A spliced intron accumulates as a lariat in the nucleus of T cells

Lian Qian, Minh N.Vu, Mark Carter and Miles F.Wilkinson*
Vollum Institute for Advanced Biomedical Research, Microbiology & Immunology Department, Oregon Health Sciences University, L220, 3181 S.W. Sam Jackson Park Road, Portland, OR 97201, USA

Received July 20, 1992; Revised and Accepted September 18, 1992
GenBank accession no. M97158

ABSTRACT

The vast majority of mammalian genes are interrupted by non-coding segments of DNA termed introns. Introns are spliced out of RNA transcripts as lariat structures, and then are typically debranched and rapidly degraded. Here, we describe an unusual spliced intron from the constant region of the T cell receptor-β (TCR-β) locus that is relatively stable in mammalian cells. This intron, IVS1C51, accumulates as a set of lariat RNA structures with different length tails in the nucleus of T cells. The accumulation of this spliced intron is developmentally regulated during murine thymocyte ontogeny. The property of stability appears to be evolutionarily conserved since the human version of this intron also accumulates in T cells. The stability is selective since other spliced TCR-β introns do not detectably accumulate in T cells. The unusual stability of this intron does not depend on T cell specific factors since non-T cells transfected with TCR-β gene constructs also accumulate spliced IVS1C51. The discovery of a mammalian intron that accumulates as a lariat in vivo provides an opportunity to elucidate mechanisms that regulate intron debranching, stability, and nuclear localization.

INTRODUCTION

RNA splicing is a process whereby exons are joined and intervening sequences (introns) are removed in the form of lariat structures (1). Following excision, intron lariats are typically debranched and rapidly degraded (1). Thus, spliced introns are generally not sufficiently stable to be detected in vivo. However, yeast intron lariats have been observed as transient species that exist either as RNA processing intermediates generated after 5' cleavage (2) or after complete excision from precursor transcripts (3,4). For example, a spliced intron derived from a yeast nuclear actin transcript can be detected as various forms along a degradative pathway, including a lariat form that possesses a tail 3' of the branchpoint, a tailless lariat form, and a debranched linear molecule (3). The intron excised from the self-splicing yeast mitochondrial ox13 transcript exists transiently as either a tailed or a tailless lariat structure (4).

The instability of yeast introns appears to be a consequence of the debranching mechanism. When the conserved branchpoint sequence of a yeast intron was mutated so that the intron was only inefficiently debranched, the intron accumulated as a stable lariat structure (5). Furthermore, mutant yeast strains that lack debranching activity accumulate high levels of lariat introns (6). Presumably, introns that are maintained in a lariat conformation are relatively stable due to the low levels of endonucleases in the nuclear environment. However, once introns are debranched these linear molecules become susceptible to exonuclease attack.

Spliced introns have also been detected in higher eukaryotic cells. A spliced SV40 intron accumulates as a lariat structure in the nucleus of injected Xenopus laevis oocytes (7). Herpes simplex virus (HSV type I) generates a latency associated transcript (LAT) that has recently been defined as a stable intron (8). The basis for the stability of LAT is not known. Furthermore, it has not been determined if LAT is a linear or a lariat molecule. A portion of this stable intron is complementary with an immediate early gene involved in trans-activation, implicating LAT as a possible regulator of viral latency. Evidence that LAT inhibits viral transactivation has been obtained in transient transfection assays (8). In contrast to these examples of stable viral introns, there is little evidence that introns derived from cellular transcripts accumulate in higher eukaryotic cells. The spliced introns that have been observed are derived from highly transcribed genes, perhaps because only under this circumstance can their transient existence be detected. For example, β-globin introns have been observed in fetal liver nuclei in vivo as lariat and linear molecules (9). The J-C intron from the immunoglobulin-κ (Ig-κ) locus has been detected as a discrete species in the nuclei of B cells (10). The conformation of the spliced Ig-κ J-C intron is not known. The heat shock gene hsc70 possesses three copies of the U14 snRNA gene within its intron sequences (11). U14 snRNA may be a ‘stable intron’ generated by RNA splicing, or alternatively, U14 snRNA may be derived from a transcriptional promoter located within the hsc70 intron. An acetylcholine receptor (AChR) intron has been localized to the nuclear membrane of myotubes by in situ hybridization (12). It has not been determined if this AChR intron is a transient intermediate, or instead selectively accumulates in the nucleus of these cells. In the present communication, we provide evidence for a mammalian intron
that selectively accumulates in the nucleus of cells. It exists exclusively as a set of lariat structures that possess different length tails. This characteristic may have relevance to the stability and possible function of this unique intron.

