2 *Alu* Elements

Prescott Deininger, PhD

Contents

Introduction
Alu Elements
Mechanism of Insertion
Evolution of *Alu*
Insertional Mutagenesis
Postinsertion Damage
Evolutionary Changes Caused by *Alu*
Future Directions
Summary
References

INTRODUCTION

Alu elements represent one of the most successful mobile elements found in any genome. They have reached a copy number in excess of one million copies, making up more than 10% of the human genome. The level of amplification required to reach this high copy number has created an enormous number of insertion mutations resulting in human disease and genome evolution. They also add extensive diversity to the genome by introducing alternative splicing and editing to a wide range of RNA transcripts. In addition, after insertion *Alu* elements contribute to a high level of genetic instability through recombination. This instability contributes to a significant number of germ-line mutations and may be an even bigger factor in cancer and/or aging.

ALU ELEMENTS

Alu elements have reached a copy number in excess of 1×10^6, representing more than 10% of the human genome *(1)*. They are widely distributed across the entire genome, with only a relatively few regions that have few of them. *Alu* elements tend to be enriched in the GC-rich, gene-rich regions, with many *Alu* elements located in the introns of genes.

The current rate of *Alu* amplification has been estimated to be in the range of one new insertion per 20–200 human births *(2,3)*. The *Alu* insertion process has the potential to damage the genome both through insertional mutagenesis, and through facilitation of unequal, homologous recombination events *(2)*. Insertional mutagenesis by *Alu* causes approx

From: *Genomic Disorders: The Genomic Basis of Disease*
Edited by: J. R. Lupski and P. Stankiewicz © Humana Press, Totowa, NJ

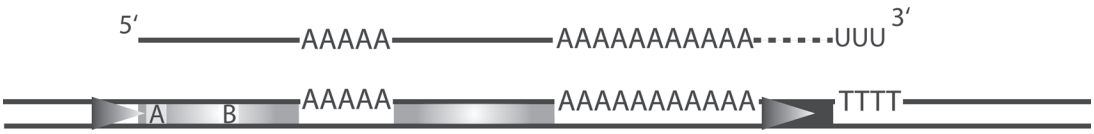

Fig. 1. Structure of an *Alu* element. A typical *Alu* element is schematized with the arrowheads representing the short, direct repeats formed at the point of insertion. The left half of *Alu* contains the A and B boxes of the RNA polymerase III promoter. The two halves of the *Alu* dimer are separated by an A-rich region and the 3' end of the *Alu* contains a variable, A-rich region. The line above represents a typical *Alu* transcript, which terminates in the downstream flanking sequence at a terminator that generally contains four or more T residues in a row. The dotted portion of the line represents sequences downstream of the *Alu* element that will be variable between transcripts from different genomic loci.

0.1% of human genetic disease, whereas recombination processes contribute to a much higher level. Little is known about the influence of *Alu* insertions in somatic or transformed cells. They have the potential to contribute to genetic instability in tumorigenesis, aging, and other somatic disorders.

MECHANISM OF INSERTION

Understanding the mechanism of *Alu* insertion is critical to understanding how they influence genetic instability. A typical *Alu* element, shown schematically in Fig. 1, contains an internal, RNA polymerase III promoter that allows transcription through the element and into the flanking sequence. *Alu* elements have a dimer structure, with both halves having an ancestral derivation from the 7SL RNA gene and have no capacity to code for proteins. At the 3' end of each *Alu* element is an A-rich sequence of variable length and the elements are generally flanked by short, direct repeats of 5–20 bp in length that are formed from duplications of the target site during insertion. It has been shown that the 3' A-rich region is routinely lengthened by the insertion process *(4,5)*, and rapidly shrinks after insertion *(6)*.

It is thought that the RNA polymerase III-transcribed *Alu* RNA must associate in some way with at least the ORF2 product of L1, which codes for endonuclease and reverse transcriptase activities. Thus, *Alu* is considered a nonautonomous mobile element, which is dependent on the autonomous L1 elements. The endonuclease then cleaves the genomic DNA at a loose consensus sequence, and the genomic site primes reverse transcription of the *Alu* RNA, using the 3' oligo-A region of the *Alu* RNA as a template. It has been hypothesized that *Alu* elements with longer A-tails are more active in the retrotransposition process *(6)*. The typical integration is then finished off by the formation of a second-strand nick and integration of the end of the cDNA (Fig. 2A). However, significant portions of the events appear to complete the integration process by recombining with another upstream *Alu* element *(7)* (Fig. 2B). This would cause sequences between the point of insertion and the upstream *Alu* element to be deleted. It also seems likely that many *Alu* elements may not complete the insertion process. Thus, the initiation of the insertion may create genomic nicks that contribute to mutation and recombination processes in the cell, but show no evidence of *Alu* insertion.

Although *Alu* amplification is clearly dependent on L1 elements for their retroposition, there are definite differences in their amplification process. The most notable difference is that, using a tagged *Alu* reporter system, *Alu* insertion was found to not require exogenous L1 ORF1 expression *(5)*.

