Trends in Genetics

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Review

# Transposable Elements: A Common Feature of Neurodevelopmental and Neurodegenerative Disorders

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**The etiology of most neurological disorders is poorly understood and current treatments are largely ineffective. New ideas and concepts are therefore vitally important for future research in this area. This review explores the concept that dysregulation of transposable elements (TEs) contributes to the appearance and pathology of neurodevelopmental and neurodegenerative disorders. Despite TEs making up at least half of the human genome, they are vastly understudied in relation to brain disorders. However, recent advances in sequencing technologies and gene editing approaches are now starting to unravel the pathological role of TEs. Aberrant activation of TEs has been found in many neurological disorders; the resulting pathogenic effects, which include alterations of gene expression, neuroinflammation, and direct neurotoxicity, are starting to be resolved. An increased understanding of the relationship between TEs and pathological processes in the brain improves the potential for novel diagnostics and interventions for brain disorders.**

## Highlights

Transposable elements (TEs) make up almost half of our genome but are mostly silenced via different epigenetic mechanisms such as histone modifications and DNA methylation.

The loss of effective silencing of TEs can lead to their activation, which in turn can lead to mutagenic and gene regulatory consequences, as well as induction of the interferon defense pathway.

The field of TE research has so far been hampered by technological limitations with recent advances in sequencing techniques greatly increasing our understanding of TE activation in various disease mechanisms.

Aberrant TE activation has been reported in both neurodevelopmental and neurodegenerative disorders, making it an interesting common denominator, which opens up possibilities for alternative diagnostic and treatment strategies.

## Neurological Disorders and Transposable Elements (TEs)

Disorders affecting the central nervous system (CNS), such as psychiatric and neurodegenerative disorders, are now the leading cause of the global disease burden [1]. The societal impact of these disorders is likely to substantially increase in the near future as the aging population continues to expand [2]. While the etiology and disease mechanism differ between various brain disorders, in most cases the cause of the disorder remains poorly understood and there are currently no effective treatments. Thus, new perspectives for disease understanding and therapeutic intervention are urgently needed. Here we propose that aberrant activation of TEs, resulting from epigenetic dysregulation during development and aging, contributes to the pathophysiology of human brain disorders.

TEs are mobile genetic elements that have colonized the genome throughout evolution and make up at least 50% of the human genome [3,4]. TEs are classified into **retrotransposons** (see Glossary) (class I) and **DNA transposons** (class II) [5] (Figure 1, Key Figure). While DNA transposons are active in bacteria, archaea, and many eukaryotes, they have become inactive in most mammals, including humans [6]. In this review, we will focus on retrotransposons, so-called due to their **transposition** via a retrotranscribed RNA intermediary. Retrotransposons are subdivided into long-terminal repeat (LTR) elements and non-LTR elements. LTR elements contribute to around 8% of the human genome, but all appear to have lost their ability to retrotranspose. The non-LTRs are the most common TEs in the human genome, where one subtype in particular, the long interspersed nuclear elements (LINEs), make up nearly 20% of the genome. We carry more than 500 000 individual LINE-1 element copies in our genome, with the majority being ancient degenerated copies unable to retrotranspose [4,7]. However, 80–100 LINE-1 elements in the human genome can still be active [8–10] and provide nonautonomous

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## Key Figure

### Transposable Elements (TEs) in the Human Genome

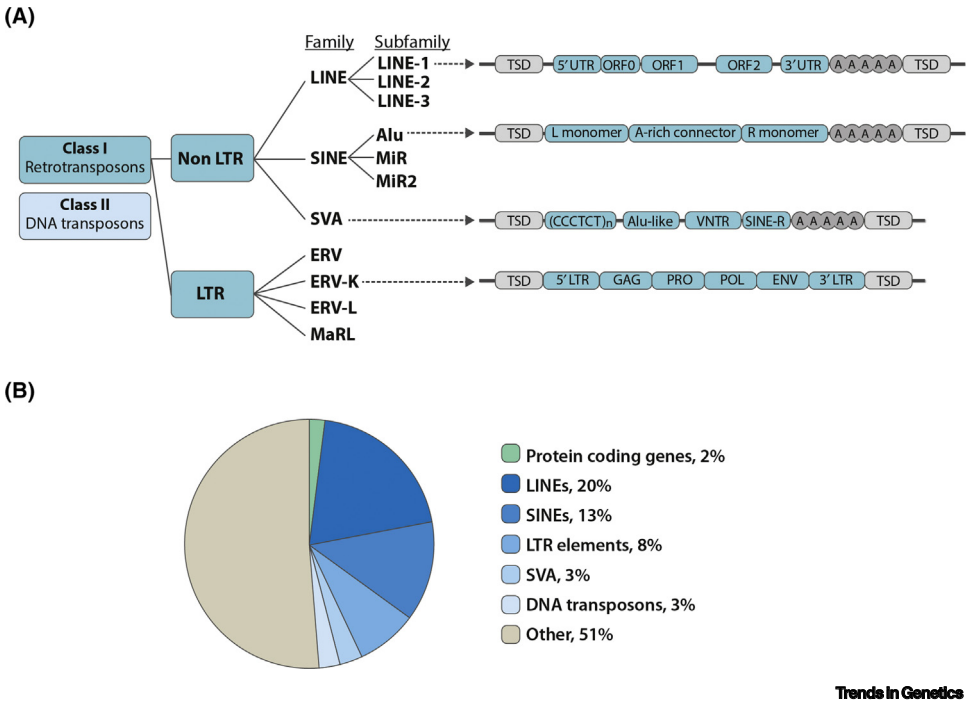


Figure 1. (A) TE classification and general structure. Full-length long interspersed nuclear element (LINE)-1 elements contain two open reading frames, making them the only active non-long-terminal repeat (non-LTR) autonomous family in humans: ORF1 encodes for an RNA-binding protein and ORF2 for a protein with reverse transcription activities. In addition, LINE-1s encode a recently discovered ORF0 that is primate specific and has a sense and antisense 5′ untranslated region (UTR) promoter. Alu elements enclose two monomers divided by an A-rich connector as 5′ UTR and a poly-A tail in the 3′ UTR. Additionally, they contain two promoters for RNA polymerase III on their 5′ region. Sine-VNTR-Alu (SVA) consists of CCCT CT repeats, an Alu-like domain, a VNTR complex, a SINE-R domain derived from the 3′ end of a HERV-K10 *env* gene, and a poly-A tail. Full-length ERV-Ks, the rest of the autonomous LTR group, contain two LTRs divided by the viral genes *gag*, *pro*, *pol*, and *env*. (B) The human genome consists of at least 47% TEs, while protein coding genes only make up 2%. The rest is composed of introns, miscellaneous unique sequences, heterochromatin, and segmental duplications. Abbreviation: TSD, target site duplication.

