Nuclear function of Alus

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Introduction

Transposable elements (TE) spread and extend genomes of various species through insertion and amplification. They are believed to play critical roles in the remodeling and controlling of genome function in response to environmental pressures and in speciation. Approximately 44% of the human genome is composed of TE (http://genome.ucsc.edu), some of which remain active at an estimated rate of 1 insertion per 10–100 live births. Over 60 years ago, Barbara McClintock discovered transposition and proposed that TE modifies and controls maize gene expression. Not till recently has the breadth of the significance of TEs in genome function come into focus. Increasing numbers of studies have explored various aspects of TE elements. These studies are well reviewed by several groups. This review focuses on the Alu family, which is a member of the small interspersed elements (SINEs), a group of RNA-mediated retrotransposable elements.

Alus are transposable elements belonging to the short interspersed element family. They occupy over 10% of human genome and have been spreading through genomes over the past 65 million years. In the past, they were considered junk DNA with little function that took up genome volumes. Today, Alus and other transposable elements emerge to be key players in cellular function, including genomic activities, gene expression regulations, and evolution. Here we summarize the current understanding of Alu function in genome and gene expression regulation in human cell nuclei.

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The Function of Non-Transcribed Genomic Alus

Active Alu transposition and amplification diversify genomes and cause diseases

Alus are believed to have retrotransposed with much higher frequency at earlier stages of primate evolution, with the rate of transposition declining from 1 per live birth then to about 1 in 20 live births today. Given the current large human population, the number of collective insertions remains to be highly significant. Over time, these insertions, together with recombination, truncations, amplifications, and mutations, allow Alus to assimilate and to co-evolve with the human genome.

Alu transposition is determined by the sequences of the Alus and their ability to interact with SRP9/14 to form unique RNPs. Transposition capable free Alus are transcribed by RNA pol III. As the older Alus continue to change over time due to mutations, losing consensus sequences required for the transposition, the current insertion events are primarily attributed to the mobility of younger Alus. It is essential for the transposition capable Alu RNAs to bind SRP9/14 proteins, which form the pre-requisite intermediates prior to the reverse transcription for transpositions.

The resulting retrotransposition generally create new insertions, most of which are deleterious to genome function. Thus, cellular mechanisms are in place to control the expression of mobile Alus. Regulation takes place both at transcription and post-transcriptional levels. At the transcriptional level, most mobile Alus remain silent through epigenetic silencing mechanisms including methylation at Cpgs within Alus and histone modifications. The transcripational silencing explains why only very few Alu genes (~150) are transcribed, many of which are not transposition competent. At the post-transcriptional level, MOV10 RNA helicase function is found to restrict transposition of Alus and other TEs through binding and targeting them to stress granules, preventing the replication and thus the insertion of TEs.

Alu insertions, while can be beneficial, are mostly disruptive, leading to lethality or diseases, accounting for approximately 0.4% of all human diseases. Over a long period of time, the slow adaptation of Alus in the genome is believed to be an engine that drives the primate evolution. The insertion, recombination, and amplification, leading to the spreading and expansion of Alus and other TEs across the genome help develop diversity and complexity along with the genome evolution, which might

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contribute to the rise of human. For example, Alu mediated RNA editing is shown to be critical for cognitive and behavioral function and defects in RNA editing predisposes people to neurodegenerative diseases. As the inverted Alu embedded in pre/mRNA are substrates for editing and the level of editing is substantially increased in human vs. mice, the editing in Alu may have direct roles in the advanced neuronal development in human. Additionally, genomic sequencing and mapping among human populations demonstrate that Alu and Alu related polymorphisms contribute greatly to the diversity among humans.

However, Alu insertions and non-allelic homologous recombination between Alus cause chromosome instability or cause disruptions of gene structures and functions, leading to diseases in surviving individuals. For example, neurofibromatosis, a genetic disorder associated with childhood neurological tumors, is caused by changes in neurofibromatosis gene, NF1. Analyses of patient genomes show that insertions of as much as 14 Alus and other TE elements are responsible for the disease causing changes in splicing patterns of the gene, rendering it dysfunctional. Through this mechanism, Alus are responsible for a large number of diseases. Interestingly, not all genes are equally targeted by Alu insertions and recombination. Some are found to be preferred hot spots for these events, such as LDLR gene for hypercholesterolemia, BRAC1 and BRCA2 for breast cancer, and so on.

