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Identification of an RNA Sequence within an Intracisternal-A Particle Element Able To Replace Rev-Mediated Posttranscriptional Regulation of Human Immunodeficiency Virus Type 1

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Human immunodeficiency virus type 1 (HIV-1) replication depends on the posttranscriptional regulation by the viral Rev protein and can be replaced with the posttranscriptional RNA control element (CTE) of the type D simian retroviruses. We have identified a sequence which shares only nucleotide sequences of the internal loops and secondary structure with the CTE and which is part of a novel murine intracisternal-A particle (IAP) retroelement, inserted within the transcribed mouse osteocalcin-related gene. This sequence, named CTE_{IAP}, can replace the Rev-mediated regulation of HIV-1, hence it is a posttranscriptional regulatory element. Related elements have been identified in other IAPs. These results suggest that insertional mutagenesis can affect gene expression by providing a functional posttranscriptional control element. The CTE_{IAP} and CTEs of the type D simian retroviruses represent a novel class of RNA elements characterized by unique sequences within the internal loops which are predicted to represent the interaction site with cellular factor(s). These findings suggest that such elements may be involved in posttranscriptional regulation of cellular mRNAs.

Human immunodeficiency virus type 1 (HIV-1) utilizes posttranscriptional regulation via the viral Rev protein to express *gag*/*pol* and *env*. Rev interacts directly with an RNA element, termed the Rev-responsive element (RRE), and is responsible for the export of the RRE-containing mRNAs from the nucleus to the cytoplasm and their expression (7, 31, 32). Simian retrovirus type 1 (SRV-1), SRV-2, and Mason-Pfizer monkey virus (MPMV), three members of the family of the type D simian retroviruses (15), contain a *cis*-acting posttranscriptional control element (CTE) located between the *env* gene and the 3′ long terminal repeat (LTR) which can replace the Rev regulation of HIV-1 (4, 41, 48). Structure-function analysis of the 173-nucleotide (nt) core SRV-1 CTE demonstrated that it forms an extensive RNA stem-loop structure (41). The features essential for its function include two internal loops (A and B) positioned at a specific distance, an AAGA bulge adjacent to loop A, the stem structures (but not the sequence), and, to a lesser extent, the sequence of the hairpin loop. The CTEs of SRV-2 and MPMV are functionally equivalent to the SRV-1 CTE and are predicted to fold into similar structures (41). The CTEs are thought to represent interaction sites for putative cellular factors, possibly acting as Rev analogs. CTE function was demonstrated in several cell types (45), which led to the speculation that CTE-like elements may exist in other posttranscriptionally regulated mRNAs.

In this study, we conducted database searches to identify CTE-like elements. We have identified a sequence that shares only nucleotide sequences of the internal loop regions and secondary structure with the SRV-1 CTE. Sequence analysis revealed that this sequence is part of a novel murine intracisternal-A particle (IAP) inserted into the transcribed osteocalcin-related gene (ORG). We show that this sequence (named CTE_{IAP}) can replace the Rev regulation of HIV-1, resulting in production of infectious virus. This is the first demonstration that a murine element can execute such functions. Since this element is active in human cells, this finding demonstrates that the cellular factors mediating CTE_{IAP} function are present in both species. Although CTE_{IAP} has homology with the posttranscriptional control element of type D retroviruses, it is clearly distinct. This finding defines a novel group of elements which may be involved in posttranscriptional regulation of cellular mRNAs. The discovery that CTE_{IAP} is part of an IAP retroelement suggests that posttranscriptional control is an important *cis*-acting function for retrotransposition. In addition, these findings show that insertional mutagenesis via retroelements has the potential to affect gene expression by providing a posttranscriptional control element.