support for the other classes of TEs that are also currently active in humans, the Alu and Sine-**VNTR**-Alu (SVA) elements. This ability to randomly insert new copies of themselves into the genome poses an obvious mutagenic threat: insertion into promoters or exons will likely lead to disruption of gene expression or function [11].

In this review we will discuss the cellular machinery that normally represses TEs in the human brain, with a focus on DNA and histone methylation. We will discuss the evidence that indicates that these pathways are dysregulated in neurological disorders and the potential pathological consequences of **TE activation**, highlighting current clinical evidence. We will discuss TE activation as a possible common pathological mechanism between seemingly unrelated neurological disorders. Finally, we will assess the particular challenges of TE analysis and how recent methodological development has greatly improved TE-related research and will continue to do so.

## Glossary

**CRISPR:** clustered regulatory interspaced palindromic repeats, an RNA-guided gene editing tool.
**DNA transposons:** a type of TE that can cut itself out of the DNA and then insert itself into another position in the DNA.
**Genomic mosaicism:** when the DNA sequence is not the same in all cells.
**Germline transposition:** a transposition event occurring in a germline cell; creating changes in cells of the individual that will be passed on to the next generation.
**H3K9me3:** epigenetic modification, where the ninth lysine of the histone H3 has been trimethylated, involved in heterochromatin and transcriptional silencing.
**Heterochromatin:** a more packed/dense form of chromatin, characterized by the presence of DNA methylation and various repressive histone modifications.
**Induced neurons:** neurons that have been reprogrammed into neurons from another cell type, often via direct reprogramming (i.e., without an induced pluripotent stem cell intermediate), thus retaining the age-dependent epigenetic landscape vital for studying age-related disorders such as neurodegenerative diseases.
**Innate immune system:** evolutionarily conserved part of our immune system that serves as the first line of defense against pathogens such as viruses and bacteria. Has the potential to become activated in the presence of TE-derived nucleic acids and/or peptides.
**Mutagenic insertion:** insertion of a TE into an exon or promoter of a gene, causing disruption of gene expression.
**Neural progenitor cells (NPCs):** a more restricted type of stem or progenitor cell that gives rise to the cells of the neural lineage (neurons, astrocytes, and oligodendrocytes).
**Noncoding RNA:** RNA that will not be translated into protein. Examples include microRNAs (miRNA, 18–22 bp) and long noncoding RNAs (lncRNA, >200 bp).
**Organoids:** *in vitro* 3D cultures that self-assemble into structures resembling the organ of interest.
**Pluripotent stem cells (PSCs):** cells grown *in vitro* corresponding to the inner cell mass stage of early human development. Thus, these cells can self-renew and give rise to all the different cell types in the body.

## TEs as Pathological Agents

It is becoming increasingly clear that TEs may play a role in human disease. As mentioned earlier, a few TEs in the human genome can still retrotranspose (i.e., move and amplify through a copy-and-paste mechanism). It is currently estimated that more than one in every 20 births results in a new **germline transposition** event, with Alu insertions being the most common (one in 20 births), followed by LINE-1 and SVA insertions (one in every 100–200 births) [12–14]. This activity has resulted in a large degree of TE **polymorphism** within the human population (Box 1) and these polymorphic alleles may, in some instances, cause detrimental effects [11]. Furthermore, **somatic transposition** events can be mutagenic and result in pathological consequences, in particular those occurring during early development [15–19].

In addition to the potential **mutagenic insertion** into the genome upon retrotransposition, the actual transcription of TEs (**TE transcription**) may have negative consequences. The TE-derived cytosolic nucleic acids can cause activation of the innate immune response [20] (Box 2) as well as serving as a source of regulatory **noncoding RNA**s [21–23]. The TE transcripts can also be translated into **TE-derived peptides**, which have been shown to be cytotoxic [24].

Finally, TEs are now also considered to be important gene regulatory elements. They can be binding sites for gene regulatory factors, thus acting as an alternative promoter, enhancer, or other regulatory

**Polymorphism:** variation in DNA sequences between individuals (germline) and between cells in an individual (somatic).
**Retrotransposon:** a type of TE that requires a reverse transcriptase and an RNA intermediate to transpose.
**Somatic transposition:** a transposition event occurring in a somatic cell, creating mosaicisms within the individual.
**TE activation:** the ensuing effects of TEs no longer being repressed. Can be mutagenic, gene regulatory, and/or immune response inductive.
**TE-derived transcripts and peptides:** the transcription of a TE, or a TE being mistakenly transcribed with a gene. If the TE is translated, it results in a TE-derived peptide.
**TE transcription:** when parts of the TEs are transcribed and this results in mRNAs and/or noncoding RNAs.
**Transposition:** the act of inserting a DNA sequence from one place in the genome into another. It could be DNA transposition (a cut-paste mechanism) or retrotransposition (a copy-paste mechanism with an RNA intermediate).
**VNTR:** variable-number tandem repeat; a DNA sequence repeated contiguously that varies in length.

### Box 1. Transposable Elements (TEs) Have the Potential to Influence Human Diversity through the Presence of Polymorphic Alleles

A polymorphic TE (polyTE) is a newly integrated TE that is not fixed in the human population. Polymorphism can occur both in germline and somatic cells. When the retrotransposition event occurs in the germline, the polyTE is passed on to the next generation. If the new insertion is harmless or beneficial for the host genome, it might increase its frequency in the population over time and perhaps become fixed.

