Chapter 8
Stress, Transposons, and the Brain Epigenome

Richard G. Hunter

Abstract Long thought to be transcriptionally silent junk, transposable elements (TEs) are emerging as sources of functional elements in mammalian genomes due to the introduction of modern deep sequencing techniques. They have begun to attract the attention of neuroscientists due to the observation that the brain appears to be a privileged environment for transposon activity. In the brain, TEs show active transposition and frequently interact with the epigenetic machinery during development and in response to environmental inputs like stress. Barbara McClintock, the discoverer of TEs, long asserted that these elements were an important part of the genomic control apparatus, particularly in response to stress to the organism. Recent work has shown that this observation was a prescient one, as stress shows the capacity to alter the activity of these elements in the brain, in some cases with both adaptive and pathogenic consequences. TEs have been recently implicated in a number of mental disorders including Rett syndrome, posttraumatic stress disorder (PTSD), and schizophrenia. TE-derived regulatory RNA may comprise one of the largest single classes of functional elements in our genome, a discovery which will have a profound effect on how gene-environment interactions are understood within the context of the nervous system and beyond.

Keywords Retrotransposon • Glucocorticoid • Steroid • Noncoding RNA • Mental disorders • Neurodegeneration

R.G. Hunter
Department of Psychology, University of Massachusetts Boston, Boston, MA, USA

Laboratory of Neuroendocrinology, The Rockefeller University, New York, NY, USA
e-mail: richard.hunter@umb.edu

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8.1 Introduction

Stress plays a significant role in a number of mental and physical disorders and can have lasting effects upon the brain and behavior. Within the brain, stress has been shown to effect neural plasticity in a variety of ways with clear behavioral consequences for the stressed organism. How stress can have such persistent effects, some of which span generations, has been the subject of intensive research much of which has focused on epigenetic mechanisms in recent years. Epigenetics offers a clear explanatory framework for how environmental inputs like stress can have long-lasting, even heritable effects without inducing changes in the structure of the genome itself. However, as research going back to Barbara McClintock's groundbreaking work in the 1940s and 1950s has shown, the genome itself is more dynamic than the classical understanding of genetics would have it. McClintock discovered a class of mobile genomic elements, which she called "controlling elements" which could reshuffle parts of the maize genome with heritable effects on plant phenotype. Most importantly for the present discussion, she noted that these effects were most often induced by stresses or "shocks" to the plant (McClintock 1951, 1984). Transposable elements (TEs) or transposons have since been found in the genomes of almost every taxon of life, including mammals where they make up roughly 50 % of the genome on average. While long regarded as "junk" DNA, these elements have recently attracted the attention of neuroscientists who have shown that they are highly active in the brain and that they appear to be under some degree of epigenetic control, findings which have substantial implications for our understanding of brain function and brain disorders (Hunter et al. 2013, 2014; Reilly et al. 2013; Erwin et al. 2014; Griffiths and Hunter 2014).

8.2 Transposons

Transposable elements (TEs) are a diverse class of genomic elements that share the capacity to move themselves from one genomic location to another. They have also played a significant role in genome evolution, genome structure, and cell fate determination. More recently it has become evident that many of them are actively transcribed and that some transposon-derived RNAs, like the Xist (X-inactive specific transcript) lncRNA, which governs X-chromosomal inactivation, play significant functional roles in both development and disease (Pontier and Gribnau 2011). Arguments asserting that these elements are merely parasitic or junk are based in part on Ohno's assertion that a maximum of 20,000 coding loci were possible based on estimates of the rate of deleterious mutations available at the time (Ohno 1972). However, these rates were likely overestimated and were, moreover, based on the assumption that protein sequence was the major molecular target of selection. It is now evident that most of the genome is actively transcribed and that much of this transcribed RNA may be the product of RNA genes, rather than the protein-coding genes circumscribed by the central dogma (Consortium 2012; Mouse et al. 2012; Fu 2014).

