Transposable elements and an epigenetic basis for punctuated equilibria

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Evolution is frequently concentrated in bursts of rapid morphological change and speciation followed by long-term stasis. We propose that this pattern of punctuated equilibria results from an evolutionary tug-of-war between host genomes and transposable elements (TEs) mediated through the epigenome. According to this hypothesis, epigenetic regulatory mechanisms (RNA interference, DNA methylation and histone modifications) maintain stasis by suppressing TE mobilization. However, physiological stress, induced by climate change or invasion of new habitats, disrupts epigenetic regulation and unleashes TEs. With their capacity to drive non-adaptive host evolution, mobilized TEs can restructure the genome and displace populations from adaptive peaks, thus providing an escape from stasis and generating genetic innovations required for rapid diversification. This ''epi-transposon hypothesis'' can not only explain macroevolutionary tempo and mode, but may also resolve other long-standing controversies, such as Wright's shifting balance theory, Mayr's peripheral isolates model, and McClintock's view of genome restructuring as an adaptive response to challenge.

Keywords: epigenetics; fitness landscape; punctuated equilibria; stress; transposable elements

Introduction

In contrast to the traditional view of evolution as a gradual process of incremental change, the theory of punctuated equilibria proposes that, on a geological time scale, evolution proceeds through bursts of rapid morphological change and speciation, followed by long-term morphological stasis.[1] Support for punctuated equilibria as a common evolutionary pattern comes from the fossil record,[2,3] long-term evolution experiments,[4] and phylogenetic analysis of DNA sequences.[5–7] Furthermore, ecological communities exhibit synchrony in stasis and diversification, implicating environmental change as a critical factor generating punctuation.[8–10] Elucidating the genetic mechanisms that maintain stasis and enable explosive diversification remains a challenge for evolutionary biology. We suggest that a key to understanding punctuated equilibria lies in the emerging field of epigenetics. Superimposed on the DNA scaffold is a complex system of regulation, involving somatically and, in some cases, transgenerationally heritable epigenetic marks (DNA methylation and histone modifications), as well as non-coding RNAs, that determines whether, where and when genes are expressed.[11] Growing evidence indicates that epigenetic regulation evolved to suppress transposable elements (TEs),[12,13] a diverse array of parasitic sequences that comprise a large fraction of eukaryotic genomes.[14] Here, we propose that vulnerability of the epigenome to physiological stress provides a parsimonious explanation for punctuated equilibria.

According to this ''epi-transposon hypothesis,'' physiological stress, associated with major climatic change or invasion of new habitats, disrupts epigenetic silencing, resulting in TE reactivation, increased TE expression[13] and/or germ-line infection by exogenous retroviruses.[15] Mobilized TEs rapidly restructure the genome and alter gene expression patterns by inserting into promoters and enhancers, and by causing chromosomal breakage, exon shuffling, sequence expansion, gene duplication, ectopic recombination, novel gene formation and expansion and re-wiring of genetic regulatory networks.[16–18] Stress-induced breakdown in epigenetic suppression enhances the spread of TEs, with potentially deleterious effects on host fitness, and increases genetically and epigenetically based phenotypic variation.[19] High mutation rate, together with increased potential for non-adaptive host evolution, enables populations to colonize new adaptive peaks, facilitating rapid morphological change and speciation. As the host genome and parasitic sequences co-evolve, epigenetic silencing mechanisms regain control, heritable variation declines, and stasis is re-established.

An essential feature of the epi-transposon hypothesis is that initial evolutionary change need not be adaptive for the individual but can stem from selection operating at the level of the nucleotide sequence.[20–22] Theoretical analyses show

Abbreviations: CNE, conserved non-coding element; LINE, long interspersed nuclear element; LTR, long terminal repeat; miRNA, microRNA; My, million years; Mya, million years ago; RISC, RNA-induced silencing complex; piRNA, piwi-interacting RNA; RNAi, RNA interference; SINE, short interspersed nuclear element; siRNA, small interfering RNA; TE, transposable element.

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that changes in heritable epigenetic marks[23] and deleterious mutations caused by TEs[24] can produce peak shifts on fitness landscapes. Reprogramming of the epigenome and unleashing of parasitic genetic elements during physiological stress can thus provide an alternative to genetic drift[25] as a potent mechanism for propelling populations across the fitness valleys that separate adaptive peaks. Indeed, TEs have been implicated in the evolution of several key innovations, including acquired immunity in vertebrates[26] and placentation in mammals.[27,28]

Support for the epi-transposon hypothesis comes from comparative genomics, molecular systematics, environmental epigenomics and adaptive radiation research. We first review evidence for the main assumptions of the hypothesis, namely that (1) TEs have been a driving force in the evolution of epigenetic silencing mechanisms; (2) TEs have been a rich source of novel genes and genetic regulatory elements; and (3) epigenetic repression of TEs breaks down in response to environmentally induced physiological stress. We then consider predictions of the epi-transposon hypothesis in the context of genome evolution and evolutionary diversification.

Transposable elements: a pervasive feature of eukaryotic genomes

With the recent proliferation of genomic sequencing studies, TEs have emerged as diverse, abundant and ubiquitous components of eukaryotic genomes, constituting up to 80% of nuclear DNA in plants, 3–20% in fungi, and 3–52% in metazoans.[29,30] These mobile genetic elements owe their abundance to their capacity to insert into new chromosomal locations, often duplicating themselves in the process. TEs are a major determinant of genome size and frequently comprise orders of magnitude more DNA sequence than protein-coding genes.[14] Despite staggering diversity involving thousands of families, TEs can be classified into two main groups based on whether transposition involves an RNA intermediate (Fig. 1).