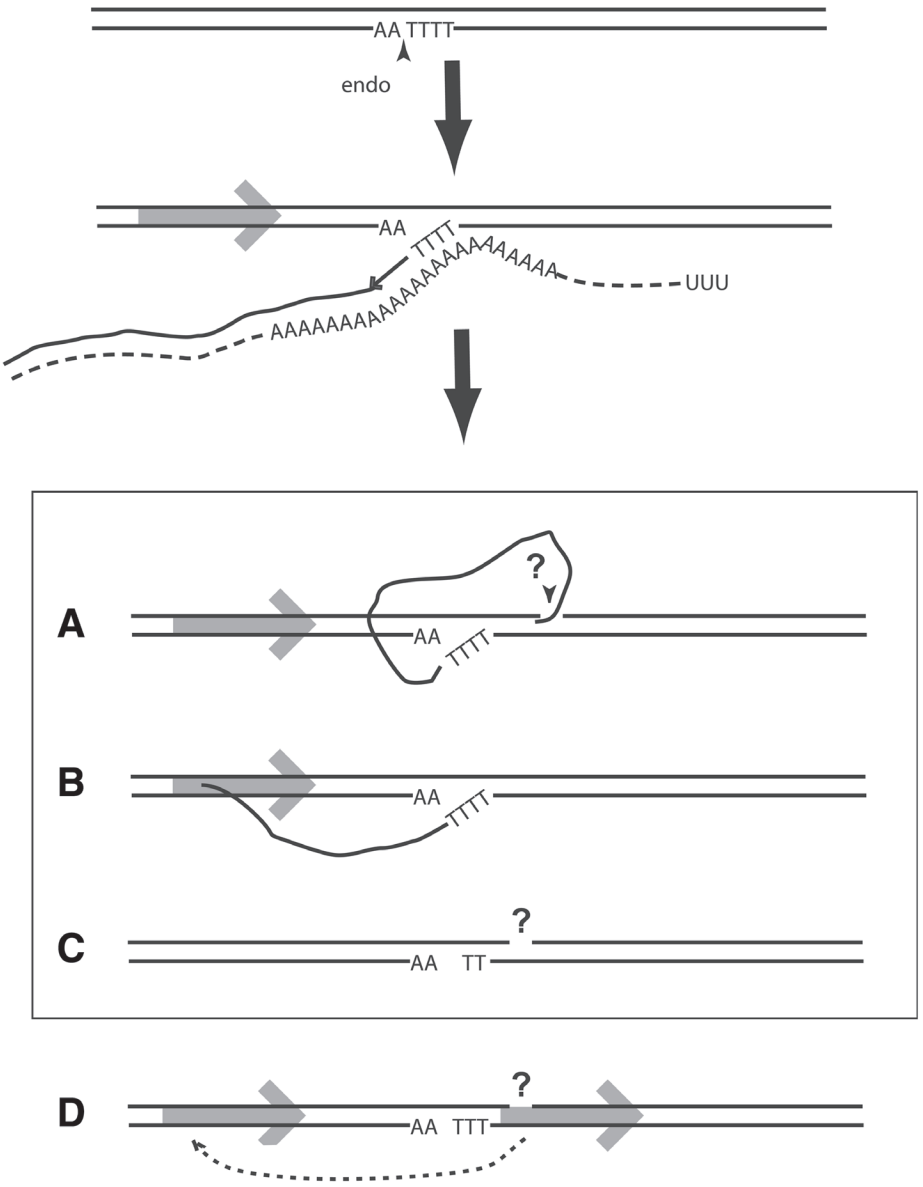

Fig. 2. Integration of *Alu* elements into genomic sites. The basic mechanism of *Alu* insertion is thought to involve a target-primed reverse transcription (TPRT) in which the endonuclease provided by L1 elements creates a nick in the genomic DNA at a consensus sequence resembling TTTT'AA. The nicked strand with the T residues can then prime reverse transcription from the 3' A-rich region of the *Alu* RNA. The second-strand integration process is poorly understood. The typical integration is schematized in (A) with a second nick occurring from an unknown source, probably allowing integration of the 3' end of the cDNA using some sort of microhomology-driven priming. (B) An alternative mechanism that occurs occasionally where the 5' end finds a homology with another *Alu* element upstream. (C) It is possible that the integration process may abort prematurely, possibly with the intervention of DNA repair processes, leading to DNA damage. (D) *Alu* elements may undergo unequal recombination with other *Alu* elements nearby. We hypothesize that nicking by L1 endonuclease at consensus sites adjacent to *Alu* elements may facilitate this recombination process.

EVOLUTION OF *ALU*

Alu elements began to amplify early in primate evolution *(8)*. A precursor to *Alu* elements may have predated the primate/rodent divergence. Rodents have B1 elements that are also derived from the 7SL RNA gene. However, the B1 elements have a monomer structure, whereas the *Alu* elements have a dimer structure that formed early in primate evolution. *Alu* elements are found even in the prosimian primates. *Alu* elements began to accumulate in primates about 65 million years ago. Because there is no specific mechanism for removal of *Alu* elements, their copy number has continued to increase throughout primate evolution.

As the copy number increased, the sequence of *Alu* elements has evolved as well. Figure 3 shows a schematic of *Alu* insertions during primate evolution. There were very high levels of *Alu* retroposition early in primate evolution. The current rate in human is nearly 100-fold lower than at the peak of *Alu* insertion. At different stages of primate evolution, the *Alu* elements that amplified had distinct sequence differences that allowed them to be classified into subfamilies *(8)*. Recent studies suggest that there has been a modest increase of *Alu* insertions in the human lineage relative to the other great apes *(9)*.

The most likely explanation for the formation of different *Alu* subfamilies at different evolutionary times is that there are extremely few "active" *Alu* elements at any one time. This allows the sequence of active elements to drift with time. Various hypotheses have been proposed for the features that limit *Alu* element activity. These include the flanking sequences of *Alu* elements influencing transcription rates *(10,11)* and subfamily-specific changes interacting with the retrotransposition machinery *(12)*. Currently, we favor the length of the A-tail as the most important factor *(6)*. The A-tail grows on insertion but shrinks rapidly in evolutionary terms, potentially silencing individual *Alu*s. In addition, older *Alu* elements accumulate mutations that lessen their expression, as well as potentially their interaction with the retroposition proteins. Thus, only progeny from the most recent insertions are likely to maintain activity.

Because the most recent inserts are also the most likely to be active elements, many of the most active elements will be polymorphic in the human population. Thus, different individuals in a population may be more or less prone to *Alu* amplification and it is the entire population that serves as a reservoir for potentially active elements.

