



Published in final edited form as:

Annu Rev Genomics Hum Genet. 2011 ; 12: 187–215. doi:10.1146/annurev-genom-082509-141802.

LINE-1 Elements in Structural Variation and Disease

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Abstract

The completion of the human genome reference sequence ushered in a new era for the study and discovery of human transposable elements. It now is undeniable that transposable elements, historically dismissed as junk DNA, have had an instrumental role in sculpting the structure and function of our genomes. In particular, long interspersed element-1 (LINE-1 or L1) and short interspersed elements (SINEs) continue to affect our genome, and their movement can lead to sporadic cases of disease. Here, we briefly review the types of transposable elements present in the human genome and their mechanisms of mobility. We next highlight how advances in DNA sequencing and genomic technologies have enabled the discovery of novel retrotransposons in individual genomes. Finally, we discuss how L1-mediated retrotransposition events impact human genomes.

Keywords

retrotransposon; polymorphism; transposable elements; genome diversity; mutation

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DISCLOSURE STATEMENT

J.V.M. is an inventor on a patent, “Compositions and methods of use of human retrotransposons,” application no. 60/006,831, issued November 2000. He has not received any money from the patent, and its issuance does not influence the opinions in this review.

INTRODUCTION

Approximately 45% of the human genome is derived from transposable elements (134). These include DNA transposons, long terminal repeat (LTR) retrotransposons, and non-LTR retrotransposons. Although most transposable elements have been rendered inactive through mutation, long interspersed element-1 (LINE-1 or L1) retrotransposition continues to diversify human genomes.

L1s comprise ~17% of human DNA (134). The L1-encoded proteins (ORF1p and ORF2p) can mobilize nonautonomous retrotransposons, other noncoding RNAs, and messenger RNAs, leading to the generation of processed pseudogenes (35, 62, 72, 81, 86, 102, 249). Thus, L1-mediated retrotransposition has generated a third of our genome.

In 1988, an examination of 240 unrelated males afflicted with hemophilia A revealed that independent mutagenic L1 insertions into exon 14 of the *Factor VIII* gene were responsible for the disease in two individuals (120). Heroic efforts to isolate an active progenitor L1 (67) and the development of a cultured cell retrotransposition assay (172) then helped elucidate the molecular mechanism of L1 retrotransposition. It now is apparent that L1s are alive and well in human populations, and that L1-mediated retrotransposition events account for approximately 1 of every 1,000 spontaneous, disease-producing insertions in man (40, 119).

Several reviews have discussed aspects of human retrotransposon biology and how the host genome defends itself from retrotransposon activity (6, 18, 51, 89, 182, 188). Here, we discuss recent progress in understanding the mechanism of L1 retrotransposition. We then highlight how new genomic technologies have illuminated the impact of L1-mediated retrotransposition events on human genetic variation and genome structure.

MOBILE ELEMENTS IN HUMAN GENOMES

Transposable elements are classified by whether they mobilize via a DNA (DNA transposons) or an RNA (retrotransposons) intermediate (Figure 1). They further are distinguished by whether they encode proteins to mediate their own mobility (autonomous elements) or rely upon proteins encoded by other elements (nonautonomous elements).

DNA Transposons

DNA transposons generally move via a cut-and-paste mechanism. They tend to have a limited life span in higher eukaryotic genomes (134, 220), which likely is due to the accumulation of nonautonomous deletion derivatives that compete for the transposase encoded by autonomous elements. In most cases, transposase binds at DNA transposon inverted repeat sequences, “cuts” the transposon from its existing location, and then “pastes” it into a new genomic location (reviewed in 55).

DNA transposons comprise ~3% of the human genome reference sequence (HGR, NCBI 36/hg18). Sequence divergence among paralogous copies indicates that virtually all DNA transposons mobilized prior to the eutherian radiation, whereas a composite method indicates they have been extinct in the primate lineage for at least 37 million years (134, 192). Nevertheless, DNA transposons have had an enduring effect on the human genome.

For example, the recombination activating genes, *RAG1* and *RAG2*, which are critical for V(D)J recombination and immune system development, likely were domesticated from the *Transib* family of DNA transposons ~500 Mya (117, 262).

Engineered DNA transposons have practical applications, and can be exploited for useful purposes. For example, a reanimated salmon DNA transposon, Sleeping Beauty, has been used to discover genes implicated in cancer progression and shows promise as a vector in gene therapy studies (48, 96, 112, 113). Similarly, an insect DNA transposon, piggyBac, has been used to create gene-specific knockouts in mouse embryonic stem cells (65, 226). Finally, a zebrafish transposon, Tol2, shows promise as a mutagen in both the mouse and zebrafish germline (118, 121).

Retrotransposons

Retrotransposons mobilize via an RNA intermediate by a copy-and-paste mechanism and remain active in most mammalian genomes. They can be subdivided into two general classes, depending on whether they contain or lack LTRs (Figure 1).

Long terminal repeat retrotransposons: human endogenous retroviruses—LTR-containing elements, such as human endogenous retroviruses (HERVs), resemble retroviruses in both their structure and mobility mechanism. Most HERVs contain a nonfunctional envelope (*ENV*) gene, which relegates them to an intracellular existence (12).

HERVs and their nonautonomous derivatives comprise ~8% of the human genome (134). Virtually all HERVs are retrotransposition defective; however, a small number of HERV-K elements [where K denotes the host lysine transfer RNA (tRNA) that presumably initiates HERV-K (–) strand complementary DNA (cDNA) synthesis] are polymorphic with respect to presence/absence status in humans, indicating that they have retrotransposed since the human-chimpanzee divergence (22, 125, 148, 176). Moreover, some HERV-K elements contain intact open reading frames (ORFs) (160), and a reanimated HERV-K virus is infectious in cultured cell assays (63, 136). Thus, it is formally possible that rare HERV-K alleles retain the ability to move in modern humans.

Recent studies indicate that HERV-K retrotransposons are expressed in certain tumors, and chromosomal rearrangements involving HERV-K sequences have been implicated in prostate cancer (176, 234). HERV expression also has been implicated in the etiology of certain autoimmune and neurological diseases; however, these data remain controversial, and causal links between HERV expression and disease require additional experiments (176).

Although apparently immobile in humans, the remnants of endogenous retroviruses also can impact the function of mammalian genomes. A growing number of examples indicate that *cis*-acting sequences derived from endogenous retroviruses can play roles in the transcription, splicing, and/or epigenetic regulation of endogenous genes (47, 151, 165, 198). Moreover, sequences derived from endogenous retroviruses have been exapted by mammalian genomes, and now play important roles in placental development [e.g., *Syncytin* and *peg10* (164, 186)].

Non-long terminal repeat retrotransposons: LINE-1 elements—L1s are the only known autonomously active human retrotransposons, and account for approximately one-sixth of our genome (134). Over 99.9% of L1s have been rendered inactive by 5' truncations, inversions, and/or point mutations within the two L1-encoded ORFs (95, 134, 189). However, a consensus sequence derived from human genomic L1s suggested the existence of full-length, retrotransposition-competent L1s (RC-L1s) (208). Indeed, the subsequent isolation of the progenitors of mutagenic L1 insertions into the *Factor VIII* and *dystrophin* genes revealed that a cohort of L1s continue to mobilize in our genome (67, 106).

The structure and mobility mechanism of retrotransposition-competent LINE-1s—RC-L1s are ~6 kb in length and contain a 5' untranslated region (UTR), two ORFs, and a 3' UTR that is punctuated by a poly(A) tail (208) (Figure 1). The L1 5' UTR houses an internal RNA polymerase II promoter that directs transcription from the 5' end of the element (227); it also contains *cis*-acting binding sites for multiple transcription factors (5, 16, 133, 169, 232, 259).

