derived CRE or TE family can be epigenetically activated by an activator protein domain (for example, VPR or p300) fused to dCas9. increase of enhancer activity is evaluated by measuring the RNA expression of the target genes (see the figure, part **c**). When performing the experiment in vivo, the manipulation may or may not result in an organism-level phenotype (see the figure, part **d**). TE sequences can be tested for their functionality as a CRE one by one or in a massively parallel manner using methods like STARR-seq[224] (see the figure, part **e**). reporter assays can be carried out with the sequences cloned into an episomal plasmid or integrated into the genome. the latter provides a chromatin environment that might be required for obtaining a reporter signal (see the figure, part **f**). To test the necessity of a transcription factor to activate a TE-derived CRE, the reporter assay can include sequences with mutant versions of the transcription factor binding sites (TFBSs). if the analysed transcription factor is involved in gene activation, the reporter activity should decrease with the introduction of the mutations (see the figure, part **g**). when performing the reporter assay in vivo, the model organism can display a positive signal or no signal (see the figure, part **h**).



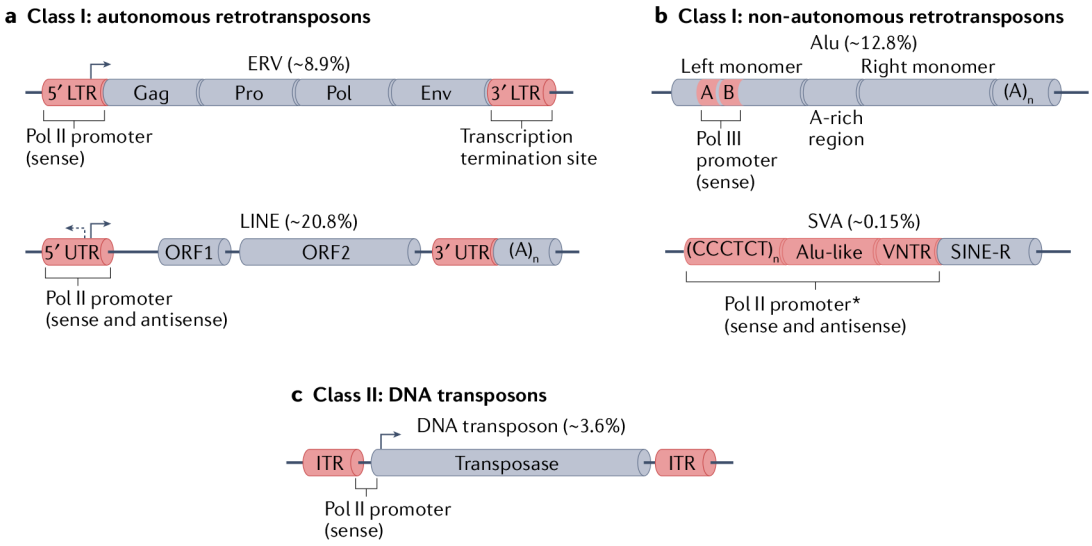


Fig. 1 |. Major types of TEs in mammalian genomes.

Transposable elements (TEs) are divided into Class I and Class II depending on their transposition mechanism[1]. Class I elements are called retrotransposons because they use RNA as an intermediate that is reverse transcribed into DNA and integrated in the genome (not shown). **a** | Autonomous retrotransposons encode all the required proteins for their retrotransposition. Endogenous retroviruses (ERVs) consist of two long terminal repeats (LTRs) flanking the open reading frames (ORFs) that encode the viral proteins. During evolution, ERVs are often reduced to a single LTR, or 'solo LTR', which renders them incapable of retrotransposition. Long interspersed nuclear elements (LINEs), such as L1, contain two ORFs that encode proteins required for their retrotransposition, which are flanked by untranslated regions (UTRs). At the 3′ end, they possess an adenines tail of variable length. **b** | Non-autonomous retrotransposons comprise those TEs that require the machinery encoded by autonomous retrotransposons to be mobilized (the TEs depicted here use the L1 machinery). Alu elements are primate-specific short interspersed nuclear elements (SINEs), and their structure consists of two monomers derived from the 7SL non-coding RNA, flanking an adenine (A)-rich region. At the 3′ end, they possess an adenines tail of variable length. On the left monomer, boxes A and B indicate a bipartite promoter for RNA polymerase III (Pol III). SINE-VNTR-Alu (SVA) elements are hominoid-specific composite non-autonomous retrotransposons comprised of a 5′ region of a variable number of repeats of the hexamer CCCTCT, followed by an Alu-like region, a variable number of tandem repeats (VNTR), and a 3′ 'SINE-R' region derived from the LTR of human endogenous retrovirus K. **c** | Class II DNA transposons encode a transposase that is required for their excision and insertion through a 'cut-and-paste' mechanism. The transposase ORF is flanked by two inverted terminal repeats (ITRs). Asterisk indicates that the precise localization of the *cis*-regulatory feature within the delimited region is unknown. Numbers in brackets indicate the percentage of the human genome (chm13-v1.0 (REF.[17])) comprised by the specific subfamily. env, envelope; pol, reverse-transcriptase; pro, protease.

