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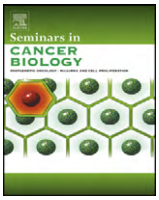

Review

SVA retrotransposons: Evolution and genetic instability

Dustin C. Hancks, Haig H. Kazazian Jr.*

Department of Genetics, The University of Pennsylvania School of Medicine, USA

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ABSTRACT

SINE-VNTR-*Alus* (SVA) are non-autonomous hominid specific retrotransposons that are associated with disease in humans. SVAs are evolutionarily young and presumably mobilized by the LINE-1 reverse transcriptase in *trans*. SVAs are currently active and may impact the host through a variety of mechanisms including insertional mutagenesis, exon shuffling, alternative splicing, and the generation of differentially methylated regions (DMR). Here we review SVA biology, including SVA insertions associated with known diseases. Further, we discuss a model describing the initial formation of SVA and the mechanisms by which SVA may impact the host.

1. Introduction

Most genomes are highly repetitive, with a large fraction of the DNA derived from transposons. Some of these transposons, in particular retrotransposons, replicate and expand through an RNA intermediate by a “copy and paste” mechanism termed retrotransposition. The non-LTR class of retrotransposons replicates by coupling reverse transcription and integration into DNA, a process termed target-primed reverse transcription (TPRT) [1,2]. Long Interspersed Element-1 (L1) [3] is the most successful non-LTR retrotransposon in mammals [4–6] and is evolutionarily old as evidenced by its presence in *Candida albicans* [7]. Human L1 is present in approximately 500,000 copies, comprising some 17% of the entire genome sequence [6]. An intact L1 encodes two proteins [8,9], one of which, ORF2, is a reverse transcriptase [10], the enzyme responsible for the reverse-transcription of retrotransposon RNA to DNA.

Despite the *cis* preference [11] of L1 proteins for their own encoding RNA, a variety of other multi-copy sequences [12–14], in particular, non-autonomous retrotransposons such as SINEs [15,16] and processed pseudogenes [17], amplify through an RNA intermediate by hijacking the L1 reverse transcriptase [18]. The factors that enable these RNAs to be preferential substrates for the L1 machinery are currently unknown.

Another interesting non-autonomous retrotransposon that likely uses the L1 machinery to enter the genome is the hominid specific SVA [19]. SINE VNTR *Alu* (SVA), as it was originally named [20], is a composite retrotransposon currently active in humans [21] and present in about 2700 copies [19] in the human genome reference sequence. SVAs were originally described as SINE-R elements [22], a retrotransposon containing 5′ GC-rich tandem repeats along with *env* (envelope) and LTR sequence from an endogenous retrovirus [23]. Since then, progress has been made, primarily through bioinformatics and sequence analysis, illuminating our understanding of SVA. Nevertheless, relatively little is known about SVA compared to L1 due to a lack of experimental data, especially an SVA retrotransposition cell culture assay. Here we review what is known about SVA biology, including what can be learned from the individual SVA domains, and examine general mechanisms by which SVA may impact the genome, and sometimes cause disease.

2. A repeat of repeats

SVA is a composite non-coding retrotransposon [24,25] (Fig. 1B) that in all likelihood relies on the L1 ORF2 reverse transcriptase for its mobilization [21], a presumption that has not yet been experimentally demonstrated. Each domain of SVA is derived from either a retrotransposon or a repeat sequence. A canonical SVA is on average ~2 kilobases (kb) but SVA insertions may range in size from 700 to 4000 basepairs (bp) [19,26] in the human genome. Starting at its 5′ end, a canonical SVA (Fig. 1B) consists of a hexameric CCCTCT repeat, followed by sequence sharing homology to two antisense *Alu* fragments, a variable number of GC-rich tandem repeats (VNTR), presumably derived from the SVA2 element [27–29] of the Rhesus macaque [30] (Fig. 1A), and sequence sharing identity to the *env* gene and right LTR of an ancient endogenous retrovirus, HERV-K10 [22], followed by a canonical polyadenylation signal (polyA), AATAAA. SVA genomic insertions exhibit the classical hallmarks of L1 mediated retrotransposition and TPRT:

* Corresponding author at: Department of Genetics, University of Pennsylvania, School of Medicine, 564 Clinical Research Building, 415 Curie Blvd., Philadelphia, PA 19104-6145, USA. Tel.: +1 215 898 3582.
E-mail address: kazazian@mail.med.upenn.edu (H.H. Kazazian Jr.).

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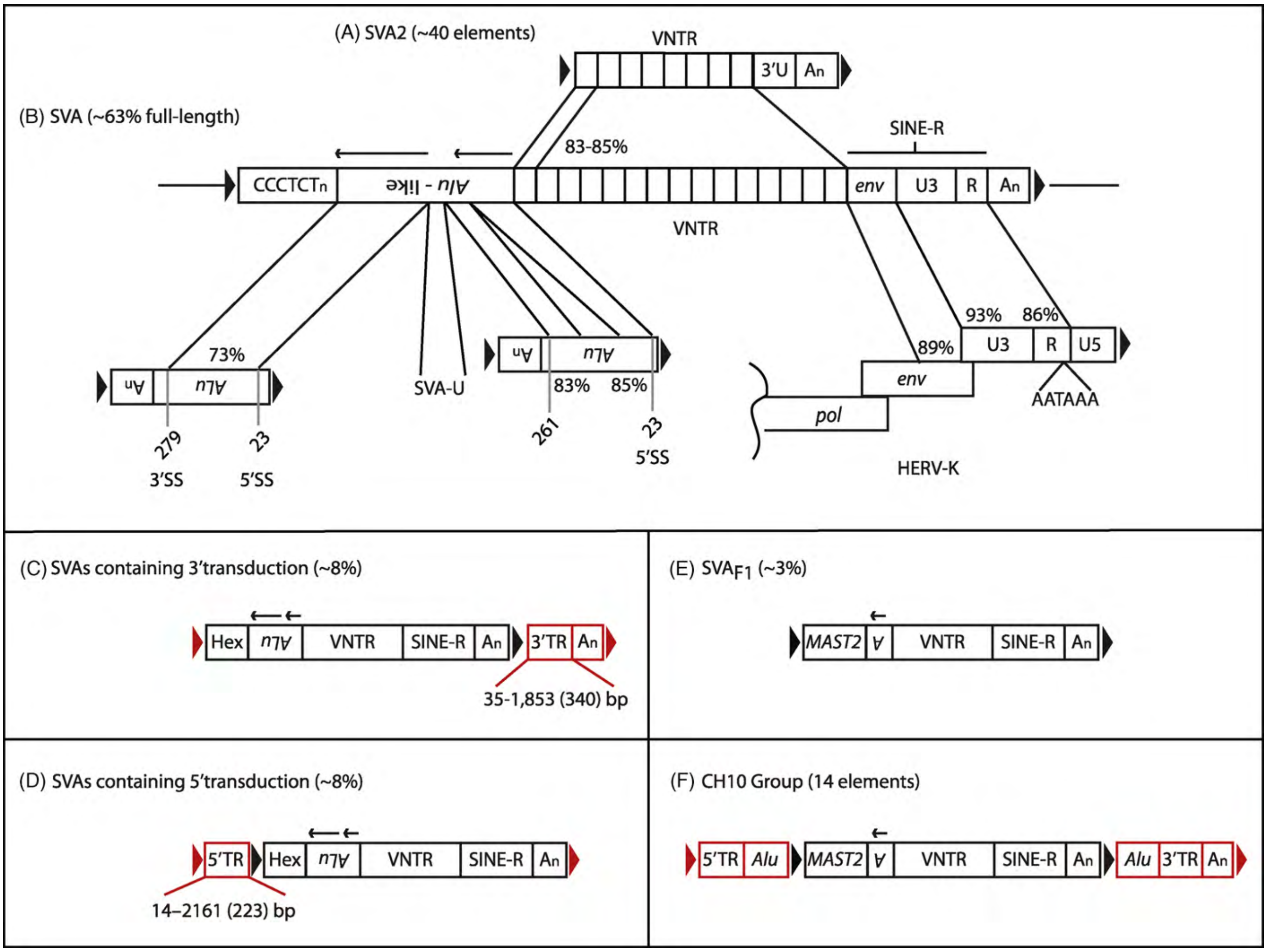