Recent studies have demonstrated that the L1 5' UTR contains a potent antisense promoter (L1 ASP). Transcription from the L1 ASP can lead to chimeric transcripts that contain a portion of the L1 5' UTR and genomic sequences flanking the 5' end of the L1 (181, 223). It is further speculated that these chimeric transcripts may either function in gene regulation or promote the formation of double-stranded L1 RNAs that regulate L1 retrotransposition by RNA interference-based mechanisms (159, 258). Interestingly, L1 ASP-derived chimeric transcripts have proven useful in the identification of expressed L1s from human embryonic stem cells, embryonic carcinoma cell lines, and somatic human tissues (77, 149, 223).

Human ORF1 encodes a ~40-kDa protein (ORF1p) required for L1 retrotransposition (107, 172). ORF1p has an amino-terminal coiled-coil domain (107), a centrally located RNA recognition motif, and a basic carboxyl-terminal domain (123, 172). Biochemical experiments with mouse and human ORF1p demonstrate that the amino-terminal coiled-coil domain facilitates ORF1p trimer formation (123, 155). Structural and biochemical analyses also suggest that the RNA recognition motif and carboxyl-terminal domains play critical roles in ORF1p binding to nucleic acids (13, 114, 123, 128). Finally, ORF1p has nucleic acid chaperone activity, which may be important for L1 integration (156).

ORF2 encodes a ~150-kDa protein (ORF2p), which has endonuclease and reverse transcriptase activities that are critical for L1 retrotransposition (71, 78, 152, 158, 172). ORF2p also contains a cysteine-rich domain of unknown function near its carboxyl terminus that is required for retrotransposition (75, 172). Recent experiments suggest that human ORF2 translation occurs by an unconventional termination-reinitiation mechanism, and that the putative ORF2 AUG initiation codon is dispensable for translation, although it is conserved in all human L1s examined thus far (1, 66, 163).

The analysis of mutagenic L1 insertions in conjunction with biochemical and genetic assays has demonstrated that human ORF1p and ORF2p preferentially associate with their encoding messenger RNA (mRNA) (67, 72, 132, 249). This association, termed *cis*-preference, leads to the generation of an L1 ribonucleoprotein (RNP) particle (105, 131,

153). ORF1p is detectable in cytoplasmic RNPs from embryonic stem cells, embryonic carcinoma cell lines, and HeLa cells that overexpress engineered L1 elements (82, 83, 105, 131, 153). ORF1p and/or L1 RNA also have been detected in human oocytes, in some human somatic cells, and at select times during human and mouse germ cell development (21, 29, 54, 85, 236). In contrast, ORF2p appears to be much less abundant than ORF1p in L1 RNPs, and its detection has relied largely upon an assay to detect L1 reverse transcriptase activity in RNPs (132). However, epitope-tagging strategies recently have allowed reliable ORF2p detection in RNPs derived from cells transfected with L1 expression constructs by both Western blotting and immunofluorescence (68, 90). L1 RNPs also associate with stress granules, although determining whether this association is important for L1 retrotransposition requires further study (68, 93).

Experiments using adenovirus-based vectors suggest that L1 retrotransposition can occur in the absence of cell division (130). Thus, some components of L1 RNPs may enter the nucleus in the absence of nuclear envelope breakdown. L1 retrotransposition then likely occurs by target-site primed reverse transcription (TPRT) (53, 78, 144). During TPRT, the L1-encoded endonuclease generates a single-strand endonucleolytic nick in genomic DNA to expose a 3'-OH (78). The liberated 3'-OH is then used as a primer by the L1 reverse transcriptase to initiate cDNA synthesis using the L1 mRNA as a template (53, 78, 132). Molecular details regarding second-strand target-site cleavage and second-strand L1 cDNA synthesis require elucidation, but insights about both these steps in TPRT have arisen from studying a related retrotransposon, R2, from *Bombyx mori* (45). The process of TPRT generates a new L1 copy that generally is flanked by ~7–20-bp target-site duplications (TSDs) (119) (Figure 2).

Nonautonomous retrotransposons: Alu and SVA elements—The human genome also contains numerous nonautonomous retrotransposons that rely upon activity of L1-encoded proteins to mediate their mobility. These nonautonomous elements consist primarily of the small interspersed element (SINE) Alu, which accounts for ~10% of sequence in the HGR (134).

Alu elements arose in mammalian genomes ~65 Mya and contain two monomeric sequences derived from the signal recognition particle (SRP) 7SL RNA (14, 238). Active Alus are ~280 base pairs (bp) in length and end in an A-rich tail. The left monomer contains an internal RNA polymerase III promoter (14) and is separated from the right monomer by an adenosine-rich sequence (Figure 1). However, flanking genomic sequences also can influence Alu transcriptional initiation and termination (46, 49, 64, 91, 143, 239). Like L1s, Alus can be stratified into subfamilies (60). Recent computational analyses and studies in cultured cells suggest that there may be thousands of active Alu “core” elements in the HGR (24, 52). However, Alu Y elements, most notably Ya5 and Yb8 subfamily members, account for the vast majority of disease-producing insertions in humans (38).

Alu elements rely upon L1 ORF2p to facilitate their retrotransposition in *trans* (62). Mutations that either block Alu transcription or interfere with SRP9/14 protein binding adversely affect Alu retrotransposition in vitro (24). Indeed, the ability of the SRP9/14

proteins to interact with Alu RNA may be intimately tied to the evolution of active Alu subfamilies (24, 203).

Alu elements can also be co-opted to play roles in gene expression. For example, inverted Alu elements within the 3' UTRs of some cellular mRNAs can result in adenosine-to-inosine RNA editing and preferential nuclear retention of the resultant edited mRNAs in differentiated cells (42). Additionally, Alu elements can be incorporated into existing transcription units by a process known as exonization, which may serve to enhance transcriptome diversification (139, 211, 217). Finally, the poly(A) tails flanking Alu elements may serve as a source for generating microsatellite sequences in human DNA (3).

SINE-R/VNTR/Alu (SVA) elements arose in primate lineages ~25 Mya and are present at ~2,700 copies in the human genome (244). They have a composite structure consisting of a variable-length hexameric repeat (CCCTCT)_n that is sequentially followed by an inverted Alu-like sequence, a variable number of tandem repeats (VNTR) region, a sequence derived from the 3' end of a HERV-K10 element (SINE-R), and a poly(A) tail (185, 187, 216) (Figure 1). Whether SVA elements contain functional promoter sequences remains an open question; however, it is likely that SVA elements are transcribed by RNA polymerase II and that the resultant SVA RNAs are mobilized to new genomic locations by the L1-encoded proteins (58, 101, 102, 187) (Figure 2). Indeed, the recent development of a cell-based assay for SVA mobilization should be instrumental in deciphering mechanistic details of SVA *trans* mobilization (102).

Nonautonomous retrotransposons: mobilization of cellular RNAs—Cellular messenger RNAs can occasionally use the L1-encoded proteins to mobilize to new genomic locations, thereby generating processed pseudogenes (72, 150, 249) (Figure 1). There are ~8,000–15,000 processed pseudogene copies in the HGR, and most are derived from genes that are highly expressed in the germline, such as housekeeping genes and ribosomal protein genes (235, 260, 261). Interestingly, some ribosomal protein processed pseudogenes (e.g., RPL21) occur at a relatively high copy number, suggesting that some property of these mRNAs allows them to recruit the L1-encoded proteins more effectively than other mRNAs (260).

Most processed pseudogenes are dead on arrival because they lack a functional promoter (242, 250). Thus, they can be used as molecular clocks to estimate mutation rates between species (94). However, some human processed pseudogenes are expressed, and a small number may encode functional genes or serve as sources of small interfering RNAs (siRNAs) with gene regulatory functions (103, 230, 242).

To date, there are no examples of *de novo* processed pseudogene retrotransposition events causing human disease. However, the expression of a processed pseudogene has been implicated in human facioscapulohumeral dystrophy patients (138, 222). Similarly, expression of an *FGF4* processed pseudogene is associated with chondrodysplasia in 19 dog breeds, consistent with the idea that selective breeding can enrich for rare mutagenic L1-mediated insertion alleles (193).