Transposons are divided taxonomically into DNA transposons and RNA transposons or retrotransposons (RTs); the former use a mechanism similar to cutting

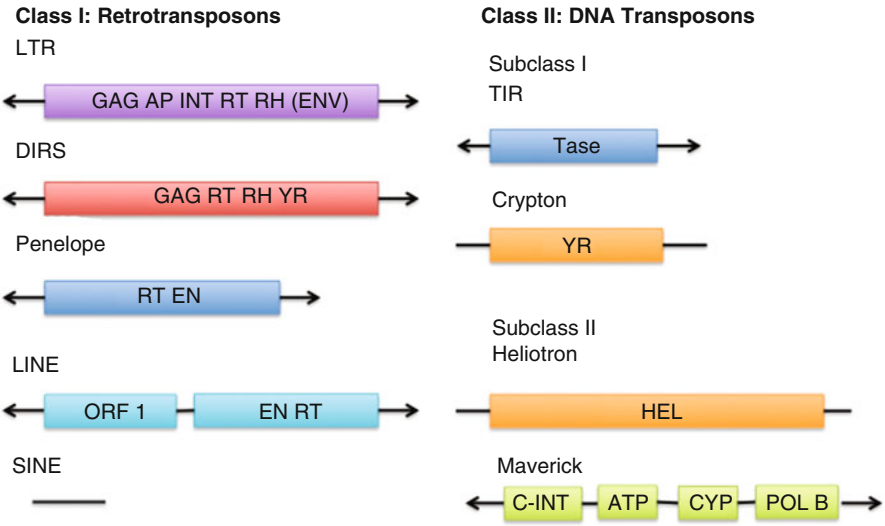

Fig. 8.1 Classification of transposable elements (TEs). TEs are subdivided into class I or II based on the requirement for an RNA intermediate in the transposition process. Class I retrotransposons are further subdivided into the LTR (long terminal repeat/endogenous retrovirus), DIRS (*Dictyostelium* intermediate repeat sequence), Penelope, LINE (long interspersed nuclear element), and SINE (short interspersed nuclear element) superfamilies based on their internal architecture and terminal repeat sequences. DNA or class II transposons are divided into two subclasses (1 and 2). Subclass 1 contains the TIR (terminal inverted repeat) and Crypton superfamilies, and subclass 2 contains the Heliotron and Maverick superfamilies. Protein-coding domains: *AP* aspartic protease, *ATP* packaging ATPase, *C-INT* C-integrase, *CYP* cysteine protease, *EN* endonuclease, *ENV* envelope protein, *GAG* capsid protein, *HEL* helicase, *INT* integrase, *ORF* open reading frame, function undetermined, *pol B* DNA polymerase B, *RH* RNAse H, *RT* reverse transcriptase, *YR* tyrosine recombinase. This classification is a simplification of the system proposed by (Wicker et al. 2007)

and pasting in text editing, while the latter typically copy and paste using RNA intermediates (See Fig. 8.1). Both varieties can be either autonomous or nonautonomous depending on whether or not they encode their own transposition machinery or not (Wicker et al. 2007; Levin and Moran 2011). Due to the relatively low fidelity of most transposases, many nonautonomous TEs are incomplete or missense duplications of autonomous TEs. Both major taxa are further subdivided into subclasses, orders, and so on down the line through superfamilies and families to individual TEs. RTs constitute the single largest class of genomic elements in mammalian genomes and are subdivided into five subclasses which include long interspersed nuclear elements (LINEs), short interspersed nuclear elements (SINEs), long terminal repeat or LTR transposons, DIRS (*Dictyostelium* intermediate repeat sequence), and Penelope-like RTs. LINEs, SINEs, and LTRs represent roughly 18 %, 12 %, and 8 % of the human genome, respectively, while DNA transposons and other varieties of RTs represent less than 3 % (Cordaux and Batzer 2009). LINEs, in their intact form, are approximately 6 kb in length and encode one or two open reading frames (ORFs), one of which codes for a reverse transcriptase and an endonuclease (Wicker et al. 2007). In the most common human

