

Awakening the dark side: Retrotransposon activation in neurodegenerative disorders

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Nearly half (45%) of the human genome is composed of transposable elements, or “jumping genes.” Since Barbara McClintock’s original discovery of transposable elements in 1950, we have come to appreciate that transposable element mobilization is a major driver of evolution, that transposons are active in the germline and the soma, and that transposable element dysregulation is causally associated with many human disorders. In the present review, we highlight recent studies investigating transposable element activation in the adult brain and in the context of neurodegeneration. Collectively, these studies contribute to a greater understanding of the frequency of complete retrotransposition in the adult brain as well as the presence of transposable element-derived RNA and protein in brain and fluids of patients with neurodegenerative disorders. We discuss therapeutic opportunities and speculate on the larger implications of transposable element activation in regard to current hot topics in the field of neurodegeneration.

Highlights

- Increased levels of retrotransposon-derived RNAs and protein products are present in several human neurodegenerative disorders.
- In ALS/FTD, TDP43 may drive retrotransposon activation by disrupting siRNA- and heterochromatin-mediated silencing mechanisms.
- In tauopathies, tau may drive retrotransposon activation by disrupting piRNA- and heterochromatin-mediated silencing mechanisms.
- Use of reverse transcriptase inhibitors to suppress retrotransposition provide a potential therapeutic strategy for patients with ALS/FTD and tauopathy.

Introduction

Transposable elements are a diverse superfamily of genomic DNA species that have the ability to either copy themselves and insert the DNA copy into a new genomic location (retrotransposons) or excise themselves from the genome and insert in a new genomic location (transposons). Over the course of human evolution, most retrotransposons and all DNA transposons have become inert, or non-mobile, due to truncation and mutation. Some human retrotransposons, however, retain mobilization potential, including specific long and short interspersed nuclear element (LINE and SINE, respectively) subfamilies [1]. Since key retrotransposons retain mobilization ability and retrotransposons outnumber DNA transposons 13 to 1 in the human genome [2], we focus on retrotransposons in the current review.

Alongside a longstanding literature focused on retrotransposition in the human brain and retrotransposon dysregulation in amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD), new studies implicate transposable element activation in the context of tau-mediated neurodegeneration, including Alzheimer’s disease. This review highlights our current understanding of retrotransposon activation in the human brain, new insights into the involvement of retrotransposition and retrotransposon-derived products in neurodegenerative disorders, including breakdown of cellular mechanisms that regulate transposable element activation in neurodegenerative disorders, as well as current and future therapeutic directions.

Retrotransposon biology

Retrotransposons are structurally akin to retroviruses (Fig. 1). Retrotransposon mobilization occurs through a copy-and-paste mechanism that involves transcription of endogenous retrotransposon DNA into RNA, reverse transcription of the nascent retrotransposon RNA into a new DNA copy, and reinsertion of the new DNA copy into the genome [1]. Retrotransposition is completed using proteins encoded by retrotransposon RNA. “Endogenous retroviruses” (ERVs) are a family of retrotransposons that are similar to some exogenous retroviruses in that they are flanked by Long Terminal Repeats (LTRs) and harbor protein-coding *gag* and *pol* domains. The *gag* protein assembles into a structural matrix in which reverse transcription by a *pol*-encoded reverse transcriptase occurs. *Pol* also encodes an endonuclease and integrase that facilitate reinsertion of the newly-formed DNA into the genome. Some intact human ERVs (HERVs) such as HERV-K(HML) also harbor an *env* domain that encodes a surface glycoprotein similar to the retroviral envelope. Intact members of the LINE family of retrotransposons encode an endonuclease and reverse transcriptase that facilitate mobilization. Current estimates suggest that 80-100 human LINE-1 elements (L1s) are mobilization-competent, with about 10% of intact L1s being highly active or “hot” [3]. Non-autonomous SINEs such as the human Alu or SVA elements retrotranspose by co-opting proteins encoded by LINES [1].