Transposable elements drive the evolution of epigenetic silencing mechanisms

Based on their phylogenetic distribution, DNA transposons apparently diverged into 10 of 12 superfamilies[30] in the common ancestor of eukaryotes.[31] Retrotransposons are similarly ancient, with long terminal repeats (LTRs), long (LINEs) and short interspersed nuclear elements (SINEs) and *Dictyostelium* intermediate repeat sequence (*DIRS*) present in protist, plant, fungal, and animal genomes.[32] Eukaryotes have therefore been coevolving antagonistically with TEs throughout most or all of their evolutionary history.[33] TEs are highly mutagenic, accounting for 50% of deleterious mutations in *Drosophila*[34] and 10% in mice.[35] Through their capacity to insert into exons, promoters and enhancers, TEs threaten host fitness by disrupting open reading frames, gene expression levels and patterns of alternative splicing.[36] In non-coding regions, TE insertions can undermine genomic integrity by acting as foci for unequal homologs recombination, resulting in duplications, deletions and translocations.[37]

In line with this longstanding threat to genomic stability is the existence of an ancient eukaryotic defense machinery for suppressing transposition. Phylogenetic, comparative-genomic and gene knockout studies reveal that fundamental mechanisms of epigenetic regulation [RNA interference (RNAi), histone modifications and DNA methylation] arose as a defense against TEs. The specifics of TE repression are complex and vary extensively between lineages. However, conservation of key proteins involved in RNAi (Argonaute/Piwi-like protein, Dicer-like protein and RNA-dependent RNA polymerase) throughout the six eukaryotic supergroups suggests that the common ancestor possessed a sophisticated silencing system involving small interfering RNAs (siRNAs).[38,39] In extant eukaryotes, defense against TEs is a three-step process in which small RNAs [siRNAs or Piwi-interacting RNAs (piRNAs)] are: (1) produced in response to TE detection; (2) selectively amplified based on sequence homology to active TEs; and (3) incorporated into Argonaute- or Piwi-containing complexes that inactivate TEs through post-transcriptional mRNA degradation and by targeting TE sequences for DNA methylation and/or histone modifications that result in a repressive chromatin environment and transcriptional silencing.[40]

To effectively defend against nucleic acid parasites, a cell must distinguish its own transcriptionally active genes from those of mobile elements. Genomic self/non-self recognition derives from small RNA/protein complexes, with RNAs conferring sequence specificity and proteins providing catalytic activity for targeted degradation of non-self transcripts.[40] In plants and yeast, TE-encoded transcripts form double-stranded RNAs that are recognized as non-self and processed by Dicer into siRNAs. These siRNAs bind to Argonaute proteins to direct source transcript cleavage. Post-transcriptional TE silencing is amplified by cycles in which cleaved RNAs provide templates for RNA-dependent RNA polymerase synthesis of additional siRNAs.

In insects and vertebrates, recognition is based on piRNA pathways that exploit TE mobility as the mechanism for transcript targeting.[41] Clusters of piRNA genes serve as ''transposon traps'' that accumulate TE sequences in proportion to their insertion frequency. These clusters transcribe piRNAs enriched in sequences antisense to expressed transposons, with piRNA amplification hypothe-