Being a large part of the genome, Alus in cis directly contribute to gene expression regulations

The idea that TEs control and regulate gene expression was first suggested by Barbara McClintock in the studies of maize genome. Since then, it is increasingly realized that TEs are the binding sites for various transcription factors and regulators. In humans, 39.4% of all transcription start sites are located within TEs. The large proportion of these sites is derived from ancient inserts that have been adapted through exaptation over a long period time co-evolving with the transcriptional regulators. Some of these elements (living fossils) are found conserved in distant species tracing back over 400 million years. Alus as a class of newer TEs (65 million years) have also been shown to create many transcription start sites. They are the binding sites for a dozen of transcription factors including SP1, PITX2, LUN1, etc., and many of these sites are associated with early developmental genes. More recently methylation and deamination of CpGs (C→G) in Alus are found to create functional binding sites for p53, myc, ANRIL, and PAX-6. These findings demonstrate that the epatation of Alus in the genome may allow for higher primate specific regulations of critical gene expressions. It would not be surprising for future analyses to implicate Alus in more transcription factor binding events.

Alus, as with other TEs, are frequently hot spots for recombination and DNA damage

The large number of copies in the genome and the transposable nature of Alus repeats provide enormous opportunities for recombination events that induce non-allelic homologous recombination among chromosomes, resulting alteration of the human genome. These events can take place at various stages of organismal development and at different physiological environments. They can be observed in germ lines, in somatic cells, and in cells under environmental stresses. For example, a focused analysis of Alu Y insertions found significant increases in recombination rate within 2 kb of the Alu among several human populations. However, not all of the events share the same efficiency. The longer of the homolog sequences in a combinatorial pair corresponds to more efficient recombination. Alu mediated recombination is one of the major cause for genome instability and chromosome translocation, underlining various diseases and malignancy. Closely related to recombination, Alus are (as with other TEs) sites prone to DNA damage. Alus are recently found enriched in chromosome common fragile sites, which tend to break, although they are part of normal chromosome structure and play an important function in sister chromatid exchange and other recombinatory functions. Another recent study shows that the majority of early replicating fragile sites in human cells are sites containing repetitive TEs, being one of them. These sites are also sensitive to damage during environmental stresses and under oncogenic pressures. Together these observations indicate that Alus (as with other TEs) play important roles in the dynamics of DNA breakage and recombination, which are part of essential genome function during development and tissues specific responses to environmental stresses. However, when they are not well controlled, the induced genome instability serves as basis for diseases, such as malignancy.

Transcribed Alu

Alus are primarily transcribed by RNA pol II and pol III. The majority of Pol II transcribed Alus are present in introns and the remaining ones are in the untranslated regions of mRNAs. Alus are rarely associated with exons. Increasingly, pre-mRNA associated Alus are found to play important roles in regulating gene expression at pre/mRNA levels. Pol III transcribes free or core unembedded Alus of about 280 bps. Much less is known regarding the function of these Alus. Additional to be the precursor of retrotransposon, they also play critical roles in cellular functions.

Alus embedded in pre/mRNA

Exonization mediated by Alus in introns can significantly influence the function of the translated proteins. Exonization is a process, in which a normally non-exon element in the intron is spliced into an exon of mRNA either in cis (within the same transcript) or in trans (between different transcripts). Alu is present in over 50% of introns and consensus Alu contains 23 potential splice sites, 19 of which are in the minor strand, consistent with the observation that 85% Alu exons come from antisense Alu elements. The abundance of Alu in introns makes it a leading force of new exon formation, contributing to 64% of new exons. Although 585 Alu exons were annotated previously, an additional 1318 cryptic exons originated from Alus have recently been identified.

Alu-mediated exonization takes place primarily through two mechanisms. The potential or cryptic splice sites in the Alu consensus sequences form pseudosplice sites. Specific mutations possibly over multiple steps turn Alu pseudosplice sites into bonafide sites. In analyses of 13 primate individuals, Singer et
al., mapped out the stepwise mutagenesis over millions of years that generated an alternative 5’ exon in the human tumor necrosis factor receptor gene.70 Additionally, insertion of inverted Alu allows for a stretch of Us incorporating into pre-mRNA, together with desirable mutations, making it into a functional splice site for exon generation. Therefore, mutations over time contribute to the conversion of pseudosplice sites within Alus into functional ones. A-I editing of Alu containing RNA is another mechanism that facilitates Alu exonization.64,71,72 Lev-Maor et al. found that the second A of the AA upstream of exon 8 of nuclear preamin recognition factor is edited from A-I, effectively turning AA into AG and making it a splice site. The editing is mediated through the formation of a double strand RNA stem loop of this Alu with an added 30 bp upstream. Incidentally, the new exon also contains TAG, a premature stop codon that is corrected also by editing A into G, 30 bp upstream. Incidentally, the new exon also contains TAG, a premature stop codon that is corrected also by editing A into G, forming harmless TGG. The newly formed exon is alternatively spliced in a tissue-specific manner.72 This is an intriguing example forming harmless TGG. The newly formed exon is alternatively spliced in a tissue-specific manner.72 This is an intriguing example