Fig. 1. The structure of a full-length SINE VNTR *Alu* (SVA) and SVA genomic variants. (A) The SVA2 element. An SVA2 element consisting of a variable number of tandem GC-rich repeats (VNTR), followed by a unique 3′ sequence (3′ U), followed by a polyA tail with the entire insertion flanked by a target-site duplication (black arrows) is shown. (B) A full-length SVA element consisting of in order from the 5′ end (1) CCCTCT hexameric repeats, (2) the *Alu*-like domain consisting of two antisense *Alu* fragments (black arrows above the *Alu*-domain indicate directionality of *Alu* sequences) and an intervening unique sequence, SVA-U, (3) a VNTR domain derived from the ancestral SVA2 element (A), (4) the SINE-R domain consisting of sequence sharing homology to the 3′ end of the HERV-K10 *env* gene and U3, R, polyA signal (right LTR), terminating with a polyA tail (A_n) with the entire SVA insertion flanked by a target-site duplication. DNA sequence identities were obtained by pairwise BLAST alignments between the individual SVA domains and ancestral repeats (*Alu*, SINE-R, VNTR identity is between individual tandem repeats). Alignments consisted of using the following Repbase [27,28] reference sequences: SVA2, SVA, *Alu*, HERV-K, and LTR5. The numbers below the antisense Alus correspond to nucleotide positions in Alu_{Rep} and whether or not this position corresponds to a known splice site within *Alu*. Different SVA variants exist within the human genome, some contain additional 3′ sequence (C, red boxes), referred to as 3′ transductions (3′ TR) or additional 5′ sequence (D, red boxes), referred to as 5′ transductions. A new target-site duplication (red arrows) flanks the SVA insertion and transduction. Transductions (C and D, red boxes) can be used to identify the source locus of a retrotransposon. Some 5′ transductions may be acquired via splicing of an upstream sequence into a downstream SVA element. This may result in novel SVA subfamilies such as $\mathrm{SVA}_{\mathrm{F1}}$ (E) that acquired the *MAST2* exon through splicing. SVA elements may also contain both 5′ and 3′ transductions (F), such as elements within the CH10 group. The number or percentage of SVAs containing a defining characteristic within the human genome reference sequence is in parentheses. Furthermore, the upper and lower limits in basepairs for reported 5′ [29] and 3′ [19] transductions is displayed below the transduction with the mean in parentheses.

(1) insertion at a consensus L1 endonuclease recognition motif 5′-TTTT/AA-3′ (where "/"denotes the cleavage site) [31], (2) a target-site duplication flanking the SVA insertion and ranging from 4 to 20 bp in length, (3) a polyA tail of varying length, (4) the occurrence of 5′ truncations, (5) internal rearrangements and inversions [21,32,33] and (6) 3′ transductions (Fig. 1C) [21,34–39]. However, one primary difference between L1 and SVA genomic insertions exists. Most SVAs are full-length, 63% and 42% in human and chimp, respectively [19]. While most (99.8%) L1 insertions are inactive due to 5′ truncations, inversions, and point mutations [6,40]. Many SVA variants exist in hominid genomes, in addition to SVAs containing 3′ transductions, SVAs may also contain 5′ transductions (Fig. 1D), upstream exons (Fig. 1E) or both 5′ and 3′ transductions [26,29] (Fig. 1F).

3. SVA lifecycle and retrotransposition

The SVA RNA is likely a RNA polymerase (Pol) II transcript based upon several sequence features [19] and SVA expression analysis (Fig. 2). SVAs contain multiple RNA Pol III terminators (TTTT) throughout the *Alu*-like and SINE-R domains. Also, the SVA RNA is ~2–3 kb, much longer than Pol III transcribed RNAs. SVA RNAs are presumably 5′ capped as indicated by the presence of guanine residues at the 5′ end of ~1/3 SVA genomic insertions, similar to L1 insertions [41], and by the ability to amplify SVA RNA by 5′ RACE [26], a technique reliant on a 5′ cap structure. Furthermore, there is a small subset of SVAs [42], including the ARH insertion [43], that contains an alternative CCCTCT hexamer ($(\mathrm{CCCTCT})_2$ $\mathrm{CCC\underline{G}TCT})_n$, where the "G" might represent an example in which the 5′ cap was

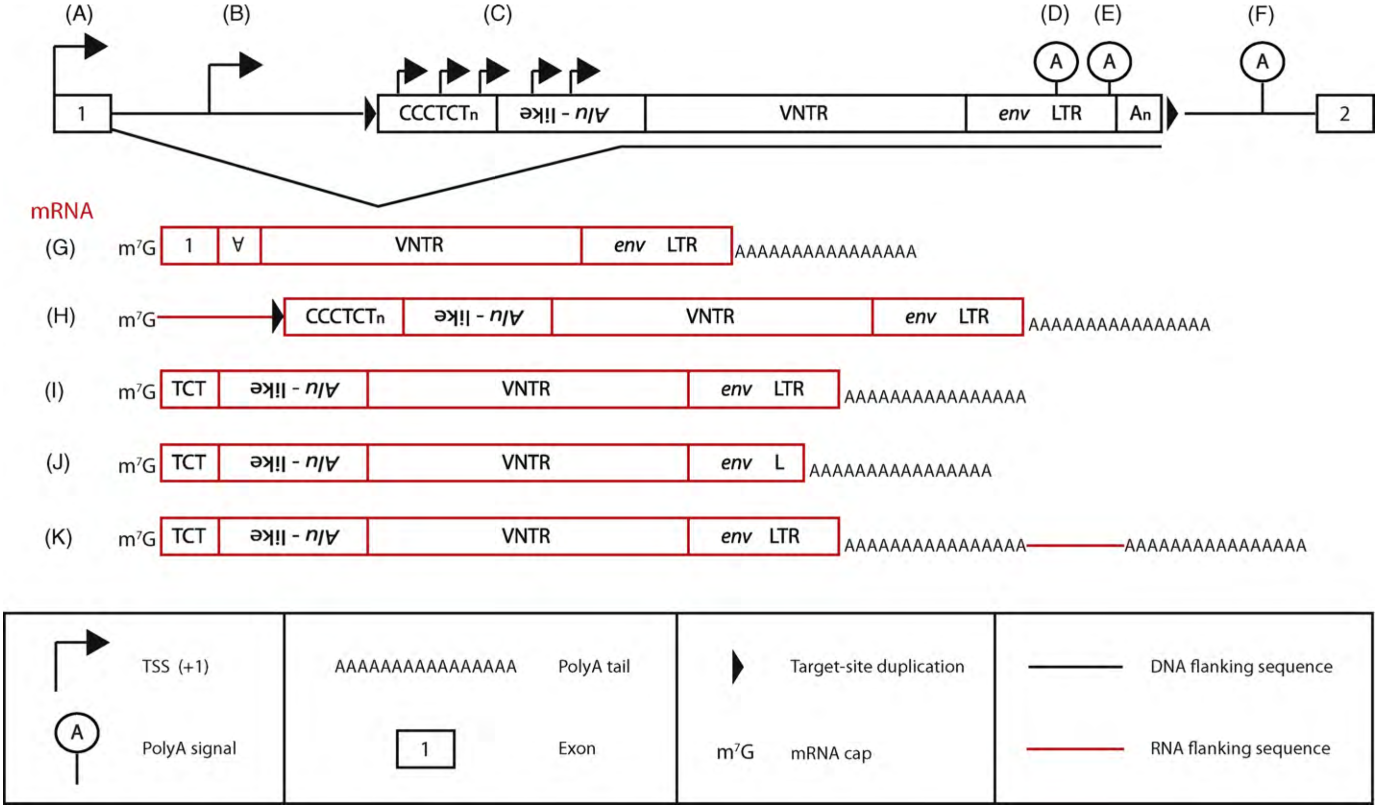
(A)
(B)
(C)
(D)
(E)
(F)
A
A
A
1
CCCTCTn
Alu - like
VNTR
env LTR
An
2
mRNA
(G) m7G 1 A VNTR env LTR AAAAAAAAAAAAAAAA
(H) m7G CCCTCTn Alu - like VNTR env LTR AAAAAAAAAAAAAAAA
(I) m7G TCT Alu - like VNTR env LTR AAAAAAAAAAAAAAAA
(J) m7G TCT Alu - like VNTR env L AAAAAAAAAAAAAAAA
(K) m7G TCT Alu - like VNTR env LTR AAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAA
TSS (+1)
PolyA signal
AAAAAAAAAAAAAAAA PolyA tail
1 Exon
Target-site duplication
m7G mRNA cap
DNA flanking sequence
RNA flanking sequence