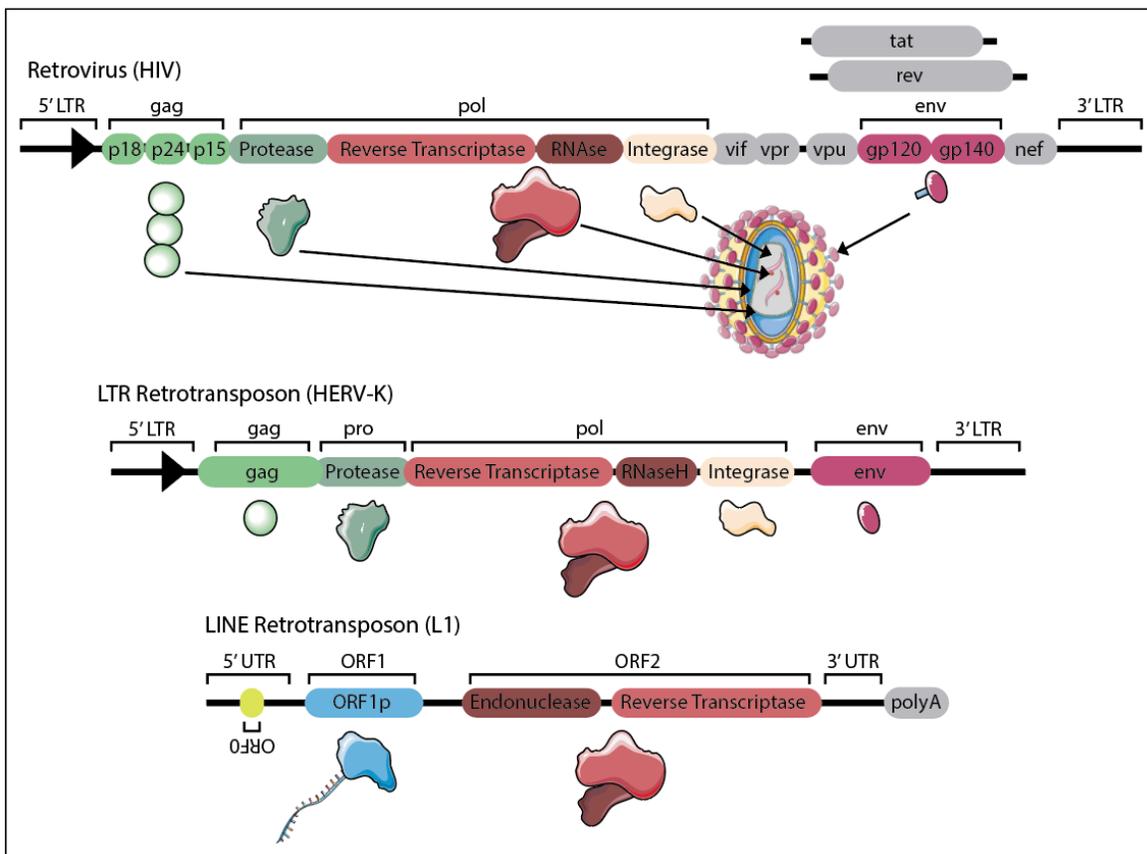


Figure 1. Comparative genomic landscape of a retrovirus, LTR and LINE element. HIV is illustrated as an example of an intact retrovirus, HERV-K is illustrated as an example of an intact LTR element, and L1 is illustrated as an example of an intact LINE. Protein products that share functional similarity among the retrovirus, LTR and LINE are represented by the same shape and color.

Based on the potentially catastrophic consequence of retrotransposition in the germline, there is a tendency to emphasize the destructive nature of retrotransposons. While this view is well-justified in many contexts, it is important to keep in mind that retrotransposons provide abundant regulatory sequences for host genomes, mediate innate immunity, and drive evolution [4]. Nevertheless, cells have developed mechanisms to keep potentially deleterious retrotransposon activation in check (Fig. 2). First, retrotransposon transcription is regulated at the epigenetic level based on the location of many retrotransposons in highly condensed heterochromatin. Second, retrotransposons are subject to post-transcriptional silencing by two classes of small RNA, endogenous small-interfering RNAs (esiRNAs) [5] and PIWI-interacting RNAs (piRNA)[6], which also have effects on promoter methylation [7]. Long-thought to be specific to the germline, piRNAs are now well-appreciated components of somatic tissues, including adult brain [8–11].