MATERIALS AND METHODS

Expression of recombinant viruses. The Rev/RRE-deficient HIV-1 molecular clone pR(−)Rev(−) contains the previously published mutations in *rev* and RRE (48) and a unique *Xho*I site (27a) which was used to insert the wild-type and the mutant CTE_{IAP}. Supernatants were collected from transfected human 293 cells and filtered. Equal amounts of virus (measured as $p24^{gag}$) were used to infect 5×10^6 phytohemagglutinin-stimulated peripheral blood mononuclear cells (PBMCs) and 3×10^6 Jurkat cells (48). Cultures were monitored by using either a commercial (Cellular Products) or an in-house $p24^{gag}$ antigen capture assay. The sequence of CTE_{IAP} in replicating virus was confirmed by sequencing. The elements from Eker rat-associated IAP (ERA-IAP), IAPE-Y, IAPE-1, and the woodchuck were PCR amplified. The IAPE-1-related elements (elements 3, 4, 8, 10, 16, 17, and 18) were amplified from genomic DNA isolated from AtT-20 cell line (ATTC CCL-89), using primers (nt 3449 to 3466 and 3635 to 3654) derived from the IAPE-1 cDNA sequence (35). The elements were introduced into pR(−)Rev(−). Upon transfection of human 293 cells with these constructs, the intracellular Gag production was analyzed, and/or the virus production was monitored after cocultivation with Jurkat cells. pSRV-1 was obtained from P. Luciw and consists of a single LTR-containing intact molecular clone of SRV-1 subcloned at the *Bam*HI site into pUC19. pSRV-ΔCTE has the CTE deleted (nt 7646 to 7818 [33]) and replaced by the unique cloning sites *Stu*I, *Not*I, and *Bss*HII followed by *Bst*EII. The 240-nt SRV-1 CTE (48) and the 176-nt CTE_{IAP} were reinserted into *Not*I-digested pSRV-ΔCTE. After *Bam*HI digestion, the purified plasmid DNAs were ligated and transfected into human 293 cells. One day later, the cells were washed and cocultivated with 2×10^6 Raji cells. Virus propagation was monitored by using syncytium formation as the indicator. The identities of the different viruses were confirmed by PCR analysis of genomic DNA.

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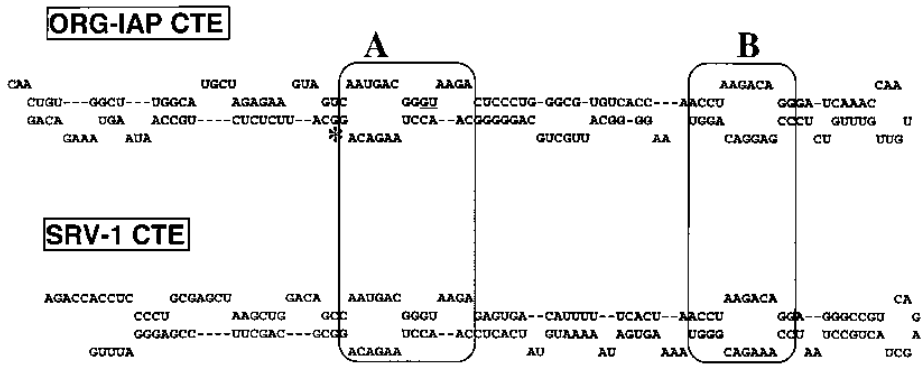

FIG. 1. Comparison of the novel murine CTE_{IAP} with the CTEs of the type D simian retroviruses. The CTE_{IAP} was identified by its sequence similarity to the 173-nt core SRV-1 CTE. The computer-predicted secondary structure of CTE_{IAP} is compared to the structure of the SRV-1 CTE, drawn after Tabernero et al. (41). The CTEs of SRV-2 and MPMV are predicted to fold into similar structures (41). The conserved internal loop regions A and B of CTE_{IAP} and CTEs are boxed. CTE_{IAP} is located in the mouse ORG. The asterisk in the CTE_{IAP} structure denotes nucleotide change G148 to A in the mutated CTEm (Fig. 2). The underlined GU denotes the location of a conserved splice donor within CTEs. Prediction of RNA secondary structure were performed with appropriate programs from the University of Wisconsin Genetics Computer Group package.

RNA PCR. To identify the splice sites, a portion of the ORG cDNA was amplified with primers in the 5′ LTR (nt 293 to 319) and in exon II (nt 3434 to 3845) and sequenced. For primer extension, a mixture of the biotinylated ORG-specific oligonucleotide (nt 4172 to 4149) in exon V and the end-labeled oligonucleotide (nt 102 to 133) in exon Ia was annealed to total kidney RNA, and the selected RNA was subjected to reverse transcription reaction and analyzed by comparison to the sequenced ORG-containing pGK1 (9). ORG mRNA containing or lacking the CTE_{IAP}-containing intron were detected by reverse transcription-PCR (RT-PCR) (29, 38). Figure 3A shows the locations of primers A (nt 2882 to 2904), B (nt 3434 to 3412), C (nt 3698 to 3666), D (nt 3845 to 3866), E (nt 3363 to 3386), and F (nt 2658 to 2678). Reactions using primers for β-actin (29) were performed to control for equal amounts of RNA in the samples; no amplification products were detected without prior reverse transcription of the RNAs.

Computer searches. Queries were designed to search for homology to the loop motifs A, A′, B, and B′, which were separated by spacers to match the lengths of the interloop stem (15 to 35 nt) and the hairpin loop: AANGACNGGT(2,1,1) AACCTAAGACAGG(1,1,1) CNANGACNGG(2,1,1) AACCTAAGACAGG (1,1,1). The numbers in parentheses indicate the number of allowed nucleotide changes, deletions, and insertions, respectively. The searches were performed with the PatScan program (http://www.mcs.anl.gov/home/papka/ROSS/patscan .html) against a nonredundant nucleotide sequence database.