Fig. 2. SVA transcription and SVA mRNA structure. A full-length SVA, with individual domains labeled, in the genome residing in an intron (top; black line) of a gene with exons numbered 1 and 2 (top; black boxes) is displayed. Black bent arrowheads indicate different sites of SVA transcriptional initiation (A–C). The different SVA RNAs present in the human and chimpanzee transcriptome (red boxes; G-K) due to variable 5′ TSSs and 3′ polyA sites (D–F) are shown below. (A) mRNA transcription may initiate at an upstream exon (black box labeled 1) that may subsequently splice into an SVA generating a "5′ truncated" SVA mRNA containing exonic sequence (G) terminating at the canonical SVA polyA signal (E). SVA-mediated exon-trapping may enable SVA evolution as is the case for the SVA$_{F1}$ subfamily. SVA transcription may initiate at an upstream TSS (B), presumably mediated by upstream promoter elements generating SVA mRNAs containing SVA 5′ flanking sequence (H; red line). Both (A and G) and (B and H) may result in retrotransposition of SVA 5′ flanking sequence, a process termed 5′-transduction. SVA RNAs may also initiate transcription internally (C) resulting in mRNAs resembling full-length genomic insertions (I), or terminate at internal non-canonical polyA sites in the SINE-R (D) resulting in 3′ truncated SVA RNAs, or bypass the SVA polyA signal, terminating at a downstream polyA signal (F), resulting in an SVA mRNA containing 3′ flanking sequence (K; red line). Note that an SVA transcript may contain both 5′ and 3′ transductions (not shown).

reverse-transcribed, and this nucleotide addition has expanded along with the hexamer.

The SVA contains a canonical polyadenylation signal, AATAAA (Fig. 2E) at its 3′ end. However, SVA transcription may occasionally bypass its own polyA signal, resulting in transcriptional readthrough, and terminate at a downstream polyA signal (Fig. 2F) [21,39]. This might result in retrotransposition of an SVA 3′ flank along with its sequence to another genomic location, a process termed 3′ transduction, with the potential for exon-shuffling [35]. Finally, SVAs have been identified that terminate at other internal non-canonical polyA sites within the SINE-R (Fig. 2D), resulting in 3′ truncated SVA genomic insertions [19,29].

The exact SVA transcriptional unit, along with its promoter and regulatory elements, is still undefined. Nonetheless, SVA RNAs are classified into three types of transcripts based upon the location of the 5′ transcriptional start site (TSS) [26]: (1) SVA sequences into which upstream exons are spliced, also referred to as SVA exon-trapping (Fig. 2A and G), (2) SVAs that initiate transcription upstream of their genomic location (Fig. 2B and H), and (3) SVAs that initiate transcription internally (Fig. 2C and I–K). SVA mRNAs can be further subdivided on the basis of polyA signal selection: (1) internal polyA (Fig. 2D), resulting in 3′ truncated SVAs (Fig. 2J), (2) canonical polyA signal (Fig. 2E), and (3) downstream polyA signal (Fig. 2F), resulting in an SVA mRNA containing 3′ flanking sequence (Fig. 2K).

It is unclear which type of SVA transcription is the preferred mode of SVA mRNA expression. Still, SVA mRNAs are expressed in a variety of ways in cell lines and *in vivo* [26,29,44]. We know that SVAs initiating transcription upstream (Group 1 and 2) are retrotransposition competent because of the identification of SVA insertions containing retrotransposed 5′ flanking sequence [26,29] due to (1) SVA exon-trapping or (2) upstream transcriptional initiation. SVAs retrotransposing 5′ flanking sequence, referred to as 5′ transductions, account for ~8% of total SVA insertions in the human genome [29]. SVA 5′ transduction as a result of upstream transcriptional initiation (Fig. 2B) is much more common than 5′ transduction by exon-trapping (Fig. 2A) as indicated by the number of distinct 5′ transduction groups relative to the number of SVA insertions identified that transduce upstream exons due to splicing. Furthermore, SVA elements are retrotransposition competent independent of polyA signal selection.

Therefore, SVAs are able to enter the transcriptome by three distinct routes (1) SVA mediated exon-trapping (Fig. 2A and 2G), (2) upstream TSS mediated in most part by upstream promoters (Fig. 2B and 2H), and (3) internal transcriptional start sites (TSS) (Fig. 2C and I–K). Three different potential models exist for SVA transcription: (1) Similar to L1 and *Alu*, SVAs rely exclusively on their own promoter and/or regulatory elements, (2) SVAs themselves contain no regulatory elements and exclusively rely on external regulatory elements, or (3) SVAs contain some regulatory elements that may act synergistically with external promoters/external regulatory elements enabling SVA transcription. Experiments to localize any internal SVA promoter activity have led to ambiguous results (Seleme and Kazazian, unpublished data).

Whether or not the internal and upstream TSSs both rely on upstream promoter units is unclear. One possibility is that many SVAs depend on upstream promoters. This would suggest that many SVA master elements, retrotransposon loci that are the source of many genomic copies, in addition to the recently described CH10 SVA master element [26,29], are present in different hominid genomes. If most of the human genome is transcribed [45,46], with different transcripts having multiple different transcriptional start sites [47,48] including transposon derived TSSs [49] and transcriptional readthrough is the primary mode of SVA transcription, then one might expect *Alu* genomic insertions to occasionally contain 5′ transductions. To the best of our knowledge, *Alus* containing 5′ transductions have not yet been identified. Moreover, if all SVAs are expressed due to upstream promoters, then SVA elements containing the CCCTCT hexamer would be 5′ truncated elements, presumably due to the inability of L1 ORF2 to reverse transcribe the CCCTCT repeat. Be that as it may, it is then difficult to reconcile how ORF2 is able to effectively reverse transcribe the VNTR of SVA.

Regarding SVA transcription model 3, SVAs may contain the inherent ability to mediate transcriptional initiation upstream of their genomic location due to an internal enhancer element. This enhancer element may be able to recruit transcription factors to upstream promoter elements, ultimately leading to transcription at or near the 5′ end of SVA. SVA elements contain many predicted transcription factor binding sites, such as SP1 binding sites in the VNTR and potential hormone response element (HRE) half-sites throughout the SVA domains [19,50–52]. The HREs and the SP1 sites may cooperate, as in one study for *Alu* [50], to drive SVA transcription. It is particularly interesting that the SINE-R contains a glucocorticoid response element and an enhancer core element [22]. Both were originally described when HERV-Ks were cloned [53] and subsequently described in SINE-R elements [22]. Notably, HERV-K mRNA expression was up-regulated in a human breast cancer cell line treated with progesterone followed by estradiol treatment [54]. The lack of an internal promoter and the presence of enhancer elements in the SINE-R may account for the variation in SVA transcript structure and is consistent with the observation that active SVA elements may lack the CCCTCT hexamer and *Alu*-like domain [26,29]. Ono et al. [22] have postulated that the glucocorticoid response element and enhancer core may act as a steroid dependent enhancer element for SINE-R elements.

After transcription, the SVA RNA needs to (1) come in contact with the L1 ORF2 protein, and (2) out-compete the L1 RNA for the attention of ORF2 reverse transcriptase. L1 and *Alu* RNA competition for ORF2 presumably takes place at the ribosome [55,56] or at the very least where *Alu* gets incorporated into the L1 ribonucleoprotein particle (RNP). Yet, the location in the cytoplasm and/or nucleus where SVA and other RNAs are incorporated by the L1 RNP and subsequently interact with ORF2 protein is unknown. The vast number, more than 2000 copies, of ribosomal processed pseudogenes [14] suggests that ORF2 competition may occur at the ribosome. To the contrary, since U6 snRNAs transit through the nucleolus, the presence of U6-L1 chimeric retrogenes [12] indicates that the nucleolus [57] may also be a site where the L1 RNP may acquire RNAs.