LINE, L1, the first ORF also codes for a nucleic acid-binding protein whose function is not completely understood (Goodier et al. 2013). Of the roughly 500,000 L1 elements in the human genome, less than 100 appear to be intact and capable of causing transposition (Brouha et al. 2003). The remainder depends on these few for the chance to mobilize, as do all of the SINE elements. These elements, of which *Alu* and SVA are most common in humans, are too short, at less than 200 bases in length, to code for their transposition machinery. LTR retrotransposons include endogenous retroviruses that have invaded genomes in cycles over millions of years. Like the LINEs, most are inactive though some retain the capacity to transpose, and a few, like the IAP elements in rodents, are still capable of forming functional virus particles (Sharif et al. 2013).

While the prevailing view has been that transposons are parasitic, and it is inarguable that some of them certainly have a parasitic origin, it has been asserted since McClintock's discovery of these "controlling elements" that they play a useful role in the genomes that contain them (McClintock 1951, 1984). It has long been evident that they have played a significant role in genome evolution (Kazazian 2004). The LTR class alone is responsible for an estimated 10 % of all spontaneous mutations in the mouse genome (Maksakova et al. 2006). Transposition is a source of exon shuffling and recombination, significant means in the creation of new genes and genomic elements (Moran et al. 1999; Abrusan and Krambeck 2006). With the advent of large-scale sequencing, it has become apparent that many of these nonprotein-coding elements are actively transcribed, and some such as Xist and HOTAIR (HOX antisense intergenic RNA) have been shown to have roles in normal physiology as well as disease (Rinn et al. 2007). Indeed, the ENCODE project now identifies many transposon-derived long noncoding RNAs (lncRNAs) as genes comprising as much as 20 % of the functional genome (Harrow et al. 2012). It remains controversial what fraction of lncRNAs are transposon derived versus having an origin as protein-coding genes or other nongenic elements of the genome. However, it is clear that a substantial fraction of vertebrate lncRNAs (65–85 %) contain TE-derived sequences and that relatively little sequence is contributed by protein-coding sequences (less than 0.5 %) or UTRs (less than 30 %) (Kelley and Rinn 2012; Kapusta et al. 2013). TEs and lncRNAs are also more sequence specific in their expression than protein-coding genes, suggesting that they could play a significant role in the determination of the extraordinary variety of neuronal phenotypes (Cabili et al. 2011; Gage and Muotri 2012; Kelley and Rinn 2012; Reilly et al. 2013). Transposons also show much higher levels of interindividual variation than protein-coding genes (see below), which means they may contribute to some of the wide variance in susceptibility and resilience seen in many complex diseases.

8.3 Transposons and the Brain

Neuroscientists have turned progressively more attention toward the role of transposable elements in the brain as the technology of next-generation sequencing (NGS) has made it possible to observe them in detail. Before the NGS era, transposons had

been connected to a variety of human disorders, such as hemophilia and cancer (Kazazian et al. 1988; Solyom et al. 2012), as well as in the proper function of the immune system where the V(D)J system responsible for antibody diversity is derived from TEs (Kapitonov and Jurka 2005) and in the telomeres that are maintained by a TE-derived enzyme, telomerase (Nakamura and Cech 1998). Telomeres also utilize many of the same types of noncoding RNA/heterochromatin interactions that obtain in TE silencing. Interest in brain TEs was low until the observation that L1 LINE transposition occurs in mammalian neurons, particularly during development and neurogenesis, and that the brain is, therefore, more of a genetic mosaic than had been thought. It has been theorized that this mosaicism provides beneficial neuronal diversity in an analogous way to the use of transposon-derived reshuffling methods present in the immune system (Muotri et al. 2005; Baillie et al. 2011; Gage and Muotri 2012). This process is regionally specific, as neurogenesis in the adult brain is restricted to the hippocampus and rostral migratory stream, whereas transposition rates in the cortex appear to be relatively low (Evrony et al. 2012). In the hippocampus, the rate of transposition is sensitive to environmental interventions like stress and exercise (Muotri et al. 2009) and to be controlled in part by elements of the molecular epigenetic machinery like MeCP2 (methyl CpG binding protein 2) (Muotri et al. 2010). It is thought that, by promoting greater fitness in the organism, TEs increase the likelihood of their propagation, which is the foundation of symbiosis (Reilly et al. 2013; Erwin et al. 2014). Indeed, it has been argued in a number of contexts that TEs exist in a symbiotic relationship with host genomes though the point remains unresolved (Ryan 2004; Upton et al. 2011; Hunter et al. 2013, 2014).