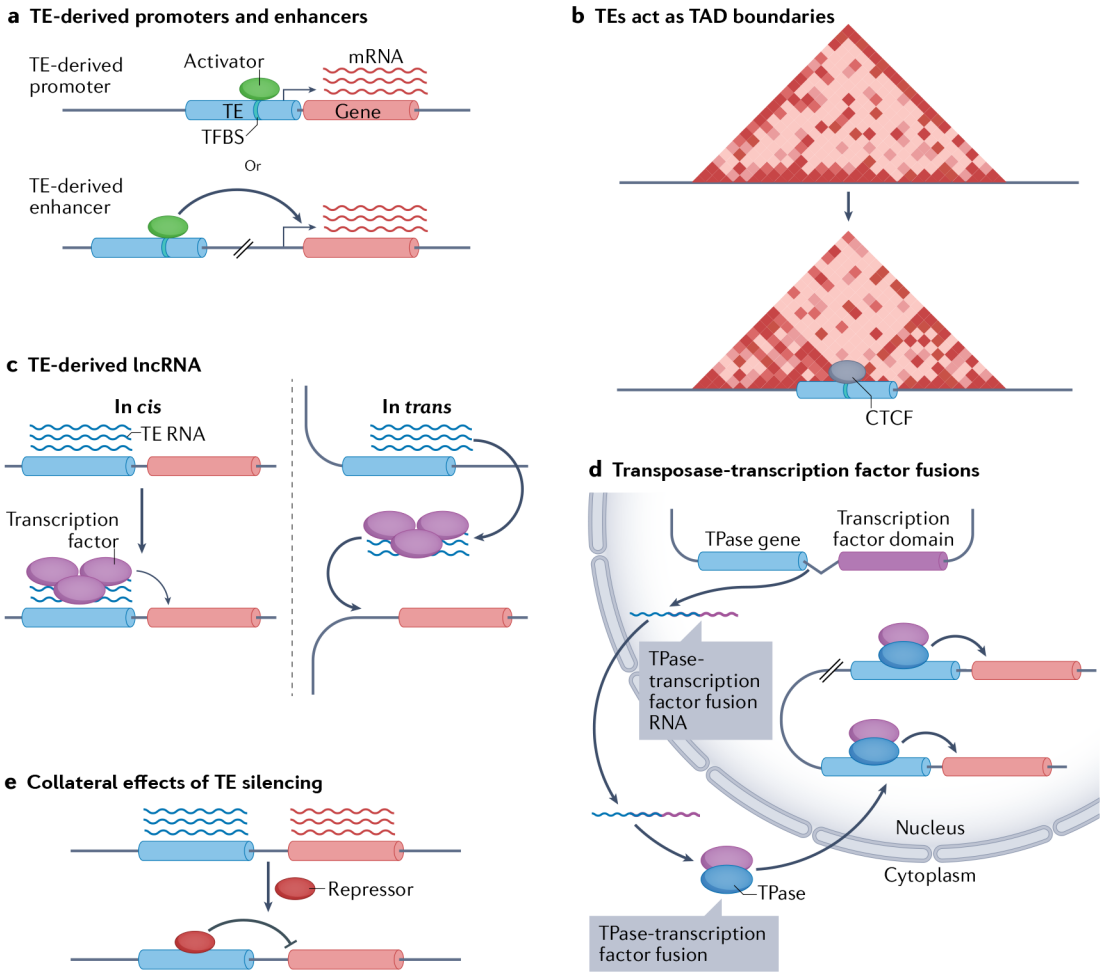


Fig. 2 |. Overview of mechanisms by which TEs influence host transcription regulation.