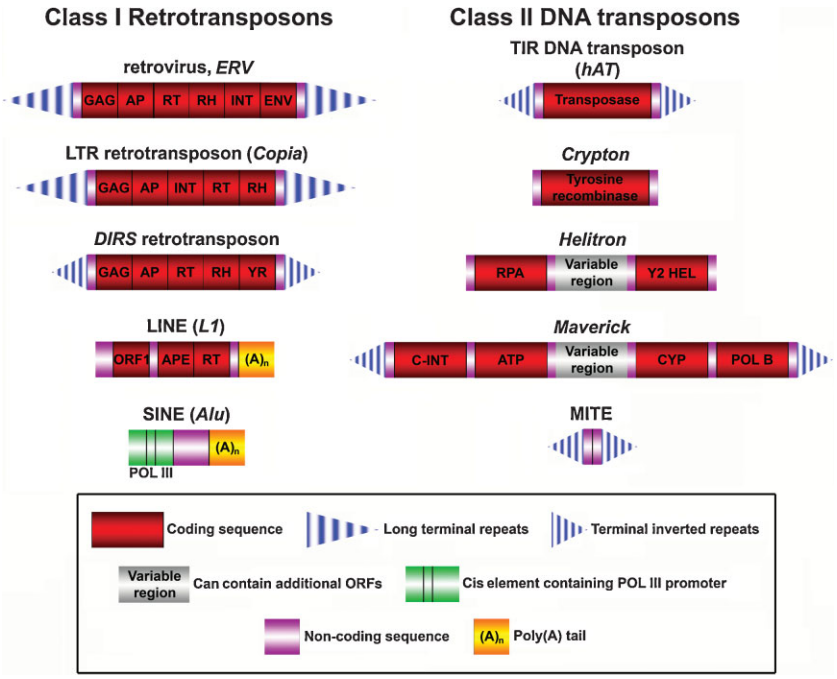

Figure 1. Structure and classification of representative examples of transposable elements (TEs). Retrotransposons (class I or type 2 TEs) are transposed through a "copy and paste" mechanism, in which a messenger RNA (mRNA) intermediate is expressed in the host cell, reversed transcribed, and inserted into the host genome as a complementary DNA copy. Class I comprises endogenous retroviruses (ERVs) and the closely related long terminal repeat (LTR) retrotransposons, non-LTR retrotransposons, including long and short interspersed nuclear elements (LINEs and SINEs, respectively), as well as the recently discovered *Penelope* and *Dictyostelium* intermediate repeat sequence (*DIRS*) retrotransposons.[30] By contrast, DNA transposons (class II or type 1 TEs) transfer to new chromosomal locations without recourse to an RNA intermediate. They include the classic "cut and paste" terminal inverted repeat (TIR) transposons, *Cryptons* and *Helitrons*, which lack terminal inverted repeats, and *Mavericks* (also known as *Polintons*), the largest and most complex transposons.[31] Both class I and II TEs include autonomous elements that encode the proteins required for transposition, and non-autonomous elements, such as SINEs and miniature inverted-repeat transposable elements (MITEs) that parasitize the replication machinery of autonomous TEs. Abbreviations: AP, aspartic proteinase; APE, apurinic endonuclease; ATP, packaging ATPase; C-INT, C-integrase; CYP, cysteine protease; ENV, envelope protein; GAG, capsid protein; HEL, helicase; INT, integrase; ORF, open reading frame; POL III, RNA polymerase III; POL B, DNA polymerase B; RH, RNase H; RPA, replication protein A; RT, reverse transcriptase; YR, tyrosine recombinase; Y2, tyrosine recombinase with YY motif.

sized to occur through a "ping-pong" mechanism. According to this model, transposon sense transcripts (those requiring destruction), are cleaved by RNA-induced silencing complexes (RISCs) that are guided by the antisense-strand piRNAs. Cleaved sense transcripts are not only degraded but also exploited as sense-strand guides for other RISCs that cleave antisense piRNA transcripts. These cleaved antisense strands are used to direct new rounds of TE transcript degradation, generating amplification cycles analogous to siRNA production by RNA-dependent RNA polymerase. Small RNA/protein complexes also guide sequence-specific genome targeting for repressive DNA methylation and/or histone modification pathways and thus silence TEs at the transcriptional level.

Disruption of any of these silencing processes may dramatically increase TE expression and mobilization, often with catastrophic consequences for host fitness. In *Arabidopsis thaliana*, mutations in the chromatin remodeling gene *ddm1* result in a 20-fold increase in transposition of the DNA transposon *AtMu1*.[42] In mice, the gene *Maelstrom* (*Mael*) encodes a protein that interacts with the piRNA pathway in the nuage,[43] a perinuclear structure involved in RNAi-mediated gene regulation in the germ line of animals.[43] Knockout of *Mael* resulted in demethylation of LINE-1 TEs and a 100-fold increase in the production of LINE-1 ribonucleoproteins in germ cells of $Mael^{-/-}$ males.[44] Accumulation of LINE-1 ribonucleoproteins was associated with extensive DNA damage, defective synapsis, meiotic arrest and male sterility.[44] Similar effects in mice have been observed in gene-knockout studies of the methyltransferase gene *Dnmt3L*.[45] In *Drosophila melanogaster*, loss-of-function alleles of *Mael* and other nuage component genes also exhibit LINE de-repression, in this case, in ovarian tissue, resulting in female sterility.[46]

Transposable elements: parasites, partners or both?

The relationship between TEs and the host genome – parasitic, mutualistic or neutral – is complex, controversial and often misrepresented.[47] In a landmark study, Hickey[20] developed a simple model in which a TE, capable of replicating itself to one new site in the genome, is introduced into a sexual, outbreeding population. He showed that such an element can spread to fixation, even if it reduces host fitness by 50%. In a sexual host, rare TEs colonize uninfected genomes through replicative transposition in the zygote or the germ line, and are thus transmitted to offspring at twice the rate of Mendelian-inherited host alleles.[12] Consequently, genic-level selection can favor TEs with highly deleterious effects on organismal fitness. However, once they reach fixation, TEs lose their transmission advantage and can enhance their own fitness only by increasing that of their host.[20]

In reality, TE dynamics are far more complex than assumed in the Hickey model. TEs can transpose to multiple sites, mutate into new, active elements, become inactivated through deletion, mis-sense or non-sense mutations, and can be reactivated by local or global alterations to the epigenetic landscape of the genome.[31,32] Nonetheless, Hickey's model provides an evolutionary perspective that reconciles divergent views of TE/host relations. Genic-level selection explains how TEs can evolve from selfish, nucleic acid parasites into mutualists that promote host fitness and facilitate adaptive evolution.

TE/host coevolution is an intriguing topic that has received little attention in theoretical population genetics, with models focusing instead on mobile element gain and loss.[48–52] The models do, however, predict a declining trajectory in transposition rate, as TEs become established in host populations. Colonizing TEs require high intrinsic amplification rates to overcome elimination by drift and selection against deleterious insertions. As TE abundance increases, fitness gains from transposition decline, and persistence is enhanced by ''self regulation.'' This is a mathematically defined term, in which insertion rate declines with TE copy number, and could stem from TE-based mechanisms or from genome self defense.[50] While instructive, these models do not address how TEs drive the evolution of host defenses, how host defenses influence the evolution of TE transposition strategies, or how this arms race might fuel the evolution of host complexity.