The L1-encoded proteins also can mobilize other noncoding cellular RNAs, such as U6 small nuclear RNA (snRNA), to new genomic locations (35). Computational and experimental evidence suggests that the L1 reverse transcriptase can switch templates to the U6 snRNA during TPRT to generate U6/L1, and less frequently U6/processed pseudogene chimeras (33, 35, 81, 86) (Figure 2). Interestingly, some U6 pseudogenes, small uracil-rich RNAs (e.g., U1 snRNA), and small nucleolar RNAs (e.g., U3 snoRNA) end in poly(A) tails and are flanked by TSDs, suggesting that they also may have been mobilized by the L1-encoded proteins (23, 61, 81, 240, 248).

TECHNOLOGIES TO IDENTIFY HUMAN-SPECIFIC LINE-1s

Overview

The ability to discriminate human-specific L1-mediated retrotransposition events from the sheer mass of defective retrotransposons in the genome is akin to finding a needle in a haystack. However, an elegant combination of phylogenetic, molecular biological, computational, and modern genomic technologies has revolutionized our ability to identify human-specific L1-mediated retrotransposition events in both reference sequences and individual genomes (Figure 3). Some of the seminal findings allowing these advances are discussed below.

A Brief Historical Perspective

L1s in the human genome have succeeded one another in a single lineage for the last ~40 million years (26–28, 122). Thus, new L1 subfamilies are continuously replacing older ones to dominate the expanding lineage of active elements (26, 28, 60, 122, 221). A majority of L1s are shared between human and chimp genomes, and likely represent retrotransposition-defective molecular fossils. However, studies in embryonic carcinoma cell lines revealed that a subset of expressed L1s contained diagnostic sequence variants within their 3' UTR (e.g., an ACA instead of a GAG trinucleotide at positions 5930 to 5932 of L1.2; accession number M80343) (219). The subset of expressed L1s were designated the Ta (transcribed, subset a) L1 subfamily (219). Interestingly, all but one disease-producing L1 insertion identified to date are derived from the Ta subfamily (18, 89). The remaining mutagenic insertion was derived from the slightly older, human-specific pre-Ta subfamily of L1 (which contains an ACG trinucleotide at positions 5930 to 5932 relative to L1.2) (120).

The identification of sequence variants peculiar to human-specific L1s and the subsequent development of a cultured cell retrotransposition assay were instrumental in allowing the identification of RC-L1s in the HGR (Supplemental Figure 1; follow the Supplemental Material link from the Annual Reviews home page at <http://www.annualreviews.org>) (15, 31, 134, 172, 179). Comprehensive computational studies revealed that the HGR contains ~90 L1s with intact ORFs (31, 179). Polymerase chain reaction (PCR)-based cloning revealed that ~44 of these show a range of activity in cultured human HeLa cells (31). Unexpectedly, six highly active, or “hot,” L1s accounted for ~84% of the retrotransposition activity in the HGR. Extrapolation of the ~44 RC-L1s to the scale of a complete diploid genome suggested that the average human genome harbors ~80–100 active L1 elements (31, 205). Interestingly, limited genotyping analyses indicated that many active and

retrotransposition-defective Ta subfamily L1s in the HGR are polymorphic with respect to presence or absence, indicating that many are recent insertions (9, 27, 31, 179).

Identification of Human-Specific LINE-1s by Mining Genome Sequences

Large-scale DNA sequencing projects have provided valuable resources to identify human L1-mediated retrotransposon polymorphisms. For example, comparative genomic analyses between the HGR and the draft chimpanzee genome allowed the identification of ~11,000 species-specific transposable elements, including 5,530 Alu, 1,174 L1, and 864 SVA elements specific to humans (167). Comparisons of DNA sequence trace files from 36 geographically diverse humans also enabled the identification of ~505 Alus, 65 L1s, 39 SVAs, 2 HERV-Ks, and 5 other polymorphic insertions (23). Similarly, comparison of a complete human diploid sequence (140) to the HGR allowed the identification of ~706 mobile element-associated structural variants, including the insertion of 584 Alus, 52 L1s, and 14 SVA elements (257). Mining 8 human genome sequences generated by next-generation sequencing yielded 4,342 Alu insertions absent from the HGR, 3,432 of which were additionally absent from a number of previous studies (108). Finally, a pilot analysis of sequence data obtained from ~200–300 individuals as part of the 1000 Genomes Project allowed the identification of over 1,000 L1 insertions that are absent from the HGR (69, 74, 168, 199). However, due to the low fold sequence coverage (2–4 fold for many genomes) and the composite nature of the HGR, it is likely that L1s are underrepresented in these analyses.

Clearly, the exploitation of human DNA sequence resources and nonhuman primate comparative genomics has revealed extensive human-specific transposable element diversity. A union of the above data has shown that these insertions are much more common in the population than once thought.

Experimental Approaches to Identify Novel Retrotransposon Insertions

The genomics revolution has revealed how structural variation contributes to interindividual genetic diversity. For example, representative oligonucleotide microarray analysis and array comparative genomic hybridization technologies have allowed the high-throughput identification of submicroscopic genetic differences of ~100 kb among individuals (110, 209). However, the size resolution of those techniques was not sufficient to permit identification of L1-mediated retrotransposition events.

More recently, a number of methods have been developed that are capable of detecting small- and intermediate-scale human structural variants (Figure 3). These methods include transposon display, array-based hybridization, second-generation DNA sequencing, and paired-end mapping of clone libraries (9, 25, 50, 69, 124, 129, 162, 168, 190, 191, 197, 215, 237, 246, 254). Together, these approaches have been instrumental in revealing transposon diversity in the genomes of geographically diverse individuals. A somewhat unexpected result is that mobile element dimorphisms account for a relatively large proportion of human genetic diversity (up to ~20%–25% of the interindividual genetic differences identified in some studies) (125, 129). The development of approaches to specifically identify these types of L1-mediated genetic variation is described below.

PCR-based display methods have allowed the identification of polymorphic L1 and Alu insertions in human genomes (9, 27, 34, 191, 200, 215). These methods exploit DNA sequence variants peculiar to human-specific retrotransposons (e.g., the ACA character present in Ta subfamily L1s) in conjunction with a pool of short, arbitrary oligonucleotides or sequences complementary to ligated linkers. PCR reactions utilizing this combination of retrotransposon-specific and degenerate or linker sequences then are used to generate complex amplicon libraries that contain the candidate retrotransposon and its immediate 5' or 3' flanking sequences. Sequencing of the flanking DNA and subsequent searches for sequence similarity then determine whether the candidate retrotransposon is present or absent in the HGR (9, 27). Together, these methods have identified numerous L1 and Alu insertion polymorphisms in individual genomes.

A derivation of classical display methods that employs suppression PCR methodology [amplification typing of L1 active subfamilies (ATLAS)] enabled the identification of nine full-length human-specific L1s from individual genomes (9). Interestingly, three out of seven tested sequences were “hot” in the cultured cell retrotransposition assay, and these three L1s were present at a minor allele frequency of less than 24% when genotyped in a panel of 90 geographically diverse individuals. Thus, these data provided additional evidence to support the hypothesis that young, human-specific L1s are underrepresented in the HGR.

Deconvolution of complex amplicon libraries generated by retrotransposon display approaches traditionally relied on electrophoretic fractionation using agarose or acrylamide gel systems and the subsequent cloning and characterization of individual molecules to map insertions to the HGR. The advent of new genomic technologies, including high-throughput DNA sequencing, now offers a means to revolutionize the discovery of polymorphic retrotransposon insertions (see Figure 3).

One method to identify dimorphic retrotransposon insertions, transposon insertion profiling by microarray (TIP-chip), employs the principles of transposon display to specifically amplify retrotransposons and their associated flanking sequences (80, 109, 253). The resultant amplicons are hybridized back to oligonucleotide arrays to identify sequences flanking the retrotransposons. TIP-chip allowed the discovery of numerous L1, Alu, and HERV-K insertion polymorphisms in genome-wide analyses. Application of TIP-chip to 69 unrelated individuals with X-linked intellectual disabilities also allowed the identification of L1 insertions within introns of the *NHS* and *DACH2* genes, and mutations in these genes are implicated in intellectual disability (109). However, future studies are needed to determine whether the L1 insertions play a causal role in the observed cognitive phenotypes in these patients.

