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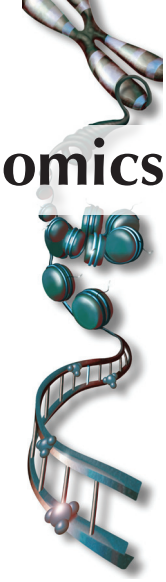
Epigenomics



The dynamic genome: transposons and environmental adaptation in the nervous system

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Classically thought as genomic clutter, the functional significance of transposable elements (TEs) has only recently become a focus of attention in neuroscience. Increasingly, studies have demonstrated that the brain seems to have more retrotransposition and TE transcription relative to other somatic tissues, suggesting a unique role for TEs in the central nervous system. TE expression and transposition also appear to vary by brain region and change in response to environmental stimuli such as stress. TEs appear to serve a number of adaptive roles in the nervous system. The regulation of TE expression by steroid, epigenetic and other mechanisms in interplay with the environment represents a significant and novel avenue to understanding both normal brain function and disease.

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When B McClintock discovered transposable elements (TEs) in maize over 60 years ago, the author noted that their activity seemed to be regulated in response to environmental stress [1,2] TEs make up about half the human genome, yet they have received just a fraction of the scientific attention that has been directed at protein-coding genes. This inattention is in part due to the belief that these elements were parasites or genomic clutter [3,4]. Historically, TE transposition was believed to be limited to germ cells, but new information provided by large consortia like ENCODE and FANTOM suggests TE expression is cell-type-dependent and affects expression of nearby genes. This discovery has led to an increased interest in the role of TEs in somatic cells [5,6]. TEs have implicated in serving both beneficial functions such as RNA splicing, editing and silencing; and in pathologies, such as hemophilia A, which may be caused by TE insertions in some cases, or macular degeneration, which results from ectopic overexpression of TEs [7–9]. The recent finding that TEs affect transposition and transcription has made TEs an emerging focus of neuroscience research [10].

TEs fall into two primary classes: DNA transposons, which are inactive in humans and function through a 'cut and paste' mechanism to change locations in the genome; and retrotransposons, which use 'copy and paste' mechanisms and RNA intermediates to create new copies of itself in the genome. Retrotransposons can be further subdivided into long terminal repeat (LTR) and non-LTR retrotransposons. The former group includes relics of previous infections acquired over the course of evolution called endogenous retroviruses (ERVs). The non-LTR retrotransposons include both short and long interspersed repeat elements (SINEs and LINEs) [11–13]. The most common TE class, LINEs, make up about 17% of the human genome and contain the necessary machinery within themselves to perform this 'copy and paste' mechanism independently by using an RNA intermediate to insert cDNA

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copy back into the genome. LINE1 (L1) contains two ORFs [14,15]. ORF1 encodes for an RNA binding protein while ORF2 encodes endonuclease and reverse transcriptase moieties that allow the TE to copy itself back into the genome at a new location [16]. For L1 to retrotranspose, a ribonucleoprotein complex must form between L1 RNA and proteins and translocate back into the nucleus [17]. The absence of the nuclear envelope during cell division eases this process, and, as would be predicted, studies have demonstrated higher rates of retrotransposition in actively dividing cell [18,19] Though most LINEs are incomplete and inactive on their own, L1 retrotransposition has been found in somatic cells, including neurons [20–24].

The transposition machinery encoded by active LINEs also permits the transposition of nonautonomous TEs, like the SINEs, which are dependent on L1 ORF2 machinery to transpose in the genome as they lack the endonuclease and reverse transcription machinery needed to retrotranspose independently [12,13]. SINEs include *Alu* elements, which make up about 11% of the human genome, and SVA elements, a 'composite' SINE made up of pieces of SINE-R, VNTR and *Alu*. This capacity to mix and match into new genomic elements is likely to be important to the capacity of the genome to evolve new noncoding RNA.