In contrast, if the event happens in a somatic cell it creates mosaicism and the effect is limited to the individual and not inherited. These types of insertions have been reported in several different types of tumors and might therefore play a role in the pathology of these diseases. However, mosaicism is also found in normal postmortem brain tissue and might therefore contribute to the functional diversity of a cell population.

The number of polyTEs present in the human population has been difficult to estimate, since reference genomes are not representative. However, newly developed long-read sequencing techniques (Pacific Biosciences, PacBio) have made reference-free genome assemblies much more accurate, increasing the sensitivity to subtle variations among the population. As a result, recent studies have estimated that there are more than 400 million polyTEs present in the human population and that any two human haploid genomes differ by around a thousand polyTEs [103,104].

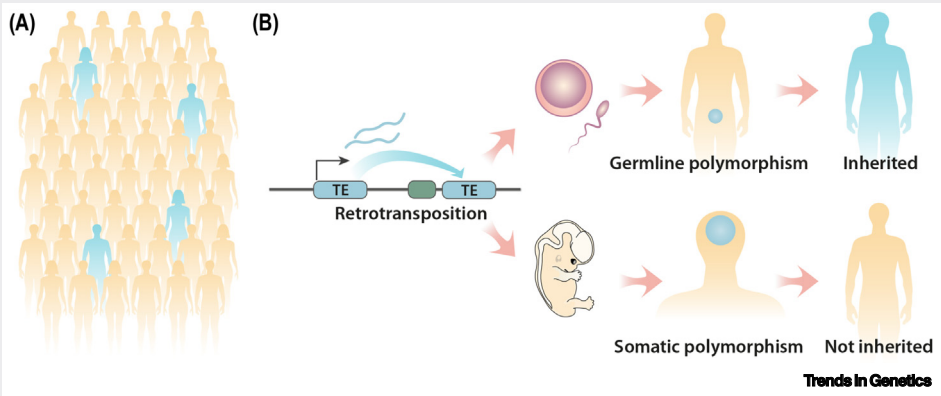


Figure I. Polymorphic TEs. (A) A polymorphic transposable element (polyTE) is a newly integrated TE that is not fixed in the human population. (B) Polymorphism can occur both in germline and somatic cells.

elements (reviewed in [25,26]). While dysregulation of such networks has the potential to contribute to pathological mechanisms, there is also evidence suggesting that this regulatory aspect of TEs might lead to beneficial effects, in particular during development (Box 3).

## Epigenetic Silencing of TEs in the Brain

Given the potential pathological consequences of aberrant TE activation, it is not surprising that the majority of TEs are transcriptionally silenced in adult tissues. It has been known for decades that this silencing correlates with the presence of DNA methylation, a covalent modification that occurs on cytosines [27]. In most cases, DNA methylation is found on CG dinucleotides, often referred to as CpGs. The establishment of this DNA modification is mediated through a family of enzymes called DNA methyltransferases (DNMTs). In humans there are three DNMTs: DNMT1, DNMT3A, and DNMT3B [28–30]. DNMT3A and 3B are mainly *de novo* methyltransferases that are responsible for establishing DNA methylation patterns, which occur mainly during embryogenesis, while DNMT1 is a maintenance methyltransferase that replicates DNA methylation patterns during cell division (Figure 2A). Intriguingly, DNMT1 is also highly expressed in adult postmitotic neurons, indicating additional roles for this enzyme in the brain [31].

CpG-DNA methylation of promoter regions correlates with transcriptional repression. However, it is not completely known how the presence of DNA methylation results in transcriptional silencing: it is thought that this occurs through several different mechanisms [32]. For example, DNA methylation can attract protein complexes that mediate the formation of **heterochromatin**. In mammals, there are five methyl-CpG-binding domain proteins, MBD1 to 4 and MECP2, of which at least some have the capacity to mediate gene silencing [33–35]. In addition, DNA

### Box 2. Transposable Elements (TEs) Have the Potential to Activate an Innate Immune Response

The innate immune system is an ancient part of our immune system and acts as a nonspecific first line of defense against different pathogens, such as viruses and bacteria. Pattern recognition receptors (PRRs) detect the infectious agent and trigger signaling cascades that lead to the nuclear activation of immune genes encoding proinflammatory effectors, such as cytokines and interferons (IFNs). The activation of the innate immune system is critical for the more specific adaptive immune responses. One of the most studied PRRs are Toll-like receptors (TLRs). They were initially identified on sentinel cells but have recently also been discovered to be expressed in neural cells.

Although TEs have become domesticated, they still retain virus-like structures (see Figure 1A in the main text) and might appear pathogenic to the host upon activation, thus triggering an innate immune response. Thus, the loss of epigenetic repression leads to transcriptional activation, when TE-derived transcripts are transported into the cytoplasm (Figure I,1), where the single stranded RNA (ssRNA) can be used as a template for cDNA in the presence of reverse transcriptase, generating RNA:DNA hybrids (Figure I,2). TLRs located in the endosomal membrane can sense these different retroviral nucleic acids. These TLRs are subsequently incorporated into an autophagosome and merged with an endosome where they are detected (Figure I,3), resulting in an activation of the innate immune system.

Alternatively, the single stranded DNA (ssDNA) generated from the reverse transcription can be used to make double stranded DNA (dsDNA) (Figure I,4) before being transported into the nucleus for reintegration into the genome (Figure I,5) (thereby completing a retrotransposition event). As a consequence, ssDNA molecules could accumulate in the cytoplasm and be detected by the cytoplasmic PRRs (Figure I,6,7), thereby triggering an IFN response.

Additionally, the TE-derived transcripts exported from the nuclei may function as mRNAs and be translated into peptides/proteins (Figure I,8). If the TE-derived proteins are released or presented on the cell membrane, the TLRs located in the cell membrane can recognize TE-derived proteins as foreign, triggering the innate immune system (Figure I,9).