It has been hypothesized that the *Alu*-like domain localizes SVA RNA to the ribosome by annealing with *Alu* RNAs [58]. However, the identification of multiple retrotransposed SVAs lacking the majority of the *Alu*-like domain, due to SVA mediated alternative splicing, in particular SVA F_1s, a human specific SVA subfamily distinguished by the presence of exon 1 from the *MAST2* gene [26,29,59], argues against an SVA-*Alu* hybridization requirement for SVA retrotransposition. Future experiments describing which RNAs and their abundance in L1 cytoplasmic and nuclear RNP complexes will assist in resolving this question.

After incorporation into the L1 RNP, SVAs are reverse transcribed in the nucleus by ORF2 probably by a template choice mechanism [18]. The present lack of any described SVA-L1 chimeras and the dearth of known retrogene chimeras [18,60], other than U6-L1 chimeras, disagrees with a template switching mechanism for ORF2 mediated SVA reverse transcription. However, template-switching by ORF2 between polyA tails [18] of L1 and SVA RNAs cannot be ruled out.

SVA elements that have been spliced into, resulting in loss of the CCCTCT hexamer and most of the *Alu*-like domain, followed by subsequent retrotransposition and those that are 3′ truncated have provided insight into requirements of SVA retrotransposition. These SVA insertions suggest much of the SVA is dispensable and unnecessary for successive rounds of retrotransposition. The VNTR is the core sequence of SVA, and the highly structured nature of the tandem repeats probably plays a yet undefined functional role. RNAs that have increased mRNA stability are over represented as processed pseudogenes [61] and the VNTR alone or within the context of SVA may increase RNA stability. The SINE-R domain is probably responsible for SVA expansion and likely enables SVA expression. The variation in TSSs may be due to the looping of the SINE-R over the VNTR to the 5′ end of SVA. Longer VNTRs may lead to internal SVA TSSs, while shorter VNTRs may lead to transcriptional initiation further upstream into flanking DNA. Recruitment of transcription factors to the SINE-R and the interaction of these factors with SP-1 proteins may assist in the assembly of the transcription pre-initiation complex.

The only obvious functional requirement for SVA retrotransposition is the polyA tail. The polyA tail is indispensable for L1 [34] and *Alu* [16] retrotransposition in cell culture. Human L1s share no sequence homology with *Alu* and SVA other than polyA stretches. However, both *Alu* [62] and SVA are highly structured RNAs and the 3′ UTR of many L1s contain GC-rich sequences that have been shown to be structured for Rat L1 [63]. Therefore, we propose in order to reconcile the lack of sequence homology among human L1, *Alu*, and SVA that RNA structure is fundamental for ORF2 substrate recognition and that RNA structure is the primary determinant of whether a RNA will be retrotransposed while the polyA tail is secondary. Work by Eickbush and colleagues has shown that the R2 retrotransposon RNA secondary structure in the 3′ UTR is required for TPRT and that the R2 protein from *Bombyx mori* is able to carry out TPRT with the 3′ UTR from *Drosophila melanogaster* R2 element *in vitro* [64]. Furthermore R2 RNAs ending in fewer adenines are more preferential substrates for target-primed reverse transcription *in vitro* [65].

Two obvious caveats exist with this model; (1) the human L1 3′ UTR is not required for retrotransposition in the cell culture retrotransposition assay [34] and (2) the presence of retropseudogenes derived from tRNAs that lack polyA tails [66] and the retroposed snRNAs [12,18], like U6, that also lack a 3′ polyA stretch. The dispensability of the 3′ UTR can be explained by human L1's intense *cis* preference [11] for its own encoding RNA. The amplification of tail-less tRNA retroelements and U6-L1 insertions can be explained by the fact that tRNAs and U6 RNAs are highly structured, and at least in the case of tRNA its ability to localize to the ribosome enhances its incorporation into the L1 RNP. Hence, successful competition for the reverse transcriptase of a non-LTR element is contingent upon a highly structured RNA or sequence containing 3′ non-LTR sequence, as in the case of tRNA derived SINEs [67] and snoRTE [68], which ultimately enables these elements to mimic retrotransposon RNA structure. Secondary to RNA structure, the ability of a RNA to localize to the ribosome determines its retrotranspositional success as indicated by *Alus*, tRNA-derived SINEs, and tailless tRNAs. Lastly, the polyA stretch of non-LTR elements is fundamentally important for retrotransposition, providing somewhat of a flat runaway for RT loading or enabling accessibility of RT for its template because the polyA lacks secondary structure. This is consistent with the longer polyA tails associated with active elements [69] and the length [70] and homogeneity [71] of the *Alu* polyA tails impacting their retrotransposition efficiency.

4. SVA origins

Although retrotransposons derived from repeat sequences are not uncommon, the structure of SVA is unique to say the least [72]. Chimeric retrotransposed sequences are present in nature and are not rare [73,74]. DNA recombination or retroelement insertion into a transcription unit may generate new sequences and increase their retrotransposition capability. Despite this fact, many retroelement chimeras are likely formed at the RNA level. U6-L1 chimeras are common in the human genome [12,18] and in primates [75]. An example of a new hominid gene, *PIPSL*, formed from a retrotransposed chimeric transcript derived from an alternative splicing event involving adjacent genes was recently described [76]. Likewise, snoRTE [68], a chimeric retrotransposon, consisting of a 5′-H/ACA-snoRNA containing the 3′ end of a BovB Plat RTE LINE, has been extremely successful, exceeding more than 40,000 copies in the platypus genome. Okada's group has shown that the 3′ ends of tRNA-derived SINEs are derived from the 3′ ends of LINEs [77]. Furthermore, it has been proposed that LTR retrotransposons and retroviruses were derived from the fusion of a DNA transposon and a non-LTR retrotransposon [78].

SVAs are evolutionarily young which enables easier identification of the origins of their multiple domains (*Alu*-like, SINE-R). SVA evolutionary analysis provides insight into (1) how non-autonomous retrotransposons are created, (2) what sequence features might enable retrotransposition of a pseudogene and (3) how genomes evolve. In all regards, SVA is a successful pseudogene. SVA is currently more active than high-copy pseudogenes, such as processed ribosomal pseudogenes, as evidenced by seven published SVA insertions associated with disease [43,79–84] (Table 1) and no disease associated pseudogene insertions. Second, each mRNA pseudogene originates from primarily one source locus, while retrotransposed SVAs are derived from many loci, as indicated by the variation in 5′ [29] and 3′ transductions [39], indicating multiple SVA source loci.

Work by Batzer and colleagues [30], as part of the Rhesus macaque genome consortium, documented the absence of SVAs from the Rhesus genome, but they noted that each SVA domain, CCCTCT hexamer, *Alu*-like, VNTR, and SINE-R was present independent of the other domains. They also reported that the VNTR was present ~40 times and contained a non-SVA sequence at its 3′ end followed by a polyA tail with the entire sequence flanked by a target-site duplication. These data suggest that the VNTR was retrotransposition-competent in the past. VNTRs similar to those described in Rhesus and referred to as SVA2 elements, had been briefly described by Repbase [27,28] and more recently in a study characterizing SVA genomic insertions [29]. Whether or not the SVA2 (VNTR) can be classified as a retrotransposon or a relatively successful pseudogene is unclear because at least 15 different non-ribosomal processed pseudogenes have more than 30 copies in the human genome [85] as compared to the 40 SVA2 copies in Rhesus.

The lack of SVAs in old world monkeys [19,30], suggests that SVAs are hominid specific retroelements [86]. Thus, SVA2 elements acquired the other current SVA domains sometime after the divergence of old world monkeys and hominids. Knowledge of the individual SVA domains has enabled us to model some of the events that likely occurred to create the present day SVA.

Alus are the most successful primate retrotransposons [87] with about one million copies in the human genome reference sequence [6]. Additionally, *Alus* are known to be frequently alternatively

Table 1
SVA insertions and disease.