Several mechanisms regulate transposition and TE expression. DNA methylation at cytosines in TEs are a primary mechanisms of silencing TE transcription. Similarly, histone modifications, such as Histone 3 Lysine 9 trimethylation (H3K9me3), can maintain TEs in heterochromatin, effectively silencing them. Post-transcriptional mechanisms also regulate TE retrotransposition. For example, premature polyadenylation has been shown to lower retrotransposition by truncating human L1 full-length transcripts [25]. Apolipoprotein B mRNA editing enzyme catalytic polypeptide, an mRNA editing enzyme, has also been shown to regulate L1 and *Alu* element retrotransposition [26]. Apolipoprotein B mRNA editing enzyme catalytic polypeptide accomplishes this by sequestering *Alu* RNA in ribonucleoprotein complexes which prevents it from interacting with L1 machinery needed for retrotransposition [27]. Interfering RNA, which is involved in post-transcriptional silencing through cleaving of dsRNA by DICER into siRNA, is another mechanism through which TES are repressed [28]. In germ cells, Piwi-interacting RNA has been shown to regulate TEs in *Drosophila* [29]. Other mechanisms are involved in somatic cells, such as sequestration of L1 and ORF1 RNA in stress granules, which associate with processing bodies containing enzymes involved in RNA turnover [30]. As we will show, TEs have a variety of functions within the genome. Regulation of TE expression is extremely important as these functions include potentially positive effects at both the evolutionary and organismal level, as well as potentially pathogenic effects including genome instability and neurodegeneration. Significant evidence continues to emerge that environmental factors (including psychological stress and trauma) influence levels of transposition and TE expression, with potentially important consequences for the organism.

Benefits of TEs: contributions to fitness & function

Benefits of genomic reshuffling

The idea that all nonprotein-coding DNA is 'junk DNA' was the dominant view in the field since it was first proposed by Ohno [4], although it has become increasingly clear recently that this view is obsolete. Research has established many examples of TEs serving functional roles in mammals and other organisms. McClintock, discoverer of TEs (which she termed 'controlling elements'), believed that TEs have positive and negative traits as insertional mutagens and regulators of gene expression, possibly helping organisms deal with environmental stressors [2,31]. While the existence of transposition has long been accepted, McClintock's ideas about the adaptive utility of TEs were not well accepted at the time she broached them and are still the subject of substantial debate among specialists in the field [32]. TEs clearly contribute to genome evolution via mechanisms like exon shuffling and recombination [33–35], so the question is whether this benefits the host organism or not.

Retrotransposition has been present in the mammalian genome throughout evolution, suggesting that TEs provide some benefits to the host, otherwise they would have been selected against [12]. In fact, the host spends an exorbitant amount of resources replicating TEs, roughly 10-fold more than is needed to replicate protein-coding DNA [36]. It is counterintuitive that TEs come at such an enormous cost to the host but still survived throughout evolution without providing any benefits to the host. Indeed, in prokaryotic organisms, where speed of replication is a significant factor in survival, these elements are rapidly deleted from the genome [36].

One hypothesis for the potential utility of TEs is aiding the host in dealing with environmental stress by providing the host with genomic diversity. In a plant like *Arabidopsis*, for example, heat shock leads to an increase in transcription and copy number of Onsen, an LTR transposon that affects local gene activation in response to heat shock [37]. Offspring of heat shocked plants also have increased Onsen copy number, indicating transgenerational transfer of the genomic 'stress

memory'. Thus, genomic diversity induced by Onsen insertions in different locations in offspring genome in response to the original heat shock will lead to activation of different genes in response to new heat shock in offspring, increasing the chances of randomly activating an adaptive response to the stressor and thereby bettering the chance of survival. However, this process is tightly controlled via the siRNA pathway, suggesting that competition between Onsen and the host genome created a symbiotic relationship from one that was initially parasitic [34].

Transposon-driven genomic diversity could be important for organisms not capable of generating genomic diversity through other means (i.e., horizontal gene transfer) and would be especially important for organisms that do not have the capacity to learn by responding to external stimuli behaviorally (i.e., plants) [36]. As would be predicted based on this assumption, 80% of plant genomes (not capable of horizontal gene transfer or learning behavioral adaptations), 50% of human genomes (not capable of horizontal gene transfer but capable of learning behavioral adaptations) and less than 5% of prokaryotic genomes (capable of horizontal gene transfer) consist of TEs [14,38–42].

In the nervous system, genomic diversity driven by stochastic retrotransposition could lead to varying cellular phenotypes, which could translate to changes in synaptic activity, neuronal circuits and by extension, an increase in potential computational complexity and adaptive cognitive capacities [43]. The benefits of having a mechanism capable of limited reshuffling of the genome is well illustrated by the use of TE-derived molecular machinery by the mammalian immune system to generate antibodies via the V(D)J recombination system [44]. Retrotransposition in immune cells can efficiently produce a wider variety of antibodies than would be possible without reshuffling of the genome or having a substantially larger genome at a lower cost. Similarly, it is possible that neuronal genomic diversity could lead to more cellular phenotypes and more varied neural networks than would be possible otherwise, an idea championed by the Gage laboratory since their groundbreaking discovery of somatic retrotransposition in the adult mammalian brain a decade ago [22,45].