Another method to identify dimorphic L1 and Alu insertion polymorphisms employs traditional Sanger capillary sequencing and 454 or Illumina-based second-generation DNA sequencing technologies to deconvolute transposon-derived amplicon libraries (73, 111). Iskow et al. (111) identified 152 L1s from 38 ethnically diverse humans and 8 cell lines using capillary sequencing. They also identified 650 L1 and 403 Alu insertions in 30 lung or brain tumors and their matched nontumor controls via 454-based sequencing. These

insertions were absent from both the HGR and a database of retrotransposon insertion polymorphisms (dbRIP) (134, 245), yielding 1,145 novel transposable elements. Ewing et al. (73) used an Illumina-based sequencing approach to identify 367 polymorphic L1s from a cohort of 25 humans that contained 15 unrelated individuals, individuals from 6 trios, and 3 pairs of monozygotic twins. Finally, Witherspoon et al. (255) used an Illumina-based sequencing approach to identify 487 Alu Yb8 and Yb9 insertions in 4 unrelated individuals that were absent from the HGR.

A third method to identify full-length or near-full-length L1 insertion polymorphisms involves paired-end DNA sequencing of fosmid libraries derived from individuals belonging to geographically diverse populations, which successfully identified intermediate-sized structural variants in human DNA (15, 124, 237). The screening of six individual libraries identified 68 L1s that were absent from the HGR. Remarkably, 37 of these 68 were “hot” L1s, 2 of which belonged to the pre-Ta L1 subfamily (15). Notably, unlike the methodologies described above, paired-end DNA sequencing of fosmid libraries does not involve PCR, allows identification of L1s in repetitive regions of the genome, and, though labor intensive, allows a comprehensive and relatively unbiased snapshot of L1 diversity. Together, the above methodologies have uncovered a virtual treasure trove of natural retrotransposon diversity in human genomes.

Allelic Heterogeneity in LINE-1 Activity

In addition to the polymorphic status of an L1, allelic heterogeneity may cause different L1 insertion alleles to exhibit different retrotransposition efficiencies in cultured cells. For example, L1.2A and L1.2B, which are likely progenitor alleles of a mutagenic insertion in the *Factor VIII* gene, exhibit a ~16-fold difference in their retrotransposition efficiencies because of amino acid substitutions near the ORF2p carboxyl terminus (67, 76, 120, 146). Similarly, the examination of three “hot” L1s from the HGR in a geographically diverse set of individuals revealed alleles with a wide range of retrotransposition activities (ranging from 0% to 390% of a reference L1) (212). Finally, a recent study identified an RC-L1 allele in an individual genome that apparently is defective in the HGR owing to a stop codon in ORF2p (15). Thus, both presence/absence of polymorphisms and allelic heterogeneity can influence L1 retrotransposition in an individual genome.

IMPACT OF MOBILE ELEMENTS ON MAMMALIAN GENOMES

LINE-1 as a Mutagen

A wealth of data is revealing the consequences of L1-mediated retrotransposition events in human genomes. Since their original discovery, approximately 65 disease-causing mutations in man have been attributed to L1-mediated retrotransposition events (18, 89). L1-mediated retrotransposition events can act as mutagens by directly disrupting exons (120). Similarly, insertions into introns can induce missplicing or exon skipping, thereby generating hypomorphic or null expression alleles (Figure 4) (19, reviewed in 89, 188). Finally, recent reports suggest the L1 endonuclease may cause double strand breaks, which in principle could lead to genomic instability (84, 142). Clearly, advances in genomics and the evolution

of DNA sequencing technologies should allow the rapid identification of other disease-producing insertions in the coming years.

Effects on Gene Expression

L1 insertions can impact gene expression by a variety of mechanisms (Figure 4). For example, experimental studies have revealed that the adenosine-rich nature of the L1 transcript can introduce premature polyadenylation and/or RNA polymerase II transcriptional pause sites into genes, thereby attenuating their expression (97, 194). Interestingly, transcriptional pausing appears to depend on both the length of the L1 insertion and whether it is in the same transcriptional orientation as its resident gene (39, 97). The L1 ASP also can generate transcripts that, in principle, could affect gene expression (159, 181, 223, 258). Finally, in rare instances, L1 insertions may disrupt genes, leading to the generation of distinct transcription units through a phenomenon known as gene breaking (Figure 4) (252).

Approximate Rates of Heritable Retrotransposition Events

Determining the rate of germline retrotransposition in the human population remains an area of ongoing investigation. Estimates suggest that Alu elements are the most active retrotransposons in the human genome, with new insertions occurring in approximately 1 out of 20 live births (51, 257). L1 insertions follow, with estimates ranging between 1 out of 20 and 1 out of 200 births, depending upon the method used in the analysis (51, 73, 109, 141, 257). SVA insertions may be the least frequent retrotransposition events, occurring in approximately 1 out of 900 births (51, 257). In spite of disparate rates of retrotransposition, the different sizes and unique characteristics of L1, Alu, and SVA elements may pose distinctive challenges to the genome.

The above estimates are subject to ascertainment biases, and may represent only minimal estimates of the actual de novo retrotransposition frequency. For example, because some studies relied on comparisons with the HGR, they may underestimate the contribution of low-allele-frequency insertions to human genetic diversity (257). In fact, Iskow et al. (111) suggested that low-allele-frequency L1-mediated retrotransposition events, representing either rare or perhaps private L1 and/or Alu alleles, may be present in “virtually all personal genomes in the human populations.” Consistently, fosmid-based paired-end DNA sequencing, TIP-chip profiling, and the preliminary examination of the 1000 Genomes Project data have readily allowed the detection of rare L1 retrotransposition events in geographically diverse individuals (15, 74, 109, 168). Clearly, the exhaustive DNA sequencing of parent/offspring trios at high coverage should provide more accurate estimates of L1-mediated germline retrotransposition events in the near future.

Somatic LINE-1 Retrotransposition: Insights from Cancer Studies

In addition to acting as a germline mutagen, studies have revealed that L1 retrotransposition also occurs in certain somatic cells. Historically, the identification of a mutagenic L1 insertion into the *adenomatous polyposis coli* (*APC*) gene in a colorectal tumor that was absent from adjacent nontumor tissue established that L1 could retrotranspose in somatic cells (166).

Recent data generated using L1 display approaches combined with 454-based DNA sequencing led to the discovery of 9 de novo somatic L1 retrotransposition events in 6 of 20 non small-cell lung cancers that were absent from matched adjacent normal tissue samples (111). Interestingly, tumors containing new L1 retrotransposition events also exhibited global patterns of hypomethylation, providing a correlative link between epigenetic changes and increased L1 retrotransposition in tumors. Hypomethylation of the L1 5' UTR also has been observed in malignant cells and cancer tissues, and is correlated with an increase in L1 mRNA and/or ORF1p expression (2, 4, 20, 89). Similarly, 5-azacytidine treatment leads to an elevation of L1-ASP-driven chimeric transcripts in nonmalignant breast epithelial cells (56). Thus, the above data suggest that the rate of L1 retrotransposition, and by proxy Alu and SVA retrotransposition, may be elevated in some cancers. Further studies should allow a greater understanding of whether and/or how often L1-mediated retrotransposition occurs in cancer and whether these events play a causal role in tumorigenesis.

Somatic LINE-1 Retrotransposition During Normal Development

Human genetic approaches, experiments conducted in transgenic animals, and cell culture models further suggest that L1 retrotransposition can occur in normal cells at discrete times during development. For example, genetic analyses have conclusively demonstrated both germline and somatic mosaicism of a mutagenic L1 retrotransposition event in the mother of a male patient afflicted with X-linked choroideremia (241). Thus, the mutagenic L1 insertion must have occurred during early embryonic development of the mother prior to partitioning of the germline. Consistent with this hypothesis, engineered L1s can retrotranspose in human embryonic stem cells (82).