The different modes of detection of the TE-derived molecules by different types of PRRs trigger signaling cascades that lead to the nuclear activation of immune genes encoding for proinflammatory effectors, such as cytokines and IFN. Producing IFN upon immune activation can establish a positive feedback loop that further upregulates IFN-stimulated genes, increasing the immune reaction. Thus, TEs hold the potential to activate the immune system in multiple ways and offer a potential trigger for the neuroinflammation found in neurodevelopmental and neurodegenerative disorders. For further reading on this topic see, for example, [113–116].

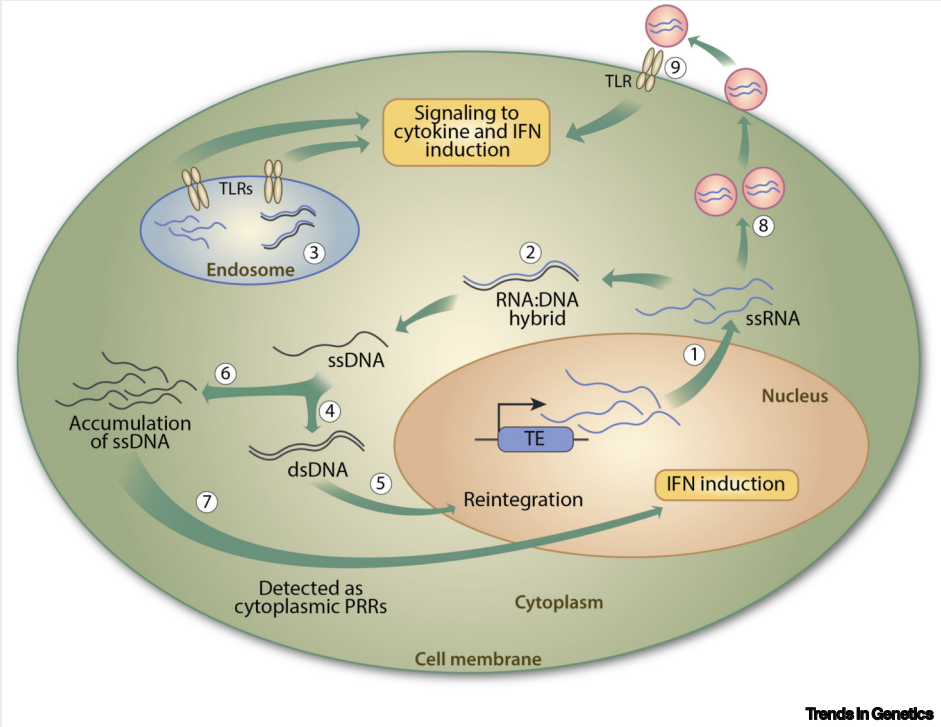


Figure I. TE-driven Activation of an Innate Immune Response. Abbreviations: dsDNA, double stranded DNA; IFN, interferon; PRR, pattern recognition receptor; ssDNA, single stranded DNA; ssRNA, single stranded RNA; TE, transposable element; TLR, Toll-like receptor.

methylation may directly influence transcription factor binding, for example, through the recruitment of transcription factors that silence gene expression (see e.g., [36]).

How DNA methylation patterns are established remains a field of intense study. In early development, DNA methylation patterns are reprogrammed through a remarkable process. During the first few days of embryonic development, DNA methylation is globally erased and reinstated a few days later, resulting in reprogramming of DNA methylation patterns with each generation [37]. During this process, TEs are silenced via transient epigenetic modifications that are characterized by the repressive histone mark **H3K9me3** [38,39]. Upon differentiation, TE silencing is then stabilized by DNA methylation, which is maintained in adult tissues [40,41].

Work by us and others has recently started to uncover the molecular mechanisms that underlie the establishment and dynamic patterning of DNA methylation of TEs in adult tissues. In **pluripotent stem cells (PSCs)**, which correspond to the inner cell mass of the early embryo, the epigenetic corepressor protein TRIM28 (also known as KAP1 and TIF1β) is a key player in the control of TEs (Figure 2B) [39]. TRIM28 is recruited to TEs via sequence-specific Krüppel-associated box zinc-finger proteins (KRAB-ZFPs), a family of transcription factors that has undergone a rapid expansion in mammalian genomes in parallel with the expansion of TEs [42,43]. A large number of KRAB-ZFPs are expressed in PSCs and directly bind to TEs, thereby recruiting the TRIM28 silencing complex resulting in H3K9me3-mediated epigenetic repression of TEs [44,45]. Deletion of TRIM28 in PSCs therefore leads to a broad upregulation of **TE-derived transcripts** [38,39]. When PSCs differentiate, TRIM28 mediates a stable transcriptional silencing where H3K9me3 is replaced by DNA methylation [41]. Consistent with this, deletion of TRIM28 in most somatic

### Box 3. Transposable Elements (TEs): Foes and Friends?

TEs can impact on the human genome in various ways. On one hand, TEs pose a threat to genomic integrity as their activation may result in transposition events that, upon integration in the genome, might result in deleterious mutations. The host has therefore evolved numerous mechanisms to prevent transposition. For example, TEs are usually covered with DNA/histone methylation marks that silence expression.

On the other hand, TEs have the potential to be co-opted and provide benefit for the host in a number of ways. For example, silenced TEs can create repressive hubs that can negatively affect the expression of nearby genes. In cases where TEs escape repression, they can be transcribed as noncoding RNA or act as *cis*- or *trans*-regulatory elements, such as enhancers or promoters.

In the 1950s it was already recognized that TEs are genetic elements that have the potential to alter the genetic landscape and influence gene expression when they integrate into new sites in their host genome [117]. Today, it is becoming increasingly clear that TEs act as important gene regulatory elements and serve as a rich source for genome innovation. For further reading on this topic see, for example, [25,26].