Chromosomal Distribution

Although *Alu* elements are spread throughout the human genome, they show some regions of higher density. In particular, *Alu* elements appear to be preferentially located in GC-rich genomic "isochores," while L1 elements are located in AT-rich isochores. However, most recent data suggest that *Alu* elements insert relatively randomly with respect to GC content *(1)*, and are selectively lost from the AT-rich regions *(13,14)*, creating the bias seen. Thus, *Alu* elements are likely to have a fairly random insertional mutagenesis potential, but are more likely to contribute to postinsertional recombination events (*see* below) in the GC- and gene-rich regions of the chromosomes.

INSERTIONAL MUTAGENESIS

Alu elements create an approx 300-bp insertion mutation at any new genomic insertion site. If *Alu* insertion occurs in a coding exon, or near a splice junction, they are likely to disrupt the appropriate expression of a gene *(2)*. Table 1 shows a collection of most of the known cases

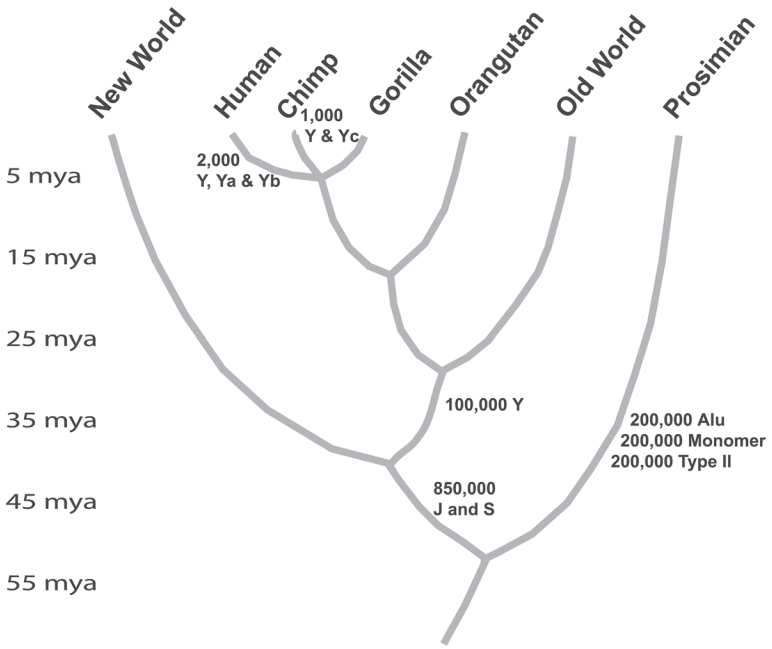

Fig. 3. Insertion history of *Alu* elements in the primate lineage. The times and approximate copy numbers of some of the major subfamilies of *Alu* elements are listed on a primate tree to illustrate the approximate times of formation of *Alu* elements, primarily for the human lineage, but also including several SINEs identified in prosimians.

of human diseases, caused fairly equally by these two types of insertions. Although these represent a number of different diseases, reflecting the broad distribution of *Alu* elements throughout the human genome, there are multiple independent cases in four different loci. This is probably largely a case of ascertainment bias because those genes have been very heavily studied. However, there is also a strong preference seen for diseases associated with the X chromosome (10/23 cases). There is much less of a bias for the X chromosome than has been seen for L1 element insertions, and probably also largely represents an ascertainment bias in favor of sex-linked diseases.

In a few rare cases, *Alu* elements have inserted into an exon coding for the carboxy-terminus of a protein and has not destroyed the function of the protein *(15,16)*. In these cases, the *Alu* element has incorporated a portion of its sequence into the coding region of the protein, likely because that portion of the protein was not critical for function, but novel proteins can evolve this way.

Insertions in the middle of introns or between genes appear to be well-tolerated and to have minimal effects on genes. Many genes have introns that include dozens of *Alu* elements that make up more than half of their DNA. However, as discussed next, many of these initially harmless *Alu* insertions can become deleterious by contributing to recombination events, or through further mutation altering their properties.

As illustrated in Fig. 2C, it is possible that *Alu* elements sometimes begin the insertion process, but that cellular repair processes remove the initial primed element. This would result in a nicked DNA that could serve as a free end in a recombination event (Fig. 2D), as well as

Table 1
***Alu* Insertions Causing Human Disease**

Locus	*CHR*	*Disease*	*Refs.*
3X HEMB (IX)	X	Hemophilia B	*3,61,62*
2X HEMA (VIII)	X	Hemophilia A	*63,64*
2X CLCN5	X	Dent disease	*65,66*
BTK	X	X-linked γ-globulinaemia	*67*
IL2RG	X	XSCID	*67*
GK	X	Glycerol kinase deficiency	*68*
BCHE	3	Cholinesterase deficiency	*69*
CASR	3	Hypocalciuric hypercalcemia and hyperparathyroidism	*70*
MLVI2	5	Leukemia	*71*
APC	5	Hereditary desmoid disease	*72*
EYA1	8	Branchio-oto-renal syndrome	*73*
2X FGFR2	10	Apert syndrome	*74*
FASL	10	Autoimmune lymphoproliferative syndrome	*75*
C1NH	11	Complement deficiency	*76*
AIP	11	AI porphyria	*77*
BRCA2	13	Breast cancer	*78*
NF1	17	Neurofibromatosis	*79*

CHR refers to the chromosome for the insertion.
2X and 3X refer to genes that have had two or three insertions, respectively.

lead to mistakes in DNA repair and other DNA damage. Such events would be the result of an attempted *Alu* insertion, but would leave no evidence of the role of the *Alu* element. Thus, at this point we believe that we underestimate the role of mobile elements in recombining and damaging the human genome.

POSTINSERTION DAMAGE

Recombination

Alu elements may continue to contribute to genetic instability even if they do not initially damage a gene. A common form of secondary damage is owing to unequal homologous recombination, but several other types of mutations can alter the properties of *Alu* elements to create damage.