Gene	Insertion (kb)	HG19	Full-length	Subfamily[a]	Associated disease	Genotype	Potential mechanism	Progenitor	Note	SVA sequence
HLA-A	2	No	Yes[b]	F_1	Leukemia	SVA/+	Deletion	3p21.31[c]	Founder insertion (JPN)	AB291067, AB291066
NF2	1.7	Yes	Yes	D	Neurofibromatosis 2	SVA/+	Deletion	N/A		hg19
BTK	0.25	No	No	N/A	X-linked agammaglobulinemia (XLA)	SVA/Y	Exon skipping	N/A	*Alu* inserton at same site	[103]
α-spectrin	0.63	No	No	E	Heriditary elliptocytosis and pyropoikilocytosis	SVA/+	Exon skipping	3q25.1[c]	Inverted 3′ transduction	dbRIP[d]
TAF1	2.6	No	Yes	F	X-linked dystonia-parkinsonism (XDP)	SVA/Y	DNA methylation	N/A	Founder insertion (PHI)	AB191243
LDLRAP1	2.6	No	Yes	E	Autosomal recessive hypercholesterolemia (ARH)	SVA/SVA	Reduced mRNA	N/A	Italian ancestry	[43]
Fukutin	3.1	No	Yes	E	Fukuyama-type muscular dystrophy (FCMD)	SVA/SVA	Reduced mRNA	N/A	Founder insertion (JPN)	AB185332

Published SVA insertions associated with disease. A full-length SVA insertion is defined as the presence of either the CCCTCT hexamer or *MAST2* sequence.

[a] SVA subfamily determined by Repeatmasker (http://www.repeatmasker.org) according to Wang et al. [19] subfamily classification.

[b] Contains *MAST2* sequence.

[c] Present in human genome (hg19) UCSC browser (http://genome.ucsc.edu/).

[d] http://dbrip.brocku.ca/.

spliced when in the antisense orientation relative to the transcriptional unit [88–90]. SVA contains sequence with identity to two antisense *Alus* orientated head to tail [20,21,24] (Fig. 1B). The 5′ most *Alu*, of the *Alu*-like domain of $\mathrm{SVA}_{\mathrm{Rep}}$ is 255 bp long and aligns with 73% identity between nucleotides 279 and 23 relative to Alu_{Rep} (Fig. 1B) [42]. Following the first antisense *Alu* is a 31 nucleotide stretch termed SVA-U [25,42]. The origin of this sequence is unclear [25]. Using BLAT and the genome sequences available on the UCSC browser website, this sequence could only be identified within SVA elements [42]. The second antisense *Alu* is shorter than the 5′ *Alu*, spanning 93 nucleotides due to an internal deletion of 152 nt. The 5′ end of the second *Alu* spans nts 261–209 followed by the deletion, an insertion of 6 nt, followed by *Alu* nts 56–23 (Fig. 1B). Overall, the entire *Alu*-domain consisting of the first *Alu*, SVA-U, and the second *Alu*, is 376 nts in length.

The *Alu*-like domain may have been formed through alternative splicing of two *Alus* [25,42] and the unknown sequence, SVA-U. It is noteworthy that 3 out of 4 of the 5′ and 3′ positions of both *Alu* fragments correspond to known 5′ and 3′ splice sites identified in antisense *Alus* [88]. *Alu* alternative splicing has been well documented in the literature [88,89]. Experimental analyses [89] have demonstrated that two primary 3′ splice sites (SS) within *Alu* are utilized, one at position 279 and referred to as the proximal AG, and the second 3′ SS at position 275 and referred to as the distal AG. The 5′ end of the first *Alu* aligns to position 279, while the 5′ end of the second *Alu* aligns to position 261. Multiple 5′ SS have been identified in antisense *Alus* with the second most common site in EST data being position 23 [88]. The 3′ end for both the *Alus* aligns to position 23. The 5′ end of the second *Alu* not corresponding to a known *Alu* 3′ SS can be explained by a deletion occurring between the junction of the 3′ end of SVA-U and the 5′ end of the second *Alu* (Fig. 3E).

The observation that the terminal nucleotides of the *Alu* fragments correspond to known splice sites, led us to propose that the SVA-U sequence is also likely the remnant of an unidentifiable alternatively spliced sequence. This is consistent with the notion that the intersection of the first *Alu* and SVA-U is a splice junction, and that SVA-U was incorporated via alternative splicing. Briefly, an mRNA containing the CCCTCT hexamer presumably spliced into the first *Alu* at position 279 and out of that *Alu* at position 23 joining the hexamer and 5′ *Alu* segments (Fig. 3A). Next, the first *Alu* spliced into downstream sequence, SVA-U, followed by splicing into the second *Alu* at an unidentifiable 3′ SS (Fig. 3B), followed by splicing out at position 23 (Fig. 3C). Two deletions to the second *Alu* involving (1) the 3′ SS to nt 262 and (2) nts 208–57 probably occurred after SVA domain acquisition by alternative splicing (Fig. 3E). The abundance of *Alus* and satellite sequences in primate genomes suggests assembly of the *Alu*-like domain by mRNA splicing is possible.

Recently, we identified 5′ and 3′ SS within the VNTR region [26]. Similar to the first *Alu* and SVA-U junction, the intersection of the 3′ end of the second *Alu* and VNTR may represent a splice junction (Fig. 3D). It is unclear which 3′ SS within the VNTR would have been utilized due to the repetitive nature of the tandem repeats. However, due to the GC-richness and asymmetry of the tandem repeats, multiple pyrimidine stretches are positioned 5′ of the canonical CAG trinucleotide splice acceptor.

The 3′ end of SVA is referred to as the SINE-R, where R indicates retroviral origin [22]. This sequence shares homology to the *env* gene and right LTR of a HERV-K10. The *env* sequence is 81 nucleotides long sharing about 88% identity with $\mathrm{HERV\text{-}K}_{\mathrm{Rep}}$. 3′ of the *env* sequence is the right LTR, consisting of the U3, R, and polyA signal derived from a HERV-K. This LTR harbors a 367 nt deletion of nucleotides 331–697 relative to $\mathrm{LTR5}_{\mathrm{Rep}}$ [22]. The 5′ portion of the LTR shares 90% identity while the 3′ portion is 87% identical to $\mathrm{LTR5}_{\mathrm{Rep}}$. Similar sequence identity and the 367 nt deletion is observed when $\mathrm{SVA}_{\mathrm{Rep}}$ is compared to the HERV-K10 in Genbank (#M14123.1).

The right LTR does not contain U5 sequence and terminates at the HERV-K polyA signal. This suggests that the SINE-R was not incorporated into SVA through a DNA based mechanism, but through a RNA based mechanism. The mechanism must be able to account for the loss of the unique 3′ SVA2 sequence [27,28], SVA2 polyA signal and polyA tail (Fig. 1A). An attractive mechanism for the acquisition of the SINE-R sequence is template-switching [18] between the HERV-K and VNTR mRNA during reverse transcription (Fig. 3F). A less appealing possibility is alternative splicing of the VNTR into the *env* sequence of the HERV-K (Fig. 3G). HERVs [91,92], and more specifically HERV-K sequences [93], are known to be spliced into mRNA and could account for the loss of the SVA2 3′ end. However, no predicted splice site in HERV-K corresponds to the nucleotide intersection of the VNTR and *env* sequences. Additionally, it is unclear whether the deletion in the LTR occurred before or after SVA incorporation.

To date, no reports of SVA intermediate structures have been identified in genomic DNA sequence. It is unclear whether SVA intermediates are rare or no intermediates may have ever existed [42]. The likelihood that six individual events, acquisition of sequence followed by retrotransposition of the (1) CCCTCT hexamer, (2) first *Alu*, (3) SVA-U, (4) second *Alu*, (5) VNTR, and (6) the SINE-R, occurred independently and sequentially may be more probable, however the lack of SVA intermediates is evidence against this model. Acquisition of each SVA domain simultaneously may be less probable, yet it only needed to occur once. It may be difficult to completely understand how SVA evolved; still, the model proposed is consistent with the data and the literature.