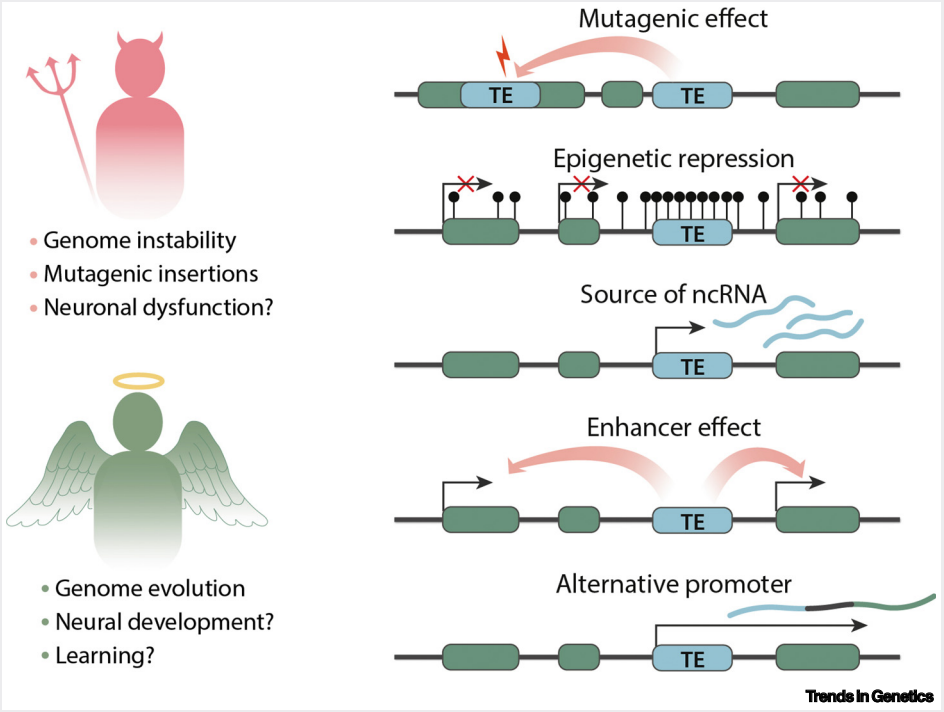


Figure I. Harmful and Beneficial Regulatory Roles for TEs. Abbreviations: ncRNA, noncoding RNA; TE, transposable element.

cells, such as fibroblasts, does not activate expression of TEs [39]. Thus, KRAB-ZFPs and TRIM28 are key players in the establishment of a stable pattern of DNA methylation on TEs observed in adult tissues.

However, emerging evidence suggests that the epigenetic regulation of TEs in the brain is more complex. For example, deletion of TRIM28 in mouse and human **neural progenitor cells (NPCs)** results in the activation of TEs [46,47]. While these TEs only represent a small proportion of those activated upon TRIM28 deletion in PSCs, many of them correspond to evolutionarily young TEs with the potential to produce long transcripts and TE-derived peptides and retrotranspose. In contrast, deletion of DNMT1 (the key maintenance DNMT) in human NPCs results in a very modest activation of TEs, despite the complete loss of DNA methylation [48]. It

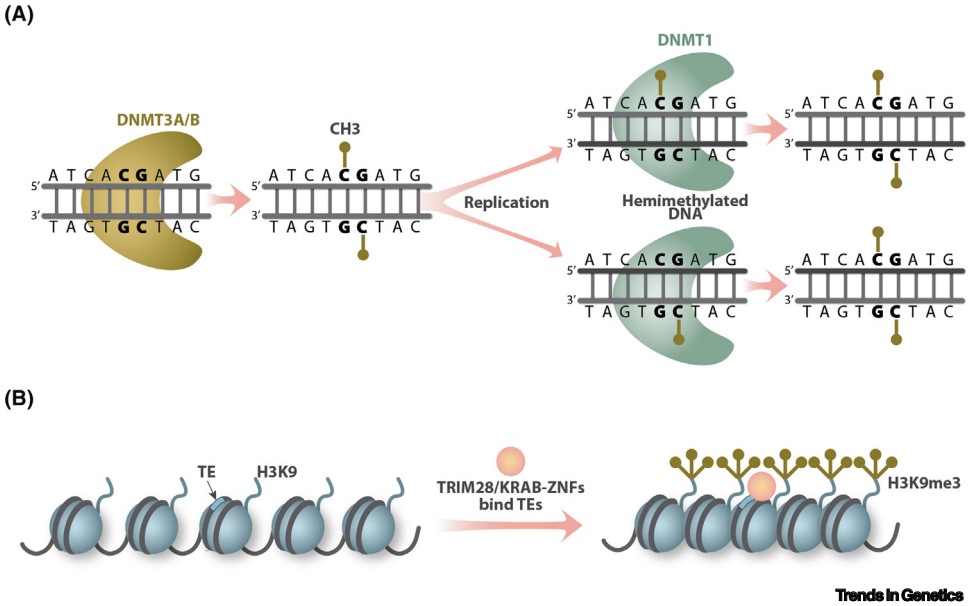


Figure 2. Transposable Elements (TEs) Are Repressed via DNA and Histone Methylation. (A) DNA methylation is an epigenetic modification where a methyl group ($CH_3$) is covalently bound to cytosine. It is most commonly found on cytosines located in front of guanine, often referred to as CpGs. The initial deposition of DNA methylation (*de novo* DNA methylation) is made by the DNA methyltransferases DNMT3A and 3B. The pattern of DNA methylation is maintained over replication by DNMT1, which recognizes hemimethylated DNA and adds a methyl group on the newly synthesized DNA strand, thereby making sure the modification is inherited over cell divisions. (B) Eukaryotic DNA is wrapped around nucleosomes, which are made up of histone proteins that can be modified in various ways to affect chromatin accessibility and thus gene expression. The presence of TEs can lead to the formation of heterochromatin by the addition of H3K9me3 upon recognition by Krüppel-associated box zinc-finger proteins (KRAB-ZNFs), which contain a TE-binding domain. The TRIM28 silencing complex binds to the KRAB-ZNFs, resulting in deposition of H3K9me3 on and around the bound TE, causing heterochromatin formation and TE repression.

appears that most TEs in NPCs carry a dual layer of repression that includes both DNA methylation and H3K9me3.