Benefits of somatic versus germline retrotransposition

Somatic retrotranspositions, as opposed to germ line retrotranspositions, do not get passed on to future generations, which seemingly contradicts the simple assertion that retrotransposition provides and evolutionary advantage [10]. However, it can be argued that the capacity for retrotransposition, specifically L1 activity, provides the advantage of genomic diversity and flexibility on a larger scale (including influences on nearby gene expression) and more rapid and directed change in an individual compared with other random sources of diversity, such as chemically induced point mutations [36,46]. It has also been suggested that L1 retrotransposition has been conserved to provide genetic diversity in germ line cells and neurons specifically throughout human evolution [10,47,48]. Therefore, the capacity for the genomic reshuffling through retrotransposition can be selected for, although individual transpositions may not. Thus, retrotransposition is of a piece, conceptually, with sexual reproduction as a means of enhancing fitness in changing environments via genomic rearrangement [49]. It is not clear that transposition has the other posited benefit provided by sex, which is purifying selection. Nonetheless it is interesting that the germline appears to be one of the sites of greatest TE activity in most organisms, suggesting that the phenomena of sexual reproduction and transposition may have some relationship. It is certainly plausible that active transposition in the germline enhances the generation of diversity that is provided by meiosis, and it may be for this reason that the germline is permissive for active TEs, but a definitive picture of this relationship awaits further research.

Benefits beyond genomic reshuffling

Of course genome reshuffling is not the only potential benefit of a properly regulated transposome; TEs provide a source of ready-made DNA and RNA motifs. TEs are a major source of promoter elements and transcription factor (TF) binding sites [50,51], particularly with regard to steroid receptors, most of which appear to be TE derived [52]. The contribution of TF binding sequences is particularly interesting in light of recent findings that heterochromatin is initiated and maintained by the binding of single TFs to specific DNA sequences, many of which appear to be retrotransposons [53,54]. This suggests that many TFs may have initially evolved in order to control TEs, and were only later co-opted to other roles in the regulation of gene transcription. If this is so, TEs have a much more significant role in genome evolution [38] and, in organisms with high genomic TE content, the mechanisms of genome evolution are less random and more driven along specific avenues than classically thought. An example of TE driven evolution in action is the mouse genome where as much as 10% of the mutations are derived from a single family of LTR class retrotransposons [55].

TEs also contribute to the evolution of noncoding RNA elements. The widespread application of next-generation sequencing has demonstrated that most of the genome is actively transcribed and much of this

RNA is predicted to be functional. Though the precise fraction of functional RNA remains undetermined, it is at least several fold higher than the fraction devoted to mRNA [56,57]. By some of the higher estimates as much as 20% of the functional genome is comprised of long noncoding RNAs (lncRNA) [56], most of which (~75%) contain TE-derived sequences, and few of which contain sequences derived from protein-coding genes (less than 0.5%) [58,59]. TEs and lncRNA show higher sequence and cell type specificity in their expression than most protein-coding genes, which may allow them to contribute significantly to the determination of the diversity of neural phenotypes [23,45,58,60,61].

TEs in the CNS

Neurons have more TE activity relative to most other somatic cells suggesting a unique role for TEs in the brain and in mammalian behavior [20,22,61]. While *de novo* L1 insertions do not appear have highly specific genomic target sequences, neuronal genes are more likely to be in open chromatin states in neurons because these are the genes being actively transcribed and because neuronal genes are typically larger in size relative to other genes [43]. Thus, new insertions are more likely to take place in or near neuronal genes more frequently due to this constraint on otherwise random insertion [61,62]. In fact, this was recently demonstrated by Bundo *et al.* [63] who found that individuals with higher L1 insertions in the prefrontal cortex often had insertions near genes important for neural connectivity and function. Since TEs can affect local chromatin state and the expression of nearby genes, it follows that insertions near neuronal genes would affect neuronal physiology.