a | Transposable elements (TEs) can introduce new enhancers or promoters of cellular genes. A TE is depicted providing a transcription factor binding site (TFBS), which influences the transcription of the gene. **b** | TEs modulate 3D chromatin structure. A TE is depicted providing a TFBS for CCCTC-binding factor (CTCF), thereby demarcating a new boundary between two topologically associating domains (TADs; represented as dark-red triangles in a Hi-C map). **c** | TEs give rise to novel nuclear long non-coding RNAs (lncRNAs). A TE-derived non-coding RNA serves as a scaffold for a transcription factor that modulates the expression of a nearby gene in *cis* or of another gene in *trans*. **d** | TEs generate new transcription factors by fusion of DNA-binding domains of their transposase (TPase) with host transcription factor domains. A transposase-transcription factor fusion is depicted modulating two genes as a result of its binding to cognate TE sequences in the genome. **e** | Collateral effects of TE silencing on host gene transcription. A TE is bound by a host repressive transcription factor, thereby causing the silencing of nearby gene.

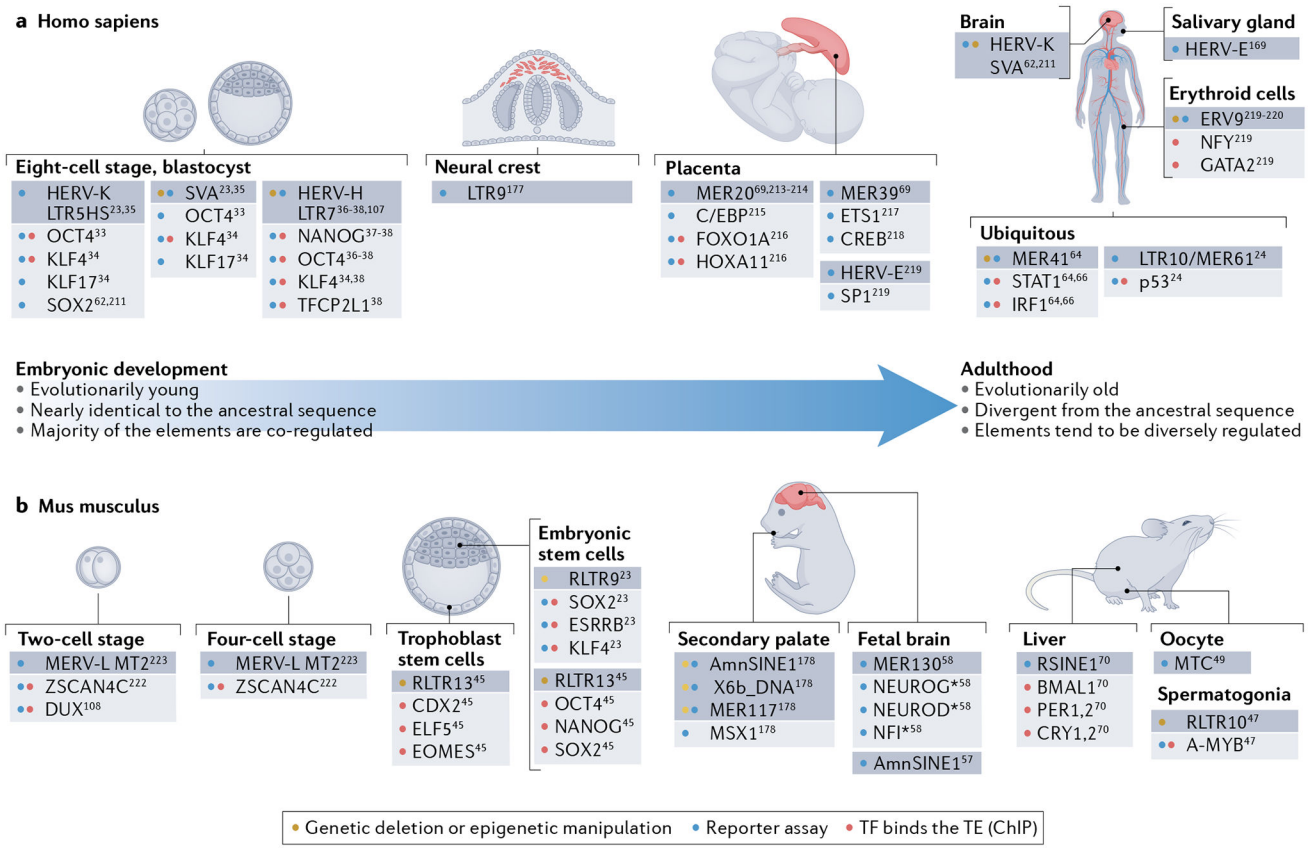


Fig. 3 |. Examples of families of TEs in humans and mice that may have *cis*-regulatory functions based on the binding of specific TFs or on functional experiments.