RESULTS

Identification of a mouse CTE-like element that can replace Rev regulation of HIV-1. To identify elements similar to the of SRV-1 CTE (41, 48), we conducted a search of the sequence databases for elements with nucleotide similarities to the 173-nt SRV-1 CTE. This search resulted in the identification of a single element which is located upstream of the coding region in the mouse ORG. ORG is a member of the murine osteocalcin gene cluster (9, 34) and is expressed in a developmental stage- and tissue-specific manner distinct from the expression of osteocalcin genes mOG1 and mOG2 (9).

The computer-predicted RNA secondary structure of this newly identified 176-nt sequence is shown in Fig. 1. The RNA stem-loop structure of this CTE-like element shares several important features with the CTE structure of SRV-1 (41), such as the conservation of the two internal loops (A and B) and the preservation of their distance, an AAGA bulge adjacent to loop A, and, to a lesser extent, the sequence of the hairpin loop (Fig. 1). RNA sequence comparisons revealed that the homology is confined mostly to the four stretches of imperfect direct repeats spanning the internal loops A and B, while the predicted stem structures of this newly identified RNA element show dramatic sequence divergence. Analyzing the SRV-1 CTE, we have shown that some of the stems have mainly structural function and that their sequences can be changed (41). This finding led to the prediction that the nucleotide changes in the stems of this element may not affect its activity. Since this element shares only the sequence of the internal loop regions and the overall secondary structure with the highly homologous group of type D retroviral CTEs, and since we demonstrate (see below) that it is part of a novel murine IAP, we named this newly identified element CTE_{IAP} to distinguish among these distinct elements.

CTE_{IAP} is a functional posttranscriptional element and can replace the Rev/RRE regulation of HIV-1. To determine the possible function of CTE_{IAP}, we inserted it into the Rev/RRE-deficient molecular clone of HIV-1, generating pR(−)Rev(−). IAP CTE. To allow virus expression, a posttranscriptional element must mediate efficient stabilization, nucleocytoplasmic transport, and translation of the Rev-dependent mRNAs (2, 8, 11, 14, 16, 24, 27, 39). Virus stocks were prepared from human 293 cells transfected with the CTE_{IAP}-containing HIV-1 hybrid clone, the wild-type virus NL4-3 (1), the Rev-independent virus that contains the SRV-1 CTE (48), and, as a negative control, the Rev/RRE-deficient molecular clone. These supernatants were used for cell-free infection of Jurkat cells and human PBMCs. Virus propagation was monitored over time, using a $p24^{gag}$ antigen capture assay.

The CTE_{IAP} was able to replace Rev-mediated activation of HIV-1 in both Jurkat cells (Fig. 2A) and PBMCs (Fig. 2B), as demonstrated by virus propagation upon cell-free infection. These data demonstrate that the CTE_{IAP} is able to mediate posttranscriptional regulation of HIV-1, analogous to the type D retroviral CTEs (4, 41, 48). The CTE_{IAP}-containing hybrid virus replicated about 10 times less efficiently than the SRV-1 CTE-containing virus. Interestingly, the cell types play an important role in the extent of propagation of the Rev-independent viruses. In Jurkat cells, there is no difference in replication of wild-type and SRV-1 CTE-containing virus (Fig. 2A), while in PBMCs, as published previously (48), the SRV-1 CTE-containing virus replicates less efficiently (Fig. 2B). It is important to note that whereas PBMCs are a more restricted cell type, they permit propagation of the CTE_{IAP}-containing hybrid virus after cell-free infection.

In both cell types, the CTE_{IAP}-containing hybrid virus replicated about 10 times less efficiently than the hybrid virus carrying the SRV-1 CTE (Fig. 2), suggesting that changes outside the conserved loop regions contribute to CTE_{IAP} function. To address this question, we generated a single nucleotide