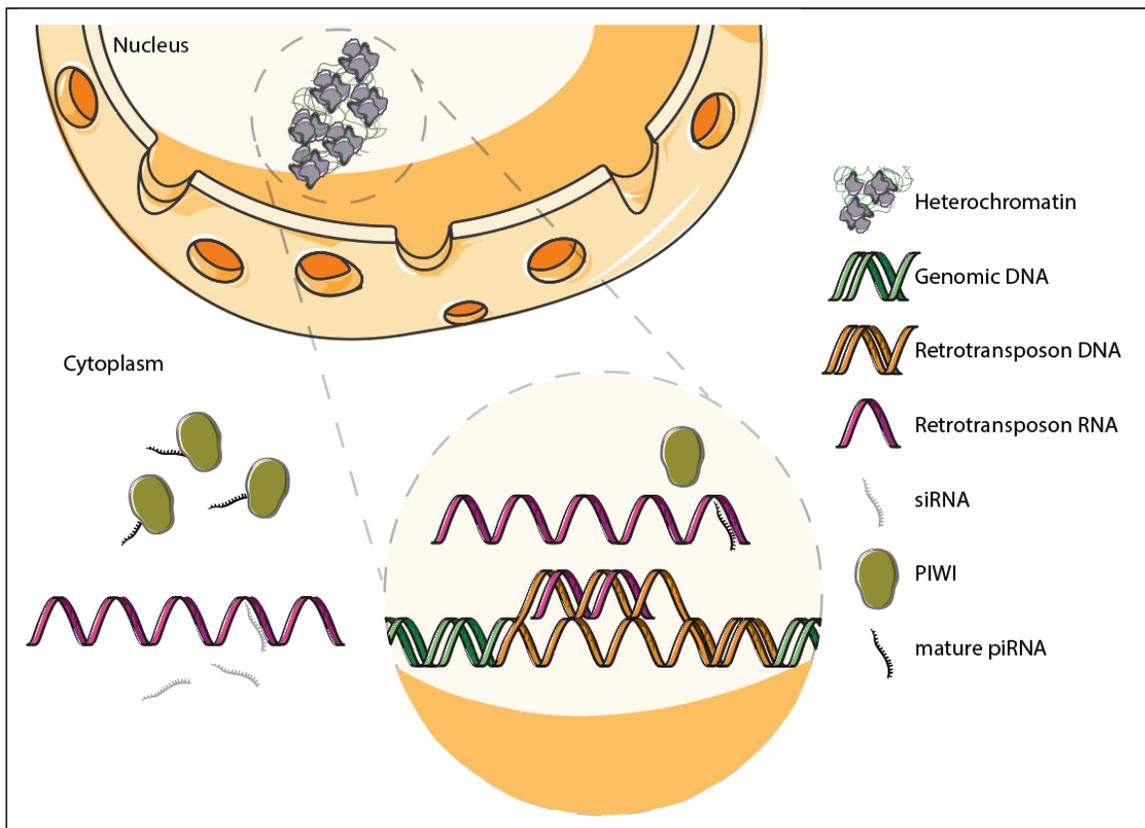


Figure 2. Two cellular layers of transposable element control. Under normal conditions in somatic cells, transposable elements are transcriptionally silenced by heterochromatin. Post-transcriptionally, retrotransposons are subject to nuclear degradation by piRNAs, and cytoplasmic degradation by siRNAs.

While much focus is placed on the consequences and frequency of *de novo* retrotransposon insertions into the genome (i.e. retrotransposon “jumping”), the RNAs and protein products generated from retrotransposons can also affect cellular function. For example, dsRNAs formed via bidirectional transcription of retrotransposons can induce an interferon response through the RNA-sensing innate immune network [12–14], and ERV-encoded proteins can drive autoimmunity [15] and motor neuron disease [16].

Evidence for transposable element mobilization in the human brain

A seminal paper from Muotri, Gage and colleagues used a fluorescent reporter of retrotransposon mobilization to detect complete transposition of human L1 in both cultured rat neural precursor cells and mouse brain [17]. This study was the first to suggest that retrotransposition can generate genomic diversity among neurons. Based on the copy-and-paste mechanism of retrotransposons, in which a single mobilization event increases the DNA copy number of a mobilizing retrotransposon by one copy, a later study detected increased DNA copy number of L1, Alu, and SVA elements in the human brain when compared to liver or heart from the same donor, and estimated the frequency of L1 mobilization at about 80 insertions per neuron [18]. While these findings were consistent with retrotransposon activation in the adult brain, both genomic and unincorporated, extra-genomic retrotransposon DNA contribute to total DNA copy number. Faulkner and colleagues thus later identified integration sites of novel somatic insertions of L1, Alu, and SVA in the hippocampus and caudate nucleus of the adult human brain [19], establishing that complete retrotransposition, including insertion of retrotransposon DNA into genomic DNA, occurs in the adult human brain.

