The Mobile Genetic Element \textit{Alu} in the Human Genome

\textit{Transposable elements represent a dramatic source of genetic variation}

Gabriel E. Novick, Mark A. Batzer, Prescott L. Deininger, and Rene J. Herrera

Genetic material has been traditionally envisioned as relatively static with the exception of occasional, often deleterious mutations. The sequence DNA-to-RNA-to-protein represented for many years the central dogma relating gene structure and function. Recently, the field of molecular genetics has provided revolutionary information on the dynamic role of repetitive elements in the function of the genetic material and the evolution of humans and other organisms.

\textit{Alu} sequences represent the largest family of short interspersed repetitive elements (SINEs) in humans, being present in an excess of 500,000 copies per haploid genome. \textit{Alu} elements, as well as the other repetitive elements, were once considered to be useless. Today, the biology of \textit{Alu} transposable elements is being widely examined in order to determine the molecular basis of a growing number of identified diseases and to provide new directions in genome mapping and biomedical research.

Only a small fraction of the human genome consists of functional, or protein-encoding, genes. If all the nucleotide information were translated, $10^9$ amino acids, or approximately 3 million average-size proteins, would be encoded. Based on the fully sequenced genomes of simple organisms and considerations of the higher complexity of humans, it is estimated that no more than 60,000 to 100,000 proteins, in addition to functional RNAs, are essential for the survival of the species (Alberts et al. 1989) and as a result are conserved throughout evolution. Considering that many genes are repetitive, only approximately 10\%-20\% of the human DNA encodes functional sequences. What is the function of the remaining DNA?

\textit{Alu} elements, responsible for innumerable genetic disorders, can be markers for human identification and are raw material for evolution.

Discovery and classification of repetitive elements

When the DNA double helix of higher organisms (species other than bacteria and viruses) is split open or denatured into two single strands by heat and left to spontaneously reanneal, the entire DNA is not likely to become double-stranded at the same rate. In contrast, all parts of bacterial and viral DNA reanneal at approximately the same rate. This phenomenon was observed in the now classical experiments of Britten and Kohne (1968) and was correctly interpreted as an indication of greater sequence complexity among eukaryotes.

These experiments distinguish three populations of eukaryotic DNA based on the time that it takes DNA to again become double-stranded. There is a quickly reannealing component representing 25\% of the total DNA, an intermediate component that represents 30\% of the total DNA, and a slow component that represents 45\% of the total DNA (Britten and Kohne 1968).

When the slow component was analyzed by DNA sequencing, it was found to be made up of single-copy sequences, which include the genes that encode proteins, while the fast and intermediate components reflected the presence of repetitive sequences. Repetitive DNA can find a complementary sequence on the other strand more rapidly than can single-copy sequences, because reiterated DNA can bind to any one of...
the copies on several sites. The fast component contains small (a few nucleotides long), highly repetitive DNA sequences, which are usually found in tandem. The intermediate component contains moderately reiterated DNA sequences (Brosius 1991).

Intermediate repetitive DNA shows two common characteristics: The repetitive units, which can be up to several thousand nucleotides long, are similar to one another but not identical, and they are interspersed along the genome (not tandemly arrayed). Within this category are the transposons, elements that are able to move from one genomic location to another. This movement can be mediated by DNA or RNA. In RNA-mediated transposition, the transposon is transcribed to an RNA intermediate, this RNA is reverse transcribed into DNA, and this copy DNA (cDNA) is reinserted into the genome. The elements mobilized in this manner are known as retroposons (Rogers 1985).

Retroposons can be divided into two superfamilies. The viral superfamily represents ancient viral integration events and includes the Copia family in Drosophila (Mount et al. 1985) and the FlI in humans (Paulson et al. 1985). The nonviral retroposon superfamily can be further divided into short and long interspersed elements (SINEs and LINES). SINEs are usually 75 to 500 base pairs (bp) in length, and LINES can be as long as 7000 bp. SINEs contain internal RNA polymerase III promoters, while LINE transcription is under the control of the RNA polymerase II promoters that are transcribed by RNA polymerase III. SINEs to remain transcriptionally active even after retroposition to new chromosomal sites. The left half of the element contains an adenine-rich region at the end, marked by an A, (at position 294 in this diagram).