The complexity, redundancy and evolvability of epigenetic regulation appear to give hosts an insurmountable advantage over their less complex nucleic acid parasites. For example, by accumulating and targeting TEs in proportion to their mobility, piRNA transposon traps possess an inherent capacity to counter evolutionary advances in transposition proficiency. However, TEs could respond through insertion site specificity. For example, regulatory regions of heat shock genes are associated with constitutively uncondensed chromatin that not only facilitates rapid up-regulation in response to thermal stress, but also enables any TEs present in the region to escape transcriptional silencing, with few options for host countermeasures. In *Drosophila*, promoters of heat shock genes are indeed enriched for P elements whose effects contribute substantially to phenotypic variation.[53] Alternatively, TEs could elevate expression during periods of greatest host vulnerability.

In mammals, global demethylation occurs during preimplantation embryonic development and coincides with dramatic increases in TE expression.[54] A similar pattern occurs during the early stages of germ cell development when the methylation marks required for genomic imprinting are reset. Developmentally regulated demethylation thus enables TEs to escape from transcriptional repression during critical stages and cause heritable transposition mutations. TE expression at these pre-meiotic stages can also yield clusters of progeny carrying identical transposition-induced mutations.[55] Such pre-meiotic clusters have major implications for transposon-mediated host evolution because they increase the fixation probability of a new mutant allele and the likelihood that the new mutation will precipitate reproductive isolation.[56]

TEs as a source of new genes and genetic regulatory networks

Transposons owe their success as selfish replicators to nucleotide sequences that encode proteins, such as transposases, endonucleases, reverse transcriptases and integrases, that cut, copy and paste DNA.[57] These same sequences endow TEs with an unparalleled capacity to promote genetic innovation in the host. TEs not only function as mediators of gene duplication and recombination, but also provide a rich reservoir of sequences that can be recruited to function for the host genome,[58] a process known as ''molecular domestication.'' Screening of genomic sequences with repetitive element detection software has dramatically increased the number of host coding sequences known to have evolved from TEs.[57–59]

Domestication of TE coding sequences has been especially important in the evolution of the mammalian placenta, with genes independently recruited from two distinct types of retro-element sequences, retrotransposon *gag* genes and endogenous retroviral envelope (*env*) sequences.[60] Therian mammals (marsupials and eutherians) diverged from the egg-laying monotremes ~170 million years (My) ago (Mya).[61] The evolution of the placenta, a short-lived, structurally simple organ in marsupials, is

associated with the acquisition of *Peg10*, a retrotransposon-derived gene with homology to the *Sushi* gag gene family of Ty3/gypsy LTR retrotransposons.[62] *Peg10* is genomically imprinted and paternally expressed in marsupials and eutherians.[63] Gene knockouts have demonstrated parent-of-origin-specific lethality in mice, with an essential role for the paternally inherited *Peg10* allele in placenta formation.[62] The evolution of more complex eutherian placentae is associated with domestication of a second retrotransposon gag-derived gene, *Rtl1*, in the common ancestor of eutherians after their divergence from marsupials. Sekita *et al.*[28] established that *Rtl1* is required for fetal capillary maintenance during late-stage fetal development. Like *Peg10*, *Rtl1* is a paternally expressed, imprinted gene. In mice, deletion of the paternally inherited *Rtl1* allele results in late fetal or neonatal lethality.

These studies indicate that paternally expressed, imprinted genes derived from retrotransposons are essential for both early development and late-stage functioning of the placenta. The critical role for *Peg10* in the origin of the placenta, together with the DNA methylation characteristics of the locus in marsupials, suggests that silencing of exogenous DNA after retrotransposition was an essential step in the evolution of the epigenetic systems involved in imprinted gene expression in mammals.[63]

Campillos *et al.*[64] identified 85 *gag* genes in humans, encoding 103 protein isoforms, suggesting that *Peg10* and *Rtl1* represent the tip of the iceberg with respect to *gag* gene domestication. In addition, genes recruited from a second retro-element type contribute to fusogenic and immunosuppressive properties of the placenta.[60] The primate syncytin-1 and syncytin-2 genes and the murine syncytin A and syncytin B genes are expressed specifically in the placenta, and were independently co-opted from endogenous retrovirus *env* genes. Retroviral progenitors of syncytin genes encode glycoproteins for cell surface receptor recognition and fusion of the viral envelope with the host cell membrane, and contain a conserved transmembrane domain that confers immunosupressive capability. Similarly, syncytin proteins mediate cell recognition, cell fusion and immunosuppression in mouse and human placental tissues.[60]

Recruitment of TE non-coding sequences as components of genetic regulatory networks may have been even more significant to host evolution than coding sequence domestication.[18] Active TEs possess numerous regulatory signals, such as POL II or III promoters, enhancers, insulators, splice sites, polyadenylation signals and transcription factor binding sites, that predispose them to play an important role in host gene regulation. Based on the abundance of TE sequences (primarily SINEs) in human promoters, it is estimated that regulation of >1,000 human genes involves *cis* elements created by TE insertion.[65] At a more global level, scaffold/matrix attachment regions, which are involved in transcriptional regulation of multiple loci, are enriched in TE sequences, particularly LINEs.[65] Comparative studies of transcription factor binding sites provide further evidence of widespread involvement of TE-derived sequences in gene regulation. In an analysis of seven mammalian genomes, Bourque *et al.*[66] showed that hundreds to thousands of binding sites for five of seven transcription factors examined occur within sequences from distinct TE families.