Estimations of the occurrence and frequency of retrotransposition based on genomic sequencing continue to be an outstanding debate in the field, and are highly dependent on genome amplification method, library preparation and sequencing platform, as well as how results are analyzed. Current estimates based on single-nucleus DNA sequencing range from <0.6-16.3 L1 mobilization events per neuron in a neurotypical brain [20,21]. Waddell and colleagues have recently called the frequency of retrotransposon mobilization in the brain into question, concluding that the majority of putative *de novo* transposon mobilization events identified by genomic sequencing result from chimeric artifacts formed during library preparation for whole genome sequencing [22]. A general consensus on the best approach for DNA sequencing-based detection of transposition is currently lacking but very much needed.

One interpretation of these studies is that the frequency of mobilization may be fairly low in a neurotypical human brain and thus potentially of little consequence to normal brain functioning. Several of these studies, however, report that retrotransposons selectively insert into genes associated with neuronal function [17,19,21,23], which may increase the impact of relatively rare mobilization events. Even in the absence of complete retrotransposition, retrotransposons generate RNAs, protein, DNA damage, and extra-genomic DNA copies that are known to affect cellular function [24].

Transposable element activation in ALS/FTD

The earliest clues pointing toward involvement of retrotransposons in a neurodegenerative disorder came from multiple studies reporting high serum levels of reverse transcriptase activity in patients with ALS [25–27]. Having ruled out exogenous retroviral infection, later detection of elevated HERV-K-derived *pol* transcripts and reverse transcriptase protein in postmortem ALS brain samples [28] led to the conclusion that ALS is associated with activation of *endogenous* retrovirus, rather than exogenous viral infection.

Investigation into a direct association between HERV activation and neurodegeneration demonstrated that expression of the env domain of HERV-K causes retraction and beading of neurites in cultured human neurons. In mice, transgenic expression of env induces a progressive motor phenotype and degeneration of motor cortex, reduced synaptic activity of pyramidal neurons, dendritic spine abnormalities, nucleolar dysfunction, and DNA damage [16], suggesting that aberrant expression of HERV-K encoded protein is sufficient to induce neurotoxicity.

Depletion of TAR DNA-binding protein 43 (TDP-43) from the nucleus and accumulation of TDP-43 in the cytoplasm of neurons and glia is a hallmark pathology of ALS and FTD [29], that also appears in 24-70% of Alzheimer's disease cases [30]. In post-mortem brains of patients with ALS, Nath and colleagues found that HERV-K reverse transcriptase is elevated in neurons harboring cytoplasmic TDP-43 inclusions [28]. Based on TDP-43 immunoprecipitation and RNA sequencing from healthy rat, mouse and human brain, TDP-43 was found to bind directly to a UGUGU pentamer motif present in multiple families of transposable element-encoded RNAs, including SINEs, LINEs and ERVs [31]. The direct binding between TDP-43 and transposable element transcripts is reduced in post-mortem brains of patients with FTD and correlates with increased transcript levels of retrotransposons that have lost TDP-43 binding. Mouse models of TDP-43 overexpression (thought to act as a dominant-negative) as well as TDP-43 knockdown are associated with elevated retrotransposon transcript levels [31], pointing toward a causal association between dysfunctional TDP-43 and loss of retrotransposon silencing. Taken together, these studies suggest that a physiological function of TDP-43 is to silence retrotransposons in neurotypical brains, and that retrotransposon silencing is compromised in ALS and, potentially, other TDP-43 proteinopathies.

While *bona fide* retrotransposition in TDP-43 proteinopathy has yet to be demonstrated *in vivo*, two recent studies suggest that TDP-43 dysfunction may cause L1 mobilization. Using neuronal nuclei isolated from ALS/FTD human brain, Lee and colleagues found that neuronal nuclei with low levels of TDP-43 have increased L1 DNA copy number compared to nuclei with high levels of nuclear TDP-43. While increased L1 DNA copy number may simply reflect extragenomic L1 DNA, accompanying experiments reveal that knockdown of TDP-43 is sufficient to induce active L1 mobilization in HeLa cells [32]. A previous study reports that wild-type TDP-43 suppresses L1 mobilization in HEK293T cells [33]. Collectively, these findings suggest that at least some portion of the increased L1 DNA content in human ALS/FTD may reflect complete retrotransposition of L1 associated with TDP-43 nuclear depletion. A summary of the retrotransposons reported as dysregulated in brain, CSF or plasma from patients with neurodegenerative disorders is provided in Table 1.