![Figure 1. The dimeric structure of the Alu element.](image)

The Alu family of repetitive elements was originally identified as a fraction of renatured repetitive DNA that was distinctively cleaved with the restriction enzyme Alu I (Houck et al. 1979). The Alu family constitutes approximately 5% of the total mass of the human genome, and there are more than 500,000 copies per haploid genome (Rinehart et al. 1981).

The structure of an Alu element, as shown in Figure 1, presents a dimeric organization in which the right monomer (heavier lines) is 31 nucleotides longer than the left one. The left half of the element contains the RNA polymerase III promoter boxes A and B. The promoter directs transcriptional initiation to a specific site upstream of box A. Although box A, located between and including nucleotides 5 and 31, seems to determine strength and accuracy of initiation, only box B, which includes positions 70–100, appears to be essential for transcription (Fuhrman et al. 1981). Alu elements possess a short A-rich sequence between the two dimers and a 3' oligo A track of up to 200 bps, which are characteristic of all SINEs (Economou et al. 1990). The repeat is flanked by direct repeats derived by duplication of target sequences at the site of integration.

Alu elements are ancestrally derived from the 7SL RNA gene (Ullu and Tschudi 1984). 7SL RNA is a small constituent of the signal recognition particle (SRP) involved in the transportation of proteins into the lumen of the endoplasmic reticulum in the cytoplasm of the cell. The SRP binds to a specific signal contained in secretory proteins and targets them to the endoplasmic reticulum (Walter and Blobel 1982). Although Alu elements have diverged, they retain approximately 90% sequence similarity to the 7SL RNA gene. However, the gene has 150 bp in its middle that is not found in the Alu family (Deininger 1989).

**Mechanism of Alu retroposition**

The mechanism by which Alu and other SINEs retropose consists of steps in which the element is transcribed, reverse transcribed, and then integrated into a new genomic location (Figure 2).

**Transcription.** Transcription of Alu elements (similar to almost all other SINES) is initiated by RNA polymerase III at a site upstream of the internal split promoter. Transcription continues along the entire element and into the flanking sequence where the polymerase terminates its reading (short poly T track). In this way, the Alu RNA transcript retains its promoter after transcription, but the promoter is not sufficient for effective transcription in a new location. In vivo studies show that the 7SL RNA gene requires sequences upstream of the promoter, which
are not carried by the SINE during retrotransposition, for efficient expression (Ullu and Weiner 1985). Because these sequences are required for Alu transcription in vivo, the vast majority of Alu elements are transcriptionally silent unless inserted into a favorable genomic location that fortuitously provides them with the 5' cis regulatory sequences.

In addition, after insertion most SINES are subject to methylation that is tissue and temporally specific. This methylation results in a tenfold higher than normal mutation rate at these sites (Batzer et al. 1990). Methylation, by itself, and the accumulated mutations within promoter sequences correlate with inhibition of transcription (Deininger and Batzer 1993). Although most SINES are transcriptionally silent (Schmid and Maraia 1993), occasionally sequences in the target site of insertion act in conjunction with the RNA polymerase III promoter to initiate transcription.

Some Alu family members inserted into genes are transcribed by polymerase II as part of nascent mRNA. Alu sequences located in introns are transcribed by polymerase II and then spliced out with the rest of the intron RNA before translation of the RNA into protein (such transcription is called piggyback transcription). Alu sequences are sometimes found in exons (translated sequences) and may become part of peptides in some cases (Makalowski et al. 1994). As part of proteins, the amino acids corresponding to Alu sequences provide raw genetic material for evolution.

Reverse transcription. The first event involved in reverse transcription is priming. It has been postulated that the uracil residues present at the 3' end of the transcript, derived from the RNA polymerase III termination signal (Figure 2), fold back, the uracil bases pair to an internal A rich area; and the stretch of uracils then functions as a primer for reverse transcription (Jagadeeswaran et al. 1981). It is also possible that an external primer may be involved in this process. A complementary strand to the RNA transcript is copied, starting at the primer binding site, by the enzyme reverse transcriptase.