5. Genomic impact of SVA

SVA has the ability to influence a genomic locus at the DNA, RNA, and epigenetic levels (Fig. 4). Retrotransposons are insertional mutagens [94] and may result in disease in humans (reviewed in Ref. [95]). SVA insertions, similar to L1, have been associated with deletion of genomic DNA: (1) a 14 kb deletion including the entire HLA-A gene [80], and (2) two different cases of neurofibromatosis 2 [82], where in one case the breakpoint exists within the SVA and in the second case the DNA breakpoint is within 400 bp of the same SVA (Table 1). The deletions may be due to non-allelic recombination (NAHR) which has been documented for L1 [96] and *Alu* [87] associated deletions (Fig. 4D). As described earlier, SVA is a repeat of repeats, therefore any of the individual repeat domains are potentially capable of misaligning with another genomic locus containing a similar SVA or repeat, resulting in NAHR. The variation in copy number both in the CCCTCT hexamer and VNTR hints that mispairing (Fig. 4D) between SVAs does occur, similar to minisatellites [97], microsatellites, and tandem repeats [98], leading to NAHR and the expansion and contraction of these SVA domains. Ostertag et al. [21] noted the relatedness between neighboring SVA VNTRs and speculated that this sequence identity was likely due to NAHR. The CCCTCT hexamer and VNTR likely evolve by additional mechanisms: (1) DNA slippage during replication [99], (2) slippage during transcription, (3) slippage during reverse transcription [29], (4) and gene conversion [100,101]. It has been demonstrated experimentally in yeast that expansion and contraction of repeats may allow quantitative and reversible functional adaptive changes [102]. It is unknown currently if the evolution of the CCCTCT hexamer or VNTR is functionally important or rather a consequence of maintaining direct DNA repeats.

The VNTR length has increased over evolutionary time [19]. The younger SVAs of subfamilies E, F, and F_1 elements tend to have longer VNTRs relative to those of the older subfamilies, B, C, and

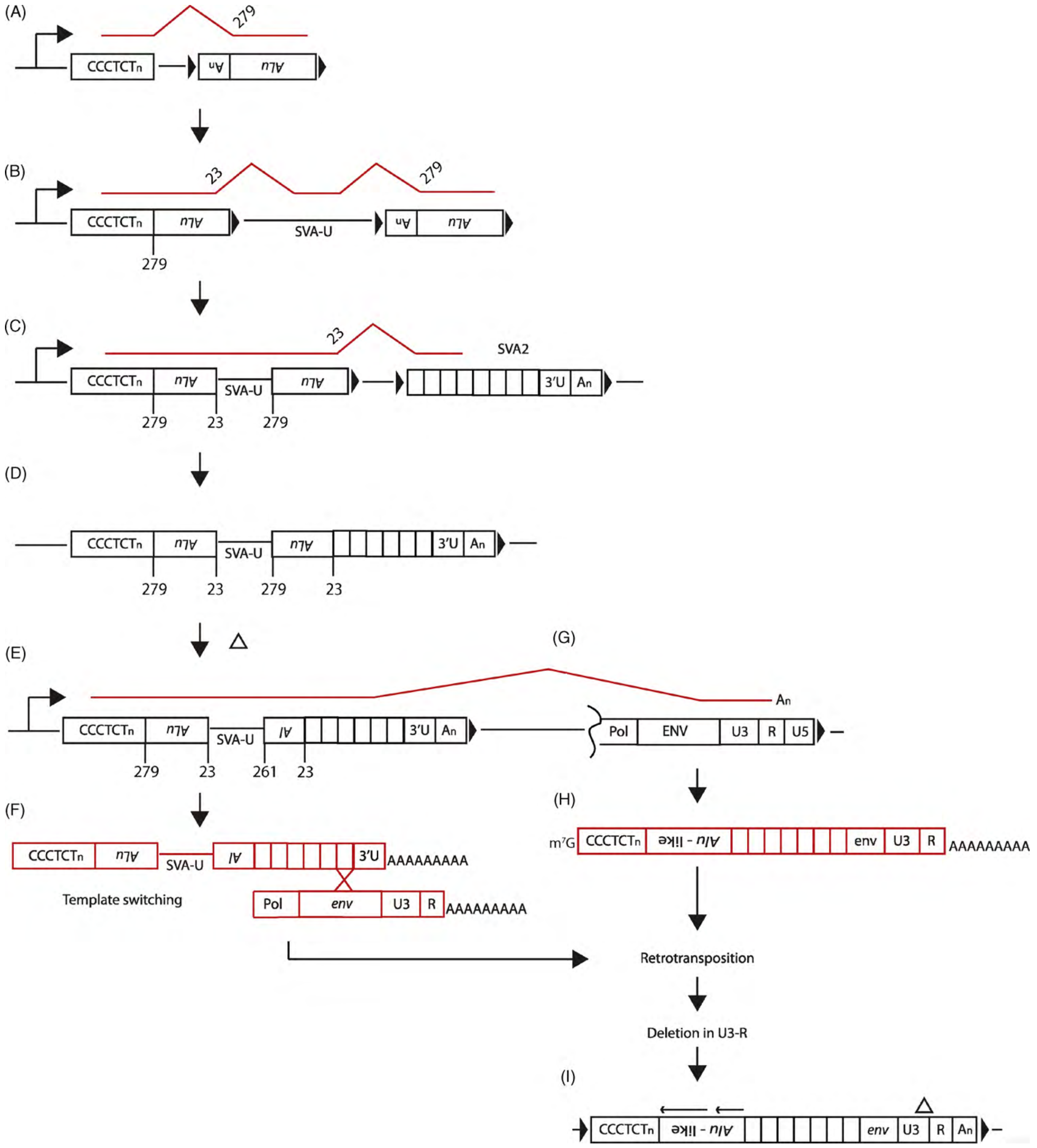

Fig. 3. The origin of SVA. A model outlining events that may have taken place to generate each junction and domain within a modern SVA is displayed. The events and junction formation are ordered from 5′ to 3′ for clarity (see text for further description). However whether the SVA domains were acquired by SVA2 independently or simultaneously is unknown. (A) An mRNA (red line) consisting of a CCCTCT hexameric repeat transcribed (black bent arrows) from a genomic location (black boxes) spliced into a downstream antisense *Alu* at the known 3′ SS position 279 relative to Alu_{Rep} creating the junction between the CCCTCT hexamer and the first *Alu* (B). Most likely in the same mRNA (B, red line), the first *Alu* was spliced out at position 23, a known *Alu* 5′ SS, followed by subsequent splicing into a sequence of unknown origin, referred to as SVA-U. SVA-U presumably spliced into the second *Alu* presumably at a known Alu 3′ SS between positions 273 and 281. Here we display *Alu* position 279 as the 3′ SS utilized in the incorporation of the second *Alu*. The CCCTCT hexamer along with the *Alu*-like domain junctions are displayed (C) prior to deletions in the second *Alu*. The 3′ end of the second *Alu* is a 5′ SS, therefore the second *Alu* and VNTR junction was likely created by alternative splicing into a genomic SVA2 (C). During its evolution, two deletions in the second *Alu* from (1) the 3′ SS to nt 262 and (2) nts 208–57 were generated (E). The SINE-R domain may have been acquired by potentially one of two RNA based mechanisms: (1) ORF2 reverse transcriptase template switching (F) or (2) alternative splicing (G) at an unidentifiable 3′ SS in the *env* sequence of a HERV-K resulting in an mRNA resembling a modern SVA (H). Subsequently, the mRNA was retrotransposed resulting in present-day SVA (I). It is unclear whether the 367 nt deletion (H; black triangle) present within the LTR occurred before or after SVA assembled.

