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Alu Elements and Human Disease

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Abstract

As a family, Alu retrotransposons compose the single largest component of the human genome [2]. They are thought to have arisen from the gene coding for 7SL RNA, a component of the signal recognition particle [14, 19, 23]. The only apparent purpose of these highly repetitive sequences is to replicate and copy themselves onto new areas of the genome; this has resulted in an estimated 10% growth in human genome size since our evolutionary divergence with the chimpanzee [2]. Normally cellular proteins methylate the cytosine and guanine rich areas of these transcripts in order to prevent the retrotransposon from displacing. When cellular conditions promote demethylation, Alu regions can be transcribed and insert into new areas of the genome via an RNA intermediate [2]. Insertion into a non-coding region is typically harmless, but introduction into a coding exon can

lead to disrupted gene transcription and altered protein synthesis. The original demethylation event is largely a result of environmental conditions and leads to heritable changes in DNA sequence [22]. It is estimated that Alu retrotransposition currently occurs at a rate of about 1 per every 200 births, and alone accounts for an estimated 0.1% of genetic disorders [8]. The recombination of Alu elements could potentially be one of the most important sources of genetic variation, but is also a major source of human genetic disease.

The Alu element

The Alu transcript is a ~300 base pair (b.p.) highly repetitive sequence rich in CpG dinucleotides (adjacent cytosines and guanines) [2]. It belongs to the category of retrotransposons arbitrarily defined as having fewer than 500 b.p., the Short Interspersed Elements (SINEs) [23, 24]. The sequence contains a functional RNA polymerase III (RNA pol III) promoter, intermediate non-coding elements, and an RNA-like poly(A) tail [11, 21]. Though Alu elements form the largest single family of non-coding elements in the genome, they paradoxically lack the necessary machinery to insert themselves into a DNA sequence [2]. While it is still not entirely clear, it is likely that they use the transposase enzyme coded for by Long Interspersed Elements (LINEs, retrotransposons with greater than 500 b.p.) [8]. The RNA pol III promoter and the borrowed transposase are sufficient to transcribe an RNA copy and insert it into a new spot in the fashion of a retrovirus. Another key aspect of this process that is still unclear is whether all Alu copies are capable of replication. The Alu elements are arranged into distinct families based on sequence homology, implying a limited number of common ancestors transposing into inactive copies [6]. Again, it is unclear whether certain sequences are inactive due to sequence variation or host silencing mechanisms [17].

Methylation of Alu Repeats

Alu retroelements, if left unchecked, would insert throughout the genome into non-coding and coding regions. The result would be mutations, interrupted protein synthesis, and eventually cell death or abnormalities. To complicate matters further, there is no known method by which Alu elements can be removed: Once they insert into a new area, they remain there permanently. Retrotransposition is held in check by methylation of the CpG dinucleotides within Alu elements. Methylation occurs on the carbon 5 position of cytosines directly adjacent to guanines [24]. Maintenance methylation by DNA methyltransferase 1 (DNMT1) exclusively targets CpG dinucleotides during replication whose counterparts on the parental strand of DNA are also methylated [7]. In this fashion, methylation patterns on Alu elements (and other CpG dinucleotides) are preserved in a cell line. Alu elements are also common

sites for de novo methylation by DNMT3a and DNMTb, especially during embryonic development [7, 13]. It is uncertain why certain CpG rich sequences are heavily methylated while others, notably the CpG islands preceeding coding genes, are not [3, 22, 24]. Methylation of Alu elements prohibits transcription (and therefore retrotransposition) in two ways: Firstly, 5-methylcytosine (m ^{5}C) directly inhibits binding of polymerase via steric repulsion. Secondly, methylated CpGs recruit methyl-domain binding proteins (e.g. Lsh) which bind and block promoter sites [13]. These proteins also recruit histone deacetylases in order to convert functional DNA into heterochromatin [22]. Once methylated, m ^{5}C eventually deaminates to form thymine [20]. The spontaneous deamination of m ^{5}C has distorted the sequence of several of the evolutionarily ancient Alu elements [24]. The number of recognizable Alu elements alone accounts for some 10% of the human genome, and retrotransposons collectively account for nearly 45% of the genome [2, 11].