MATERIALS AND METHODS

Cell culture and transfection
All cells were cultured as described (13). Transfection of DNA into HeLa and rat1 cells was performed by calcium phosphate precipitation (14). HeLa-β and rat1-β cells were generated by stable transfection of the following constructs: a 12kb HindIII Vβ2cβ2 genomic fragment (5C.C7) that possesses a functional Vβ promoter was inserted into the pcDNA-neo vector (Invitrogen Corp.) and transfected into HeLa cells; a 1.0kb Stul Cβ2 fragment (15), that includes IVS1, was inserted in pcDNA-neo so that it is under the control of the CMV immediate early promoter, and transfected into rat1 cells.

RNA preparation and northern blots
Total cellular RNA was prepared by lysis in guanidinium isothiocyanate, followed by ultracentrifugation over a 5.7M CsCl cushion, as described (ref. 16; protocol 1). Cytoplasmic and nuclear RNA was prepared as described (ref. 16; protocols 6 & 7). Briefly, the cells were lysed in a tris-saline buffer containing 0.5% Nonidet P-40, 0.25% sodium deoxycholate and 50μg/ml dextran sulphate, followed by immediate centrifugation to generate a nuclear pellet and a cytoplasmic supernatant—these two fractions were completely denatured in guanidinium isothiocyanate buffer and the RNA was prepared as described for the total cellular RNA. Poly(A)+ RNA was prepared directly from cell lysates as described (ref. 16; protocol 9). The RNA was electrophoresed in 1–1.5% agarose gels in the presence of formaldehyde, and capillary blotted onto Nytran membranes (14). RNA was also electrophoresed in denaturing polyacrylamide gels containing 7M Urea and 1 x TBE (14). The RNA was prepared from polyacrylamide gels was electroblotted onto Nytran membranes (14). RNA was also electrophoresed in denaturing polyacrylamide gels containing 7M Urea and 1 x TBE (14). The RNA from polyacrylamide gels was electroblotted onto Nytran membranes in 10M Tris (pH 7.8), 5mM sodium acetate, 0.5mM EDTA overnight at 50mA, followed by 1 h at 200mA. All the blots were stained with methylene blue (17) to demonstrate equivalent loading of RNA and to mark the migration of 18S rRNA, 28S rRNA, and RNA molecular weight ladders (Bethesda Research Laboratories, Bethesda, MD). The blots were hybridized with random oligomer primed 32P-labeled cDNA inserts in the presence of 10% dextran sulphate and 50% formamide for 12–18 h at 42°C, followed by washing with 0.1 x SSPE /0.1% SDS at 50–60°C (14). Blots were stripped for sequential hybridization by placing them in boiling 0.1 x SSPE/0.1% SDS, and then gently agitating them while they cooled to room temperature.

RNA manipulation
The 0.5kb RNA fraction was prepared from gel slices derived from 1% agarose gels containing formaldehyde (14) by the method originally designed for DNA purification (18), except that the gel slices were melted in gel running buffer instead of TE, and extracted with phenol/chloroform prior to isopropanol precipitation. RNase H mapping was performed as follows: RNA was first incubated with 5μM oligonucleotide in 10mM Tris (pH 7.6), 1mM EDTA in a total volume of 10μl for 30 minutes at 45°C. A RNase H cocktail was then added (15μl) that consisted of 2.5μl 10× RNase H buffer [0.2M Hepes (pH 8.0), 0.5M KCl, 0.1M MgCl2], 2.5μl 10mM DTT, 1 unit of RNase H, and H2O. Following an incubation of 30 minutes at 37°C in this cocktail, the RNA was extracted and ethanol precipitated.