Engineered human L1s can also retrotranspose during the early stages of mouse and rat embryonic development; however, most of the resultant insertions are not heritable (116). These findings are remarkable and suggest that L1 mRNA may be transferred from the gametes to the zygote to undergo retrotransposition at a later time in development, generating somatic mosaicism in the resultant offspring.

Unexpectedly, recent studies also revealed that engineered human L1s can retrotranspose at discrete times during neuronal development (54, 177, 178). These retrotransposition events could, in principle, generate somatic mosaicism in the nervous system and have the potential to affect intraindividual neuronal variability (54, 177, 178). In fact, sensitive TaqMan PCR-based approaches suggest that certain regions of the brain have an increase in human-specific L1 content relative to heart and/or liver tissues isolated from the same individual (54, 178). The observed increase in L1 DNA copy number in the brain suggests elevated levels of endogenous L1 retrotransposition in brain. However, other non-mutually-exclusive mechanisms must be considered when accounting for these L1 copy number differences (195, 251). Indeed, formal proof of endogenous L1 retrotransposition in brain will require the characterization of new L1 insertions from individual neurons. Although this remains a daunting task, advances in DNA sequencing technologies—including the ability to comprehensively characterize genome sequences from single or small pools of cells—should allow rigorous testing of these interesting observations.

Together, the above findings overturned the long-held dogma that L1-mediated retrotransposition could occur only in germ cells, and has led to speculations about how L1-mediated retrotransposition events may affect intraindividual genetic variation (116, 154, 177, 178, 218). Time and rigorous experimentation will tell whether somatic L1 retrotransposition events represent stochastic genetic noise that is tolerated by the host genome or whether these events have functional consequences in disease pathogenesis and/or neuronal development.

LINE-1 AS AN AGENT OF GENOME DIVERSIFICATION

LINE-1 Retrotransposition by Target-Site Primed Reverse Transcription

Canonical TPRT generally results in the insertion of an L1 or nonautonomous retrotransposition event at a new genomic location flanked by short TSDs (Figures 2 and 4). On occasion, TPRT also can lead to small deletions of target-site DNA that vary from approximately 2 to 50 bp in length, and/or to the addition of nontemplated or filler nucleotides at the 5' genomic DNA/L1 junction sequence (5, 135, 180). Similar target-site alterations were observed upon examining engineered L1 retrotransposition events in transformed cell lines, human embryonic stem cells, and neuronal progenitor cells (54, 82, 86, 87, 177, 228).

Approximately 35% of Ta subfamily L1 retrotransposition events represent full-length insertions (26). The remaining L1s are 5' truncated (95) and often contain short micro-homologies at the 5' genomic DNA/L1 junction sequence (8, 86, 87, 157, 228, 263). Additionally, approximately 25% of L1s contain L1 inversion or deletion events that likely are generated by a process termed twin priming (189) (Figure 4). The high frequency of 5' truncation associated with new L1 retrotransposition events remains enigmatic and may reflect host defense or DNA repair processes that act to either dissociate the L1 reverse transcriptase from the nascent L1 cDNA or degrade the L1 mRNA template prior to the completion of reverse transcription.

LINE-1 Retrotransposition-Mediated Deletion Events

In addition to acting as an insertional mutagen, L1-mediated retrotransposition events can lead to various forms of human structural variation. For example, studies conducted in HeLa and HCT116 cells revealed that ~10% of retrotransposition events derived from engineered human L1s are associated with the formation of chimeric L1s accompanied by intrachromosomal deletions, intrachromosomal duplication or inversions, and perhaps interchromosomal translocations (86, 87, 228). Comparisons of the pre- and postintegration sites of chimeric retrotransposition events suggested that DNA recombination processes such as single-strand annealing, synthesis-dependent strand annealing, and perhaps nonhomologous end joining are involved in the formation of these aberrant structures (86, 87, 228).

L1 retrotransposition-mediated genomic deletions are not peculiar to transformed human cells. For example, comparative biological approaches between the human and chimpanzee reference genomes enabled the discovery of 30 L1 and 19 Alu retrotransposition-mediated

deletion events, which together account for a loss of ~26 kb of human genomic DNA in the past six million years (36, 100, 202).

L1 retrotransposition-mediated deletion events have also been observed in human genetic diseases (41). For example, a full-length L1 insertion that was accompanied by a deletion of ~46 kb, including 7 exons of the *PDHX* gene, led to a sporadic case of pyruvate dehydrogenase complex deficiency (170). Similarly, a ~4-kb L1 insertion was accompanied by a ~17-kb deletion that disrupted 3 exons of the *EYA1* gene, leading to a sporadic case of branchio-oto-renal syndrome (173); however, the structure of this event suggests that it may have occurred by an endonuclease-independent (ENi) L1 retrotransposition mechanism (see below).

Alu and SVA retrotransposition-mediated deletion events also have impacted the human genome. For example, an Alu retrotransposition-mediated deletion approximately 1 Mya resulted in the loss of a 92-bp exon of the *CMP-Neu5Ac hydroxylase* gene, leading to a loss-of-function frameshift mutation in humans (104). Similarly, an SVA retrotransposition-mediated deletion apparently resulted in the loss of a 14-kb region of genomic DNA encompassing the entire *HLA-A* gene in three Japanese families containing patients afflicted with leukemia (229). Thus, although relatively rare, retrotransposition-mediated deletion continues to impact the human genome and represents a mechanism that can lead to both human structural variation and disease.

Postintegration LINE-1- and Alu-Mediated Recombination Processes

The abundance of L1 and Alu retrotransposons in the human genome provides numerous potential substrates for postintegration recombination events that can lead to disease and/or structural variation in human genomes (reviewed in 51, 89). For example, nonallelic homologous recombination (NAHR) events between two inverted Alu elements originally were observed in a ~5-kb deletion that included exons of the *Low-Density Lipoprotein Receptor* gene, resulting in a case of familial hypercholesterolemia (137). Similarly, NAHR between genomic L1s has been implicated in sporadic cases of phosphorylase kinase deficiency, Alport syndrome, and Ellis–van Creveld syndrome (32, 210, 233). Finally, comparative biological and genomic studies have revealed that NAHR events between L1s or Alus have generated structural variants in the chimpanzee and human genomes (98, 99, 125, 145, 213). Notably, young Alu elements are significantly enriched in regions flanking segmental duplications, suggesting that Alu-mediated recombination events may be involved in some segmental duplication expansions observed in humans (11). Personal genomics and advances in DNA sequencing likely will continue to reveal that inter-retrotransposon recombination events represent a significant portion of structural variation in human genomes.

Endonuclease-Independent Insertions

Experiments in XRCC4-deficient and DNA protein kinase catalytic subunit (DNA-PKcs)-deficient Chinese hamster ovary cell lines, which are defective in the nonhomologous end-joining pathway of DNA repair, revealed an alternative mechanism of ENi L1 retrotransposition distinct from conventional TPRT (174, 175). Characterization of ENi

insertions revealed that they generally integrated at noncanonical L1 endonuclease cleavage sites, lacked TSDs, and frequently were truncated at both their 5' and 3' ends. In addition, some ENi retrotransposition events were associated with genomic DNA deletions and/or contained cDNA fragments derived from non-L1 cellular RNAs that likely were reverse transcribed during the integration process. L1 insertions bearing the hallmarks of ENi retrotransposition events have been identified in the human genome, again showing how cultured cell models can predict events that occur in nature (214, 224). Together, these findings suggested that ENi retrotransposition might bypass the requirement for endonuclease activity by initiating reverse transcription at endogenous lesions in genomic DNA, thereby acting as a molecular bandage (175, 243). Consistent with the above hypothesis, some ENi retrotransposition events in DNA-PKcs-deficient Chinese hamster ovary cells integrate at dysfunctional telomeres (174). Thus, ENi retrotransposition shows curious similarities to the action of telomerase and may represent a form of RNA-mediated DNA repair utilized by retrotransposons that lack an endonuclease domain (57, 88, 174).