Not only is the rate of insertions different among somatic tissues, but the rate of new TE insertions also varies between brain regions [64]. Estimations of insertion rate of LINE elements vary between fewer than 0.6 insertions per neuron in the cortex and caudate nucleus to 80–800 new insertions per neuron in hippocampal neurons [20,64]. A more recent study found 13.7 somatic L1 insertions in hippocampal neurons using single cell RG–Seq [65]. These insertions were found near genes specially related to hippocampal neurons and glia supporting the hypothesis that somatic insertions influence function [65]. Accurately detecting insertion rate between single cells in the brain constitutes a technical challenge, and differences in estimation of L1 insertions between these studies can be attributed to differences in methodologies, as well as genuine differences in insertion rates between brain regions. The estimation of less than 0.06% insertion rate by Evrony *et al.* [64] using genome-wide L1Hs insertion profiling of 300 single neurons provides a lower bound estimation while the estimation put forth by Upton *et al.* [66] using RG-Seq provides a likely upper bound. Kurnosov *et al.* [66] conducted the first study comparing rate of insertions between various brain regions of one individual using sequencing and mapping methods and found that the rate of L1 insertions were similar between the cerebellum, cortex, subventricular zone and myocardium (0.058–0.063%). However, the rate of new insertions was higher in the dentate gyrus (DG) of the hippocampus (0.093%), implicating the DG as hotspot for generating new L1 insertions, in line with previous observations [22,67]. These findings differ from previous observations in some of their particulars [64,68] and suffer from a limited sample size, but they do support the idea that the hippocampal formation is a particularly active brain region in terms of retrotransposition.

TE activity can be influenced by environmental factors, such as stress, cocaine, alcohol, heat and exercise, which may also lead to differences in rates of insertions in the aforementioned studies [67,69–74]. Future studies are needed to look at differences both between brain regions in the same individual and compare the same brain regions across individuals to gain a better sense of what the typical insertion rate is.

The finding that the rate of insertions is higher in the hippocampus supports other research implicating L1 transcription during differentiation from neural progenitor cells (NPCs) into neurons and glia, given that the DG of the hippocampus is a site of neurogenesis in adults [20,22,75]. The L1 promoter contains binding site for several factors also involved in neurogenesis (e.g., SOX2, T cell factor/lymphoid enhancer factor), so interaction between these factors during neurogenesis and differentiation may lead to increased L1 transcription [76]. L1 retrotransposition during neurogenesis leading to unique genomes in new neurons would contribute to overall genomic diversity in the brain which may affect gene expression and neuron phenotype [43].

Epigenetic regulation of TEs

While high levels of TE retrotransposition increase genomic diversity, it also increases the chance that a TE copy gets inserted into or near a gene necessary for cell survival, possibly inhibiting gene expression or proper protein function. Transposition and TE expression are highly regulated through epigenetic and other regulatory mechanisms, including post-transcriptional silencing by DICER and siRNA, to prevent uncontrolled retrotransposition from leading to genomic instability. In stem cells and the germline, both histone methylation and DNA methylation predominate in the control of TE expression [77]. In adult cells, regulatory mechanisms appear to vary between different tis-

sues and brain regions [62,78]. TEs are often suppressed through DNA methylation at CpG sites, but are also regulated through small RNA-mediated regulation and post-transcriptional modifications [7,62,76,79].

Chromatin state through histone modifications is another mechanism by which TE transcription and retrotransposition is controlled. H3K9me3 is a well-established histone modification associated with facultative heterochromatin and transcriptional silencing, including silencing of TEs. Both H3K9me3 and Histone H3 lysine 27 trimethylation have been associated with silencing of TEs in a number of cell types, but appear to target different classes of TEs with H3K9me3 targeting the more active classes of retrotransposons [73,80]. This is associated with the transcriptional repression of TE expression in the rat brain after environmental stress, where the Suv39h2 methytransferase appears to be the active methyltransferase [73]. A recent study demonstrated that Setdb1, an H3K9-specific methyltransferase important for maintenance of stem cells [81], also seems to play an important role in the establishment and/or maintenance of H3K9me3 and DNA methylation at some LTR and L1 elements in germline and stem cells [82–84]. In the latter paper, a previously established association between H3K9 methylation and the KAP-1/TRIM28 repressor complex was observed. TRIM28 has been shown to regulate the expression of TEs, like the intracisternal A particle (IAP) elements in mouse stem cells [85]. TRIM28 deletion was associated with reduction in H3K9me3 and an increase in H4 acetylation. Furthermore, DNA methylation was shown to work synergistically with TRIM28 to repress IAP transcription. This effect is only seen in embryonic cells; TRIM28 deletion in differentiated somatic cells does not affect ERV expression presumably because DNA methylation during embryogenesis permanently repressed those elements [86]. However, TRIM28 may have a unique function in NPCs relative to other somatic cells because TRIM28 continues to be required for ERV suppression through histone modifications in NPCs [86]. The mechanism by which H3K9me3 might be able to suppress TE transcription in differentiated neurons may bear similarity to that recently proposed by the Jenuwein group for TF targeted and Suv39h-mediated suppression of active transposons in mouse embryonic stem cells [53,54]. They state that this effect is restricted to stem cells, but their observations fit with our own in the adult rat hippocampus [73] though the details of the picture in the adult brain are as yet less clear than in stem cells.