It is worth noting that upon deletion of DNMT1 in human NPCs, which leads to global DNA demethylation, evolutionarily young full-length LINE-1 elements are activated. TRIM28-deletion in NPCs does not, however, activate these young LINE-1s. Thus, DNA methylation appears to be particularly important for silencing this family of TEs in the brain [48]. In line with this, recent data suggest that certain polymorphic LINE-1 elements that have escaped DNA methylation are active in the human brain, further linking DNA methylation and LINE-1 repression [49].

In addition to epigenetic repression, mechanisms that repress TEs at a post-transcriptional level have also been reported, including, for example, RNA binding proteins and small RNAs. In the *Drosophila* brain, small RNAs appear to play a key role in silencing TEs and disruption of this pathway has been linked to aging and pathological mechanisms [50–53]. However, many questions concerning small RNA silencing of TEs in the brain remain unresolved and it is still to be determined if it is small interfering RNAs (siRNAs) or piwi-interacting RNAs (piRNAs) that are central for this process [54]. It has been challenging to demonstrate the presence of corresponding mechanisms in the mammalian brain (see e.g., [23,55]) and the importance of post-transcriptional silencing of TEs in the human brain, and its potential link to human disease, remain unclear and under debate.

Taken together, the silencing of TEs in the developing brain is characterized by a multilayered machinery, including DNA and histone methylation. However, most of these studies have been performed on proliferating cell types such as NPCs or similar. How TEs are controlled in the adult brain remains largely unexplored. It is, for example, not known if TEs are controlled in the same way in different cell types of the brain, including the postmitotic neurons. It is worth mentioning that recent evidence demonstrates that the DNA methylation landscape in the adult brain is unique compared with other somatic tissues, characterized by the presence of abundant non-CpG DNA methylation [56]. How this unique epigenetic landscape affects the state of TEs remains unknown. It is also likely that there are species-specific differences, suggesting that mechanisms observed in model organisms are not always relevant for humans. This is demonstrated, for example, by the absence of DNA methylation in the *Drosophila melanogaster* genome, indicating a completely different machinery for TE silencing, likely based on small RNAs, compared with the human genome.

## Alterations of TE Silencing in Neurodevelopmental Disorders

The concept that many psychiatric disorders are a consequence of neurodevelopmental alterations is now well established. Along with genetic factors, it is likely that environmental exposure during pregnancy and early life are crucial for the pathogenesis of these disorders [57,58]. Exposures such as diets, toxins, inflammation/infection, and drugs are all thought to contribute to the appearance and progress of diseases such as anxiety disorders, depression, bipolar disorders, and schizophrenia, indicating that epigenetic alterations play key roles in the disease process of psychiatric disorders (see e.g., [59,60]). In line with this, several recent studies have documented broad alterations in DNA methylation profiles in schizophrenia as well as autism spectrum disorders and attention deficiency hyperactivity disorder (see e.g., [61–63]).

The epigenetic dysregulation reported in several psychiatric disorders indicates that TEs may become aberrantly activated and contribute to the disease progress. Proof-of-principle data of this notion come from the neurodevelopmental disorder Rett syndrome (RTT), which is caused by mutations in MECP2 [64]. Data from both mouse models, human cell-culture models, and human postmortem tissue suggest that the activation of LINE-1 elements, including the presence of new somatic transposition events, is a part of the disease phenotype in RTT [65]. Similarly, increased LINE-1 activation was found in mouse models and human postmortem tissue from cerebellar ataxia, a disease caused by mutations in ATM, a gene involved in DNA repair [66]. Increased LINE-1 insertions have also been reported in neurodevelopmental and psychiatric disorders with a less defined genetic component, such as schizophrenia and depression [67–69]. In addition, studies on detrimental environmental exposure, such as prenatal maternal stress, have demonstrated an activation of LINE-1 in the mouse brain [15]. Although many of these studies suffer from limitations, such as choice of methodology and low statistical power, they point in the same direction, suggesting that LINE-1s, and perhaps other TEs, can become activated in psychiatric disorders as a consequence of genetic and/or environmental factors.

The transcriptional activation of LINE-1 elements in the human brain has been linked to somatic transposition events [16–19,70]. These events create **genomic mosaicism** in the human brain and have been suggested to contribute to functional diversification within the neuronal population in an individual, thereby contributing to individual differences and perhaps triggering pathology (Box 1). However, due to technical challenges associated with estimating the rate of transposition in somatic cells, it remains a matter of debate how frequent somatic LINE-1 insertions actually occur in the human brain (see e.g., [16,19,71–73]). Some recent estimates suggest that the insertion rate in human neurons is very low, making it questionable if these events have a physiological impact. An alternative mechanism may be that epigenetic changes causing

transcriptional activation of LINE-1 elements cause alterations in transcriptional networks. We recently found that when DNA methylation is experimentally removed in human NPCs, many activated LINE-1 elements act as alternative promoters, thereby influencing the expression level of many protein-coding genes [48]. These observations suggest that the presence of LINE-1s scattered throughout the mammalian genome provides a template for a widespread gene regulatory network. LINE-1s appear to be central for the regulation of transcriptional networks in the developing brain and the disruption of this mechanism has the potential to influence the expression of many coregulated host genes. With this in mind, it is interesting to note that many of the genes where LINE-1 acts as an alternative promoter, upon loss of DNA methylation, have previously been implicated in neurodevelopmental disorders [48].

Interestingly, many psychiatric disorders are known to have an immune component. An activated immune system has been found in, for example, autism, posttraumatic stress disorder, depression, and schizophrenia, including both a dysregulated immune response in the periphery as well as microglia and astrocyte activation in the brain [74]. The underlying cause of this immune response remains largely unknown, but is thought to play an important role in the disease process. The combination of epigenetic dysregulation and immune activation in neurodevelopmental disorders raises the intriguing possibility that TEs are directly involved in this phenomenon, since TEs are known to be able to activate immune pathways (Box 2).