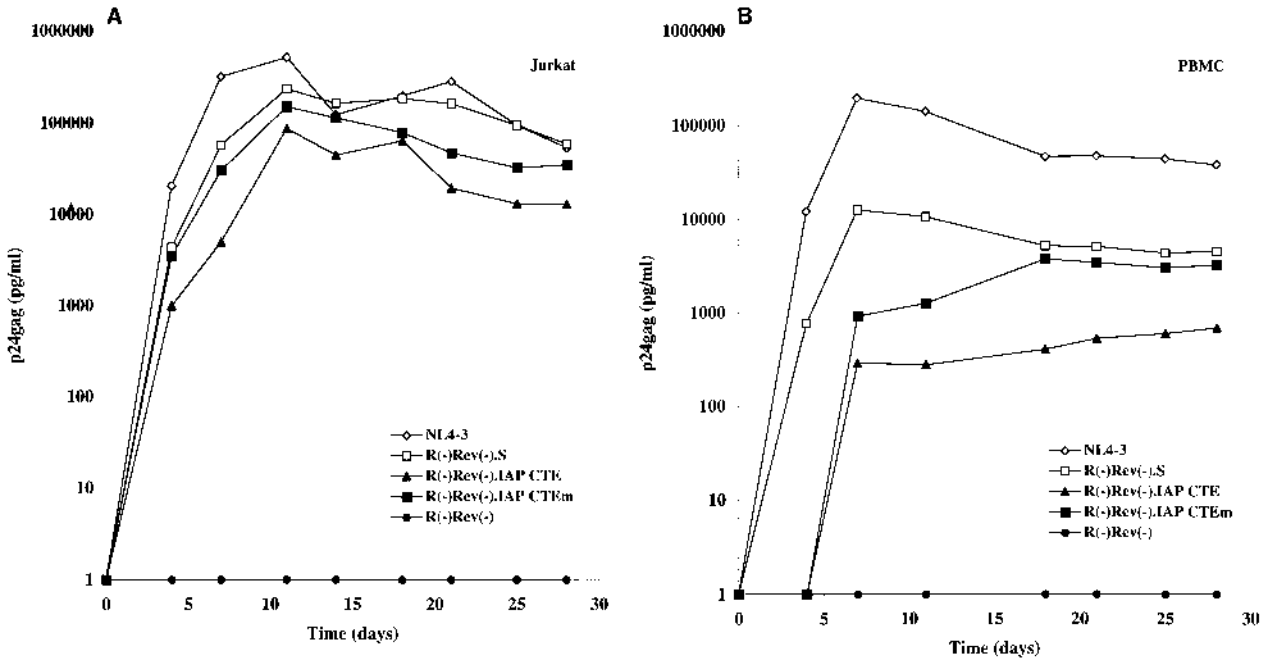

FIG. 2. The CTE_{IAP} replaces Rev/RRE in HIV-1 and generates infectious virus. Jurkat cells (A) and human PBMCs (B) were infected, and virus propagation was monitored by measuring *gag* expression. Jurkat cells are known to support replication of Rev-independent, CTE-containing HIV-1 hybrid virus (45). NL4-3 is the wild-type virus (1), whereas R(−)Rev(−) is the Rev/RRE-deficient derivative that contains 2 point mutations in *rev* and 37 point mutations in RRE that do not affect the overlapping reading frames (48). R(−)Rev(−).S contains the 240-nt SRV-1 CTE (48). R(−)Rev(−).IAP CTE and R(−)Rev(−).IAP CTEm contain the wild-type and mutant CTE_{IAP}, respectively. The conserved GCC/GGC stem structure preceding loop A is GUC/GGC in CTE_{IAP} (Fig. 1). CTEm was generated by introduction of a nucleotide change (G148 to A) into CTE_{IAP}, which generates a stronger predicted stem structure (GUC/GAC).

change in CTE_{IAP} (Fig. 1). The GCC/GGC stem structure preceding loop A, which is conserved in all CTEs, is changed to GUC/GGC in the CTE_{IAP} (Fig. 1). Introduction of a point mutation that changed G148 to A in CTEm results in a stronger predicted stem structure (GUC/GAC). This mutant CTE_{IAP} was inserted into the Rev/RRE-deficient HIV-1 clone to test its function. In both Jurkat cells (Fig. 2A) and PBMCs (Fig. 2B), the CTEm-containing virus, R(−)Rev(−).IAP CTEm, propagated more efficiently than the virus carrying CTE_{IAP}. This result indicates that the secondary structure of the element may influence its activity.

In conclusion, the CTE_{IAP} and the type D retroviral CTEs form a novel group of posttranscriptional elements. These elements are distinct but share nucleotide sequences of the internal loop regions and the overall secondary structure and replace the Rev regulation of HIV-1 in different cell types.

The newly identified CTE belongs to a novel IAP retroelement inserted into the mouse ORG. We sequenced the region 5′ of the newly identified CTE (GenBank accession number U53820) and found that this element is part of a novel IAP retroelement (termed ORG-IAP), integrated upstream of the coding region of ORG in the same transcriptional orientation (Fig. 3A). ORG is a member of the mouse osteocalcin gene cluster (9, 34) containing also the genes for osteocalcin 1 (mOG1) and osteocalcin 2 (mOG2), which encode similar proteins. Sequence analysis (the numbering starts with the first nucleotide of U3 as +1) revealed the insertion of a 3,402-nt IAP between the bone-specific promoter and the coding region of ORG (GenBank accession number U53820).