Despite the significant evidence connecting ALS/FTD and TDP-43 to retrotransposon activation, two recent studies failed to detect differential expression of HERV-K encoded *gag*, *pol*, or *env* transcripts in post-mortem brain samples from sporadic ALS [34,35]. Greater insight into the discrepancy among studies may be gleaned from two recent publications reporting that increased retrotransposon transcript levels are limited to cases of ALS associated with *C9orf72* [33,36], a GGGGCC repeat expansion in a non-coding region of chromosome 9 open reading frame. *C9orf72* expansion is the most

common genetic abnormality in ALS, and was not included as a biological variable in studies that failed to detect HERV-K activation in ALS [34,35]. Indeed, a mouse model of *C9orf72*-associated toxicity features elevated transcript levels of many classes of repeat elements, including LINEs, LTRs, and SINEs [37].

Mechanism

Mechanistically, there is evidence for disruption of small RNA-mediated silencing of retrotransposons in the context of ALS/FTD. Studies from Dubnau and colleagues report that expression of TDP-43 in either glia or neurons of the *Drosophila* mushroom body increases retrotransposon transcript levels and reduces siRNA-mediated transcript clearance. Importantly, genetic manipulation of the siRNA pathway modifies TDP-43-induced toxicity in *Drosophila* [38], suggesting a direct association between TDP-43-induced disruption of siRNA-mediated transposable element clearance and neurodegeneration. ATAC-seq of neuronal nuclei isolated from patients with ALS reveals that heterochromatin is decondensed in nuclei lacking TDP-43, with intergenic repeats and L1 elements being particularly affected [32]. In human ALS/FTD brain tissue, *C9orf72*-associated poly(PR) dipeptide repeats localize to chromatin. In mice, expression of poly(PR) dipeptide repeats affect posttranslational modification of histone 3 and decrease levels of heterochromatin-associated protein 1 [37]. While these data link *C9orf72* expansion with changes in heterochromatin, a causal association between *C9orf72* expansion-induced heterochromatin decondensation and transposable element activation has yet to be established.

Transposable element activation in tauopathy

We have recently identified transposable element activation as a novel driver of neuronal death in tauopathies [10], a group of age-related neurodegenerative disorders that are pathologically defined by deposits of tau protein in the brain [39]. RNA-seq analysis of brain lysates from post-mortem human controls, Alzheimer's disease and progressive supranuclear palsy, a "primary" tauopathy, revealed elevated levels of specific L1, HERV, and SVA transcripts, and decreased levels of Alu family members [10]. Coincident with our work, Shulman and colleagues reported a significant association between decreased cognitive performance in the year prior to death and elevation of specific HERV subfamilies in human Alzheimer's disease brain, as well as an association between tau tangle burden and increased transcript levels of select L1 and HERV elements [40]. It is currently unknown if the increase in L1 and HERV transcripts translates to an increase in L1 and HERV-encoded protein, or if retrotransposition frequency is increased in human Alzheimer's disease and associated tauopathies compared to neurotypical aged controls.

Mechanism

Experiments in *Drosophila* provide greater mechanistic insight into transposable element mobilization and the cell biology mediating transposable element activation in tauopathy. We have reported that pan-neuronal transgenic expression of human tau in *Drosophila* disrupts two arms of transposable element control – heterochromatin and piRNA-mediated retrotransposon silencing [10]. Consistent with the increase in DNA copy number of select retrotransposons in heads of

tau transgenic *Drosophila* [40], we demonstrated active retrotransposition as a consequence of human tau in neurons of the adult *Drosophila* brain. We found that genetic manipulation of retrotransposon regulatory machinery modifies tau-induced neurodegeneration, which suggests a causal link between transposable element dysregulation and neurodegeneration. Together with reports of heterochromatin relaxation [41,42] and piRNA dysregulation [9,43] in post-mortem human Alzheimer's disease brain, these studies suggest that tau-induced heterochromatin decondensation, piwi/piRNA dysregulation and consequent transposable element activation is a novel, conserved driver of neurodegeneration in tauopathy.