The source of this enzyme is still a matter of controversy. RNA viruses called retroviruses use their own reverse transcriptase to synthesize a cDNA intermediate before insertion into the host genome. Retroviral infections or endogenous retroviral-like sequences (Martin et al. 1981) may provide the enzyme for SINE reverse transcription. Most L1 elements (the most important LINE family in humans) are truncated; however, some of them are known to code for a functional reverse transcriptase (Mathias et al. 1991). Because mammalian genomes contain up to 50,000 copies of L1 elements, these elements constitute a prolific source of endogenous reverse transcriptase.

Integration. Integration of the cDNA into a new location requires nicks in the genomic DNA (one in each DNA strand) at the target site for integration. Repair of the nick after insertion results in the formation of flanking direct repeats.

The integration process is fairly random. However, there appears to be alternating distribution of SINES and LINEs along the chromosome, based on in situ hybridization studies of Giemsa-stained metaphase chromosomes (Korenberg and Rykowski 1988). The target sites for SINE integration tend to be A+T rich regions (Batzer et al. 1990). Because SINES are highly G+C rich elements, their insertion creates small G+C islands in A+T rich regions.

Origins and evolution of Alu elements

Although it is generally accepted that the Alu family originated from a modified RNA transcript of the
7SL RNA gene (Ullu and Tschudi 1984), two models have been proposed to explain the expansion of the repetitive element family. These models are called the transposon model and the master gene model.

The transposon model postulates that many SINEs generate new elements, each with the same chance of being transpositionally active. This mechanism would generate sequences that diverge from the original sequence as the multiplication process continues. The nucleotide differences among Alus would include every possible mutation along the entire length of the elements. The sequences of the members of the family would diverge independently as different members multiply and accumulate mutations.

Data shows that the amplification rate (number of insertions as a function of time) is not exponential as would be expected if the transposon model is correct and other factors (e.g., availability of reverse transcriptase) were not limiting. In fact, Alu amplification rate is highly variable, with periods of high and low amplification rates. The recent Alu amplification rate, estimated at one insertion fixed in the population per 5000 years, is approximately 100-fold slower than at its peak approximately 40–50 million years ago (Shen et al. 1991).

The observation of a few clearly defined subfamilies of elements also does not support the transposon model. Subfamilies are defined according to the presence of certain characteristic (diagnostic) mutations. For example, a given Alu subfamily may be characterized by the presence of a G, instead of the more typical T, at nucleotide position number 136.

The master gene model (Deininger et al. 1992) proposes that most SINEs are derived from one or a few active loci. This model predicts a linear amplification rate controlled by the amplification of the master genes. Mutations in the master genes would generate new subfamilies and might cause variations in the amplification rate (Deininger and Batzer 1993).

For example, at time zero an unmutated master gene would generate a family of elements with a sequence identical to itself. Over time, this master gene would mutate, and subsequent copies of the sequence would constitute a subfamily, which is slightly different from the original group. Each new mutation in this master gene would not only be added to the previous one and generate a new subfamily, but it might also affect the rate of amplification of the family.

In the case of Alu elements, subfamilies can be arrayed in a sequential manner (Britten 1994, Britten et al. 1989, Shen et al. 1991) in which the older the subfamily, in evolutionary terms, the more random variations are observed within the subfamily members. For example, the number of differences present among the older primate-specific (PS) subfamily members is greater than the number observed among a human-specific (HS) subfamily.

A recently inserted human specific (HS-1) subfamily was identified in humans with only a few copies located in chimp and gorilla genomes (reviewed in Batzer et al. 1995). Another human specific subfamily (HS-2) was exclusively found in humans. In addition, a third subfamily, the Sb-2, has human-specific members. Some of the members of HS-1, HS-2, and Sb-2 subfamilies have inserted so recently that not all humans have the insertion in a particular locus. This observation represents the basis for the use of polymorphic Alu insertions for population studies of geographically diverse groups (Batzer et al. 1991, 1993, 1994, in press, Novick et al. 1992, in press). In light of all this evidence, even if full exponential amplification is halted by limiting factors, the existence of a small number of subfamilies provides strong support for the master gene model.