TEs have certainly contributed to the evolution of gene promoters, particularly with regard to steroid response elements, the binding sites where steroid hormone receptors interact with DNA (Cotnoir-White et al. 2011). The brain, like other steroidogenic tissues (such as the placenta and gonads), seems to be a privileged site for TE activity (Pillai and Chuma 2012; Hunter et al. 2014; Ross et al. 2014). In humans and other primates, Alu SINEs have contributed response elements for vitamin D, progesterone, and glucocorticoid receptors (Gombart et al. 2009; Jacobsen et al. 2009). Steroid receptors themselves have been implicated in chromosomal rearrangements similar to those produced by transposons and may act in concert with TEs to do so (Lin et al. 2009; Holzman 2010). This suggests that steroids may act to activate some TEs, as indeed they do in the few studies that have sought to examine this phenomenon. Androgens increase LINE expression, and the L1 LINE ORF-1 protein seems to interact directly with the androgen receptor itself in prostate cancer cells to activate AR gene targets (Morales et al. 2002; Lu et al. 2013). TEs represent a source of both genomic and phenotypic variance at the cellular level that steroids may interact with to shape and canalize across the course of development and within the emergency life stage represented by stress. Indeed, it recently has been suggested that TEs may act in this way in the sexual differentiation of the brain as well as in gonadal development (McCarthy et al. 2015). Glucocorticoid stress hormones have been shown to upregulate the expression of SINE elements in the liver as well (Sun and Frankel 1986). Steroids are in part responsible for orchestrating responses to environmental conditions across the organism, so their potential

to mobilize TEs as sources of genomic restructuring, as well as potential RNA diversity, is an exciting area deserving further exploration, nowhere less so than with regard to stress and the stress response.