By favoring the evolution of RNA-mediated silencing in ancestral eukaryotes, TEs have also been instrumental in the origin and evolution of post-transcriptional mechanisms of gene regulation. This is particularly apparent in the case of microRNAs (miRNAs), short, non-coding RNAs that are transcribed by the genome and regulate gene expression by degrading mRNA or repressing its translation. Of the 462 human miRNA genes analyzed by Piriyapongsa *et al.*,[67] 55 (12%) originated from TEs. Given the capacity of individual miRNA genes to target multiple transcripts, TE-derived miRNAs are likely to participate in the regulation of thousands of human genes.[67]

Transposons unleashed: stress-induced breakdown in epigenetic silencing

McClintock recognized that physiological stress, induced by X-irradiation, viral infection or tissue culturing conditions, can substantially increase transposon activity.[68] Indeed, many plant TEs were discovered because of their stress-induced phenotypes.[69] Subsequent research has revealed stress-induced activation and/or increased TE expression in fungi,[70] corals,[71] insects,[72] crustaceans,[73] and mammals.[74] In *D. melanogaster*, transposition rate of the TE Dm-412 increased by two orders of magnitude after heat shock treatment.[72] In the shrimp *Penaeus monodon*, exposure to thermal, hypotoxic, and osmotic stress dramatically elevated TE expression, with ~30% of differentially expressed genes exhibiting high similarity to *pol* sequences of non-LTR retrotransposons.[73] In fission yeast, CENP-B proteins, domesticated from ancient DNA transposons, promote the clustering of *Tf2* retrotransposons into transcriptionally silent "Tf bodies." Oxidative stress results in disassembly of Tf bodies, leading to retro-element expression and mobilization.[75]

An epigenetic basis for stress-induced breakdown in TE repression is supported by growing evidence that the epigenome is highly sensitive to stress-related environmental perturbations. Factors such as air pollution,[76] temperature extremes,[77] endocrine disrupting chemicals,[78] dietary regimes,[79] famine,[80] and psychological stress[81] can cause epigenetic modifications that influence disease susceptibility and have other important fitness-related consequences. Exposure to epigenome-modifying agents during critical, early phases of development can result in stable transmission of altered epigenetic states across multiple

generations.[11] Genomic instability and epigenetic changes are hallmarks of cancer and have been linked to increased TE mobilization.[82]

The clearest example of environmental effects on epigenetic silencing of TEs comes from studies of the influence of diet on expression of the viable yellow allele (A^{vy}) of the *agouti* signaling protein (ASP) gene in mice.[11] This allele possesses an intracisternal A-particle retrotransposon that, when active, acts as a promoter that usurps transcriptional control of *agouti*, resulting in ectopic expression of ASP and a phenotype of yellow fur, obesity, type II diabetes and predisposition to tumors. When the retrotransposon is silent, agouti expression is normal and only transiently expressed in hair follicles, producing a subapical yellow band and the wild-type, agouti (brown) coat color. Activity of the retrotransposon varies widely between genetically identical mice carrying the A^{vy} allele, with phenotypes ranging from yellow and obese to lean and fully agouti. In offspring of pregnant females whose diets had been supplemented with methyl donors, methylation of the A^{vy} allele significantly increased, resulting in reduced retrotransposon expression and a dramatic shift in A^{vy} offspring towards agouti coat color and lean body mass.[79] These environmental effects on the epigenome were heritable, with methyl donor supplementation shifting the A^{vy} phenotype spectrum not only in mice exposed as fetuses but also in their offspring.[11]

The A^{vy} example reveals a simple mechanism in which diet directly influences TE methylation and silencing. Epigenetic regulation is, however, an extremely complex process involving multiple, interconnected pathways that silence TEs at the transcriptional and post-transcriptional levels. *Caenorhabditis elegans*, for example, possesses 27 distinct Argonaute proteins that act sequentially during RNAi.[83] In mammals, transcriptional silencing of TEs encompasses both DNA methylation and histone modifications.[54] Five DNA methyltransferases participate in the methylation of cytosine nucleotides, and at least five histone methyltransferases mediate repressive chromatin via methylation of the histone tail protein H3K9.[54] DNA methylation effects further depend on methyl-CpG-binding proteins. Other non-methyltransferase enzymes, such as lymphoid-specific helicase 1, also function in TE silencing.[84] Given this complex network of interactions, the mechanisms responsible for stress-induced epigenetic deregulation may be almost as varied as the environmental factors that generate stress.

While several authors have argued for stress-induced evolutionary innovation (reviewed in[9,85]), the basis for this relationship remains controversial. Genome reorganization resulting from unleashed TEs can provide the raw material for the evolution of novel phenotypes. However, McClintock's interpretation of TE activation as an adaptive response by the host genome to challenge[68] contradicts neo-Darwinian principles, since genome reorganization is likely to be disruptive in the short term, and natural selection should favor current function over future evolutionary potential. There is no such selective constraint, however, if TEs are activated, not as an adaptive host response, but rather because stress disrupts their epigenetic suppression. In the evolutionary tug-of-war between TEs and the host genome, physiological stress shifts the balance of power in favor of parasitic sequences, resulting in genomic modifications that may not be of immediate benefit to the host. Such physiological stress is likely to be experienced by any species that invades a new habitat or is subjected to periods of major climatic and/or geologic change.