An interesting related issue concerns the potential factors that regulate Alu copy number. Because Alu insertions are for the most part deleterious, it is logical to expect a maximal number of insertions after which the genetic burden or load would not tolerate additional Alu insertions. The 100-fold decrease in Alu insertion rate over the last 40–50 million years may be indicative of a limit to the number of Alu copies that our gene pool can withstand.
The force responsible for establishing and maintaining such a limit may be a selection against individuals experiencing de novo insertions, especially if they significantly disrupt the function of critical genes. In theory, once the number of Alu copies in the human gene pool reached this limit, only a few de novo insertions could become fixed in the population, replacing the Alu copies lost, for example, during unequal crossing over.

**Alu insertions and the genomic environment**

Do Alu elements have any function? How does their presence affect neighboring sequences and the genome as a whole? Transposable elements were initially considered mere parasites of the genome with no major effect on the organism (Orgel and Crick 1980). This perception resulted in what is known as the selfish DNA hypothesis (Orgel and Crick 1980), wherein repeated DNA sequences, and transposable elements in particular, have no cellular function or genetic effect but to increase their copy number. The phrase **selfish DNA** has become so popular that it is included in *The Oxford English Dictionary* (1989), which defines it as “genetic material tending to be perpetuated or to spread although of no effect on the phenotype.”

Initially, transposable elements were viewed by some as being either molecular fossils or inconsequential both to the cell and to the evolution of the species. New molecular evidence regarding the structure and evolution of this class of sequences, however, suggests that although repeated DNA sequences may have originated as innocuous elements, they can have dramatic effects on genome organization, function, and evolution (Makalowski et al. 1994). Although the notion of Alu elements as mutagens causing deleterious effects is well recognized, there are also innumerable examples of Alu insertions involved in normal DNA structure and function. To cite a few examples, representative and recent studies have demonstrated the role of Alu elements as DNA organizers (Englander and Howard 1995), enhancers (Almenoff et al. 1994), and silencers (Hanke et al. 1995) in gene expression. Over the past decade, the list of studies pointing to roles performed by SINEs is extensive (see von Sternberg et al. 1992 for review of the literature). Therefore, whether or not Alu elements and SINEs in general, are considered selfish DNA, they can result in functional sequences.

As an alternative to the selfish DNA hypothesis, we proposed a model called genome canalization (von Sternberg et al. 1992). Genome canalization is the process whereby once a pattern of genomic organization is established, all further evolutionary changes must adapt to the prevailing genetic context. The basic concept of genome canalization is not a novel one, coevolution being the underlining principle. In the same way that birds and plants coevolved specific peak and flower morphology for their mutual benefit, so it is that different types of DNA coevolved to perform a function.

At the DNA level, for example, there is an intimate relationship between genes and their transcription regulatory sequences brought about by the process of molecular coevolution. Alu elements and other SINEs are just one source of genetic material that is often placed in physical proximity to other DNA sequences, dramatically fueling the coevolutionary process.

The various types of mutations (transposition events being among

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**Figure 4.** Agarose gel electrophoresis and codominant segregation of polymorphic Alu PCR products for the angiotensin converting enzyme locus in a family. The pedigree studied is represented on top of the figure, and the electrophoretic separation is found immediately under each individual on the pedigree. Lanes 1 and 9 represent molecular size standard Hae III digested OX174 DNA. Arrow A indicates the insertion band (490 bp for this locus), arrow B points to the lack of insertion band (190 bp for this locus), and arrows C and D represent primer-dimer and primer, respectively.
can, in turn, influence the fate of genomic environments. Other factors act at the population level and effects on gene structure and function may yield diverse novel transcription regulatory sequences, that into an adaptive one.

Table 1 illustrates the retroposon mode of insertion, potential genomic insertion sites, immediate consequences on gene function and organization, and some of the possible adaptive outcomes within structural genes and regulatory sequences. *Alu* insertions are capable of affecting the structure and function of the rest of the genome in many ways.

This finding is not surprising considering that these elements contain genetic information, including transcription regulatory sequences, that insert themselves indiscriminately all over the genome. Subsequent to insertion, coevolution with different types of neighboring sequences ultimately may yield diverse novel functions for the DNA region in question.

Table 1 focuses on the immediate effects on gene structure and function of *Alu* insertions in different genomic environments. Other factors like genetic drift, migration, or polymorphisms near the insertional sites act at the population level and can, in turn, influence the fate of *Alu* elements as well.