LINE-1-Mediated Transduction

The examination of disease-producing L1 insertions and experiments using the cultured cell retrotransposition assay revealed that L1s are able to mobilize genomic DNA sequences flanking their 3' (and less commonly their 5') ends by a process termed L1-mediated transduction (Figure 4). The transduction of 3' sequences is relatively common, and suggests that RNA polymerase II frequently bypasses the natural L1 polyadenylation site and instead uses a site in flanking genomic DNA (106, 171, 172). Computational analyses and the examination of L1 structural variants in individual genomes have revealed that 3' transductions flank ~20% of human-specific L1s in the HGR and that some 3' transductions are over 1 kb in length (15, 92, 125, 196). Extrapolations based on these data suggest that L1-mediated 3' transductions may comprise as much as ~19–30 Mb of the human genome (92, 196).

L1 3' transductions create recognizable sequence tags, which can be used to infer parent/offspring relationships between full-length L1s and their progeny. For example, a ~489-bp region of genomic DNA flanking a mutagenic L1 insertion into the dystrophin gene was instrumental in identifying an active L1 progenitor allele (106). Similarly, shared 3' transduction sequences were recently used to identify minifamilies of L1s that contain active, rare alleles in the human population (15, 125). L1s from two of these 3' transduction families, LRE3 and L1_{RP}, are responsible for disease-producing insertions in sporadic cases of chronic granulomatous disease and X-linked retinitis pigmentosa, respectively (30, 127, 207). Thus, the examination of transduction families that include highly active elements may be used as a way to discover novel, rare, and highly active L1s in human populations.

The cultured cell L1 retrotransposition assay revealed that 3' transduction could, in principle, lead to the mobilization of exons and/or regulatory DNA sequences to new genomic locations, and may represent a mechanism of exon shuffling (171, 172). To date, there are no *in vivo* examples of L1 3' transduction leading to the formation of a new gene. However, SVA elements are also frequently associated with 3' transductions (187), and SVA-mediated 3' transduction events prior to the divergence of humans and great apes led

to the dispersion of the *AMAC* gene to three distinct places in the human genome (256). Whether these copies of the *AMAC* gene are functional requires validation, but the potential for genomic diversification through mobile element-mediated dispersion is clear.

The ability of the L1-encoded proteins to mobilize non-L1 RNAs to new genomic locations in *trans* provides another potential mechanism for exon dispersal and/or the creation of new genes. For example, retrotransposition of a *cyclophilin A* cDNA into the *TRIM5- α* locus of New World monkeys led to the creation of a chimeric protein that confers HIV resistance to owl monkeys (206). Similarly, a novel testes-specific hominoid gene, *PIPSL*, is a chimeric ubiquitin-binding protein derived from a fusion of the *PIP5K1A* and 26S proteasome subunit RNAs that underwent retrotransposition (7, 184). The *PIPSL* gene appears to have been subject to positive selection and is conserved among humans, suggesting a functional role in modern genomes. Finally, it appears that exons from the *CFTR* and *ATM* genes have been mobilized in *trans* to new genomic locations by the L1 retrotransposition machinery (70, 201). However, it remains possible that some of these examples represent L1-mediated 3' transductions that were so severely 5' truncated that they lack L1 sequences (70, 171).

In principle, L1 5' transduction can occur if a transcript initiating upstream of an L1 undergoes retrotransposition. Because 5' transduction can only be found by examining full-length L1s, they appear to be much less common than 3' transductions. However, potential examples of L1 5' transduction have been identified in the HGR, in cultured cell experiments, and in a mutagenic mouse insertion (39, 134, 228, 249). In contrast, 5' transduction events frequently accompany SVA retrotransposition events, which is consistent with the idea that some SVA elements rely on host promoter sequences for their transcription (58, 101). Indeed, a 5' transduction derived from the *MAST2* gene was used to identify a family of SVA elements (SVA_F) that likely are amplifying in the human genome (58, 101).

Epigenetic Phenomena Related to LINE-1s

L1s may also play critical roles in the epigenetic regulation of host genes (Figure 4). For example, recent research suggests that indicator cassettes delivered into the genome by engineered L1 retrotransposons can be epigenetically silenced either during or immediately after their integration (54, 83, 177). It will be interesting to determine whether silencing is specific for the retrotransposed L1 sequence or whether it can affect the epigenetic status of adjacent genes.

The ability of L1s to affect the epigenetic regulation of genes may not be restricted to new retrotransposition events. For example, the ability of L1s to accumulate on sex chromosomes over evolutionary time led Mary Lyon (147) to speculate that L1s might function as *cis*-acting booster elements to aid in the spreading of heterochromatin formation observed during X-inactivation. Consistent with this notion, some genes that escape X-inactivation are in relatively L1-poor regions of the X-chromosome (10, 37), and L1 density appears to correlate positively with heterochromatin spread in X/autosomal translocations (231). Indeed, recent experiments suggest that L1s might play an active role in nucleating heterochromatin formation on the inactive X chromosome (44). Clearly, although these

findings are still in their early stages, further research should allow even more discoveries about how L1-associated epigenetic changes impact gene expression.

CLOSING REMARKS

It is now clear that transposable elements are an integral part of our genomes. In the coming years, technological breakthroughs in DNA sequencing and genome annotation will undoubtedly uncover many more examples of how transposable elements impact biological processes. For example, high-coverage, longer-read sequencing of trios and monozygotic twins and/or the ability to sequence the genomes of single or small populations of cells will yield more information about the actual rate of L1-mediated retrotransposition events in both germline and somatic cells, and should clarify the role of L1-mediated retrotransposition events in certain types of human cancers. It will be interesting to determine whether genetically distinct populations or particular individuals are more prone to L1-mediated retrotransposition events.

Because transposable elements can be considered intracellular genomic parasites, they also provide an ideal means to study host-parasite interactions. The availability of new experimental reagents, such as epitope-tagged engineered human L1s, combined with cell-culture-based retrotransposition assays now allows a way to identify host factors that act to combat the constant assault of transposable elements on the genome. We probably will discover even more examples where genetic differences in host factors correlate with variation in L1 retrotransposition rates (e.g., 43, 89, 126, 183, 225).

Finally, future studies will enlighten us about how transposable element-derived sequences serve as seeds for evolutionary change. There is an ever-growing number of examples of regulatory elements derived from transposable elements that are required for proper gene expression (e.g., 17, 115, 204, 247; reviewed in 79), and we anticipate this list will expand. Indeed, transposable elements may provide a mechanism to amplify and distribute *cis*-acting DNA sequences to new genomic locations, which after selection may function in gene regulation—a potentially prescient hypothesis put forth by Davidson & Britten (59) over 40 years ago.

We have come a long way since Barbara McClintock's (161) discovery of mobile genetic elements in maize over half a century ago. In the past decade alone, we have witnessed an exponential growth in knowledge about how transposable elements have impacted the human as well as other mammalian genomes. Though much progress has been made, our work has just begun. The coming years likely will identify how transposable elements contribute to phenotypic variation and human-specific traits. Clearly, the "junk" in our genomes is stepping into the limelight. It is an exciting time for transposable element research; the human genetics community should take greater notice of these dynamic elements.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Drs. Haig Kazazian and Mark Batzer for their critical and insightful review of the manuscript. We thank Aurélien Doucet, Billy Giblin, Nancy Leff, John Moldovan, Sandra Richardson, and other members of the Moran lab for helpful comments and discussions. C.R.B. was supported in part by NIH training grants T32GM7544 and T32000040. J.V.M. is supported by NIH grants GM060518 and GM082970, and is also an investigator of the Howard Hughes Medical Institute. J.L.G.-P. is supported by an ISCI-CSJA (FEDER/EMER07/056), a Marie Curie IRG action (FP7-PEOPLE-2007-4-3-IRG), CICE (P09-CTS-4980), Proyectos en Salud PI-002 from Junta de Andalucía (Spain), and the Spanish Ministry of Health (FEDER/FIS PI08171). R.M.B. was supported by a Wellcome Trust project grant (075163/Z/04/Z) to R.M.B. and Prof. Sir Alec Jeffreys, FRS. Finally, we thank our spouses for their support and patience while writing this review. Henry Levin and J.V.M. have recently written a review that also discusses various aspects of L1 biology.