Alu elements may contribute to recombination events in several ways. The best understood is through either a deletion using the single-strand annealing reaction (Fig. 2D) or a reciprocal, unequal homologous recombination (Fig. 4A), which can cause either duplications or deletions of the segments between the *Alu* elements that recombine. The recombination event does not have to be reciprocal. For instance, if cells use the single-strand annealing pathway of recombination, nearby homologous *Alu* elements may recombine causing only deletions. These types of homologous recombination events have been estimated to cause at least 0.3% of human genetic disease *(2)*. However, as the majority of larger genomic rearrangements have not been characterized to this level, it seems likely that this represents an underestimate.

There are several studies suggesting that *Alu* elements located near one another in an inverted orientation may be even more destabilizing than those in a direct orientation *(17–19)*. How-

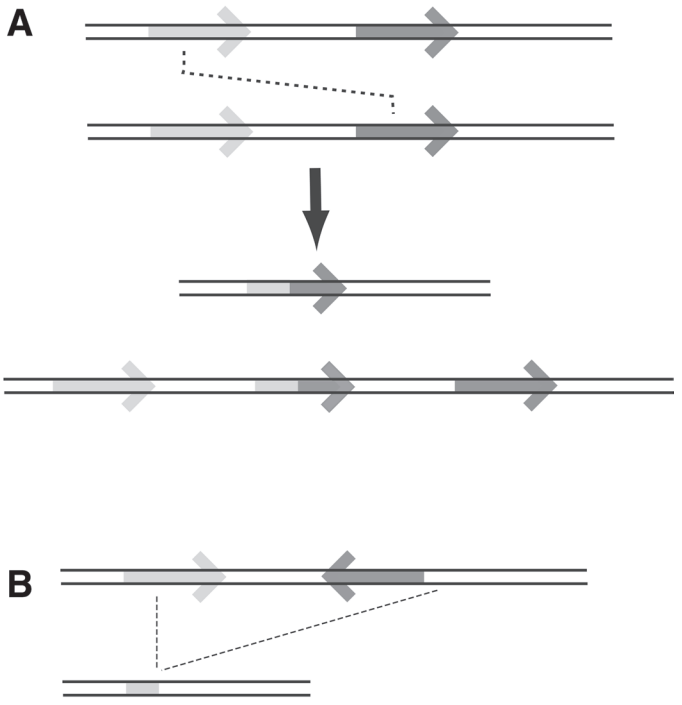

Fig. 4. Unequal *Alu–Alu* recombination. (A) When *Alu* elements are located in the same orientation near one another in the genome, it is possible for an unequal homologous recombination event to occur that can give rise to either a duplication or deletion of the sequences between the *Alu*s. A hybrid *Alu* element is usually formed at the point of recombination. (B) When *Alu* elements are in the inverted orientation, they also seem to trigger recombination in their vicinity, but the recombination junctions do not seem to be driven by recombination so that the resulting products can be variable.

ever, the instability caused by the inverted *Alu* elements (Fig. 4B) does not create a predictable junction that allows individual recombination events to be definitively defined as being caused by *Alu* elements. Recombination can occur at various locations around both *Alu* elements. Thus, it is difficult to distinguish between *Alu*-induced recombination events of this type, and *Alu*-unrelated nonhomologous end-joining events. However, it has been suggested that secondary structures may contribute to the majority of recombination junctions *(20)*, and *Alu* elements represent one of the most abundant elements that could consistently contribute to such secondary structures.

We have previously reported a broad range of genes that have undergone homologous recombination events leading to genetic defects *(2)*. These represent a broad variety of genes, as *Alu* elements are spread throughout essentially all genes. However, there are a few genes with unusually high levels of *Alu–Alu* recombination events. This includes a large number of events in the LDLR and C1 inhibitor loci. However, there are also seven different recombination events leading to breast cancer in the *BRCA1* gene *(21–25)*, three mutations in the *MSH2* gene that may represent as much as 10% of the defects in that gene *(26,27)*, and a duplication and a deletion in *MSH6 (28)*. The majority of cases of acute myelogenous leukemia that do not involve a visible translocation have been shown to involve *Alu–Alu*-mediated duplication events in the *MLL* gene *(29)*. Although inter-chromosomal translocations generally do not

Fig. 5. Map of the *Alu–Alu* recombination junctions found in an assortment of recombination events. The darker dashed lines represent those occurring in the *LDLR* gene. The lighter dashed lines represent those occurring in the globin genes. The solid gray lines represent those that occurred in the *MLL* gene leading to acute myelogenous leukemia and the solid dark lines represent recombination events in an assortment of other genes. The length of the lines represents the uncertainty in the exact point of the recombination events relative to the schematic map of the *Alu* dimer shown. The arrows in the dimer portions represent the A-rich regions.

involve *Alu–Alu*-mediated translocations, there is now some evidence that many of these cases have additional, smaller rearrangements in the *MLL* gene that may be *Alu*-mediated *(30)*. Recently, it has been suggested that up to 30% of mutations in the Fanconi anemia gene (*FANCA*) may be caused by *Alu–Alu* recombination events and that these types of events are not typically detected using the polymerase chain reaction strategies commonly in use *(31)*. Thus, there is likely a strong ascertainment bias against detecting *Alu*-mediated genomic rearrangements.

Most of the *Alu–Alu* recombination events are between nearby elements, generally spanning distances of less than 50 kb and often only a few kb in length. There are also a number of cases of chromosomal translocations that suggest some involvement of *Alu* elements *(20,32)*, but very few that involve *Alu–Alu* homologous recombination.