### Table 1. Potential effects of *Alu* insertions.

<table>
<thead>
<tr>
<th>Insertion</th>
<th>Potential effect on gene organization and function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Into promoters</td>
<td>Trans-acting factors and/or RNA polymerase cannot recognize the promoter sequences leading to transcription failure.</td>
</tr>
<tr>
<td>Between promoters</td>
<td>The distance between promoters is modified and their function altered or obliterated preventing transcription initiation.</td>
</tr>
<tr>
<td>Into enhancers</td>
<td>The action of enhancers over promoters is altered affecting transcription levels.</td>
</tr>
<tr>
<td>Into exons</td>
<td>A new product is produced with similar or different function(s). Rerangements are promoted leading to addition/deletion and frame shift mutations.</td>
</tr>
<tr>
<td>Into introns</td>
<td>The splice site(s) are modified generating a new gene product with similar or different function(s). The <em>Alu</em> element is removed.</td>
</tr>
<tr>
<td>Into heterochromatic domains</td>
<td>New <em>Alu</em> elements tend to cluster in the area affecting expression of nearby genes.</td>
</tr>
</tbody>
</table>

### Alu insertions and human biology

Some representative examples of *Alu* insertions causing human disorders are:

- **Hemophilia**—a series of disorders that are caused by mutations in any of a number of different blood-clotting factors. A de novo insertion of an HS *Alu* family member into the blood coagulation factor IX locus has resulted in hemophilia (Vidaud et al. 1993). This *Alu* repeat inserted into exon V of the factor IX gene results in the generation of a premature stop codon. This insertion gives rise to a nonfunctional factor IX, which is inherited as an X-linked disorder.

- **Meningiomas**—tumors of the membranes that surround the central nervous system. Meningiomas are frequently associated with complete or partial deletions of chromosome 22, and the gene most frequently affected is the *c-sis* gene, which encodes one of the two polypeptide subunits of the platelet-derived growth factor (Dalla Favera et al. 1982). The deletion of an *Alu* element located in the fifth intron of *c-sis* is associated with the development of this type of tumor (Smidt et al. 1990). Therefore, this human malady represents an *Alu* element necessary for normal gene function.

- **Hereditary angioneurotic edema**—a genetic disorder caused by the deficiency of an inhibitor called *Cl INH*. Seventeen *Alu* repeat sequences were identified within the *Cl INH* gene (Carter and Fothergill 1989), and one study demonstrated that partial deletions, observed in two families with hereditary angioneurotic edema, are the result of *Alu*-*Alu* recombination by unequal crossing-over within the *Cl INH* gene (Ariga et al. 1990). The most frequent breakpoint for recombination lies on the left half of the *Alu* elements between two A and B boxes of the RNA polymerase III promoter.

- **Familial hypercholesterolemia**—an autosomal dominant disease caused by mutations in the gene for the low density lipoprotein (LDL) receptor. A defective LDL receptor in a member of an affected family was found to be 50,000 daltons larger than normal. The anomalous protein was found to be the result of a 14-kilobase duplication due to unequal crossing-over between homologous *Alu* elements in introns 1 and 8 (Lehrman et al. 1987). Again the most frequent breakpoints were found in the area of the RNA poly
merase III promoter.
- Gyrate atrophy of the choroid and retina—an autosomal recessive chorioretinal degeneration caused by deficiency of ornithine-k-aminotransferase. In one patient with typical symptoms and consanguineous parents, the molecular defect was found to be a 142-nucleotide insertion in the mature ornithine-k-aminotransferase mRNA due to a splice-mediated insertion mutation of an Alu element in opposite orientation to the affected gene. This Alu element is normally located in intron 3 of the gene (Mitchell et al. 1991).
- Severe combined immune deficiency—a disorder caused by a dysfunction of the gene that codes for adenosine deaminase (ADA). Two independent cases of unrelated origin showed that the defect was due to a 3.2-kilobase deletion in the promoter and first exon of ADA. This deletion was confirmed to be the result of homologous recombination between Alu repeats. The breakpoint was mapped to the left arm of the Alu, in the area of the polymerase III promoter (Berkvens et al. 1990).
- Neurofibromatosis type 1 (NF1)—a common autosomal dominant disorder characterized by neurofibromas, other cutaneous disorders, bone deformities, and learning disabilities. A mutation found in an affected adult male is a de novo Alu insertion into an intron of the NF1 gene (Wallace 1991). The target site for insertion was found to be a 26-base pair A+T rich region that is known to be a preferential site for Alu insertions. The presence of the element causes intron sliding in which the following exon is removed along with the intron during splicing and the reading frame is shifted (Wallace 1991).
- Glanzmann thrombasthenia (GT)—an autosomal recessive bleeding disorder due to a defect in the platelet fibrinogen receptor, which causes an inability of the platelets to either bind to fibrinogen and aggregate. Both binding to fibrinogen and aggregation are crucial for normal coagulation. In one affected family, the molecular defect was identified as a 1-kilobase deletion immediately preceding a 15-kilobase inversion due to breaks in two antisense (opposite orientations) and one sense Alu sequences within the gene of one of the glycoproteins (GPIIb) that constitute the receptor (Lu and Bray 1993).