IAPs represent a family of retroelements that have been identified in mouse, hamster, and rat genomes (6, 23). The ORG-IAP is flanked by two imperfect direct repeats with partial homology to the LTRs of the ERA-IAP (46) (Fig. 3B). The LTRs contain a predicted TATA box (nt 226) and polyadenylation signal (nt 325) and are flanked by a GG dinucleotide duplication. A putative polypurine tract lies next to the 3′ LTR. The ORG-IAP underwent major deletion and mutation events, and although it does not contain any intact open reading frames, it shows homology to the putative *gag*/*pol* and *env* regions of other IAPs (Fig. 3B). Interestingly, the CTE_{IAP} is located between the putative *env* gene and the 3′ LTR of this retroelement, which is similar to the CTE location in type D retroviruses.

We next investigated the role of ORG-IAP insertion in ORG expression. Sequence analysis of the region 5′ to the IAP identified a putative osteocalcin promoter (9) (Fig. 3A). We identified the start of transcription 47 nt downstream of the ORG TATA box in the 5′ LTR of the ORG-IAP (Fig. 3A). No transcript initiating at the LTR start site was identified. These results indicate that the integration of the ORG-IAP results in the displacement of the functional promoter by 3.4 kb, interfering with its normal activity. This, we hypothesize, may be at least partially responsible for distinct expression pattern of ORG (9).

Further analysis of the ORG transcripts revealed that most of the IAP sequence was removed by three splice events utilizing the canonical GU/AG splice sites (Fig. 3A). Interestingly, one of the splice donors is located within CTE (Fig. 1), and therefore, upon splicing, CTE_{IAP} is removed from the terminally spliced mRNA (9). To determine, whether ORG mRNAs including the intron containing the CTE_{IAP} exist, we analyzed the transcripts from spleen, bone, and kidney by RT-PCR (Fig. 3C and D), using the primers shown in Fig. 3A. Using the sense primer A, overlapping the CTE, and primer E, located toward the 3′ end of the CTE-containing intron, resulted in the detection of ORG mRNA containing the CTE_{IAP}