8.4 Transposons and the Epigenome

Molecular epigenetic mechanisms include histone modification, DNA methylation, and noncoding RNA. Given that many ncRNAs are transposon derived, the epigenetic role of these elements is established. However, this is not the limit of transposon-epigenome interactions. Transposons are important determinants of heterochromatin domains, and their RNAs are directly involved in the regulation of chromatin state (Bodega and Orlando 2014). Perhaps the best-known example of the interaction of transposable elements with the epigenome is the agouti locus in the mouse. The DNA methylation status of this locus controls coat color in the mice that carry it, and the core methylation site is derived from an IAP ERV/LTR retrotransposon (Michaud et al. 1994; Morgan et al. 1999). IAP elements, unlike the majority of ERVs, retain some capacity for producing infectious virus particles and are highly active in the mouse genome (they are thought to be responsible for as much as 5 % of the mutations) (Maksakova et al. 2008; Ribet et al. 2008). Because IAP elements are incompletely tamed, they are under tight epigenetic control, particularly during conditions of stress. In addition to DNA methylation, these elements also seem to be marked with the repressive histone H3 lysine 9 trimethylation (H3K9me3), which appears to be the major brake on their expression as deletion of the repressor, TRIM28/KAP1, results in IAP overexpression. TRIM28 (tripartite motif-containing 28) is responsible for maintenance of H3K9me3 as well as DNA methylation at IAP and other ERV/LTR loci (Rowe et al. 2010, 2013; Hunter et al. 2012). With regard to heterochromatic control of TEs, it appears that both facultative and constitutive heterochromatic marks, histone H3 lysine 27 trimethylation (H3K27me3) and H3K9me3, are involved, though they appear to target different classes (Day et al. 2010). Similarly, L1 retrotransposition is under epigenetic control in the brain via the actions of histone deacetylase and the methylated DNA-binding protein MeCP2. In patients with Rett syndrome, a neurodevelopmental disorder caused by mutations in the MeCP2 gene, L1 retrotransposition rates appear to be higher (Muotri et al. 2010). This is also the case in schizophrenia, though it is less clear what the mechanism might be for increase retrotransposition in the disorder (Bundo et al. 2014) These TE control mechanisms have been co-opted to form the basis of the developmental silencing of areas to the genome in order to specify cell fate (Bodega and Orlando 2014). Disruption of developmentally appropriate epigenetic silencing by an expansion of non-transposon repetitive elements is the pathogenic mechanism in fragile X syndrome (Mirkin and Mirkin 2014), and similar processes may be at work in Huntington's disease as well (Evans-Galea et al. 2013), which suggests that this mechanism may obtain in other neurodevelopmental disorders and neurodegenerative disorders.

The X chromosome imprinting machinery uses the lncRNA Xist to guide the heterochromatin-mediated inactivation of one X chromosome in female mammals. Xist is derived from both the exons of a decayed protein-coding gene and a number of TEs likely cobbled together in a process like exon shuffling (Elisaphenko et al. 2008). Xist is transcribed from the inactive X chromosome and serves as a scaffold for the facultative heterochromatin machinery, notably the polycomb repressive complex, PRC2, which trimethylates H3K27. Xist is itself regulated by the lncRNA Tsix, which is Xist antisense transcribed by the active X chromosome and Xist. Tsix also appears to bind to the PRC2 complex via the same repeat motif utilized by Xist suggesting that it may inhibit inactivation of the active X chromosome both by acting as an interfering RNA and as a competitor for PRC2 binding (reviewed in (Pontier and Gribnau 2011)). Tsix also appears to bind to the PRC2 complex using the same repeat motif utilized by Xist, and this appears to be the first identified case of a more general phenomenon where lncRNA serves to recruit chromatin-modifying enzyme complexes. Other TE-derived lncRNAs have been shown to interact with different chromatin-modifying complexes, such as CoREST (REST corepressor) and trithorax proteins (Khalil et al. 2009; Schuettengruber et al. 2011; Casa and Gabellini 2012). Both of the aforementioned complexes play significant roles in development and cell fate determination. Many transcribed lncRNAs (many TE derived) were discovered by scanning for a chromatin sequence characteristic of actively transcribed protein-coding genes (H3K4–H3K36 trimethylation), providing evidence that these elements share the same sort of epigenetic regulation as classically defined genes (Khalil et al. 2009).

Recruitment of repressive complexes to transposable elements makes sense if they are purely parasitic, and indeed there is evidence that repressive histone H3 lysine 9 and H3 lysine 27 trimethylation are associated with distinct classes of transposable elements (Day et al. 2010; Rowe and Trono 2011; Hunter et al. 2012). However, as the example of actively marked lncRNA genes above makes plain, some of these elements are targeted by activating epigenetic writers. It has been proposed that TEs are a source of functional domains for the creation of lncRNA genes and that these domains enable the variety of interactions between nucleic acids and proteins of which noncoding RNAs are capable (Johnson and Guigo 2014). This supports the idea that TE-derived genomic elements are important for the structure and function of the epigenome itself.