a | During human embryonic development and in human differentiated tissues[24,33–38,62,64,66,69,107,169,177,211,213–221]. **b** | During mouse embryonic development and in mouse differentiated tissues[23,24,45,47,49,57,58,70,108,178,222,223]. The figure shows how the transposable elements (TEs; dark grey boxes) that exhibit *cis*-regulatory activity at different stages of the life of an organism (namely humans and mice) differ in their characteristics. TEs that are active during early embryonic development belong to evolutionarily young families, whose sequences are nearly identical to their ancestral TE sequence. As a consequence, these elements share transcription factor (TF) binding sites and are often co-regulated. As development proceeds, the TEs that become active belong to evolutionarily older families and their DNA sequences frequently diverge from the ancestral sequence. In contrast to the co-regulation of the young TEs, only specific copies of these older TEs become active in the different tissues. Asterisk indicates that the specific member of the family of TFs that activates the reporter was not specified. Only the *cis*-regulatory activity of TEs and TFs demonstrated by genetic manipulation, reporter assay or chromatin immunoprecipitation (ChIP), is depicted. CDX2, caudal type homeobox 2; ELF5, E74-like factor 5; EOMES, eomesodermin; FOXO1A, Forkhead box O1; HERV-K: human endogenous retrovirus type K; KLF4, Krüppel-like factor 4; LTR, long terminal repeat; LTR5HS, long terminal repeat 5 human specific; MER41, medium reiterated sequence 41; MTC, mouse transcript family type C; OCT4, octamer-binding transcription factor 4; SOX2,

SRY-box transcription factor 2; SVA, SINE-VNTR-Alu; TFCP2L1, transcription factor CP2-like 1.

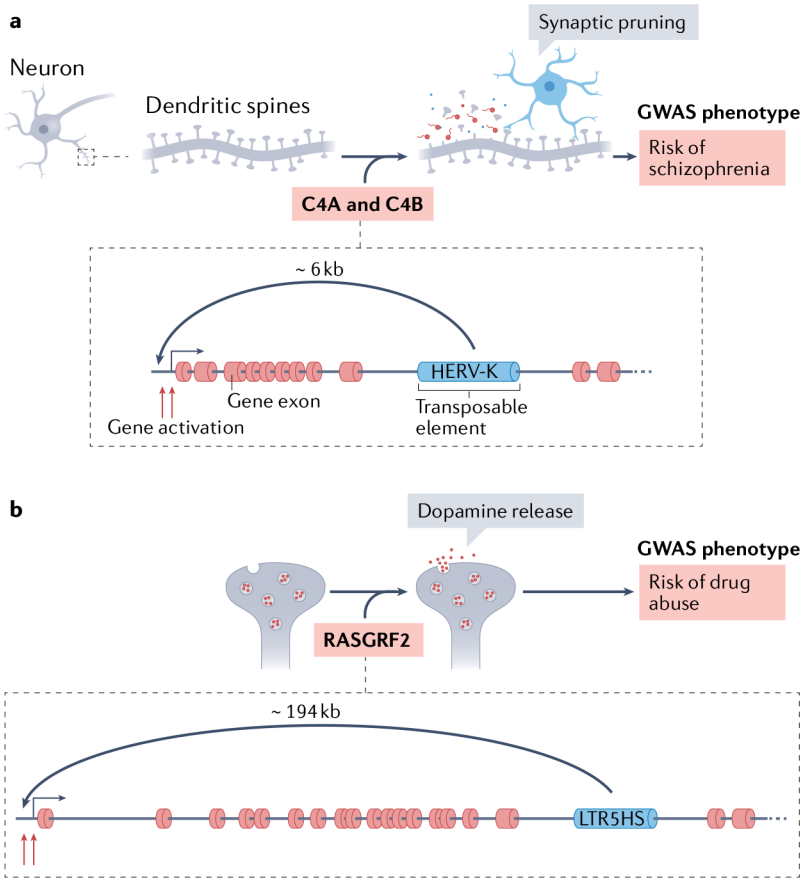


Fig. 4 |. Transposable element insertional polymorphisms that drive *cis*-regulatory changes and their phenotypic association in GWAS.