![Diagram](image)

Figure 5. Summary of scores obtained from the members of the pedigree represented in Figure 4 after the Alu PCR polymorphism assay for each of the five loci studied. HF: homozygous for the insertion; H: heterozygous; HL: homozygous for the lack of insertion.

**Alu elements as research tools**

**Alu** repeats as markers for human population genetics and DNA fingerprinting. We have already described the way in which subfamilies of Alu elements might have arisen from sequential mutations of a master gene. Recently inserted **Alu** elements (like some HS-1, HS-2, and Sb-2 subfamily members) are only found in some of the insertion sites in a given population (Batzer et al. 1995). Therefore, they represent insertion polymorphisms within populations. Given that **Alu** elements are generally highly stable after integration (Sawada and Schmid 1986), and each element is absent from nonhuman primates, the lack of insertion can be considered the ancestral state and the presence of the **Alu** element in that location the recent character state.

We have developed a polymerase chain reaction (PCR) assay to detect the presence or absence of **Alu** insertions (Batzer et al. 1991). PCR reproduces in vitro the events of
DNA replication in a cyclic manner, resulting in the exponential amplification of the original target sequence (Saiki et al. 1988). The principle of the Alu polymorphism assay is summarized in Figure 3. The assay uses oligonucleotide primers directed to the 5' and 3' single-copy sequences flanking the specific Alu element to be studied. This assay generates three potential outcomes when the PCR products are separated by electrophoresis on an agarose or acrylamide gel. Homozygous individuals for the Alu insertion amplify, for example, a large fragment that represents the Alu insertion between the two primers; an individual homozygous for the lack of insertion shows only a small fragment; and a heterozygous individual amplifies both products—the small fragment from the allele that lacks the insertion and the large fragment from the insertion allele (see lower panels in Figure 3).

When this assay is used to screen geographically distinct populations, a genetic profile of each group can be constructed and compared. All the characteristics of Alu elements described thus far (mechanism of retroposition, relatively nonspecific site of insertion, rate of amplification, and variability of the oligo dA track) suggest that it is unlikely two populations of unrelated origin have the same insertion frequencies for several Alu insertions. The stability and dimorphic distribution of Alu elements make them ideal markers for population studies. For example, we recently presented evidence for an African origin of modern human populations using only four polymorphic Alu insertion loci (Batzer et al. 1994), and we demonstrated admixture involving African Colombian and American Indian populations (Novick et al. in press) as well as African Caribbean and African American groups (Batzer et al. in press).

In the area of DNA fingerprinting, multiple approaches are currently being used and new strategies are in constant development. Restriction fragment length polymorphisms or RFLPs (Kan and Dozy 1978) and hypervariable minisatellite regions (Jeffreys et al. 1985) are the most widely used DNA typing methods. Recently, polymorphic Alu insertions have been employed successfully in human identification studies (Novick et al. 1994).