8.5 Transposons and Stress

TEs are more likely to transpose under conditions of stress, as their discoverer noted (McClintock 1951, 1984). Endogenous retroviruses, which comprise a substantial portion of mammalian RTs, are also more likely to replicate under conditions of stress to the host (Cho et al. 2008). Psychostimulants, which produce many of the same endocrine and neurochemical effects as a stressor, have been shown to alter both TE transcription and transposition (Maze et al. 2011; Okudaira et al. 2014). Due to the

danger that active viruses and genome reshuffling pose, organisms have developed a number of mechanisms to ensure that these elements are well controlled. Many of the epigenetic silencing mechanisms that are responsible for chromatin structure and the regulation of cell fate appear to have evolved in part to control potentially parasitic TEs (Fedoroff 2012). This static level of TE control via heterochromatinization has long been observed and provided some of the evidence for the view that TEs were transcriptionally inactive, a view we now know to be incomplete at best. The relationship between TEs and the epigenetic mechanisms involved in their control is not as simple as the suppression of parasitism, as we have explained above. In most if not all organisms examined, TEs and the mechanisms of genome regulation have evolved together, co-opting these elements in order to increase their utility to the organism. This seems particularly true with regard to increasing the capacity of the organism to adapt to environmental conditions, particularly aversive ones. At the evolutionary level, TEs provide a source of on-demand capacity for genome reshuffling and gene creation via duplication, deletion, and insertion events. Examples of the adaptive effects of TEs on genomes span the range from bacterial insertion sequences to the TE-derived enhancer sequences in the mammalian pro-opiomelanocortin gene and include a number of cases where TE activity has increased in response to specific environmental conditions such as temperature or rainfall (Casacuberta and Gonzalez 2013). Some TEs may confer stress sensitivity to nearby genes, as is the case with the rice DNA transposon, *mPING* (Naito et al. 2009). We have proposed elsewhere that TEs might represent a store of potentially useful genetic code in complex organisms which has less access to the horizontal gene transfer networks present in prokaryotes and unicellular eukaryotes (Hunter et al. 2014).

But rapid adaptation to population level stress is not the only space in which transposons and the stress response interact. Many transposons are acutely activated by stress signals (Casacuberta and Gonzalez 2013), and the yeast retrotransposons Ty5 completely alters its activity and transposition targets in response to environmental stress (Dai et al. 2007). Another example is CDT-1 (chromatin licensing and DNA replication factor 1), which was originally identified as a desiccation tolerance gene in plants but later shown to be a TE. Consistent with the emerging theme of co-opting TEs into noncoding RNA genes, CDT-1 produces RNA, which helps orchestrate the desiccation tolerance response (Hilbricht et al. 2008). Another significant example is the upregulation of SINE elements in response to heat shock in both mice and humans (*Alu* and *SINE B2*, respectively). Heat shock response is one of a number of evolutionarily conserved mechanisms of cellular stress response present in eukaryotes. In both man and mouse, these TEs are actively transcribed by RNA pol II in response to heat shock, and their RNA binds to RNA pol II to block transcription of protein-coding genes (Mariner et al. 2008; Yakovchuk et al. 2009). Suppression of transcription is an important part of the heat shock response, which is dedicated in part to the prevention of potentially cytotoxic protein misfolding (Verghese et al. 2012). More than any other example, this work clearly demonstrates the adaptive utility of TEs in the stress response of individual organisms.

In this context, the recent findings that stress can rapidly, over the course of an hour or so, downregulate the transcription of numerous RTs in a stress-sensitive brain