Fitness landscapes and the epi-transposon hypothesis

In simulations incorporating both point mutations and transposon-induced mutations of large phenotypic effect, McFadden and Knowles[24] showed that evolutionary diversification of digital organisms on fitness landscapes can occur as a two-stage process. In the pivotal first stage, transposon-induced, deleterious mutations displace populations from local optima, thus enabling colonization of alternative peaks. In the second stage, populations gradually ascend new peaks, driven by selection favoring non-transposon mutations with small, beneficial effects on host fitness.

The epi-transposon hypothesis fills a critical void in the McFadden/Knowles fitness landscape model by incorporating stress-induced breakdown in epigenetic regulation of TEs, thereby specifying the environmental and molecular mechanisms underlying episodes of TE mobilization (Fig. 2). The model's conclusion that TE-driven mutations facilitate rapid evolution of novel phenotypes is supported by studies of protein and bacterial evolution. In directed evolution studies of *Escherichia coli*, production of genetic variation through DNA sequence shuffling accelerated evolution of antibiotic resistance ~50-fold over point mutation strategies.[86] Because of their capacity to recombine nucleotide sequences, TEs are uniquely suited for generating the complex mutational change required for rapid and divergent evolution.[47] Indeed, gross chromosomal rearrangements have been detected in yeast strains cultured in glucose-limited environments. These rearrangements were bounded in nearly every case by TEs or TE remnant sequences, suggesting that TE-based mechanisms were the primary cause of genomic change in these physiologically stressed strains.[87]

Predictions of the epi-transposon hypothesis

The hypothesis that epigenetic deregulation of TEs underlies punctuated equilibria yields testable predictions regarding

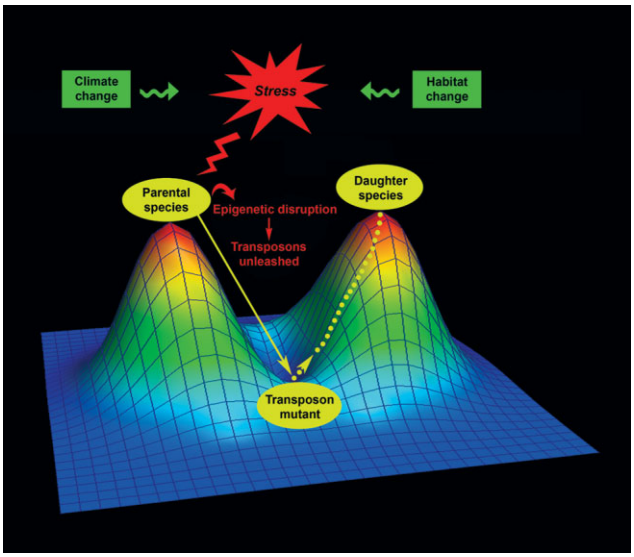

Figure 2. The epi-transposon hypothesis for punctuated equilibria. Physiological stress in the parental species, induced by climate change or invasion of a new habitat, disrupts epigenetic regulation and unleashes TEs. With their capacity to drive non-adaptive host evolution, mobilized TEs restructure the genome and displace the population from its adaptive peak (solid yellow arrow) on the fitness landscape, thereby providing an escape from stasis, and generating the genetic innovations required for rapid phenotypic evolution and speciation. The population then gradually ascends a new fitness peak (dotted yellow line), driven by natural selection favoring non-transposon mutations with small, beneficial effects on host fitness. As the host genome and the parasitic sequences coevolve, epigenetic regulatory mechanisms regain control over TEs, level of heritable variation declines, and stasis is reestablished in the daughter species. It should be recognized that the model is a simplification, since environmental changes also have the potential to alter contours on the fitness landscape.

patterns of selection acting on repetitive elements, historical patterns of TE expansion, and the role played by TEs in the diversification of new lineages. Specifically, the hypothesis predicts that (1) episodes of TE mobilization should seed the genome with non-coding regulatory elements co-opted by the host and conserved in evolution; (2) the history of TE mobilization and expansion should itself follow a punctuational pattern, with genomes subjected to repeated episodes of TE bombardment; (3) the evolution of new lineages should be associated with bursts of TE activity, resulting in lineage-specific patterns in expansion and domestication of TE families; and (4) adaptive radiations should be triggered by episodes of TE mobilization.

Episodes of TE expansion give rise to conserved non-coding elements

Consistent with the hypothesis that stress-induced episodes of TE amplification provide the raw material for subsequent adaptive evolution, a sizeable fraction of TE-derived repetitive sequences has been subjected to strong purifying selection throughout the radiation of eutherian mammals.[29,88] Proliferation of TEs enriches the genome with non-coding sequences that can then be recruited as cis-regulatory elements and components of gene regulatory networks.[18,36] In a survey of the human genome, Lowe *et al.*[88] identified ~10,000 TE-derived, non-exonic elements that have been conserved in evolution for >100 My. These conserved, non-coding elements (CNEs) are enriched near genes regulating transcription and development. Comparison of eutherian and marsupial genome sequences has shown that all but 1% of protein-coding sequences conserved among eutherians are also present in the opossum genome. By contrast, 20% of eutherian CNEs evolved after the divergence from metatherians, with at least 16% of these unambiguously derived from TEs.[29] The high proportion of TE-derived, eutherian-specific CNEs indicates that a burst of novel and then strongly conserved sequence elements was introduced into the eutherian lineage either during or soon after its divergence from marsupials.