It has been proposed that *Alu* elements may contain specific sequences that trigger recombination *(33)*. An analogy has been made to "chi-like" sites that may cause targeting of the recombination events within the upstream portion of the *Alu* element. The original data that triggered that hypothesis were mostly from recombination events in the *LDLR* gene that appear to cluster (Fig. 5). However, when a broader range of *Alu–Alu* recombination events are mapped, they occur dispersed throughout the *Alu* element, with only a modest predisposition to the left end. This includes both germline recombination events, as well as somatic events like those that contribute to AML *(29)*. The apparent predisposition for the upstream end of *Alu* may be caused by numerous factors. One is that none of the positions that define the *Alu* subfamilies occur toward the upstream end of *Alu* and therefore *Alu* elements from different subfamilies may show fewer mismatches with one another at that end. Also, that region includes the RNA pol III promoter for the element and may have a more open structure, particularly with specific *Alu* elements that may be in the appropriate chromatin environment to favor expression *(11)*.

Finally, whatever sequence features favored the insertion of the *Alu* at that location in the first place may lead to higher recombination rates near the end of the element.

Alternative Mutagenic Changes

In addition to a major contribution to genetic instability through insertion and recombination, *Alu* elements may also contribute to other forms of DNA damage. There are a growing number of reports that *Alu* elements may influence splicing of genes. *Alu* elements have inserted in a number of loci without causing any apparent defect, but point mutations in the *Alu* elements have led to activation of cryptic splice sites causing genetic defects *(34,35)*. In addition, there is growing evidence that even *Alu* elements that are tolerated in genes may be contributing to a significant level of alternative and aberrant splicing *(36–38)*. This leads to the inclusion of parts of *Alu* elements in a proportion of transcripts coming from the gene. In most cases these would be expected to result in defective RNAs and would, therefore, decrease the overall expression from those genes.

Although the A-tails of new *Alu* insertions appear to generally be homogeneous, they have been shown to commonly lead to the creation of more complex microsatellites over time *(39)*. Although such changes would lead to frequent genetic polymorphism, the vast majority would be expected to be relatively harmless. One important example of a disease-causing change occurred in the middle A-rich region of an *Alu* in the frataxin gene *(40)*. In one particular human, this region mutated into a GAA microsatellite. This GAA microsatellite created the permutation allele that grew through triplet repeat instability to a size where it somehow blocked transcription of the frataxin gene *(41–43)*, leading to Friedreich ataxia in those carrying this microsatellite.

Other changes within these A-rich regions commonly generate the potential polyadenylation signal, AATAAA *(44)*. This could lead to truncation of transcripts and disruption of gene expression.

EVOLUTIONARY CHANGES CAUSED BY *ALU*

In order to create the more than 1 million *Alu* elements fixed today in the human genome, there have had to be massive numbers of other insertions events that were not fixed. Thus, *Alu* elements have been a major contributor to genomic instability and evolution throughout primate history. One such event was an insertion-mediated deletion that inactivated the CMP-*N*-acetylneuraminic acid hydroxylase gene only in humans *(45)*. This led to altered protein glycosylation, which may have been a significant change helping to result in the speciation of humans from chimpanzees.

Alu–Alu recombination events have also played an important role in chromosomal evolution and potentially speciation. It appears that an *Alu–Alu*-mediated recombination in the gulonolactone oxidase gene occurred after the divergence of prosimians from the other primates *(46)*. This enzyme is a critical late step in the synthesis of vitamin C and resulted in the inability of primates to synthesize this vitamin, leading to the possibility of scurvy.

In a broader sense, *Alu* elements appear to have been involved in a number of recombination events that have helped lead to segmental duplications on human chromosomes *(47,48)*. These segmental duplications have in turn been associated with a number of different syndromes through instability of the segmental duplications *(49)*. Thus, *Alu* elements appear to have contributed to an overall rearrangement of the genome and chromosomes that has given rise

to extra copies of genes, which may be beneficial for evolution, which on the other hand, can have negative impacts on the long-term genomic stability and function.

Gene Regulation and Stability

In addition to the evolutionary changes that *Alu* may cause that are related to its role in genetic instability, *Alu* elements have also been suggested to cause changes in gene structure and regulation. Transcriptional regulatory elements have been mapped to *Alu* elements near the promoters of genes (reviewed in ref. *50*), as well as *Alu* elements having been shown in several reporter systems to be able to contribute transcription factor binding sites to stimulate gene expression *(51,52)*, as well as insulator sequences to isolate genes from other nearby elements *(53)*. Thus, *Alu* elements have probably influenced expression of many genes through insertion near their promoters.

It also has been suggested that expression of *Alu* elements may contribute to a selective regulation of the initiation of translation *(54)*. Because expression of *Alu* elements is stimulated by viral infection *(55)*, transformation *(56,57)*, chemotherapeutic DNA-damaging agents *(58),* and a number of cellular stresses *(59)*, leading to speculation that this may help regulate the translation process in those situations.

Alu elements may make up a large portion of the intronic sequence in RNAs, as well as being presented in 3' non-coding regions. It has recently been noted that there are high levels of adenine-to-inosine RNA editing in human cells, and that more than 90% of it occurs within *Alu* elements *(60)*. This is likely because of the ability of *Alu* elements in various orientations in the RNA to form duplex structures that make excellent substrates for the editing enzyme (ADAR). Whether there is a role for this editing of *Alu* elements, or whether they just compete with other RNA substrates, is currently not known. Through RNA editing, differential splicing, and other mechanisms, *Alu* elements may influence the processing and stability numerous cellular transcripts.

FUTURE DIRECTIONS

Although our understanding of both the mechanisms of *Alu* element amplification and their role in human disease have increased greatly, there are a number of important issues still to be resolved. In terms of human disease, one of the current problems is that many of the polymerase chain reaction-based strategies used to identify human disease mutations are biased against the detection of large sequence insertions or deletions. Thus, we are probably not detecting a significant proportion of the *Alu* element-induced damage in human disease.