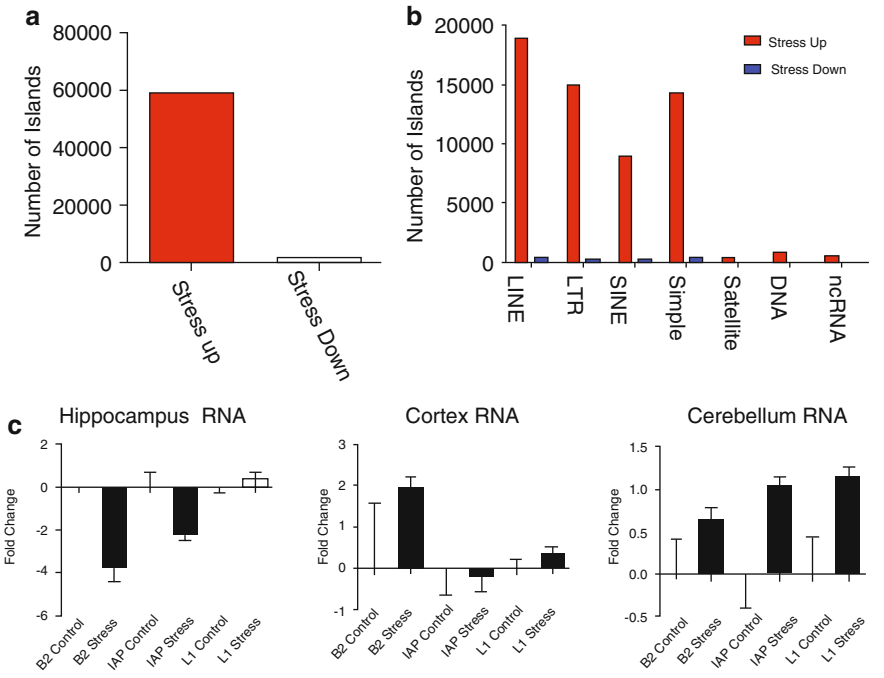

Fig. 8.2 Stress-induced epigenetic regulation of retrotransposons in the hippocampus. (**a**) shows the total number of genomic elements (arbitrary) that showed increased (stress up, *red*) or decreased (stress down, *blue*) H3K9me3 in rat hippocampus after an acute stress exposure. (**b**) breaks down the changes in H3K9me3 shown in A, by repeat class; retrotransposons showed pronounced increases in H3K9me3. (**c**) shows the regulation TE RNA after stress of a few individual examples of SINE (B2), LTR (IAP), and LINE (L1) retrotransposons. Downregulation is specific to the hippocampus, while in the cerebellum, where the H3K9me3 response was not observed, TE expression was increased (*$-p<0.05$, **$p<0.005$) (Adapted from Hunter et al. (2012))

region are particularly striking. In the rat hippocampus, via rapid increases in heterochromatic histone 3 trimethylation at lysine 9 (H3K9me3), a single immobilization stress represses the transcription of numerous retrotransposons at as many as 60,000 different genomic loci (See Fig. 8.2) (Hunter et al. 2009, 2012). Interestingly, this downregulation, which included B2 SINE elements, was hippocampus specific, with most tissues observed showing no significant change in expression, while one area, the cerebellum, showed an increase in TE RNA expression after acute stress (Hunter et al. 2012). Rapid downregulation of RT expression would, of course, be superfluous if they were silent; however, they are not. In contrast, hippocampal H3K9me3 levels decline with repeated stress, while amygdala expression of at least some LINEs is increased in more chronic models of stress and alcoholism (Ponomarev et al. 2010, 2012). The observations that B2 elements are reduced in expression after an acute psychological stress while they are increased after heat shock suggest that these elements and others like them are subject to fine-scale regulation in response to environmental conditions, rather like genes, and like genes they have a functional role to play

in the cells that express them. The available evidence suggests that many if not most of these elements are transcribed at some level in the brain and other tissues. In fact, their expression is highly tissue and cell type specific (Faulkner et al. 2009; Reilly et al. 2013). Given this set of facts, it is unsurprising that they are stress regulated.