Disease	Increased		Decreased	
	Transposable Element	Subfamily	Transposable Element	Subfamily
Alzheimer's Disease (AD)	ERV	ERV17, ERV9, ERVH48I, ERVK22I, ERVKC4, ERVL [10] ERVFc_1 [41]		
	LINE1	L1PA7_5 [10] L1MB4_5 [41]		
	LTR	LTR12C, LTR14 [10] LTR77, PRIMA4_LTR [41]		
	SINE	AluYh9, AluYc5, AluSp [41]	SINE	AluYa5, AluYb8, AluYc1, AluYi6 [10]
	Other	SVA_B, SVA_C, TIGGER2 [10] THER2, PB1D11 [41]		
Progressive Supranuclear Palsy (PSP)	ERV	ERV17, ERV9, ERVH48I, ERVK22I, ERVKC4, ERVL, ERVH, ERVP71A_I [10]		
	LINE1	L1PB2c [10]		
	LTR	LTR12C, LTR14 [10]		LTR14A [10]
			SINE	AluYa5, AluYb8, AluYc1, AluYi6, AluSp, AluY, AluYd8, AluYe5, AluYg6, AluYk11, AluYk12 [10]
Amyotrophic Lateral Sclerosis (ALS)	ERV	ERVK <i>pol</i> and RT protein [29] ERV-K <i>gag</i> , <i>pol</i> , <i>env</i> transcripts and <i>env</i> protein [17]		
	LINE1	L1MA9 [35]		
	LTR	LTR2, LTR70, MER21B, MER51C [35]		
	SINE	AluYk12, AluYa5, FRAM [35]		
	Other	Reverse transcriptase activity [26-28]		
Frontotemporal lobar degeneration (FTD)	LINE1	L1MA9 [35]		
	LTR	LTR2, LTR70, MER21B, MER51C [35]		
	SINE	AluYk12, AluYa5, FRAM [35]		
Sporadic Creutzfeldt-Jakob Disease (sCJD)	ERV	ERV-W, ERV-T, ERV-FRD, ERV-L, ERV-9 [51]		

Table 1. Retrotransposons reported as dysregulated in brain, cerebrospinal fluid (CSF) or plasma from patients with neurodegenerative disorders. A comprehensive summary of the retrotransposons identified in human neurodegenerative diseases, including retrotransposon subtype. Font in blue: transposable elements detected in human brain tissue, grey: transposable

elements detected in human cerebral spinal fluid, and **red: transposable elements detected in human serum**. Refer to references for full details of each study indicated.

Transposing elements into treatment

Based on the similarities between exogenous retroviruses and ERVs, numerous studies have investigated the therapeutic efficacy of anti-retroviral medications, including nucleoside analog reverse transcriptase inhibitors (NRTIs), to prevent transposable element expression and mobilization [10,44–46]. Current clinical trials for anti-retroviral therapy in the context of neurodegeneration include the “Lighthouse” study, an open label, multi-center study to investigate the safety and tolerability of Triumeq, a combination anti-retroviral therapy (dolutegravir, abacavir, and lamivudine) in ALS patients (Clinical Trials ID NCT02868580).

We originally became interested in the utility of lamivudine (3TC) [47], an NRTI that is FDA-approved for HIV and Hepatitis B, to suppress tau-induced retrotransposition based on studies reporting that lamivudine suppresses L1 retrotransposition at concentrations within the standard dosing range for HIV [44,48,49]. Similarly, we found that lamivudine suppresses tau-induced retrotransposition and consequent neurodegeneration in *Drosophila* [10]. Lamivudine is also reported to suppress consequences of L1 activation including the interferon-1 (IFN-1) response and senescence-secretory phenotype response *in vitro* and *in vivo* [45], suggesting that, beyond their effects on dampening retrotransposition, NRTIs limit production of retrotransposon-derived RNA or protein products.

Concluding thoughts

Despite continued controversy in transposable element biology and computational analyses, the studies described make a strong case that retrotransposition and retrotransposon-derived RNA and protein products are involved in human neurodegenerative disorders and are potentially pharmacologically targetable. We further propose that active retrotransposons provide reverse transcriptase activity that is required for somatic mosaicism of the amyloid precursor protein (APP) recently discovered by Lee and colleagues [50], and speculate that retrotransposon activation contributes to the anti-viral response in Alzheimer’s disease that has also been attributed to exogenous viral infection [51]. Continued studies focusing on the prevalence of retrotransposition in human neurodegenerative disorders, toxicity of retrotransposon-derived RNA and protein products, and the utility of anti-retroviral therapy will allow us to better understand the darker half of our genome and its involvement in neurodegenerative biology.

Conflict of interest statement

Nothing declared

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