As we learn more about the mechanism of *Alu* amplification, it will be important to focus on which cell types are involved in the amplification; germ line, somatic, and tumor cells. Also important are questions of how *Alu* elements interact with cellular gene products that may modulate, or respond to, their amplification. This will help determine whether different individuals are more or less susceptible to damage by these elements. Similarly, with evidence that expression of *Alu* elements being stimulated by numerous environmental factors, the possibility that different individuals are more or less prone to damage by *Alu* elements in response to genomic exposures will be an important issue. Finally, *Alu* elements apparently utilize the L1 machinery for their amplification more efficiently than does L1 itself. It will be important to delineate both the commonalities and differences in the actual mechanism of amplification of these elements, as well as their respective impacts once inserted in the genome, in order to fully assess the roles these elements play in

human genetic instability. Ultimately, we must determine whether there is anything we can do to minimize the genetic instability caused by these elements.

SUMMARY

Alu elements have caused insertional mutation events throughout primate evolution and continue to cause a significant level of sporadic human genetic disease. Furthermore, the high copy number of *Alu* elements in the human genome contributes to secondary events, such as recombination events, that also contribute extensively to human disease. There are also a number of other ways in which *Alu* elements contribute to human genetic diversity (i.e., causing alternative splicing and changes in gene regulation).

REFERENCES

1. Lander ES, Linton LM, Birren B, et al. Initial sequencing and analysis of the human genome. International Human Genome Sequencing Consortium. Nature 2001;409:860–921.
2. Deininger PL, Batzer MA. Alu repeats and human disease. Mol Genet Metab 1999;67:183–193.
3. Li X, Scaringe WA, Hill KA, Roberts S, et al. Frequency of recent retrotransposition events in the human factor IX gene. Hum Mutat 2001;17:511–519.
4. Hagan CR, Sheffield RF, Rudin CM. Human Alu element retrotransposition induced by genotoxic stress. Nat Genet 2003;35:219–220.
5. Dewannieux M, Esnault C, Heidmann T. LINE-mediated retrotransposition of marked Alu sequences. Nat Genet 2003;35:41–48.
6. Roy-Engel AM, Salem AH, Oyeniran OO, et al. Active Alu element "A-tails"; size does matter. Genome Res 2002;12:1333–1344.
7. Xing J, Salem AH, Hedges DJ, et al. Comprehensive analysis of two Alu Yd subfamilies. J Mol Evol 2003;57:S76–S89.
8. Deininger P, Batzer MA. Evolution of retroposons. In: *Evolutionary Biology* (Heckht MK, et al., eds. New York, NY: Plenum Publishing, 1993; pp. 157–196.
9. Salem AH, Ray DA, Xing J, et al. Alu elements and hominid phylogenetics. Proc Natl Acad Sci USA 2003;100: 12,787–12,791.
10. Chesnokov I, Schmid CW. Flanking sequences of an Alu source stimulate transcription in vitro by interacting with sequence-specific transcription factors. J Mol Evol 1996;42:30–36.
11. Roy AM, West NC, Rao A, et al. Upstream flanking sequences and transcription of SINEs. J Mol Biol 2000;302:17–25.
12. Sinnett D, Richer C, Deragon JM, Labuda D. Alu RNA transcripts in human embryonal carcinoma cells. Model of post-transcriptional selection of master sequences. J Mol Biol 1992;226:689–706.
13. Brookfield JF. Selection on Alu sequences? Curr Biol 2001;11:R900–R901.
14. Deininger PL, Batzer MA. Mammalian retroelements. Genome Res 2002;12:1455–1465.
15. Makalowski W, Mitchell GA, Labuda D. Alu sequences in the coding regions of mRNA: a source of protein variability. Trends Genet 1994;10:188–193.
16. Britten RJ. Mobile elements inserted in the distant past have taken on important functions. Gene 1997;205: 177–182.
17. Gebow D, Miselis N, Liber HL. Homologous and nonhomologous recombination resulting in deletion: effects of p53 status, microhomology, and repetitive DNA length and orientation. Mol Cell Biol 2000;20:4028–4035.
18. Stenger JE, Lobachev KS, Gordenin D, Darden TA, Jurka J, Resnick MA. Biased distribution of inverted and direct alus in the human genome: implications for insertion, exclusion, and genome stability. Genome Res 2001;11:12–27.
19. Lobachev KS, Stenger JE, Kozyreva OG, Jurka J, Gordenin DA, Resnick MA. Inverted Alu repeats unstable in yeast are excluded from the human genome. Embo J 2000;19:3822–3830.
20. Chuzhanova N, Abeysinghe SS, Krawczak M, Cooper DN. Translocation and gross deletion breakpoints in human inherited disease and cancer II: Potential involvement of repetitive sequence elements in secondary structure formation between DNA ends. Hum Mutat 2003;22:245–251.