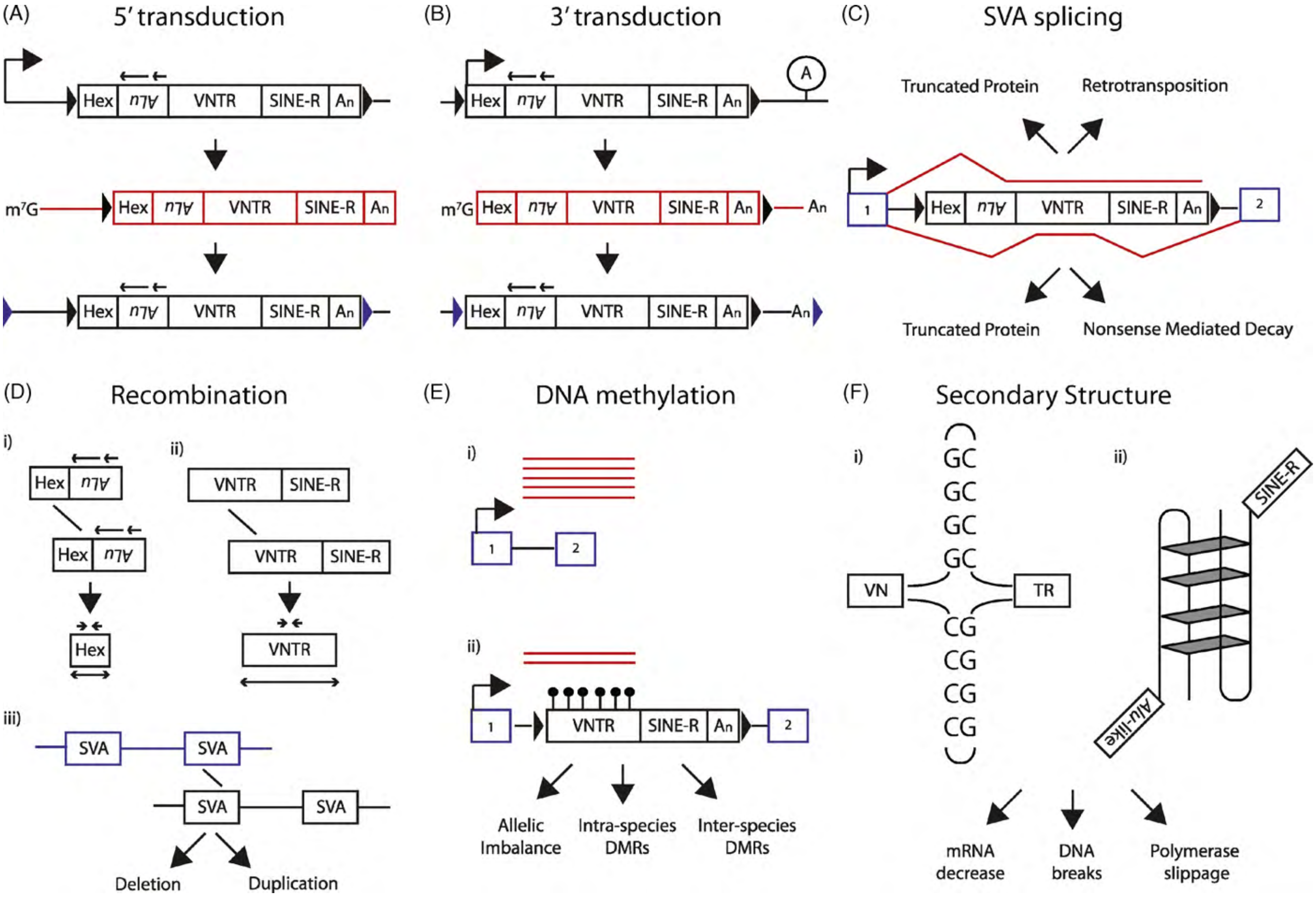

Fig. 4. Genomic impact of SVA. (A) SVAs are known to contain upstream transcriptional start sites (top, black bent arrow). This upstream transcriptional initiation will lead to an SVA mRNA (middle, red boxes) containing 5′ flanking sequence and sometimes result in retrotransposition of this sequence to a new genomic location (bottom), a process termed 5′ transduction. 5′ transduced sequence can be identified by the location of the target-site duplication positioned (blue arrowheads). (B) SVAs are known to bypass their polyA signal and terminate mRNA transcription at a downstream polyA signal (lollipop A). This transcriptional readthrough will result in an SVA mRNA (middle, red boxes) containing 3′ flanking sequence and sometimes result in retrotransposition of this sequence to a new genomic location, a process termed 3′ transduction. 3′ transduced sequence can be identified by the location of the target-site duplication (blue arrowheads). The same SVA element is able to transduce 5′ and 3′ flanking sequences (not shown). (C) SVA alternative splicing reduces host gene expression. SVA splicing may result in exon trapping (top, red line) that may result in truncated proteins or retrotransposition of upstream exons. SVA exonization (bottom, red line) may introduce nonsense codons into the mRNA leading to truncated proteins or nonsense-mediated decay. (D) SVA elements may result in non-allelic homologous recombination within the CCCTCT hexamer (i) or the VNTR (ii), or (iii) between different SVA elements leading to deletion of genomic DNA. (E) SVA VNTRs are known to be densely methylated (bottom, black lollipops) which may result in reduced mRNA expression (red lines) leading to allelic imbalance and inter- and intra- species-specific differentially methylated regions (DMR). (F) The SVA VNTR is composed of GC-rich tandem repeats. Imperfect palindromes within individual tandem repeats may lead to the formation of cruciform structures in DNA (i). The VNTR may also result in more complex DNA structures, such as G-quadruplexes (ii). Structures formed due to the GC-richness of the VNTR may lead to decreases in mRNA, DNA breaks during replication, and DNA polymerase slippage, resulting in VNTR deletions. However, the RNA secondary structure of the VNTR is presumed to be functionally important in SVA retrotransposition.

D. There is a linear relationship between VNTR size and subfamily age if the oldest subfamily, $\mathrm{SVA_A}$ is excluded [19]. The VNTR size of SVA orthologues differs between species, as indicated by the size variation of the duplicated SVA_{RHOT1} insertions [26]. SVA_{RHOT1} insertions are a group of three SVAs where the original SVA retrotransposed following an SVA- mediated alternative splicing event. A fusion mRNA containing six exons and SVA sequence retrotransposed to CH13 sometime after our last common ancestor (LCA) with the orangutan. This SVA containing the *RHOT1* exons inserted into a larger copy number variant which subsequently duplicated twice, once since our LCA with gorilla to CH18, and once since our LCA with chimp to CH21 [26]. It is noteworthy, that the 3 human SVA_{RHOT1} insertions also differ in VNTR size [26], consistent with a DNA-based mechanism for VNTR evolution.

Two SVA insertions have also been associated with exon-skipping (Table 1). An inherited SVA insertion into the Bruton's tyrosine kinase gene (BTK) interrupted an exon leading to exon skipping identifiable in the patient's cDNA [81]. The SVA insertion was not *de novo* in the patient as it was inherited from grandmother to mother to patient. This insertion disrupted the 5′ SS of BTK exon 9 resulting in loss of protein and X-linked agammaglobulinemia (XLA). This SVA insertion was 253 bp long, contained only SINE-R sequence, with a 92-bp polyA tail, and was flanked by a 16-bp target site duplication. Oddly, an *Alu* insertion has been identified in a different patient that occurred at the exact same site, to the nucleotide, also resulting in XLA [103].

An additional example of an SVA insertion disrupting an exon led our lab to become interested in SVA (Table 1). A report describing a family with hereditary elliptocytosis and pyropoikilocytosis was associated with a truncated α-spectrin protein [83] (OMIM #182860). Further analysis revealed a 632 bp insertion interrupting exon 5 of α-spectrin. The insertion contained a polyA tail with the entire insertion flanked by a target-site duplication, but this sequence shared no homology to known retrotransposons. At the time that the insertion was first described, it was unknown that L1s [34–38] and SVAs [19,21] were able to retrotranspose sequences 3′ of their genomic location (Fig. 4B). It turned out that the unidentifiable retrotransposon insertion in α-spectrin was a severely truncated SVA insertion that lacked SVA sequence. The insertion sequence represented a secondary SVA 3′ transduction event that was inverted and contained a 22 bp deletion at the site of inversion [21].

Three examples have been described where an SVA insertion has been associated with loss of mRNA expression (Table 1). The first, is a 2.6 kb SVA insertion into intron 32 of the TAF1 gene that has been hypothesized to cause X-linked dystonia-parkinsonism (XDP) [79] in individuals originating from the Philippine island of Panay [104] (OMIM #313650, #314250). Reduced TAF1 mRNA expression in the caudate nucleus of XDP patients was associated with hyper-DNA methylation of the SVA as indicated by *HpaIII*/*MspI* restriction analyses [79]. SVA DNA is known to be methylated [24,105] and this methylation presumably occurs in the GC-rich VNTR (Fig. 4E). One study that performed a genome-wide screen analysis for DNA methylation sites, described SVA sequence as comprising 70% of their library and they noted that the SVA VNTR was completely methylated in adult tissues [24]. Due to SVA insertional polymorphism in humans [19,106,107] and across species [108], SVA represents differentially methylated regions (DMR). The functional significance of SVA DNA methylation still needs to be demonstrated but it may lead to a decrease in gene expression (Fig. 3E).