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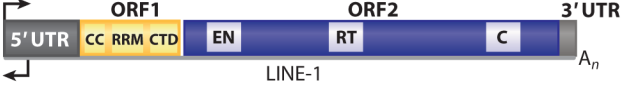



Type of mobile element	Example structure	HGR percentage	Active?
DNA transposons			
Transposons	 Mariner	~3%	No
Retrotransposons			
Autonomous retrotransposons			
LTR retrotransposons	 HERV-K	~8%	Uncertain (none known)
Non-LTR retrotransposons	 LINE-1	~21%	Yes
Nonautonomous retrotransposons			
SINEs	 Alu (e.g., Ya5 and Yb8 subfamilies)	~10%	Yes
	 SVA	<1% ~2,700 copies	
Processed pseudogenes	 RPL21	<1% ~11,000 copies	

Figure 1.

The classes of mobile genetic elements in the human genome, showing the type of mobile element, the structure of representative elements, the percentage of each element in the human genome reference sequence (HGR), and whether each class of elements is currently active (134). Abbreviations for human endogenous retrovirus-K (HERV-K): LTR, long terminal repeat; Gag, group-specific antigen; Pol, polymerase; Env, envelope protein (dysfunctional). For LINE-1: UTR, untranslated region; CC, coiled coil; RRM, RNA recognition motif; CTD, carboxyl-terminal domain; EN, endonuclease; RT, reverse transcriptase; C, cysteine-rich domain. For Alu: A and B, component sequences of the RNA polymerase III promoter; AR, the adenosine-rich segment separating the 7SL monomers. For SINE-R/VNTR/Alu (SVA): VNTR, variable number of tandem repeats; SINE-R, domain derived from a HERV-K. A_n signifies a poly(A) tail.

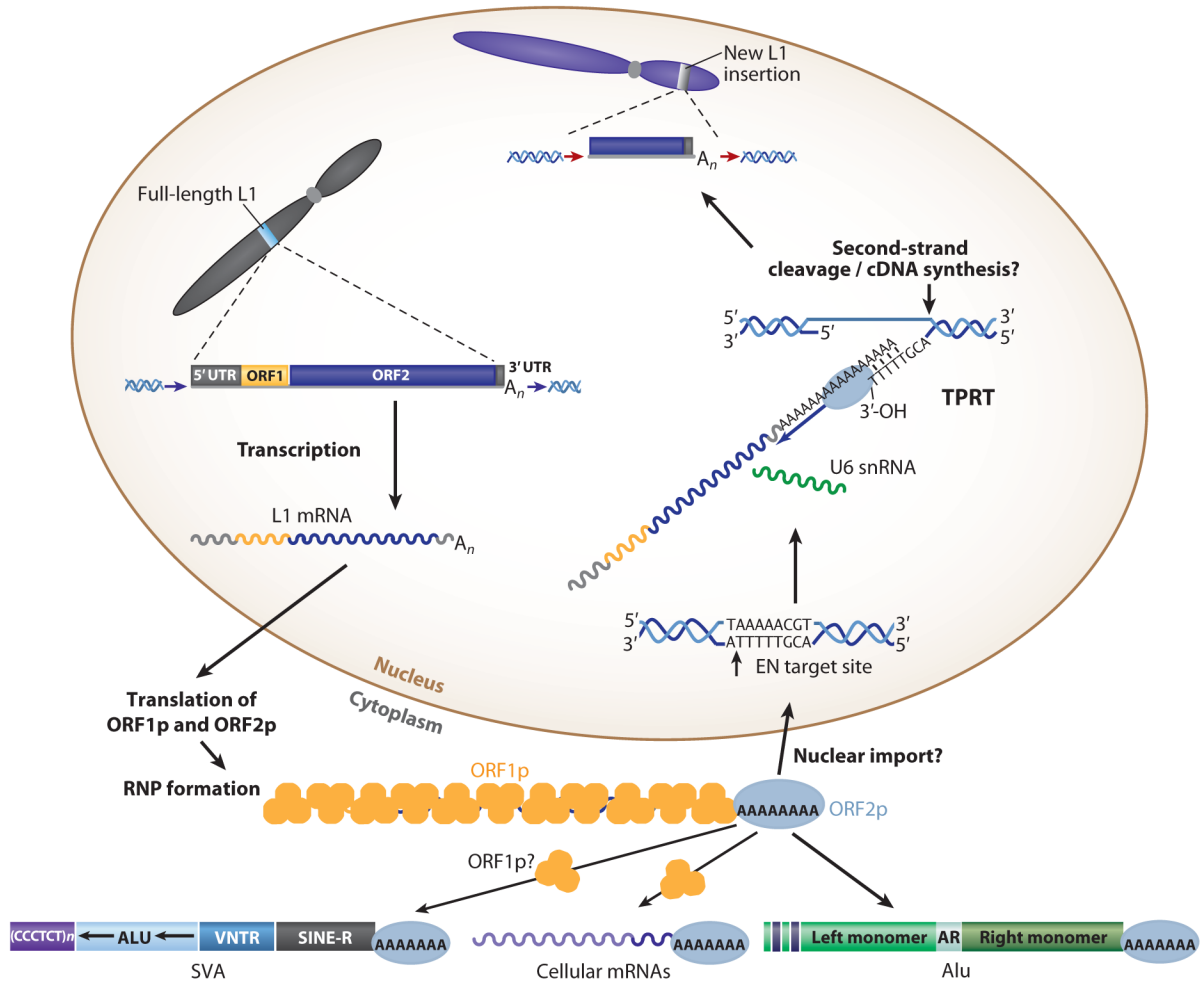
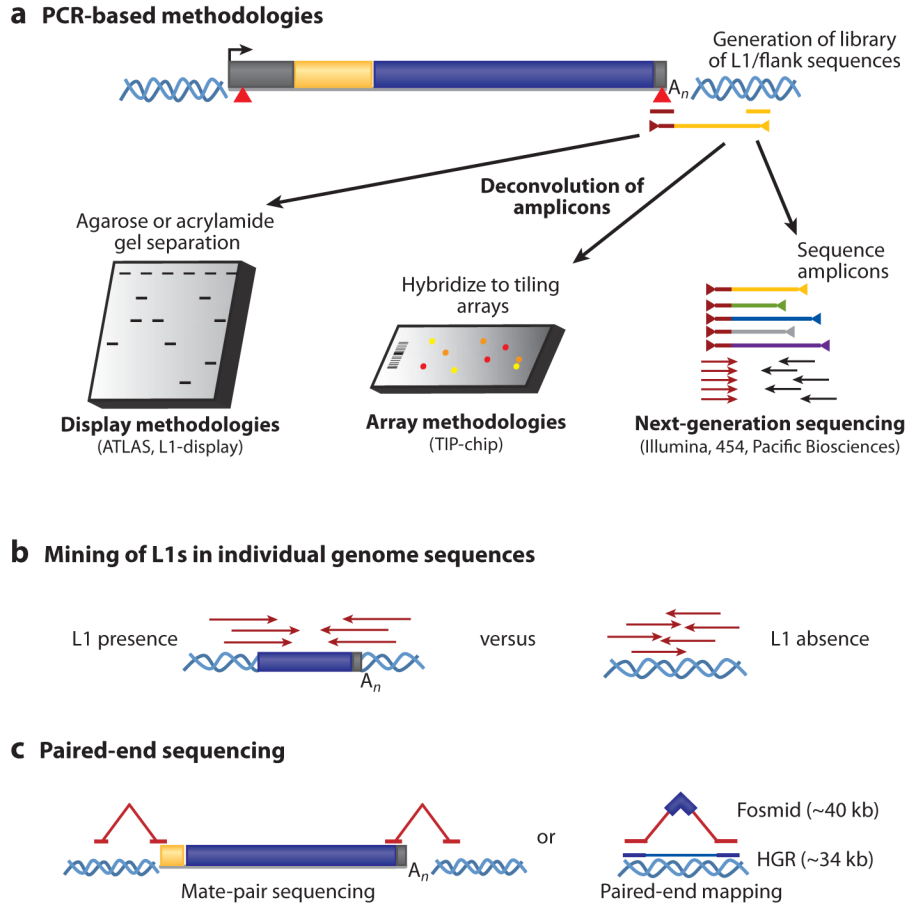