21. Puget N, Sinilnikova OM, Stoppa-Lyonnet D, et al. An Alu-mediated 6-kb duplication in the BRCA1 gene: a new founder mutation? Am J Hum Genet 1999;64:300–302.
22. Puget N, Torchard D, Serova-Sinilnikova OM, et al. A 1-kb Alu-mediated germ-line deletion removing BRCA1 exon 17. Cancer Research 1997;57:828–831.
23. Rohlfs EM, Chung CH, Yang Q, et al. In-frame deletions of BRCA1 may define critical functional domains. Hum Genet 2000;107:385–390.
24. Rohlfs EM, Puget N, Graham ML, et al. An Alu-mediated 7.1 kb deletion of BRCA1 exons 8 and 9 in breast and ovarian cancer families that results in alternative splicing of exon 10. Genes Chromosomes Cancer 2000;28:300–307.
25. Montagna M, Santacatterina M, Torri A, et al. Identification of a 3 kb Alu-mediated BRCA1 gene rearrangement in two breast/ovarian cancer families. Oncogene 1999;18:4160–4165.
26. Wang Y, Friedl W, Lamberti C, et al. Hereditary nonpolyposis colorectal cancer: frequent occurrence of large genomic deletions in MSH2 and MLH1 genes. Int J Cancer 2003;103:636–641.
27. Thiffault I, Hamel N, Pal T, et al. Germline truncating mutations in both MSH2 and BRCA2 in a single kindred. Br J Cancer 2004;90:483–491.
28. Plaschke J, Ruschoff J, Schackert HK. Genomic rearrangements of hMSH6 contribute to the genetic predisposition in suspected hereditary non-polyposis colorectal cancer syndrome. J Med Genet 2003;40:597–600.
29. Strout MP, Marcucci G, Bloomfield CD, Caligiuri MA. The partial tandem duplication of ALL1 (MLL) is consistently generated by Alu-mediated homologous recombination in acute myeloid leukemia. Proc Natl Acad Sci USA 1998;95:2390–2395.
30. Kolomietz E, Meyn MS, Pandita A, Squire JA. The role of Alu repeat clusters as mediators of recurrent chromosomal aberrations in tumors. Genes Chromosomes Cancer 2002;35:97–112.
31. Callen E, Tischkowitz MD, Creus A, et al. Quantitative PCR analysis reveals a high incidence of large intragenic deletions in the FANCA gene in Spanish Fanconi anemia patients. Cytogenet Genome Res 2004;104:341–345.
32. Wei, Y., Sun, M., Nilsson, G., et al. Characteristic sequence motifs located at the genomic breakpoints of the translocation t(X;18) in synovial sarcomas. Oncogene 2003;22:2215–2222.
33. Rudiger NS, Gregersen N, Kielland-Brandt MC. One short well conserved region of Alu-sequences is involved in human gene rearrangements and has homology with prokaryotic chi. Nucleic Acids Res 1995;23:256–260.
34. Knebelmann, B., Forestier, L., Drouot, L., et al. Splice-mediated insertion of an Alu sequence in the COL4A3 mRNA causing autosomal recessive Alport syndrome. Hum Mol Genet 1995;4:675–679.
35. Mitchell, G. A., Labuda, D., Fontaine, G., et al. Splice-mediated insertion of an Alu sequence inactivates ornithine delta-aminotransferase: a role for Alu elements in human mutation. Proc Natl Acad Sci USA 1991;88:815–819.
36. Sorek R, Shamir R, Ast G. How prevalent is functional alternative splicing in the human genome? Trends Genet 2004;20:68–71.
37. Lev-Maor G, Sorek R, Shomron N, Ast G. The birth of an alternatively spliced exon: 3' splice-site selection in Alu exons. Science 2003;300:1288–1291.
38. Kreahling J, Graveley BR. The origins and implications of Aluternative splicing. Trends Genet 2004;20:1–4.
39. Arcot S, Wang Z, Weber J, Deininger P, Batzer M. Alu repeats: a source for the genesis of primate microsatellites. Genomics 1995;29:136–144.
40. Montermini L, Andermann E, Labuda M, et al. The Friedreich ataxia GAA triplet repeat: premutation and normal alleles. Hum Mol Genet 1997;6:1261–1266.
41. Ohshima K, Montermini L, Wells RD, Pandolfo M. Inhibitory effects of expanded GAA.TTC triplet repeats from intron I of the Friedreich ataxia gene on transcription and replication in vivo. J Biol Chem 1998;273:14,588–14,595.
42. Bidichandani SI, Ashizawa T, Patel PI. The GAA triplet-repeat expansion in Friedreich ataxia interferes with transcription and may be associated with an unusual DNA structure. Am J Hum Genet 1998;62:111–121.
43. Patel PI, Isaya G. Friedreich ataxia: from GAA triplet-repeat expansion to frataxin deficiency. Am J Hum Genet 2001;69:15–24.
44. Roy-Engel AM, El-Sawy M, Farooq L, et al. Human retroelements may introduce intragenic polyadenylation sites. Cytogenet Genome Res 2004, in press.
45. Hayakawa T, Satta Y, Gagneux P, Varki A, Takahata N. Alu-mediated inactivation of the human CMP-N-acetylneuraminic acid hydroxylase gene. Proc Natl Acad Sci USA 2001;98:11,399–11,404.
46. Challem JJ, Taylor EW. Retroviruses, ascorbate, and mutations, in the evolution of Homo sapiens. Free Radic Biol Med 1998;25:130–132.