In the brain, the L1 promoter repressor complex includes SOX2 and HDAC1, which is known to associate with the transcriptional repressor methyl CpG binding protein 2 (MeCP2). During neuronal differentiation, there is a switch from this repressor complex to T cell factor/lymphoid enhancer factor which promotes L1 transcription through chromatin remodeling [75,76]. Lower levels of MeCP2, induced through siRNA or full knock out, lead to increased L1 transcription and retrotransposition, implicating MeCP2 as an important L1 repressor [76]. Promyelocytic Leukimia Zinc Finger protein (PLZF, also known as ztb16) is a member of the POZ and Kruppel zinc finger family and is known to orchestrate local epigenetic changes resulting in repressed transcription through chromatin remodeling. PLZF recruits nuclear corepressors, histone deactylases and DNA methyltranferases to accomplish this [87–89]. Puszyk *et al.* [90] demonstrated that PLZF induces DNA methylation and, using ChIP, showed that 57.5% of PLZF binding is within L1 elements. The authors go on to suggest that PLZF may help 'guide' MeCP2 to the L1 promoter. However, because this study was conducted in hematopoietic cells and not NPCs, it is unknown whether this mechanism is generalizable to the brain.

Stress & epigenetic regulation of TEs

DNA methylation, histone modifications and TFs certainly seem to play a role in regulating TEs but external factors are also important to consider. For instance, exercise influences L1 retrotransposition in NPCs [58] and stress exposure alters L1 transcription [63], providing evidence of changes in regulation of due to environmental factors. Given evidence that stressful stimuli can induce epigenetic changes which affect gene expression, perhaps stress influences TE expression via similar epigenetic mechanisms.

The hippocampus is not only a region with high rates of retrotransposition, it is also particularly susceptible to the effects of stress because of its high level of glucocorticoid receptors (GRs). GRs in hippocampus are part of the negative feedback system for the hypothalamic pituitary axis that is responsible for physiological stress response. GRs, once bound with CORT (cortisol or corticosterone) dimerize, enter the nucleus and act as transcription factors to regulate gene expression. Chronic stress, marked by chronically high levels of CORT, can lead to an overall reduction in hippocampal volume explained by dendritic atrophy [91]. This volume reduction is accompanied by deficits in memory and cognitive flexibility [92,93]. Acute stress response is influenced by past experiences with stress. For example, Gray *et al.* [94] showed that animals with no stress history shared only 10% of the change in gene expression following a stressful swim task with animals previously exposed to chronic stress. Therefore, it seems that the 'memory' of stressful experiences

are somehow maintained and affects response to subsequent stressors. Epigenetic marks are an ideal target for coding stress memories as they are sensitive to environmental stimuli but also stable enough to be maintained and impact future stress reactivity [36,95–96].

Acute stress leads to an increase in H3K9me3 in the hippocampus for up to 7 days following stress exposure [97]. On the other hand, the effect of chronic stress (21 days daily stressor) on H3K9me3 mark was small, suggesting habituation after the first 6 days of stress exposure [97]. H3K9me3 increase implies a local reduction in transcription, a change that has been suggested to help provide genomic stability following stress exposure [36,98]. This finding contrasts with a study that found that a heat shock stressor actually lead to increased transcription of B2 and *Alu* [70,99–100]. The distinct effects of different stressors on SINE expression are perhaps unsurprising given our observations that different stressors produce substantially different patterns of transcriptions within the same tissue [94]. Perhaps more importantly, given the capacity of B2 and *Alu* SINEs to globally block RNA polymerase II mediated transcription [8], the difference makes functional sense. During heat shock, blocking protein production before translation prevents the production of misfolded proteins, which have been implicated in a variety of neurodegenerative disorders. However, during a stressful event, the adaptive priority in the hippocampus the would be the preservation of memories of the stressor and associated stimuli so they can be remembered and avoided in the future, requiring the continued function of the protein synthesis machinery [101].