Stress and stress-related disorders show large variance across individuals in terms of their impact. PTSD, for example, affects roughly 7 % of the population, while a majority of the population is exposed to trauma at some point in their lifetime (Kessler et al. 1995; Breslau et al. 1998), raising significant questions about what makes some individuals vulnerable and others resilient. TEs show much wider variability across individuals than protein-coding genes, and most of us have at least one that may be "private" and may not exist in another member of our species (Iskow et al. 2010); thus, they may represent a means to understand both interindividual variance in stress resilience and the "missing heritability" observed in many stress-related psychiatric disorders (Manolio et al. 2009; Crow 2011). Indeed, dysregulation of the brain and peripheral transposon expression has been observed in animal models of PTSD and alcoholism as well as in human subjects suffering from the same disorders (Ponomarev et al. 2010, 2012; Rusiecki et al. 2012). Ectopic overexpression of TEs, like chronic stress, may contribute to brain aging and neurodegenerative disorders. In *Drosophila*, as in the mammalian brain, TEs contribute to brain mosaicism (Perrat et al. 2013), and they are under the control of epigenetic mechanisms including the dsRNA processing machinery including Argonaute proteins and TDP-43 (transactive response DNA-binding protein 43 kDa). In aging flies, transposon expression expands producing a "transposon storm" which contributes to age-associated neuronal deficits (Li et al. 2013; Reilly et al. 2013). A similar process has been implicated in human frontotemporal dementia (Li et al. 2012). Loss of control of Alu SINE RNA expression due to declining dicer1 expression has been shown to be a mechanism in certain varieties of age-related macular degeneration, suggesting that decline in the ability to control the expression of TE RNA may be a common culprit in brain aging (Kaneko et al. 2011; Tarallo et al. 2012). Stress also contributes to brain aging and to biological aging in general; chronic stress reduces telomere length, for example (Mora et al. 2012; Shalev et al. 2013). The work showing that stress is also involved in the control, or lack of control, of TE expression suggests that the two may be mechanistically connected with regard to brain aging and neurodegeneration. Much remains to be explored about how exactly TEs play a role in both normal physiology and pathology. It will also be necessary to disentangle and clarify the differences between transposons and the ncRNA genes, which are to a large extent composed of TEs if such a distinction can be clearly made.

8.6 Conclusions

Transposons are ubiquitous but poorly understood genomic elements, which represent a potentially enormous store of information and regulatory mechanisms. While research into the pathogenic potential of transposable elements is several decades old, much remains to be discovered. The beneficial functions of these elements are more

controversial, and for the most part much more recently described. Though the discoverer of TEs, Barbara McClintock, argued from the outset that their role was an adaptive rather than a parasitic one, that view was overridden until recently by eminences such as Francis Crick and Susumu Ohno, who argued that they were junk. Evidence from large-scale sequencing projects such as ENCODE has helped to change this view, particularly by demonstrating that most of the genome is transcribed at one level or another. It is clear from the examples given here that these elements are not without function nor are they without the potential to induce pathology.

With regard to the effects of stress upon the brain, these elements are particularly exciting as a potential means of explaining the wide individual variation in stress response and in stress-related diseases from PTSD to type II diabetes. That being said, much work needs to be done to establish a mechanistic relationship between these elements, the epigenome and the lasting effects of stress upon the brain and behavior. With some exceptions, most of the work done to date is correlational in nature, and we do not have an integrated understanding of all of the potential interactions and mechanisms in which TEs might be involved. In particular it is important to work toward understanding the mechanisms by which TE expression is regulated and to what physiologic signals they respond. Stress plays a significant role, so too might sex steroids, what other systems might be involved? It is probable that signals of cellular distress, such as elevated reactive oxygen species or caspase activation might act as switches, but as yet the question has not been addressed. Given that TEs represent a tenfold larger fraction of our genetic material than the protein-coding genes that have been at the center of molecular biological attention for the better part of a century, this dearth of knowledge should not be surprising. The level of complexity this implies means that we will have to develop not only new bioinformatics tools but new ways of thinking about cellular, epigenome, and genome function. However, the potential to explain phenomena like missing heredity (or complex causality) in the complex diseases that now represent most of the disease burden in the developed world makes this daunting task clearly worthwhile.

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