One of the best-characterized roles of TEs in inflammation comes from studies of the rare genetic disorder Aicardi-Goutieres syndrome (AGS), a spectrum of disorders characterized by severe neurological impairments. AGS is caused by loss-of-function mutations in genes (e.g., TREX1, MDA5, and ADAR1) that normally limit the presence of cytosolic nucleic acids. Data from multiple labs have demonstrated that the presence of these mutations results in the accumulation of TE-derived cytosolic nucleic acids leading to activation of the **innate immune system** [75–77] (Box 2). Thus, the host cells misinterpret the activation of TEs as a viral infection and this viral mimicry drives the immune response. This indicates that accumulation of TE-derived nucleic acids may also contribute to the inflammatory response in disorders where the genetic component is less defined but where epigenetic alterations are prevalent, including, for example, schizophrenia, depression, and autism, as well as various neurodegenerative disorders.

## Alterations to TE Silencing in Age-Related Disorders

Age is the overall largest risk factor for neurodegenerative disorders such as Alzheimer's disease (AD), Parkinson's disease (PD), and amyotrophic lateral sclerosis (ALS). These disorders are characterized by the presence of protein aggregates in the brain parenchyma, which are linked to neuronal cell death, resulting in motor and/or cognitive disturbances. Similar to neurodevelopmental disorders, these diseases also display epigenetic alterations and a prominent inflammatory component, raising the possibility that TEs are part of the disease process.

## ALS and TEs

The most well-studied relationship between TEs and neurodegenerative disorders is the one observed in ALS, a progressive adult-onset neurogenerative disorder characterized by the selective death of motor neurons. Several studies from independent labs have reported an increased reverse transcriptase activity in serum from ALS patients. During the last decade this has been linked to human endogenous retroviruses (HERV), which are TEs belonging to the LTR family. HERV-derived transcripts and proteins (which have been shown to be neurotoxic) have been detected in serum, cerebrospinal fluid, and postmortem brain tissue of ALS patients [24,78–82].

ALS, as well as the closely related neurodegenerative disorder frontotemporal dementia (FTD), is pathologically characterized by intraneuronal protein aggregates of the RNA and/or DNA binding protein TDP-43. The cytoplasmic aggregation of TDP-43 that occurs in ALS and FTD results in neuronal nuclei lacking this protein [83]. The loss of nuclear TDP-43 is associated with changes in heterochromatin leading to an activation of TE expression [84]. In addition, TDP-43 directly associates with TE-derived RNAs, thereby providing a post-transcriptional control of TE-expression [85]. Another link between ALS and TEs is C9orf72, which is the most commonly mutated gene in familial forms of ALS as well as FTD. Transcriptome analyses suggest that ALS/FTD patients carrying a mutated C9orf72 allele display elevated levels of TE expression, and mechanistic studies have indicated that small peptides originating from C9orf72 impairs HP1-mediated heterochromatinization, ultimately resulting in TE activation [86,87].

Together, these studies point to an important role for TE activation in ALS. However, there is currently a large discrepancy in the literature regarding if and how HERVs and other TEs are truly activated in ALS, and the potential contribution of this phenomenon to the disease process is still being debated [88,89]. Therefore, more work is needed to elucidate the exact role of TEs in ALS [90].

## Activation of TEs in Aging

Interestingly for the field of neurodegeneration, several recent studies have described direct links between aging and TE activation. Epigenetic changes, including alterations in DNA methylation, are central in the aging process. For example, it is possible to use only DNA methylation patterns of different tissues, including the brain, to accurately estimate the age of humans [91]. These age-dependent epigenetic changes may also result in the activation of TEs. A protein central to this process appears to be SIRT6, a chromatin-associated protein involved in DNA repair. Loss of SIRT6, either via experimental modeling or through age-dependent downregulation, results in increased levels of LINE-1-derived RNA and DNA [92,93]. The presence of LINE-1-derived nucleic acids has the ability to activate the interferon pathway, thereby resulting in an age-dependent inflammatory response [92]. Given the important role of aging and neuroinflammation in neurodegenerative disorders, it is tempting to speculate that TEs are broadly involved in the disease process through age-dependent chromatin relaxation. However, the majority of the SIRT6-related observations come from peripheral tissues and it remains unclear if these findings are relevant for the brain as well as if this phenomenon contributes to neurodegeneration. Still, the well-documented presence of inflammation and epigenetic alterations in, for example, AD and PD, together with emerging studies that report on an increased expression of TEs in these disorders, provide a strong foundation for further studies in this area [53,94,95].

## AD and TEs

AD is an age-dependent progressive neurodegenerative disorder, characterized by a loss of neurons in the CNS, as well as the accumulation of intracellular neurofibrillary tangles of Tau protein and extracellular amyloid plaques. The vast majority of AD cases are sporadic in nature, without a known underlying cause. Recently, three independent large-scale RNA-seq efforts found increased levels of HERVs, and other TEs, in the brain of AD patients [53,95,96]. While these studies display some discrepancies, they point to elevated TE expression as being part of the AD pathology.

Mechanistic studies in *Drosophila* models suggest that the activation of TEs is linked to Tau pathology [53,95]. Overexpression of Tau results in a global loss of heterochromatin, which in turn activates TE expression [53,97]. Tau overexpression also appears to inhibit post-transcriptional silencing of TEs in fly brains by downregulating the expression of PIWI-proteins [53]. Notably,

by blocking TE activity through the use of reverse transcriptase inhibitors, it is possible to rescue disease-related phenotypes in *Drosophila* AD models [53]. While it remains unclear how relevant these results are for the human disease, it is noteworthy that human AD brains display alterations of heterochromatin, including a diffuse H3K9me2 staining and an altered distribution of heterochromatin protein 1 (HP1) [97].

Another interesting phenomenon in AD brains is the reported increased mosaic copy number of the amyloid beta precursor protein gene (APP) [98,99]. A recent study demonstrates that these new copies of APP display molecular signs of being transposed, including, for example, lack of intronic regions [99]. This raises the possibility that APP, which is directly linked to the disease process through the formation of amyloid plaques, undergoes somatic transposition and copy number expansion in AD brains. This is a phenomenon that potentially could be mediated by TEs expressing reverse transcriptase, such as LINE-1 elements.