Alu

The results demonstrate that hnRNP C plays a key role in protecting mechanism is to restrict access to the Alu cryptic splice sites by splicing factors.67 For general splicing, U2AF65 binds exonal signals to initiate the recruitment of splicosomes.75 A recent study shows that hnRNP C competes with U2AF65 for splice sites by splicing factors.67 In the absence of hnRNPC, U2AF65 binding to Alu exonal signal significantly increases, directly corresponding to the significant increases of Alu mediated exonization globally. The results demonstrate that hnRNPC plays a key role in minimizing Alu exon inclusion under normal circumstance by blocking the access of U2AF65 to cryptic splice sites.67

Over time, the adapted Alu initiated exonization survives66 and directly contributes to the complexity of human genome function. For example, ADAR, an RNA editing enzyme, has 4 alternative spliced forms in human cells. One of which derives from the inclusion of an Alu-initiated exonization, leading to 40 extra amino acids in the protein. This inclusion has since been adapted as a part of isoforms of the deaminase and is highly expressed in neuronal tissues.76 Mattick and Mehler72 proposed that the adaptation of Alus through selection could contribute to the higher order of cognitive function in humans. The Alus could be the critical element that sets human apart from other primates.8,47

Alu at the 3′ UTR is a critical regulatory element for mRNA metabolism. While majority of the intragenic Alus are in the introns, approximately 5% of mRNA contains Alus and 82% of them locate at the 3′ UTR.77 Alus elements at 3′ UTR have been shown to regulate mRNA functions through several mechanisms, including RNA editing, nuclear retention, polyadenylation, RNA stability, miRNA function, and translational regulation in the cytoplasm. As this review focuses on the Alu function in the nucleus, we will discuss mostly their roles in the nuclear retention of mRNA, editing, and polyadenylation.

Alu at the 3′ UTR are found to regulate the nuclear retention of mRNA. mCAT2, a cation transporter protein, encodes two isoforms through the use of two promoters. The short form is transported into the cytoplasm and is translated at steady-state. The longer form of the mRNA is predominantly retained in the nucleus.74 It is only released into cytoplasm under stresses, such as viral infection and IFN gamma responses. The nuclear retained RNA is primarily enriched in the par splice bodies. The 3′ UTR contains 3 reverse and 1 forward Alu elements. Their presence is essential for nuclear retention.79 The forward repetitive element is a substrate for editing and is hyper-edited. It is believed that the editing of these repetitive elements helps explain the nuclear retention of these RNA. Subsequently, Chen et al. find that inverted Alu at 3′ UTR is responsible for editing and nuclear retention using ectopically expressed reporter constructs.79 However, it is not entirely clear whether editing alone can explains the nuclear retention of the inverted Alu containing mRNA. Two more recent studies show that an endogenous mRNA or a reporter mRNA with edited inverted Alus at the 3′ UTR exports to the cytoplasm rather than being retained in the nucleus.80,81 These findings suggest that editing may not be the key to nuclear retention of at least some mRNA with inverted Alu in 3′ UTR. In ES cells without detectable par splice bodies, mRNAs with extensive editing at the 3′ UTR with inverted Alus, are effectively transported into the cytoplasm.82 A recent study begins to shed light regarding the export of mRNA with inverted Alus containing 3′ UTR. Elbarbary et al.83 demonstrate that binding of inverted Alus at 3′ UTR with STAU1 proteins (dsRNA-binding protein Staufen1) inhibits the nuclear retention of these RNA, thus promoting the export of the mRNA.83 These findings together suggest that the nuclear retention of mRNA with Alus at 3′ UTR could be the results of the regulated balance between exporting forces (binding to STAU1, etc.) and retention power (editing, and other yet to be revealed functions).

Reversed Alus with two stretches of A rich sequences embedded in coding genes can also be potential polyadenylation signals. A single point mutation turning As to AATAAA, a conserved polyadenylation signal, can induce premature termination of transcription and generates truncated proteins. While this can be harmful, it is also a way to generate tolerated diversity.17 Analyses of human genome indicates that some of the genes use Alus as polyadenylation sites84,85 and among them, some of which distinguish human transcripts from other primates,86 suggesting a role of these adapted Alus polyadenylation sites in human evolution.