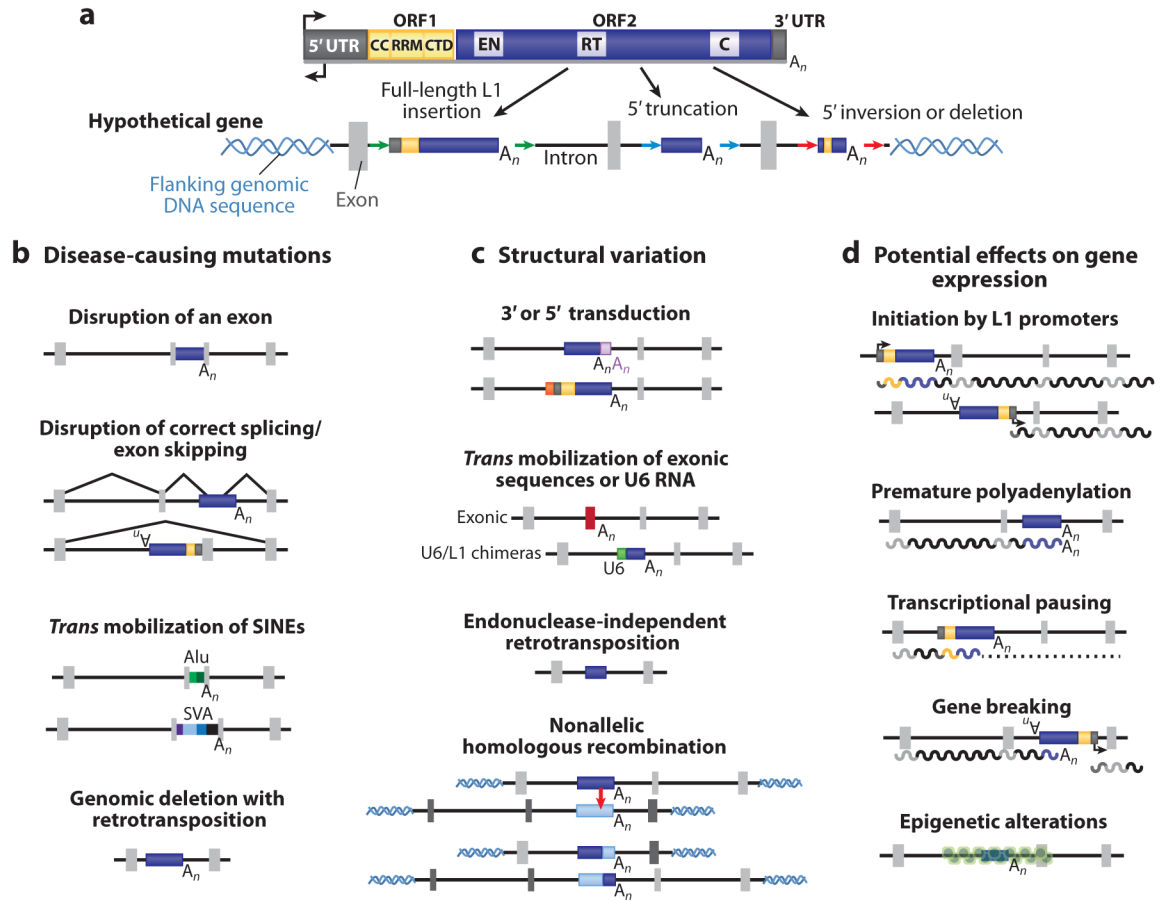
Figure 2.

A LINE-1 retrotransposition cycle. A full-length L1 (light blue bar on gray chromosome) is transcribed, the L1 messenger RNA (mRNA) is exported to the cytoplasm, and translation of ORF1p (yellow circles) and ORF2p (blue oval) leads to ribonucleoprotein (RNP) formation. Components of the L1 RNP are transported to the nucleus, and retrotransposition occurs by target-site primed reverse transcription (TPRT). During TPRT, the L1 endonuclease (EN) nicks genomic DNA, exposing a free 3'-OH that can serve as a primer for reverse transcription of the L1 RNA. The processes of second-strand cleavage, second-strand complementary DNA (cDNA) synthesis, and completion of L1 integration require elucidation. TPRT results in the insertion of a new, often 5'-truncated L1 copy at a new genomic location (gray bar on purple chromosome) that generally is flanked by target-site duplications (red arrows). Alu, SINE-R/VNTR/Alu (SVA), and cellular mRNAs may hijack the L1-encoded protein(s) in the cytoplasm to mediate their *trans* mobilization. U6 small nuclear RNA (snRNA) may be integrated with L1 during TPRT. Question marks denote steps in the retrotransposition pathway of unknown mechanism.

**Figure 3.**

Methods to detect LINE-1-mediated polymorphic human retrotransposition events in individual genomes. Modifications of these assays can also be used to identify other polymorphic retrotransposons in human DNA. (a) Polymerase chain reaction (PCR)-based methodologies. PCR using primers specific to diagnostic sequence variants in the L1 (*red triangles* and the corresponding *maroon primer*) and arbitrary oligonucleotides or primers complementary to ligated linkers (*yellow line*) can be used to amplify human-specific L1s and their associated flanking sequences (*maroon/yellow line* flanked by *triangles*). The amplicon libraries are then resolved using electrophoresis, and individual products are cloned and sequenced (*left*). Alternatively, the amplicons can be hybridized to genome tiling microarrays (*center*), or directly characterized using high-throughput sequencing methodologies (*right*). Abbreviations: ATLAS, amplification typing of L1 active subfamilies; TIP-chip, transposon insertion profiling by microarray. (b) Mining of L1s in individual genome sequences. Whole-genome sequences, comparative genomics, or mining trace sequence databases can discover dimorphic L1s in individual genomes that are absent from reference genome assemblies. (c) Paired-end sequencing. Mate-pair reads containing one sequence from a uniquely mapping portion of genomic DNA and one sequence from an L1 can be used to identify novel retrotransposons (*left*) in individual genomes. Paired-end sequencing of fosmid inserts with restricted size distributions (~40 kb) allows the discovery of novel ~6-kb insertions (*right*) as well as deletions and inversions relative to a reference

sequence. Fosmids containing insertions can then be screened for the presence of human-specific L1s. Abbreviation: HGR, human genome reference sequence. These methods are also described in another recent review (182).

**Figure 4.**

Schematics highlighting the various ways that LINE-1-mediated retrotransposition events can impact the human genome. (a) A hypothetical wild-type gene locus. Light-gray rectangles represent exons, black lines represent introns, and helical lines represent flanking genomic DNA sequence. A full-length (*left*), 5'-truncated (*center*), and inverted or deleted L1 formed by twin priming (*right*) (189) are shown as intronic insertions. The arrows indicate target-site duplications (TSDs), and for simplicity are shown only in this panel. (b) Examples of L1-mediated processes that may result in disease. (c) Examples of structural variation caused by L1 insertions. The transduction figures show a 3' transduction in light purple with its own poly(A) tail, and a 5' transduction in orange. The nonallelic homologous recombination figure shows L1s at different loci (*light* and *dark gray* exons) acting as substrates for aberrant recombination (*red arrow*). (d) Potential effects on gene expression caused by L1 insertion. Note that L1s in all the depicted events would generally contain TSDs, with the exception of endonuclease (EN)-independent retrotransposition events and some genomic deletions. Abbreviations: C, cysteine-rich domain; CC, coiled coil; CTD, carboxyl-terminal domain; RRM, RNA recognition motif; RT, reverse transcriptase; SVA, SINE-R/VNTR/Alu; UTR, untranslated region.