However, much remains unclear about the role of TEs in AD and there are conflicting reports on the topic (see e.g., [100]). It is not known if TE activation and somatic transposition have a role in the disease process, or if they are by-products of other more important pathological processes. Additional studies are required to address a number of questions (i.e., if TEs are truly activated in AD brains, in which cells this occurs, when it happens, and if, and how this contributes to the disease process in humans).

## X-Linked Dystonia Parkinsonism (XDP)

Solid proof-of-concept data on additional mechanisms by which TEs can directly contribute to neurodegenerative diseases come from studies on the rare genetic disorder XDP (Figure 3). This Mendelian disease is most likely caused by a rare polymorphic SVA insertion into intron 32 of TAF1, a gene involved in transcriptional control [101]. XDP is an adult-onset neurodegenerative disorder, characterized by dystonia and parkinsonism. Genetic linkage analysis, in combination with induced pluripotent stem cell-modeling and genetic editing, has demonstrated that the SVA insertion is directly involved in the disease process by mediating transcriptional interference [101]. Interestingly, the length of the VNTR repeat within the SVA element is variable and appears to play an important role in the disease process, since a longer repeat is associated with an earlier

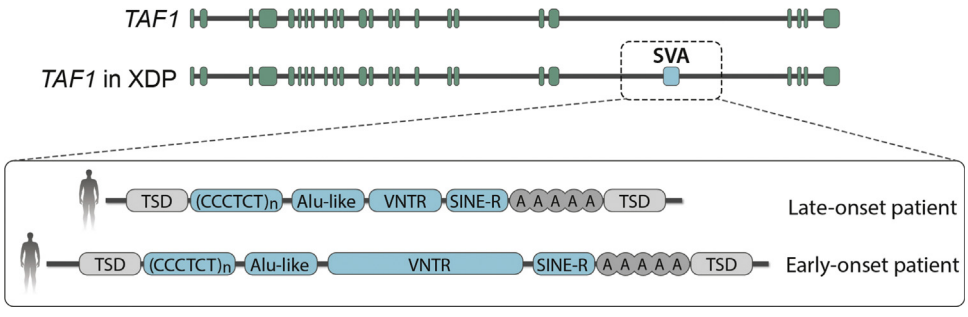


Figure 3. Transposable Elements (TEs) Can Drive Individual Variation. An example of a polymorphic TE causing a neurodegenerative disorder is X-linked dystonia parkinsonism (XDP), a heritable disease endemic to the Philippines. Although variable, patients experience the onset of the disease at around 40 years old, with a combination of the dystonia and Parkinson's disease-like phenotypes. The disease has been linked to a Sine-VNTR-Alu (SVA) insertion localized in the gene *TAF1*. *TAF1* encodes for a TATA-box binding protein that, in the presence of the SVA insertion, retains part of the intron, resulting in a decrease of *TAF1* expression. One important characteristic of SVA elements is the presence of a VNTR with a variable number of repeats among patients. Interestingly, in XDP, the length of the VNTR in the SVA inversely correlates with the age of onset.

age-of-onset [102]. While many questions regarding the underlying molecular mechanisms remain unresolved, these observations demonstrate that a polymorphic TE insertion can cause a neurodegenerative disorder. This also suggests that rare polymorphic TE insertions may be important in other more common neurodegenerative disorders, where the genetic component is less understood, such as AD or PD.

## Concluding Remarks

Over the last few years, there has been an increase in the number of studies on TEs and their role in both the healthy and diseased brain. The rapid development of novel sequencing techniques, including new long-read approaches, is responsible for this progress. Other crucial elements moving the field forward include specialized bioinformatical pipelines and software that allow for accurate analysis of TEs. Still, the repetitive nature of TEs challenges genomic analyses and many observations made earlier need to be confirmed as the technologies continue to evolve. Until now, most studies investigating a role for TEs in neurological disorders using human material have suffered from shortcomings, including the lack of specificity of the sequencing approach and bioinformatical analysis adopted, coupled with low power and lack of single-cell resolution. To date, high-throughput single-cell RNA and DNA analysis focused on TEs remains extremely challenging, with its further development proving essential for the clarification of the expression and activity of TEs in the brain. Furthermore, it appears essential to address the challenge of polymorphic TE insertions. It has been estimated that each human genome differs by around a thousand TE insertions [103,104]. This genomic diversity is likely to greatly impact the output of current bioinformatical pipelines based on the use of the human reference genome. Noteworthy, this issue also relates to studies of model organisms, such as the mouse or *Drosophila*, where different strains harbor a large degree of genomic differences in their TE composition (see e.g., [105]).

Advances in genomic approaches applied to human patients must also be linked to improved causal experiments in animal models and human *in vitro* culture systems. The progress in advanced genetic editing allows for unprecedented possibilities to mechanistically link TEs to physiological events. For example, it is now possible to use **CRISPR**-based approaches to delete single or multiple TEs from the genome, or to transcriptionally activate and repress whole TE families (see e.g., [106–109]). By applying these systems to *in vivo* models, such as the mouse brain, it will be possible to mechanistically link, for example, activation of TEs to inflammation, something that has not yet been achieved (see Outstanding Questions). Furthermore, by using these technologies in novel human cell culture models, such as **organoids** or aged **induced neurons** [110–112], it will probably become possible to uncover findings relevant for humans. Together, these kinds of approaches hold the potential to deliver, at a single-cell resolution, a detailed understanding of how TEs are linked to psychiatric and neurodegenerative disorders, and possibly provide novel targets for future diagnostic tools and drug development.

### Outstanding Questions

Can aberrant DNA methylation and TE activation be functionally involved in the pathogenesis of neurodevelopmental and neurodegenerative disorders?

How do we develop single-cell sequencing approaches with sufficient resolution to study TEs in terms of both sample size and sequencing depth?

How can we identify individual variations and polymorphisms within the population and their involvement in the etiology of neurological disorders?

How can the knowledge of TE and pathological processes in the brain be utilized to improve diagnostics and interventions?

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