a | In neurons, a human endogenous retrovirus type-K (HERV-K) polymorphic insertion located between exons 9 and 10 of the gene encoding the C4A and C4B complement components promotes its transcriptional upregulation. The complement component signalling pathway is associated with synaptic pruning, and the HERV-K-containing C4A and C4B gene variants have been associated with schizophrenia[193]. **b** | Also in neurons, a sole long terminal repeat (LTR5HS) of HERV-K located at the *RASGRF2* gene (encoding Ras-specific guanine nucleotide releasing factor 2) ~194 kb away from the promoter, leads to an increase in *RASGRF2* expression. RASGRF2 is required for dopamine release, and this LTR5HS polymorphism has been associated with drug abuse[194]. GWAS, genome-wide association studies.

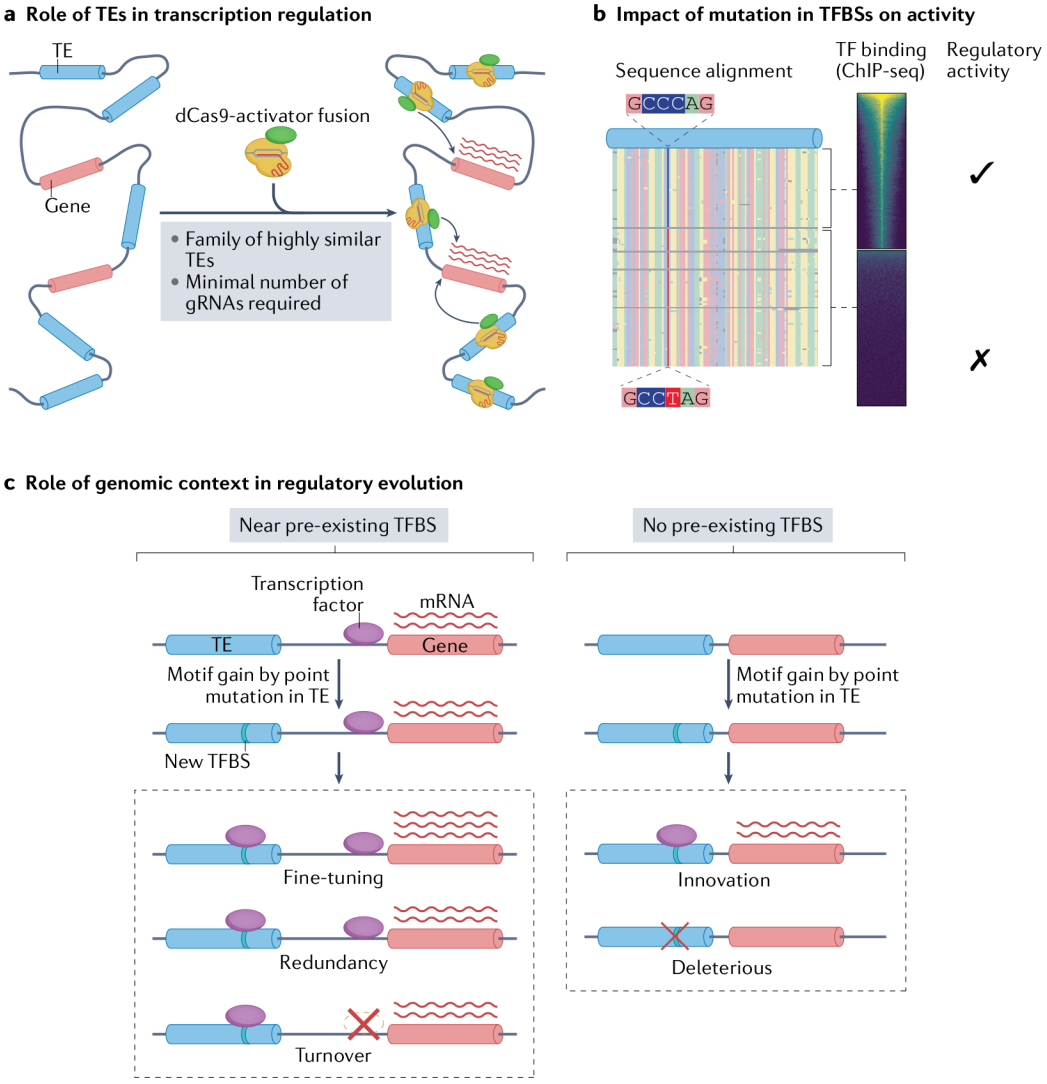


Fig. 5 |. Use of TEs as a model system for studying transcription regulation.