47. Bailey JA, Liu G, Eichler EE. An Alu transposition model for the origin and expansion of human segmental duplications. Am J Hum Genet 2003;73:823–834.
48. Babcock M, Pavlicek A, Spiteri E, et al. Shuffling of genes within low-copy repeats on 22q11 (LCR22) by Alu-mediated recombination events during evolution. Genome Res 2003;13:2519–2532.
49. Bailey JA, Yavor AM, Massa HF, Trask BJ, Eichler EE. Segmental duplications: organization and impact within the current human genome project assembly. Genome Res 2001;11:1005–1017.
50. Britten RJ. DNA sequence insertion and evolutionary variation in gene regulation. Proc Natl Acad Sci USA 1996;93:9374–9377.
51. Norris J, Fan D, Aleman C, et al. Identification of a new subclass of Alu DNA repeats which can function as estrogen receptor-dependent transcriptional enhancers. J Biol Chem 1995;270:22,777–22,782.
52. Vansant G, Reynolds WF. The consensus sequence of a major Alu subfamily contains a functional retinoic acid response element. Proc Natl Acad Sci USA 1995;92:8229–8233.
53. Thorey IS, Cecena G, Reynolds W, Oshima RG. Alu sequence involvement in transcriptional insulation of the keratin 18 gene in transgenic mice. Mol Cell Biol 1993;13:6742–6751.
54. Rubin CM, Kimura RH, Schmid CW. Selective stimulation of translational expression by Alu RNA. Nucleic Acids Res 2002;30:3253–3261.
55. Panning B, Smiley JR. Activation of expression of multiple subfamilies of human Alu elements by adenovirus type 5 and herpes simplex virus type 1. J Mol Biol 1995;248:513–524.
56. Carey MF, Singh K, Botchan M, Cozzarelli NR. Induction of specific transcription by RNA polymerase III in transformed Cells. Mol Cell Biol 1989;6:3068–3076.
57. Chu WM, Wang Z, Roeder RG, Schmid CW. RNA polymerase III transcription repressed by Rb through its interactions with TFIIIB and TFIIIC2. J Biol Chem 1997;272:14,755–14,761.
58. Rudin CM, Thompson CB. Transcriptional activation of short interspersed elements by DNA-damaging agents. Genes Chromosomes Cancer 2001;30:64–71.
59. Li TH, Schmid CW. Differential stress induction of individual Alu loci: implications for transcription and retrotransposition. Gene 2001;276:135–141.
60. Levanon EY, Eisenberg E, Yelin R, et al. Systematic identification of abundant A-to-I editing sites in the human transcriptome. Nat Biotechnol 2004;22:1001–1005.
61. Vidaud D, Vidaud M, Bahnak BR, et al. Haemophilia B due to a de novo insertion of a human-specific Alu subfamily member within the coding region of the factor IX gene. Eur J Hum Genet 1993;1:30–36.
62. Wulff K, Gazda H, Schroder W, Robicka-Milewska R, Herrmann FH. Identification of a novel large F9 gene mutation-an insertion of an Alu repeated DNA element in exon e of the factor 9 gene. Hum Mutat 2000;15:299.
63. Sukarova E, Dimovski AJ, Tchacarova P, Petkov GH, Efremov GD. An Alu insert as the cause of a severe form of hemophilia A Acta Haematol 2001;106:126–129.
64. Ganguly A, Dunbar T, Chen P, Godmilow L, Ganguly T. Exon skipping caused by an intronic insertion of a young Alu Yb9 element leads to severe hemophilia A Hum Genet 2003;113:348–352.
65. Claverie-Martin F, Gonzalez-Acosta H, Flores C, Anton-Gamero M, Garcia-Nieto V. De novo insertion of an Alu sequence in the coding region of the CLCN5 gene results in Dent's disease. Hum Genet 2003;113:480–485.
66. Thakker RV. Molecular pathology of renal chloride channels in Dent's disease and Bartter's syndrome. Exp Nephrol 2000;8:351–360.
67. Lester T, McMahon C, VanRegemorter N, Jones A, Genet S. X-linked immunodeficiency caused by insertion of Alu repeat sequences. J Med Gen Suppl 1997;34:S81.
68. Zhang Y, Dipple KM, Vilain E, et al. AluY insertion (IVS4-52ins316alu) in the glycerol kinase gene from an individual with benign glycerol kinase deficiency. Hum Mutat 2000;15:316–323.
69. Muratani K, Hada T, Yamamoto Y, et al. Inactivation of the cholinesterase gene by Alu insertion: Possible mechanism for human gene transposition. Proc Natl Acad Sci USA 1991;88:11,315–11,319.
70. Janicic N, Pausova Z, Cole DE, Hendy GN. Insertion of an Alu sequence in the Ca(2+)-sensing receptor gene in familial hypocalciuric hypercalcemia and neonatal severe hyperparathyroidism. Am J Hum Genet 1995;56:880–886.
71. Economou-Pachnis A, Tsichlis PN. Insertion of an Alu SINE in the human homologue of the Mlvi-2 locus. Nucleic Acids Res 1985;13:8379–8387.
72. Halling KC, Lazzaro CR, Honchel R, et al. Hereditary desmoid disease in a family with a germline Alu I repeat mutation of the APC gene. Hum Hered 1999;49:97–102.
73. Abdelhak S, Kalatzis V, Heilig R, et al. Clustering of mutations responsible for branchio-oto-renal (BOR) syndrome in the eyes absent homologous region (eyaHR) of EYA1. Hum Mol Genet 1997;6:2247–2255.

74. Oldridge M, Zackai EH, McDonald-McGinn DM,et al. De novo Alu-element insertions in FGFR2 identify a distinct pathological basis for apert syndrome. Am J Hum Genet 1999;64:446–461.
75. Tighe PJ, Stevens SE, Dempsey S, Le Deist F, Rieux-Laucat F, Edgar JD. Inactivation of the Fas gene by Alu insertion: retrotransposition in an intron causing splicing variation and autoimmune lymphoproliferative syndrome. Genes Immun 2002;3:S66–S70.
76. Stoppa-Lyonnet D, Duponchel C, Meo T,et al. Recombinational biases in the rearranged C1-inhibitor genes of hereditary angioedema patients. Am J Hum Genet 1991;49:1055–1062.
77. Mustajoki S, Ahola H, Mustajoki P, Kauppinen R. Insertion of Alu element responsible for acute intermittent porphyria. Hum Mutat 1999;13:431–438.
78. Miki Y, Katagiri T, Kasumi F, Yoshimoto T, Nakamura Y. Mutation analysis in the BRCA2 gene in primary breast cancers. Nat Genet 1996;13:245–247.
79. Wallace MR, Andersen LB, Saulino AM, Gregory PE, Glover TW, Collins FS. A de novo Alu insertion results in neurofibromatosis type 1. Nature 1991;353:864–866.

http://www.springer.com/978-1-58829-559-0

Genomic Disorders
The Genomic Basis of Disease
Lupski, J.R.; Stankiewicz, P.T. (Eds.)
2006, XVIII, 426 p. 112 illus., 1 illus. in color., Hardcover
ISBN: 978-1-58829-559-0
A product of Humana Press