Punctuated equilibria in TE mobilization and expansion

The evolutionary history of TE mobilization has been extensively investigated in primates and bats. Genomes in both orders have experienced repeated episodes of bombardment by a succession of TE families.[37,89,90] DNA transposons, which exist largely as fossils in extant mammals, are inferred to have been intensely active during the emergence of primates at the end of the Cretaceous, 80–65 Mya.[89] It is estimated that 74,000 of the 98,300 primate-specific DNA transposons fixed in the human genome integrated during this interval.[89] Consistent with the epi-transposon hypothesis, the late Cretaceous was a period of dramatic climate change and mass extinction. Primate DNA transposon activity ceased by ~40 Mya, but was replaced by waves of transposition in other TE families, most notably in the primate-specific SINEs known as *Alu* elements.

Comparative analysis of *Alu* sequences indicates that all major bifurcations in primate phylogeny coincide with episodes of SINE expansion.[37,91] The first of these, involving *AluJ* elements, commenced ~65 Mya, and is associated with the divergence between anthropoid (apes, old and new world monkeys) and strepsirrhine (mouse lemurs, lemurs and galagos) primates. Anthropoid genomes then experienced a proliferation of *AluS* elements beginning ~45 Mya, followed by a surge in *AluY* activity starting ~30 Mya. Bailey *et al*[91] estimated that >33% of all fixed retroposition events in the human genome occurred during amplification of *AluS* sequences ~35–40 Mya, a period coinciding with divergence

between new world monkeys and other anthropoids.[92] The beginning of *AluY* expansion, in turn, is contemporaneous with the split between apes and old world monkeys. An intriguing pattern in primate TE expansion is the twofold increase in *Alu* insertions in humans compared to chimps, mediated largely by *AluYa5* and *AluYb8*.[93] The elevated rate in humans resulted from a burst of activity 2.5–3.5 Mya. Perhaps not coincidentally, this phase of *Alu* expansion occurred in the context of major geologic uplifting, paleo-environmental change, and rapid and divergent morphological evolution in hominins, including the emergence of *Homo*.[94]

With ~1,100 species, a fragmentary fossil record and the capacity for self-powered flight, bats are a diverse and enigmatic mammalian order. This peculiarity extends to mobile elements present in bat genomes.[90,95] Beginning with an explosive amplification of *Helitrons* ~40 Mya, vespertilionid bat genomes have experienced waves of TE expansion by a progression of DNA transposon families. As in primates, episodes of TE expansion are associated with cladogenesis. The early radiation of Vespertilionidae, the most species-rich bat family, coincides with the burst in *Helitron* activity.[95] In addition, a major episode of diversification in *Myotis*, the most species-rich mammalian genus, corresponds to a period of intense activity of *Tc1*-like elements ~12–13 Mya.[90] These findings suggest that TEs played a role in a unique event in mammalian evolution, the colonization of an aerial adaptive zone.

TE expansion and evolutionary diversification

The recurring pattern in which bursts of mobile element expansion occur in concert with new lineage formation, implicates TEs as a causative agent in evolutionary diversification. A cause-and-effect relationship seems unequivocal for several coding sequences derived from TEs, such as *Rag1* in jawed vertebrates,[26] *Peg10* in therian mammals, *Rtl1* in eutherians, syncytin-1 and syncytin-2 in primates, and syncytin A and syncytin B in murine rodents. Somatic selection acting on *Rag1*-mediated recombinant lymphocytes levels the playing field in the battle between rapidly evolving microbial pathogens and their long-lived vertebrate hosts. The evolution of a complex placenta, which involved domestication of *Rtl1* and lineage-specific *env* and *gag* genes, in addition to *Peg10*, was a key innovation in the adaptive radiation of mammals. Eutherians comprise 94% of extant mammalian species and are far more diverse than either their sister group, the marsupials, or their more distant relatives, the monotremes.[61]

The impact of non-coding, TE-derived sequences is more difficult to assess. Because of the genetic code, simple DNA sequence comparisons can identify functionally significant changes in coding sequences.[96] By contrast, deciphering the effects of non-coding sequences requires detailed functional and biochemical studies. Two recently investigated examples, SINE involvement in mammalian-specific brain formation, and *Alu*-based expansion of an miRNA gene family in primates, provide evidence that TE-mediated regulatory changes precipitate evolutionary innovations. AmnSINE1 is a newly identified family of ~100 highly conserved, mammalian-specific SINEs.[97] In mice, 32 of 124 identified loci are located near genes influencing brain development.[98] The mammalian forebrain is unique in its layering of the neocortex. Sasaki *et al.*[98] performed mouse enhancer assays on ten candidate AmnSINE1 loci, demonstrating that two act as distal transcriptional enhancers during embryonic development. Both enhancer effects are restricted to the developing forebrain, and their functions are specific to mammals.