Many technologies, aimed at human identification, have been developed. These technologies include the use of monoclonal antibodies for the identification of body fluids (Martin and Parkin 1988), protein polymorphisms (Hobart 1979), human leukocyte antigen (HLA) typing (Ishihara and Hirota 1988), short tandem repeat-PCR polymorphisms (Edwards 1991), amplified fragment length polymorphisms (Eisenberg and Maha 1991), and minisatellite variant repeat-PCR (Jeffreys et al. 1991).

These techniques can be used to answer different types of questions, and the degree of variability observed by the different procedures is likely to limit their optimal range of usage. For example, ABO typing may not be a conclusive method to differentiate between individuals, because different individuals may share the same blood group, while variable number tandem repeats, on the other hand, would not be ideal to study population differences, because the same allelic variant can arise multiple times in different populations and their hypervariability generates so many alleles that clear differences between populations may be evident.

The nature of Alu insertions makes it improbable that the same insertion occurred independently within human evolutionary time at exactly the same genomic location in two different individuals. In addition, there is no known mechanism to precisely delete just the repetitive elements without deleting flanking sequences or leaving residual Alu sequences behind. Therefore, polymorphic Alu insertions represent a distinct, easy-to-measure genetic change resulting from an event that occurred one time within the human lineage. These characteristics make the polymorphic Alu insertions valuable tools for human population genetic studies and DNA typing, while providing information for the window of genetic variability left by highly variable sequences on the one hand and more conserved single and multiple copy genes on the other.

The use of polymorphic Alu insertions in paternity testing. We applied the principle of PCR-based Alu polymorphism testing to ascertain paternity using five polymorphic Alu insertions. The five HS Alu elements examined are unique to the human lineage. Their genomic localizations have been described (reviewed in Novick et al. 1994). In one study, we compared our strategy with the two approaches most widely used in paternity testing: HLA molecules and RFLP analysis.

The HLA complex is the most polymorphic set of genetic markers in humans, so it is a powerful system to resolve cases of disputed paternity (Lamm et al. 1983). RFLP analysis is based on differential digestion of the DNA with a restriction enzyme. This analysis results in a distinctive banding pattern that reveals not only the virtually unique genetic profile of an individual but also allows the parental origin of each band to be determined (Helminen et al. 1988).

Figure 4 displays the outcome of the analysis of one family for one locus. The results of the paternity testing using HS Alu polymorphism for the five loci in the same family is shown in Figure 5. To determine the likelihood of parentage of the two parents with respect to the five offspring of the pedigree, the paternity index and paternity probability were calculated using standard formulas as previously described (Novick et al. 1994).

The RFLP and HLA analysis of this pedigree yielded a probability of maternity of 99.99% and 85%, respectively. According to the HS Alu polymorphism assay, the probability of maternity obtained was 89%, and the probability of maternity was 96%. There are hundreds of potential polymorphic Alu markers for this type of analysis. Currently, our laboratories are in the process of characterizing additional polymorphic Alu insertions. Five to ten additional polymorphic Alu markers would allow probability determinations in the order of 99.99%.

Conclusions

DNA structure was initially viewed as a chain of rigid building blocks...
and the sequence DNA-to-RNA-to-protein was the sole and central dogma describing gene function. Unexpected discoveries in the field of gene structure and function have opened new perspectives, and old paradigms are replaced by continuously emerging hypotheses. Within this context, transposable elements, and Alu sequences in particular, constitute a large portion of the genome. Their insertion is not entirely random and, rather than being useless sequences, they represent a dramatic source of genetic variation.

Like any other type of mutation, Alu sequences are in most cases deleterious but not useless to the population as a whole. As retrotransposons and facilitators of unequal crossing over, they reshuffle and duplicate the DNA, thereby generating a significant amount of genetic variability while providing genetic flexibility and increasing the population’s survival in a changing environment. As a result, there are innumerable cases in which Alu elements and SINEs, in general, function in normal gene structure and activity. Transposable elements constitute a remarkably active component of a fluid and constantly changing genome pool, having only partially understood functions, and can be reckoned as a force helping to shape the evolutionary path of the entire genome.

Acknowledgments
This research was supported by grants from the National Institutes of Health (RR08205) to Rene J. Herrera and (R01 HG 00770) to Prescott L. Deininger. We would like to thank Ralph L. Diaz-Wagner and Frank Verde for their editorial assistance.

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January 1996