The last two examples of SVA insertions associated with disease resulted in almost complete loss of gene expression in the patients (Table 1). The first is a patient that had autosomal recessive hypercholestrolemia (ARH) and was homoyzygous for a full-length SVA insertion into the first intron of the *LDLRAP* gene [43]. This individual had no mRNA expression as indicated by Northern blot. Strangely, this SVA insertion was not detected in any other individuals, and whether consanguinity was present in this family is unknown. The homozygosity of this patient, and the inability to detect it in other individuals, suggest that the SVA insertion is relatively old or that there might be loss of heterozygosity at this locus in this individual.

Finally, another ancient SVA insertion has been described that results in Fukuyama-type muscular dystrophy (FCMD) (OMIM# 607440, #253800) [84]. FCMD is one of the most common autosomal recessive disorders in Japan. Patients are homozyogous for a full-length SVA insertion in the 3′ UTR of the *fukutin* gene. Unexpectedly, both patients and carriers display little to no expression of the *fukutin* gene. The mechanism by which SVA mediates loss of mRNA expression in the ARH patient and FCMD patients is currently unknown. Unlike the TAF1 insertion, both the ARH and FCMD insertions are in the sense orientation relative to the disease gene.

Recently, it was shown that SVAs contain many functional 5′ and 3′ SS on the sense strand of the element [26]. SVA-mediated alternative splicing defined as exon-trapping (Fig. 4C; top) or SVA exonization (Fig. 4C; bottom) may result in a decrease of mRNA output from a gene. Both SVA exon-trapping and SVA exonization may lead to nonsense-mediated decay (Fig. 4C). An alternative mechanism that may explain the loss of *fukutin* mRNA is that the SVA insertion may have resulted in elongation of the 3′ UTR resulting in NMD [109]. In summary, SVA insertions may result in exon-skipping, generate a novel DMR, or decrease mRNA output, potentially due to SVA mediated alternative splicing.

SVAs may also create genetic instability through other mechanisms, primarily related to the GC-richness of the SVA VNTR and its potential to form stable structures (Fig. 4F). The presence of imperfect palindromes within the VNTR, GGGGGGTCAGCCCCCC, may potentially generate cruciform structures [23] that may present problems during DNA replication resulting in VNTR deletions, resulting in variation in VNTR copy number. VNTRs may also have the capability to form G-quadruplexes [110]. Using a G-quadruplex prediction website [111], seven G-quadruplexes are predicted in SVA_{Rep}, with five having modest scores [42]. Interestingly, a structured GC element has been previously characterized in the Rat L1 3′ UTR [63]. The secondary structure of the VNTR may lead to a reduction in mRNA output or DNA breaks during DNA replication (Fig. 4F). Nevertheless, the VNTR makes it difficult to PCR amplify and sequence SVA DNA as observed by the numerous gaps in the chimpanzee and orangutan reference genome draft sequences corresponding to SVA VNTRs.

6. SVA retrotransposons and cancer

The precise role human retrotransposons play in cancer is unknown. The negative impact of retrotransposons in cancer may or may not rely on whether these elements are actively retrotransposing. For example, LINE-1 is likely more deleterious as an insertional mutagen relative to SVA in cancer. However, how “active” are retrotransposons in human cancer is still of great debate and interest.

Here, we have described multiple mechanisms by which an SVA may alter gene expression. Of particular interest is the ability of SVA-mediated alternative splicing to result in either (1) the production of a dominant negative product or (2) an overall decrease in mRNA output from a specific gene. Furthermore, it is well established that DNA methylation patterns are disrupted in cancer resulting in inappropriate silencing or activation of genes. Therefore, it is worthwhile to investigate the DNA methylation state of specific SVAs and the role of this methylation or lack thereof on local gene expression in cancer.

Notably, as described in Section 3, SVA elements contain a HRE within the SINE-R. Since the HERV-K from which the SINE-R is derived is inducible upon addition of progesterone followed by estrogen, it is likely that SVA mRNA expression may also be induced upon addition of hormone. Nevertheless, SVA contains HREs that are likely functional, and these HREs in polymorphic SVAs may be oncogenic or contribute to cancer progression.

7. Human variation and evolution

Human SVA elements can be divided into six families, A thru F, based upon point mutation and indels within the SINE-R [19] or in the case of the F_1 subfamily, presence of the *MAST2* first exon [26,29,59]. Similar to L1 [112], phylogenetic analyses suggests one dominant retrotransposing SVA family at a time [19]. SVAs from the D and human-specific subfamilies E, F, F_1 are polymorphic in humans [19,29,106]. A recent study identified 14 SVA insertions present in the HuRef genome not shared with HGWD [107]. Previous studies estimate that ∼40% of SVAs [106] are polymorphic, in particular 37% of E and 27% of F SVA elements [19]. The personal genome era combined with high-throughput DNA sequencing technologies will enable a better estimate of SVA polymorphism levels.

SVAs residing in genes are potentially disruptive in either orientation. About 1/3 of all SVAs in the human genome reside in genic regions [19], with about 20% of those SVAs being the same orientation as a gene [26]. The depletion of SVAs on the coding strand suggests selection against insertions on the sense strand of a gene. A similar pattern is observed for chimp SVAs. This underrepresentation may be due to SVA-mediated alternative splicing or SVA induced DNA methylation. Considering SVA’s ability to transduce genomic sequence along with its ability to mediate alternative splicing and the high degree of SVA polymorphism, SVA is capable of generating considerable inter-individual variation in gene expression at loci in which it resides.

It has been estimated that ∼80% of SVA insertions occurred after the human-chimp split ∼6 mya [106]. A more recent study comparing draft genomes identified 800 SVA insertions as human-specific and about 400 SVAs as chimp-specific [108], while the chimpanzee genome project estimated about 1000 lineage specific SVA insertions [113]. A higher coverage chimpanzee genome draft sequence along with an exhaustive genotyping approach will provide better insight into the number of SVA elements fixed and polymorphic between the two species. Furthermore, the authors of the chimpanzee genome analysis speculated that SVAs may generate species-specific differences due to multiple CpGs and potential transcription factor binding sites.

SVAs evolved from repeats and are currently evolving in humans, as indicated by the acquisition of *MAST2* sequence via splicing forming the SVA_{F1} subfamily (Fig. 1E) and the many transduction groups identified recently. The F_1 subfamily comprises at least 32% of all SVA_{F}s [29]. SVA_{F1}s have further evolved by acquiring 5′ and 3′ Alu transductions forming a group that contains at least 13 elements in the HGWD and a non-reference insertion associated with disease derived from an SVA master element locus on chromosome 10 [26,29]. How the *MAST2* sequence or the *Alus* enhance SVA retrotransposition is unknown. On the other hand, it is clear that SVAs may acquire additional sequence such as novel TSSs through retrotransposition of upstream exons as indicated by the expression of SVA_{TPTE} in chimp testes from transcription initiated in the first exon transduced by SVA [26]. Alternatively, genes may pick up SVA sequence as in the case of *LEPR* [114]. Here, an SVA is expressed as the C-terminal coding exon of the leptin receptor isoform RNA. SVA sequence incorporation into the transcriptome is probably common, however the multiple nonsense codons in each SVA reading frame presumably prohibit SVA sequence from being translated into protein. SVA may also create new gene families, as described for the retrotransposon-mediated duplication of the *AMAC* gene due to SVA 3′ transduction [39]. In this instance, three *AMAC* copies were duplicated by SVA 3′ transductions and at least in humans maintain intact ORFs.

8. Concluding remarks

In summary, SVA is alive and well and its activity impacts the human genome by the mechanisms reviewed here along with other unknown mechanisms. It is of critical importance to develop a robust SVA cell culture retrotransposition assay to further study SVA. The lack of a *bona fide* progenitor element to a *de novo* SVA insertion has impeded development of the assay. On a more fundamental level, it is necessary to understand not only what enables SVA retrotransposition, but what enables its transcription. How active SVA is in humans is presently unknown, but the current DNA sequencing technologies will provide unprecedented opportunities for retrotransposon research. Good estimates of L1, *Alu*, and SVA retrotransposon activity in humans will exist within the next couple years. Progress on characterizing SVA biology has been slow and difficult at times; however more recent progress has revealed exciting findings and new directions. As other genomes are finished, such as the gorilla and orangutan, along with individual humans, the impact of SVA on individual variation and disease will slowly be revealed.

Conflict of interest

None.

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