DNA Demethylation

There are thought to be two general mechanisms for DNA demethylation. The first involves direct removal of the methyl group from m ^{5}C using water, resulting in a demethylated cytosine and a methanol byproduct [25]. This process is not likely to occur spontaneously, and would require a DNA demethylase enzyme. There are controversial reports of such an enzyme, but no solid evidence as to its existence yet [25]. The second method is excision of the entire m ^{5}C, leaving the phosphate backbone intact. The base is then replaced by an endonuclease, restoring the original sequence [25]. Known chemical demethylating agents act in either a replication dependent or independent manner. Drugs such as 5-Azacytidine are cytosine analogs and incorporate into newly synthesized DNA and RNA strands [4]. 5-Azacytidine ring structure prohibits addition of methyl groups, preventing future methylation in a transcription dependent manner. Other compounds such as valproate can reverse methylation in non-dividing cells (independent of transcription) [9]. Drugs that block methylation are important as potential chemotherapy drugs. In tumor cell lines, there is a global hypomethylation, but tumor suppression genes are hypermethylated and hence not expressed [10, 24]. The aim of demethylation drugs is to promote expression of innate tumor suppression machinery by removing excess methylation.

Mechanism of Retrotransposition

Alu retrotransposition requires both the demethylation of the Alu elements themselves as well as LINEs coding for transposase enzymes [8]. The reactivated Alu element attracts RNA pol III which transcribes the sequence along with a stretch of several thymines, which act as a RNA pol III termination signal [2]. The nick in the new insertion spot is likely made by a LINE-encoded endonuclease at the 3'-AA/TTTT-5' consensus site [2]. The poly(A) tail of the Alu RNA binds the TTTT, and a second nick is made in the DNA to allow insertion [2]. The strand then inserts and is ligated by an unknown mechanism.

It is likely that once one strand is inserted, host repair machinery is responsible for ligating the strand and polymerizing the complimentary strand [1].

Alu elements and Disease

The hypomethylation of both LINE and Alu elements promotes insertion of Alu elements, affecting both transcription as well as splicing. Interruption of either process results in aberrant protein synthesis or malignancies. Diseases directly associated with Alu insertion into coding regions include neurofibromatosis, haemophilia, agammaglobulinaemia, leukemia, breast cancer and ovarian cancer [8]. Any malignancy caused by Alu insertion is both heritable along somatic cell lines as well as in germ lines Recent research shows that insertion of Alu elements can also indirectly affect coding regions: Proximity to Alu elements is a predictor of the length and methylation resistance capacity of CpG islands preceeding coding regions [10, 15]. In this fashion, spontaneous insertion of an Alu element causes nearby promoters to be hypomethylated, increasing gene expression. This mechanism could account for some of the hypomethylation patterns seen in tumour cell lines, making it an important direction for researchers.

Variation in Alu mobility is a factor of the demethylation levels of both Alu elements and of LINE elements. It is probable that the main causes of demethylation are environmental, results of chemical (demethylating agents) and physical (e.g. irradiation) influences along with the actions of cellular machinery [4, 9, 16]. Hypomethylation of Alu elements constitutes epigenetic change in DNA transcription, which can indirectly result in heritable and permanent change in the DNA sequence [22].

Conclusion and Outlook

The mechanism by which Alu elements mobilize should serve as a caution. Chemotherapy drugs that cause global demethylation would indeed reactivate tumor suppressor genes, but may also increase the mobility of Alu elements. This process could result in the development of disease or malignant cells, as well as fully heritable epigenetic changes. While in the short term Alu retrotransposition is responsible for a variety of disease, they are also a major source of useful genetic recombination [18]. An estimated 2.8 million years ago, a spontaneous Alu insertion inactivated a gene necessary for the synthesis of N-acetylneuraminic acid [5]. It is theorized that the subsequent build-up of the precursor corresponds to the evolutionary expansion of the human brain, and may have played a role in the evolution of human intelligence. Other heritable Alu insertions have been linked to protection against heart disease as well as Ewing's Sarcoma among certain populations [8, 26]. While Alu insertions have repeatedly been implicated in causing human disorders and mutations, they are a source of the genetic recombination that fuels evolution. Retrotransposons in general comprise a large and currently unpredictable force. Elucidation of their

mechanisms of action may facilitate treatment of genetic disease and prevent inadvertent activation.

Endnotes

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