TE involvement in expansion of a primate-specific family of miRNA genes (Chr19) has been investigated in a comparative study of nine species spanning the range of primate diversity.[99] The number of Chr19 loci ranges from 10 in the slow loris to 85 in the spider monkey. Analysis of the sequence characteristics of a 140 kb region encompassing Chr19 revealed a consistent pattern in which *Alu* elements occur at the junctions between miRNA duplication units. In addition, unlike the internally located *Alu* elements, these boundary *Alus* show evidence of recombination, indicating that *Alus* are responsible for amplification of the Chr19 miRNA family. Expression studies have demonstrated that many of the 46 Chr19 genes in humans are functional and expressed predominantly in placenta and brain.[99,100] As this example illustrates, TEs contribute to miRNA-based regulation both directly through domestication of transposon sequence[67] and indirectly via recombination-mediated gene duplication.

TE expansions and adaptive radiations

SINE insertions have been described as ''nearly perfect'' characters,[101] especially for resolving relationships of rapidly diversifying lineages, such as cetaceans,[102] hominids[103] and, most notably, African cichlids.[104] SINEs are assumed to be neutral markers whose integration at a specific genomic location represents a derived homologous character. It is becoming clear, however, that not all SINE insertions are neutral. We suggest that SINE insertions are highly informative markers in rapidly evolving lineages precisely because it is TE mobilization that triggers bursts of evolution, with some fraction of SINE insertions playing a role in the colonization of new fitness peaks. Such mutations should be particularly phylogenetically informative, since they are agents in the diversification process.

Arguably the most spectacular example of punctuated equilibria involves the adaptive radiations of haplochromine

cichlid fishes in East African Rift lakes. Cichlidae, the largest vertebrate family with ~3,000 species, originated 121–165 Mya[105]; yet it is estimated that 1,000 to 2,000 speciation events occurred within just the last 5 My in Lakes Tanganyika, Malawi and Victoria.[106] The ~2,000 cichlid species inhabiting these lakes exhibit remarkable diversity in morphology, feeding mode and behavior, despite limited genetic differentiation. In a study of five species from Lake Malawi, screening of >32,000 single nucleotide polymorphisms found no fixed differences between species or even between major lineages, and less nucleotide diversity than detected among laboratory strains of the zebrafish.[107]

To critically evaluate the various hypotheses for African cichlid diversification, Seehausen[106] conducted an investigation that included failed as well as successful adaptive radiations. His analysis showed that putative key innovations, such as the decoupled pharyngeal jaw and maternal mouth brooding, could not account for the success or failure of a radiation. The study did reveal, however, that the propensity for diversification increased along a single branch of the cichlid phylogeny, a lineage of haplochromine cichlids that evolved in Lake Tanganyika and went on to colonize and diversify explosively in Lakes Malawi and Victoria. As predicted by the epi-transposon hypothesis, analysis of 75 SINE loci indicates that this haplochromine lineage has experienced repeated bouts of cichlid-specific SINE insertions followed by extensive radiations within each lake.[108] Moreover, geological evidence of aridification in East Africa 2.5–3 Mya[94] and extreme lake level fluctuations of several hundreds of meters over the last 1.1 My[109] is consistent with epigenetic regulation of cichlid TEs breaking down in response to environmentally induced physiological stress.

Conclusions

We have proposed that epigenetic silencing of TEs perpetuates developmental homeostasis but breaks down during periods of environmentally induced stress, unleashing TEs and thereby creating the genomic mutability required for rapid morphological change and speciation. Obviously, no single mechanism can account for all cases of punctuated equilibria. However, the strength of the epi-transposon hypothesis lies in its ability to explain both long-term stasis and rapid diversification by providing an epigenetic link between environmental change and TE-driven genomic innovation. Our preliminary test of the epi-transposon hypothesis relies heavily on vertebrate examples. As more genome sequences become available, extension of this research to non-vertebrate taxa, in combination with experimental evolution studies, should enable rigorous testing of the hypothesis.

Although the epi-transposon hypothesis was developed to explain macro-evolutionary tempo and mode, stress-induced breakdown in epigenetic regulation of TEs has broader implications. The temporary liberation of TEs from epigenetic constraints generates genome-wide mutation rates much greater than those typically assumed in population genetics. In small populations, high TE-based mutation rates, relaxed selection on deleterious insertions, and the capacity of TEs to yield novel coding and regulatory elements promote evolvability and maintain heritable variation. This provides a potent alternative to pure genetic drift as a mechanism for Wright's shifting balance theory[25] and Mayr's "genetic revolutions",[110] and can account for extremely rapid evolution in small, peripheral isolates, such as the house mouse population on Madeira.[111]

Similarly, rapid evolution has occurred in the domestic dog. Although dogs were derived from wolf ancestors only ~15,000 years ago and have experienced extreme genetic bottlenecks over the last few centuries, they nonetheless exhibit greater phenotypic variation than any other mammal. The canine genome is replete with SINEs, and dog breeds display remarkable diversity in insertions of the SINE_Cf family, with an estimated 10,000 loci bimorphic for insertions.[112] Even more striking is TE-based genomic divergence between inbred mouse strains. In a study of five strains, 10,000 genomic variants were identified, 85% of which were attributable to recent mobilization of predominantly LINE-1 TEs.[113] LINE-1 insertions altered the structure and expression of numerous genes, demonstrating the rapidity with which transposon mobilization can generate genetic and phenotypic divergence.

While TEs have contributed substantially to host diversification, it should be recognized that the epigenetic path to new fitness peaks is a precarious one. In contrast to McClintock's view of TE mobilization as an adaptive response to challenge, breakdown in epigenetic suppression of TEs may frequently have short-term deleterious consequences. Mass mobilization of transposons may therefore frequently result not in evolutionary innovation but in population decline and extinction.

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