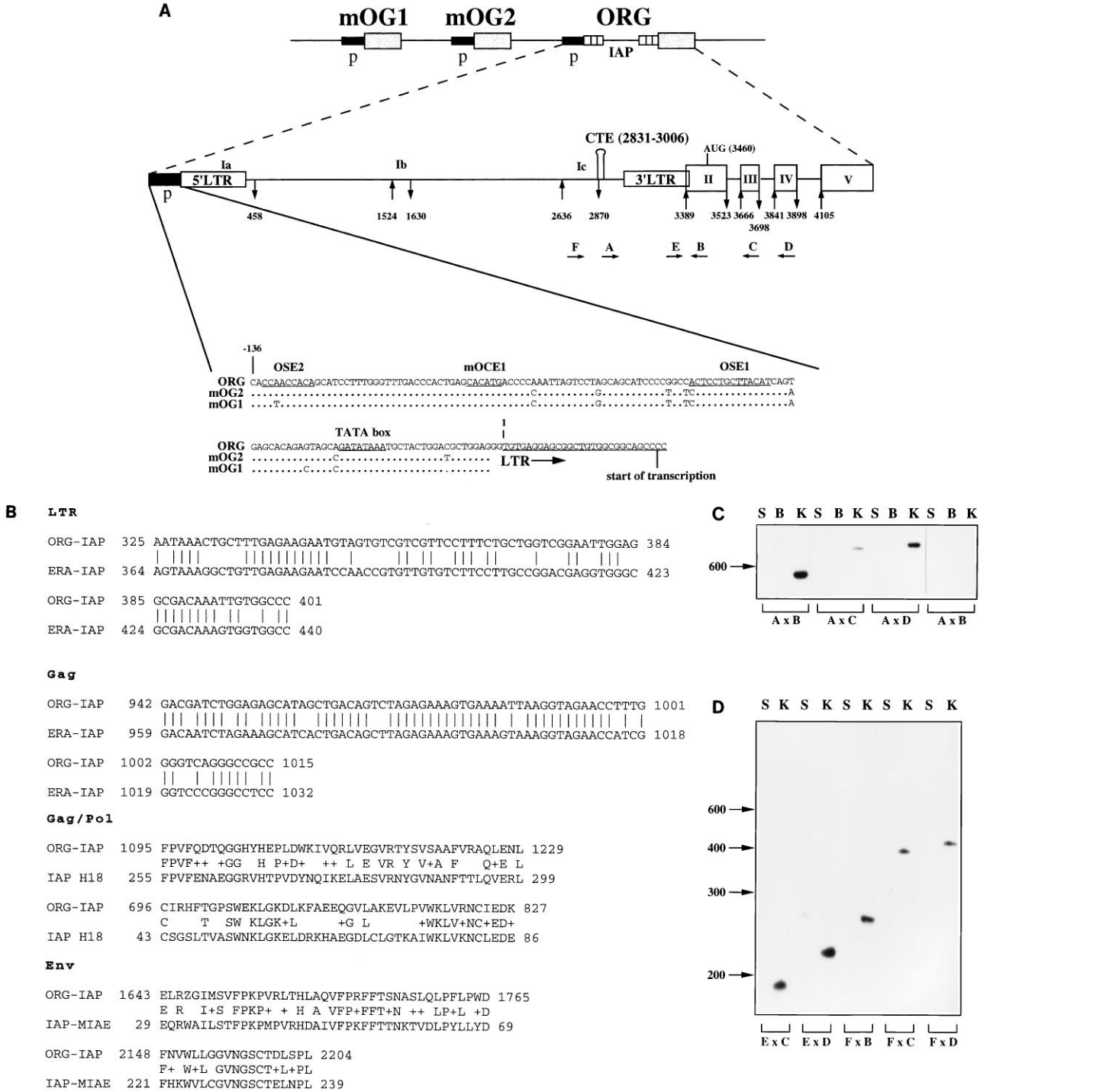

FIG. 3. The newly identified CTE is part of an IAP inserted into the transcribed ORG (A). The murine osteocalcin gene cluster consists of mOG1, mOG2 and ORG. The newly identified IAP inserted between the promoter and the coding portion of ORG. To maintain the exon assignment (9), exon I was renamed exon Ic and the newly identified noncoding exons were named Ia and Ib. The sequence of the ORG promoter, the nucleotide changes in mOG1 and mOG2, and the identified *cis*-acting OG2 elements are shown (10). The CTE, the mapped ORG transcription start site, and the splice sites are indicated. (B) The ORG-IAP shows homology to other IAPs such as to the 3′ portion of ERA-IAP LTR (46) (top panel), to the *gag* gene of ERA-IAP (middle panel), and to translation products Gag/Pol and Env of the hamster IAP H18 (GenBank accession number M10134) and IAP-MIAE (GenBank accession number P31790) (bottom panels). This observation supports the classification of ORG-IAP as an *env*-containing IAP. (C and D) Polyadenylated RNAs from spleen (S) and kidney (K) and total bone RNA (B) were subjected to RT-PCR. The locations of the primers are shown in Fig. 3A. The sense primers A and E are located in the CTE_{IAP}-containing intron, while the primer F is located in exon Ic. Antisense primers B, C, and D are located in exons II, III, and IV, respectively. Both the CTE_{IAP}-containing and terminally spliced ORG mRNAs are present in kidney but not in spleen or bone RNA. The last three lanes in panel C contain PCR amplifications without prior reverse transcription of the RNAs, demonstrating that the RNA samples do not contain DNA. Arrows indicate the end-labeled 100-bp DNA ladder (GIBCO/BRL).

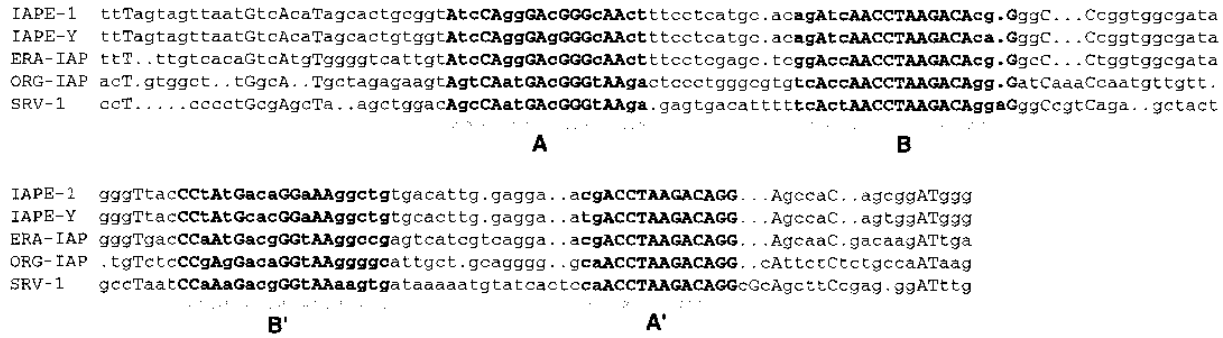

FIG. 4. Sequence alignment of the CTE-like elements from different IAPs. The CTE of SRV-1, CTE_{IAP}, and the CTE-like elements from IAPE-1 (35), IAPE-Y (GenBank accession number X87638), and ERA-IAP (46) are compared. The single-stranded regions of internal loop regions A and B (indicated as A and A′ and as B and B′, respectively) are underlined and shown in boldface. Lowercase letters indicate nonconserved nucleotides. Multiple sequence alignments, phylogenetic analyses, and prediction of RNA secondary structure were performed with appropriate programs from the University of Wisconsin Genetics Computer Group package.