The role of steroid receptors like GR in the regulation of transcription is well described. As mentioned above, many steroid response elements are TE derived, and response elements for GR, progesterone and vitamin D receptors all derive from *Alu* class SINEs in the human genome [102,103]. The fact that a primate lineage specific TE like *Alu* is responsible for most steroid binding sites in our genome suggests promoter evolution is less constrained than coding sequence evolution, as it indeed is, but lineage-specific exaptation of TEs for the production of novel regulator sequences may be a significant mechanism of speciation. Interestingly, steroid receptors, like TEs, have also been implicated in chromosomal translocations, and they may act synergistically with TEs (like LINE elements) to do so [104,105]. The steroid–transposon association extends to the tissue level, steroidogenic tissues like the brain, gonads, adrenals and placenta which all show high levels of transposition and TE transcription [36,106–108]. Steroids can activate TE transcription in a number of tissues [36]. It is plausible that sex hormone receptors activated by sex hormones can influence chromatin state and TE transcription and activity. Differences in TE activation or abnormal steroid-receptor-dependent regulation of TEs may provide understanding as to why one sex is more vulnerable to the development of a specific disorder, such as autism spectrum disorder, which is diagnosed at least four-times more often in males than in females [109]. This hypothesis is supported by the finding that schizophrenic brains show higher levels of L1 activity than controls [63]. Schizophrenia shows substantial differences in age of onset between males and females and is also influenced by early life stress and inflammation, both of which can involve altered glucocorticoid activity [36]. This area is not well researched but may offer important insights as mechanisms behind observed sex differences in prevalence of developmental disorders.

Dys-regulation of transposon activity in disease

Abnormal TE activity in individuals could manifest as altered transcription of TEs or as an increase in number of new insertions which may then affect gene expression either by modifying local chromatin state or inserting near or within a gene. Understanding the mechanistic role of TEs in mental disorders cannot only help elucidate the ontogeny of these disorders, but may also expose the role of TEs in the normally functioning brain. Similarly, we can learn from studies of TE function in aging. Some of these studies suggest that TE expression and insertion increases with age and results in decreased function of neurons with new insertions [43]. In *Drosophila*, increased expression of LINE-like elements R1, R2 and Gypsy (an LTR transposon) is associated with typical aging [79]. The increased expression of Gypsy is also associated with increased transposition [79]. TDP43 is a transcriptional repressor that can bind to both DNA and RNA and abnormal function has been implicated in amytrophic lateral sclerosis. TDP43 has been demonstrated to bind to transcripts from LTR, LINE and SINE TEs, repressing their activity [110]. Furthermore, frontotemporal dementia was associated with reduced TDP43-TE binding in human cortex relative to healthy controls [110]. TEs have been implicated other age-related degenerative disorders. For example, loss of control over *Alu* RNA expression by DICER1 during aging produces macular degeneration [7]. Together, these studies suggest multiple mechanisms involved in regulation of TEs and changes in these mechanisms with neuronal decline during aging and neurodegeneration.

At the other end of the lifespan, experience during development can have lasting effects on physiological stress response throughout life and may influence epigenetic regulation of TEs and account for some individ-

ual variation in vulnerability of developing some mental disorders. Stressful experiences during early life lead to stable epigenetic marks on stress-related genes. Experiences including abuse, neglect and more subtle stressors, also increases vulnerability to developing mental disorders (such as anxiety and depression) and health issues (like cardiovascular disease and diabetes) [111]. In fact, exposure to adverse experiences in childhood has been shown to be a predictor for the development of post-traumatic stress disorder (PTSD) in combat troops [112]. However, there are variable levels of resilience between individuals since not all individuals who experience adverse events during early childhood end up with these stress-related health issues. Genetic studies have attempted to point to gene variants that account for this variation in vulnerability. Although a few have identified genetic risk factors for disorders such as schizophrenia and PTSD, there is still a large amount of variability unaccounted for. This missing variability is also reflected in studies that use organisms with high genetic similarity but that have variable stress resilience. TE activity affecting gene regulation could be a missing piece, either due to novel insertions of TE or due to the regulatory effect of TE RNA. Differential methylation of LINE and other TEs has been implicated in PTSD risk in the combat exposed veterans [113] and in an animal model of the disorder [71], support this idea at least in variability of PTSD resilience.