a | The dispersed, repetitive nature of transposable elements (TEs) enables large-scale sequence-specific transcriptional alterations using catalytically dead Cas9 (dCas9) fused to a transcription factor (domain). In the case of dCas9 fusion to a transcription activator, a minimal number of guide RNAs (gRNAs) complementary to a highly repetitive TE family can enable simultaneous transcriptional perturbation of many individual TE loci. **b** | The repetitive nature of TE families allows discrimination between the effects of subtle changes in sequence on transcription factor binding and regulatory activity. **c** | TEs enable the study of how the genomic context of a regulatory element influences its activity and evolution. A TE that inserted near a pre-existing transcription factor binding site (TFBS) gains an additional TFBS for the same transcription factor by point mutation. This process can result in either *cis*-regulatory fine-tuning, owing to the addition of a second binding site with similar activity; *cis*-regulatory redundancy, which can buffer against harmful regulatory changes introduced by future mutations; or *cis*-regulatory turnover, where introduction of the second TFBS relaxes selection on the pre-existing TFBS, which then decays by mutation

and leaves the new nearby TE-derived TFBS in its place. A TE that gains a TFBS with no pre-existing TFBS nearby has two outcomes: the introduction of a *cis*-regulatory innovation, where new activity arises de novo, or the activity is deleterious and is purged by selection. ChIP, chromatin immunoprecipitation.

Table 1 |

Genome editing-driven advances in the study of TEs

Technique	Brief description	Adaptations for TEs	Examples of key findings
Targeting individual TEs by nucleases: TALEs and CRISPR–Cas9	Highly versatile genome-editing technologies based on the generation of double-strand breaks at a desired genomic locus for the introduction of genetic modifications[8]	Target the unique flanking regions of the TE sequence to limit off target effects on other TEs of the same family	An MTC retrotransposon drives the expression of an oocyte-specific Dicer isoform[49]; LINE1 transcription is required for mouse embryo pre-implantation[127]; MER41 elements have been co-opted as innate immunity-responsive enhancers[64]; mouse liver LTR and LINE1 elements are required for proper gene function[208]; the regulatory capacity of ERVs is buffered by HSP90 (REF[209]); a HERV-K polymorphism regulates *RASGRF2*, a gene-related to dopaminergic activity[194]; specific insertions of the RLTR13 and RLTR9 subfamilies of TEs function as enhancers in mESCs and mTSCs[45]; HERV-H demarcates TADs in hESCs[107]
Large-scale TE perturbations with CRISPRi or CRISPRa	Variation of the CRISPR–Cas9 technology that uses dCas9 fused to transcription activators or repressors to modify the functional status of a target region	The guide RNA must bind to a sequence that is common to all copies of the targeted TE subfamily; successful perturbation might require the combined action of multiple guide RNAs and molecules of dCas9-effector at the target region	LTR5HS insertions function as ape-specific early embryonic enhancers[35]; the RLTR13D6 TEs minimally contribute to gene regulation despite containing histone modifications characteristic of enhancers[45]; LTR5HS and SVA TEs function as enhancers in naive hESCs[34]; the HERV-K envelope protein promotes neurodegeneration[210]; ZNF417 and ZNF587 repress LTR5HS and SVA elements in neurons[211]; RLTR10 elements function as enhancers during mouse spermatogenesis[47]; HERV-K activation impairs cortical neuron differentiation[212]

CRISPRa, CRISPR activation; CRISPRi, CRISPR inhibition; dCas9, catalytic mutant of Cas9; ERVs, endogenous retroviruses; HERV-K: human endogenous retrovirus type K; hESCs, human embryonic stem cells; HSP90, heat-shock protein 90; LINE1, long interspersed nuclear element 1; LTR, long terminal repeat; LTR5HS, long terminal repeat 5 of HERV-K; MER41, medium reiterated sequence 41; mESCs, mouse embryonic stem cells; MTC, mouse transcript family type C; mTSCs, mouse trophoblast stem cells; SVA, SINE-VNTR-Alu; TAD, topologically associating domain; TALEs, transcriptional activator-like effectors; TEs, transposable elements.