in kidney (primers A × B, A × C, and A × D in Fig. 3C; primers E × C and E × D in Fig. 3D). The ORG mRNA is absent in spleen (Fig. 3C and D) and bone RNA (Fig. 3C), as shown previously by Desbois et al. (9). On the other hand, terminally spliced ORG mRNA is also detected in the kidney, using sense primer F, located in exon Ic, in combination with different antisense primers, B, C, and D, located in exons II, III, and IV, respectively (Fig. 3D). In conclusion, this analysis revealed the presence of two species of polyadenylated ORG mRNA in the kidney: RNA containing the CTE_{IAP} and the previously identified (9), terminally spliced ORG mRNA lacking CTE_{IAP}. The location of the osteocalcin promoter in relation to the 5′ LTR of the ORG-IAP and the acquisition of a posttranscriptional control element are most likely responsible for the distinct expression pattern of ORG.

Modified database queries identify additional related elements. The results of the comparative analysis of the CTE_{IAP} and the SRV-1 CTE (Fig. 1) allowed us to define the requirements of this class of posttranscriptional elements to perform a more thorough search for additional elements in the mammalian genome. We constructed a set of queries based on the conserved sequences of the internal loop regions A and B, the distinct length of the stem structure separating these loops, and a hairpin loop (see Materials and Methods). Only sequences predicted by computer-simulated folding to form CTE-like structures were further analyzed.

This search revealed that in addition to the CTEs, such elements are also present in several previously identified IAPs, such as the murine IAPE-1 (35) and IAPE-Y (GenBank accession number X87638) and the rat ERA-IAP (46). Sequence alignment of these elements shows that the homology to CTE_{IAP} (and SRV-1 CTE) is mostly confined to the internal loop regions (Fig. 4). However, while the nucleotide sequences of the upper strand of loop B and the lower strand of loop A are highly homologous, the sequences of the opposite strands and the bulge adjacent to loop A are less conserved. It is important to note that their primary sequences, except for the internal loops, are divergent. These elements fold into similar computer-predicted structures (data not shown) sharing several features with CTE_{IAP} and the type D retroviral CTEs, such as the secondary structures and spatial arrangement of internal loops A and B, the bulge adjacent to loop A. The CTE-related sequences are located between the putative *env* coding sequence and the 3′ LTR in these retroelements, which is similar to the location of CTE in the ORG-IAP. These findings suggest that CTE_{IAP} or related elements are a common feature of retroelements.

Using these queries, we also identified a related sequence in the woodchuck genome (47). Since no retroelement-related sequences were found near this element, it may represent the remainder of a retrovirus-like insertion that underwent severe rearrangements. Since IAPE-1-related sequences are widespread in the mouse genome (35), we designed primers to amplify related elements from DNA isolated from mouse AtT20 cells by PCR. Seven closely related but distinct elements (which may belong to other members of the IAPE family) were cloned (elements 3, 4, 8, 16, 17, 18, and 10; GenBank accession numbers U53813 to U53819). These findings demonstrate that additional related elements are present in rodent genome.

We tested whether these CTE-related elements could replace Rev function in the Rev/RRE-deficient HIV-1 clone. Some of the hybrid viral constructs were analyzed for Gag production after transient transfection. The presence of two of these elements (from IAPE-1 and the related element 18, which differs by one nucleotide change of G101 to A), led to measurable activation of *gag* expression, yielding about 5 to 10% of the activity of the CTE. Upon cocultivation of Jurkat cells with the transfected 293 cells, no virus propagation was observed, which may be the result of the low activity of these elements. Sequence inspection of their internal loop regions shows several nucleotide changes compared to the consensus sequence, which may explain their impaired activity.

CTE is essential for SRV-1 propagation and can be replaced by CTE_{IAP}. Our data also demonstrate a novel link between type A retroelements and type D retroviruses since both contain functional posttranscriptional control elements that share nucleotide sequences of the internal loop regions and have similar secondary structures. This relationship further supports the previously described evolutionary link based on the homology of *gag/pol* regions (23). To demonstrate the role of such regulatory elements for expression of the retroelements and type D retroviruses, we replaced the CTE of an infectious molecular clone of SRV-1 with the CTE_{IAP} and tested the hybrid virus for propagation. Human 293 cells were transfected with the intact SRV-1 molecular clone, the CTE-deleted clone, and CTE_{IAP}-containing hybrid SRV-1 clone and cocultivated with the human B-cell line Raji. The cultures were monitored for virus propagation by syncytium formation. The CTE-deleted clone did not produce virus, whereas insertion of the CTE_{IAP} or, as a positive control, the CTE of SRV-1 resulted in virus propagation as measured by syncytium formation. The ability to produce infectious hybrid virus was further shown by cell-free transmission of the CTE_{IAP}-containing hybrid SRV-1. These data indicate that the presence of a post-

transcriptional control element is essential for SRV-1 propagation and demonstrate further that the CTE_{IAP} can substitute for the SRV-1 CTE.