Alus at the 3′ UTR can also serve as targets for miRNA regulations. There is a significant number of miRNAs with sequences that are complementary to Alus.87-89 A primate specific gene cluster in chromosome 19 is found to encode miRNAs that
target the most conserved part of sense Alus. The majority of the 3′ UTR-located Alus carries potential target sites for at least 53 miRNAs.

Transcribed free Alu by pol III

Although there are more than a million copies of Alu spread in the human genome, very few are transcribed independently. Most of transcribed Alus are those embedded in pre-mRNA and transcribed by RNA polymerase II. RNA pol III is responsible for transcribing free Alu (~280 bps), which contains an internal promoter for class two pol III transcription. As Alus are ubiquitous throughout coding sequence, RT-PCR is not representative of free Alu transcription. Over the past few years, the genome-wide ChIP for pol III transcription machinery from three groups mapped the active Alu transcribing loci in genomes of several human cell lines, revealing for the first time the specific Alus that are transcribed. Over the past few years, the genome-wide ChIP for pol III transcription machinery from three groups mapped the active Alu transcribing loci in genomes of several human cell lines, revealing for the first time the specific Alus that are transcribed. More than 150 loci are found to be active in various cells. While many loci are overlapping among different cell lines, some are unique to specific cell lines. The majority of pol III associated loci are those of older families including Alu S and Alu J and most of these transcripts lack sequences required for retrotransposition, suggesting that these are not the main source of active retrotransposition.

What then is the function of the non-transposable Alu RNA? Heat shock and translation inhibitors have been shown to activate the transcription of free Alus. The similar elevation was also found during viral infection. The transcription enhancement is highly regulated and the levels of Alu RNA return to the basal level upon relief from the stresses. The increases in the free Alu RNA upon stresses directly correspond to the inhibition of RNA pol II transcription in the nucleus. The mechanistic studies show that Alu RNA directly binds RNA pol II as tested both in vitro and in vivo, suggesting that Alu RNA integrates into the transcriptional complex, disrupting the interaction between pol II and promoter DNA and blocking transcriptional initiation. Additionally, elevated levels of free Alus may contribute to cellular senescence. Transcription of Alu in reverse orientation can also act as a cis natural antisense transcripts (NAT). The NATs regulate gene expression at both transcriptional and post-transcriptional levels. Transcription at opposite orientations on the same DNA template slows down progression of polymerases possibly to avoid collision. At the post-transcriptional levels, NATs can act through antisense RNA or small RNA interference mechanisms. Recently, studies of a retinoic acid responsive and pol III transcribed DR2 Alus show that the small RNAs derived from dicer processed DR2 Alus play critical role in the degradation of stem cell specific RNAs during cellular differentiation.

Cytoplasmic free Alu RNAs have multi-facet functions. Alus are found to regulate translation of mRNA, in which Alu RNAs form RNP with SRP protein, SRP9/14. The resulting Alu RNP's inhibit double strand RNA-dependent protein kinase (PKR), leading to translational stimulation. The free Alu RNAs are found to help prevent miRNA targeting to targets that are within Alus at the 3′ UTR, possibly by blocking access to the targets, thus reducing miRNA regulatory function to the specific RNAs. Furthermore, free Alu pairing with 3′ UTR Alus in trans can activate mRNA decay. These findings demonstrate that Alus have multiple and unique functions in human cells. As the human genome contains a large number of Alu genes, we will probably see more cellular functions to be assigned to Alu RNAs and perhaps they are critical in making us human.

Summary

Alu, although relatively young in the evolution history (65 million years), is the most successful transposable element in
the human genome, occupying over 10% of the genome. They function in two forms, as genomic elements or as transcribed RNA (Fig. 1). *Alu*-mediated transposition, DNA damage, mutations, and recombination directly contribute to the complexity and instability of the genome. *Alu*, as with other TEs play key roles in gene expression at the transcriptional level. They are frequently the transcription start sites and transcription factor binding sites. These sites appear to co-evolve with transcription and replication machineries over time to allow for specific regulations, which could be critical for speciation. When embedded in pre/mRNA, *Alu* regulates the diversity of mRNA through editing and exonization and influence the stability and translatability of the mRNAs. Furthermore, free *Alu* RNAs regulate genome functions from pol II transcription and retrotransposition to antisense regulation of gene expression under normal and stressed conditions. Altogether, these highly diverse functions of *Alu* mediate genetic drift, increase complexity of the genome function, and are likely to play critical roles in human evolution.

**Disclosure of Potential Conflicts of Interest**

No potential conflict of interest was disclosed.

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