Substantial evidence suggests that stressful experiences can lead to changes in TE activity, particularly in the hippocampus, the primary site of neurogenesis. The hippocampus contains many GRs as part of the stress hypothalamic pituitary axis negative feedback loop and is also involved in encoding memories of stressful experiences so stressful events and associated stimuli can be avoided in the future. Reduced hippocampal volumes have been linked to PTSD, further implicating the hippocampus as a region potentially involved in the development of PTSD [114,115], though it must be said that the connection between hippocampal volume and PTSD is neither linear nor likely to be causal. The upregulation of H3K9me3 seen in response to acute stress may be a part of the normal genomic stress response, silencing genes and presumably TEs in the hippocampus [36,73,98]. This typical genomic response to stress may help to properly encode the memory associated with the stress. If this mechanism of genomic silencing is dys-regulated in individuals with PTSD, proper memory encoding may be inhibited leading to the behavioral symptoms associated with the disorder. Contrastingly, stress-enhanced fear learning, a model of PTSD, resulted in upregulation of L1 of transcripts in the amygdala in rodents [71]. The difference in findings between these studies could be due to differences in type of stressor, differences in magnitude of stress or could suggest differing genomic stress responses in the amygdala and hippocampus.

Further support for the hypothesis that TE activity is dys-regulated in mental disorders comes from a recent study that used whole-genome sequencing to demonstrate increased L1 insertions in individuals with schizophrenia in the prefrontal cortex [63]. This effect was replicated in mice using Poly-IC injection, an accepted model of schizophrenia which activates a maternal immune response [116] and in neonatal primates using EGF, which has a similar impact on neural development [117]. The resulting increase in L1 copy number in offspring prefrontal cortex of Poly-IC treated mice and prefrontal cortex of EGF treated primates provides evidence that early exposure to some environmental insults could alter levels L1 retrotransposition [63]. Interestingly, no change in MECP2 or SOX2 was seen, indicating that another regulatory mechanism is likely behind the observed increase in L1 activity, than those previously described by Moutri and Gage [63,67,76]. These mechanisms could include either histone modification or the RNA silencing machinery, though the question awaits further experimental analysis. Environmental influences, such as viral exposure, are important in inducing vulnerability to developing schizophrenia [118]. Perhaps environmental factors and genetic factors, which either increase or decrease vulnerability, interact and lead to loss of regulation of L1 activity, which contributes to the pathogenesis of schizophrenia. We have argued elsewhere that the inflammatory process that appears to precede the development of psychotic symptoms in schizophrenia [119] could result from the over activation of cellular immune responses due to elevated levels of TE RNA, a hypothesis which is compatible with the increased levels of L1 activity reported by Bundo *et al.* [36,98]. Interestingly, the Bundo paper observed less pronounced elevations in L1 transposition in both major depression and bipolar disorder, suggesting that dysregulation of transposition might be a common feature of major mental disorders. If this is so it will have to be determined if this is an epiphenomenon of another process or a causative mechanism in these disorders.

The importance of developmental timing of exposure to environmental risk factors and consequences for TE regulation in the brain has yet to be explored. Likewise, based on evidence suggesting different mechanisms for TE regulation in different brain regions, the impact of environmental insults on TE regulation across brain regions has not yet been determined. Although it is attractive to propose that TE dysregulation induced through specific environmental factors leads to the development of mental disorders, in

reality the process is likely exponentially more complicated and dependent on timing, brain region, presence of compensatory mechanisms and changes in chromatin state that lead to more global epigenetic regulation of TEs and genes.

Conclusion & future perspective

Given their genomic ubiquity, it is surprising that the potential functional significance of TES has remained largely unexplored for so long. Recent years have marked a sea change in this regard, particularly in the neurosciences, where observations of active transposition in the adult mammalian brain have opened the door to the serious examination of these elements in the function of the nervous system. It seems clear, even at this early stage, that these elements do have a number of functions in the brain and that more roles for them await discovery. It is also clear, that along with other steroidogenic tissues, the brain is unusual in its level of TE activity, which implies that the brain has specialized uses for transposons.