DISCUSSION

In this study, we identified a posttranscriptional regulatory element, CTE_{IAP}, which is part of a novel murine IAP retroelement. We demonstrate that CTE_{IAP} can replace Rev-mediated activation of HIV-1 and the CTE-mediated regulation of SRV-1 in human cells. CTE_{IAP} and the type D retroviral CTEs are related RNA elements since they share only nucleotide sequence within two internal loop regions and a similar secondary structure. Therefore, these elements represent a novel class of RNA regulatory elements which may be also involved in posttranscriptional regulation of cellular mRNAs. Since CTE_{IAP} and CTE are proposed to act via related cellular factors, the elucidation of their mechanisms of function will greatly aid our understanding of posttranscriptional regulation. Notably, the CTE_{IAP}, a murine element, is functional in human cells, suggesting that the putative cellular factors necessary for CTE_{IAP}-mediated expression are present in both species. Such factors most likely recognize the conserved internal loop regions (Fig. 1) in the context of the conserved secondary structure.

We further demonstrated that these elements function at several posttranscriptional steps. CTE_{IAP} (and type D retroviral CTEs) are thought to act via stabilization, nucleocytoplasmic transport, and efficient expression of HIV-1 mRNAs, resulting in the production of infectious virus. CTE of SRV-1 was also shown to promote expression of the human papillomavirus L1 mRNA in undifferentiated cells, while L1 production is otherwise strictly limited only to differentiated keratinocytes (42).

The conservation of a functional CTE_{IAP} in a retroelement may suggest a *cis*-acting function essential for retrotransposition. We propose that the CTE_{IAP} is essential and acts in addition to the other *cis*-acting elements (such as the LTRs, the primer-binding site, the packaging signal, and the polypurine tract). Since most retroelements are heavily mutated, rendering them inactive, it is expected that the function of the CTE is also affected. Functional tests in human cells of several of the additionally identified elements showed that they are indeed severely impaired or inactive, although we cannot exclude that several of these CTE-like elements may function in murine cellular background. On the other hand, the CTE_{IAP} is able to replace Rev regulation of HIV-1 and CTE regulation of SRV-1 in human cells. This observation raises the possibility that the ORG-IAP represents a recently transposed retroelement, since it contains a functional CTE and probably all other *cis*-acting elements necessary for transposition. Alternatively, CTE_{IAP} may be advantageous for ORG expression, resulting in conservation of its function.

Analyzing the ORG mRNA, we observed that CTE_{IAP} is present in the primary transcript but can be spliced out. This splice site is conserved in the CTE_{IAP} and the type D retroviral CTEs. A role of splice factors in posttranscriptional regulation was proposed for the Rev-dependent HIV-1 mRNA (5, 25) and a bovine papillomavirus late mRNA (3). Therefore, the splice site in the CTEs might overlap with a crucial interaction site of the putative binding factor(s). However, since point mutations eliminating this splice site in the SRV-1 CTE did not affect its function (mutant 35 [41]), we conclude that CTE most likely act independent of splicing.

Our data also demonstrate the presence of a CTE-like elements in many type A retroelements. This offers the possibility that insertional mutagenesis via IAPs alters mouse gene expression by providing a posttranscriptional control element that may affect regulation of the transcript at multiple steps from nucleocytoplasmic transport to polysomal loading. Unexpectedly, using even the more sophisticated databank searches, we did not identify related sequences in genomes other than the rodents. However, since this class of elements functions in a wide spectrum of cell types and species (45), it is likely that related elements exist in cellular mRNAs.

From the works of several groups, it became apparent that posttranscriptional regulation is an important regulatory step employed by several DNA and RNA viruses. For DNA viruses, such as human papillomavirus (42, 43) and bovine papillomavirus (3), this regulation is thought to be mediated via keratinocyte-specific factors. Hepatitis B virus expression has been shown to be mediated via a posttranscriptional control element in hepatoma cells (19, 20). Avian leukosis viruses are thought to use species-specific factors (28, 30), while lentiviruses (11, 12, 14, 16, 26, 37, 44) and some oncoretroviruses (13, 17, 18, 21, 22, 36, 40) use their viral Rev and Rex proteins, respectively, to mediate posttranscriptional regulation. Interestingly, IAP retroelements and the simian type D retroviruses utilize putative cellular factor(s) for posttranscriptional regulation. It is important to note that although posttranscriptional regulation is a common feature of many viruses, the RNA elements and cellular or viral factors involved in this regulatory step are clearly different.

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