It has also become evident that TE function in the brain is regionally specific and complex. The hippocampal formation in particular shows much higher levels of activity than the other brain regions thus far examined. The hippocampus is particularly sensitive to stress and glucocorticoid stress hormones, and these factors are also able to influence the behavior of TEs within the hippocampus in a fashion that has some of the hallmarks of an adaptive response to environmental inputs. The mechanisms of steroid receptor–TE interaction, still only circumstantially described, deserve

Executive summary

Benefits of transposable elements: contributions to fitness & function

- Genomic reshuffling in the brain through retrotransposition, which could lead to changes in cellular phenotype, is a potential advantage of active transposition in coping with environmental stressors.
- Somatic retrotranspositions may not get passed on to future generations, however, the capacity for reshuffling is, and this advantage of genomic diversity and flexibility can be selected for throughout evolution.
- Transposable elements (TEs) likely had a significant role in the evolution of the genome with contributions to the evolution of transcription factors and noncoding RNA elements.

TEs in the central nervous system

- Higher rates of retrotransposition have been found in the brain relative to other somatic tissues
- The estimates of retrotransposition in the brain range from less than one to hundreds of new insertions in neurons. Regional differences, differences in methodologies between studies and environmental influences are likely to contribute to this variance.
- Studies have found evidence of increased rates of L1 retrotransposition in the hippocampus, lending support to the hypothesis of increased retrotransposition during neurogenesis to generate genomic diversity.

Epigenetic regulation of TEs

- TEs are regulated through DNA methylation and histone modification, including H3K9me3 and H3K27me3.
- The epigenetic regulators MeCP2, TDP-43 and TRIM28 have recently emerged as possibly having roles in regulating TEs in the brain.

Stress & epigenetic regulation of TEs

- Past experience with stress influences subsequent genomic response to stress as evidenced by changes in gene expression after multiple stressors.
- Epigenetic regulation of TEs in response to stress appears to be dependent on type of stress.
- Understanding the role of steroid receptor in regulation of TEs may help explain differences between sexes in prevalence of certain developmental disorders.

Dys-regulation of transposon activity in disease

- Abnormal TE expression, TE transposition and abnormal levels of regulating factors such as TDP43 and DICER1 have been found to be associated with aging, neurodegeneration and mental disorders such as post-traumatic stress disorder and schizophrenia.
- Effects of TE regulation, expression and transposition are promising avenues of research to explain the variability in vulnerability to developing mental disorders such as post-traumatic stress disorder not accounted for by genetic variants

Conclusion & future perspective

- Research on the functional significance of TEs is in its infancy, although emerging evidence suggests an important role for TEs, especially in the brain where TE function seems to be regionally specific and responsive to environmental factors like stress.
- Understanding the role of TEs in the brain has significant potential to help clarify our understanding of the development of mental disorders not explained by genetic variants.
- More mechanistic experiments are needed to elucidate the relationship between transcription factors and TEs.

further exploration. It is certainly plausible to argue that steroid hormones, which are responsible for directing global physiological and developmental programs within an organism, would also play a role in regulating the activity of TEs (or TE-derived ncRNA) in the global regulation of somatic genomes.

TEs have long been implicated in diseases like cancer but more recently evidence has emerged implicating TEs in disorders of the nervous system as well, notably in schizophrenia and PTSD. The interaction of these elements with stress and sex steroids is congruent with a role for TEs in the effects of sex and stress in the expression of mental disorders. However, none of this evidence has clearly delineated a causal role for TEs in these disorders. Describing such links is an important goal for the field going forward, as TEs, due to their heritability and their individual diversity, offer a potential avenue to explain at least part of the problem of 'missing heritability' in mental disorders [120]. They may also provide us with a novel mechanism for brain aging, as a number of lines of evidence now implicate TEs in neurodegenerative disease [7,10,79,110,121].

The regulation of TEs remains incompletely understood, but it is clear that many of the epigenetic mechanisms, which have garnered scientific attention in recent years are directed in part toward the control of these elements. DNA and histone methylation, noncoding RNA and RNA interference mechanisms have all been implicated in the control of TEs in one context or another. TEs also appear to be regulated by a number of transcription factors, like GR and Sox2, but a comprehensive picture has yet to emerge. The inferential nature of much of the data we have is a limiting factor, and more mechanistic experiments will help clarify the picture. What has begun to emerge is the importance of these elements for the evolution of both the genome and the epigenome, casting a light on an evolutionary process within the genome every bit as complex as the evolution of the species to which it belongs in the larger world without.

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