DNA probes and DNA sequence analysis
The murine IVS1cβ2 0.5kb DNA fragment used as a probe for blot analysis was prepared by the polymerase chain reaction (PCR) with a murine Cβ1 genomic template, an oligonucleotide that corresponds to nucleotides 17–33, and oligonucleotide C (see Fig. 2). 5' and 3' specific IVS1cβ1 probes were prepared by cleaving the full length fragment with HpalII. The Cβ1 exon probe is a 0.3kb EcoRI/HindIII fragment from the 3' untranslated region of murine Cβ1 genomic DNA (15). The Cβ2 exon probe is a 0.2kb Nsil/Hpal fragment from the 3' untranslated region of murine Cβ2 genomic DNA (15). The human IVS1cβ1 fragment was prepared by PCR amplification from a human Cβ1 genomic template (20,21) with an oligonucleotide that corresponds to sequences starting 22 nucleotides from the exon1/IVS1 border (5'-GCCTGGAGGAGAGATAG-3') and another oligonucleotide complementary to the 3' terminus of IVS1 (5'-CTGAAAGAAGAGCGG-3'). This 0.4kb fragment was then cleaved with PvuII to generate 5' and 3' specific probes. The nucleotide sequence of murine IVS1cβ1 was obtained from a 5C.C7 genomic clone (B10.A mouse strain; ref. 22) by dyeoxy-sequencing methods with Sequenase reagents (U.S. Biochemical Corp., Cleveland, OH).

RESULTS AND DISCUSSION

Identification of a spliced TCR-β intron that accumulates in the nucleus of T cells
The murine genome contains two TCR-β constant genes (Cβ1 and Cβ2) which are similar in genomic structure and encode similar proteins (23). Each Cβ gene contains three introns: IVS1, IVS2 and IVS3 (23). These introns are present in TCR-β pre-mRNAs that accumulate in the nucleus of T cells (15; Qian et al., submitted). During the course of characterizing these splicing intermediates in the murine T-lymphoma cell clone, SL12.4, we noted that in addition to large pre-mRNAs, that a small 0.5kb RNA species hybridized with an IVS1cβ1 probe on Northern blots (Fig. 1A). The size of this small RNA transcript was identical to the size of IVS1cβ1 alone. Both 5' and 3' specific IVS1cβ1 probes (which do not contain sequences in common) hybridized with the 0.5kb RNA species. This transcript was enriched in poly(A)+ RNA (Fig. 1A), as expected, since two poly(A) tracts are present in the 5' portion of the intron (Fig. 2). Taken together, these results suggest that IVS1cβ1 accumulates as a free intron following excision from TCR-β pre-mRNA.

The 0.5kb IVS1cβ1 transcript was present in the nucleus of SL12.4 cells; little or none accumulated in the cytoplasm (Fig. 1A). Large IVS1cβ1-containing pre-mRNAs were also confined to the nuclear compartment. In contrast, JCβ2 (1.0kb) transcripts, which are fully spliced in SL12.4 cells (15), were present in both the nuclear and cytoplasmic compartments, as expected (Fig. 1A).

It is possible that the accumulation of free IVS1cβ1 RNA in SL12.4 T-lymphoma cells is an oddity of this cell line, rather than a general property of this intron. We tested normal thymocytes and found that they also accumulated 0.5kb IVS1cβ1. Fig. 1B shows expression of IVS1cβ1-containing transcripts during fetal thymocyte ontogeny. The upper panel
pre-mRNAs (1.5-2.5kb and 5-7kb transcripts during fetal ontogeny that is different than other TCR-β introns that the spliced rVSlc intron exhibits a pattern of expression at different stages of thymic development. The results indicate autoradiographic exposure to show relative levels of expression transcript at each stage, whilst the second panel displays a single developmental stage to allow visualization of the 0.5kb IVSlc intron does different autoradiographic exposures for each displays different autoradiographic exposures of the individual lanes. The same blot was also hybridized with the 0.4kb transcript in Jurkat cells (see materials and methods).

Figure 1. Northern blot analysis of rVSlc in murine and human T cells. All panels show RNA electrophoresed in agarose gels, blotted, and hybridized with the probes indicated. A. Left panel: blot containing 2µg SL12.4 poly(A)+ (pA+) RNA hybridized with the murine IVSlc probe; right panel: blot containing 10µg nuclear (N) and cytoplasmic (C) RNA from SL12.4 cells sequentially hybridized with murine C2 exon and IVSlc probes. The blot shown provides results with a full length IVSlc probe; the same hybridization pattern was observed with either 5' or 3' specific probes (see materials and methods). B. Blot containing total cellular RNA (5µg) from thymus derived from fetal mice (days of ontogeny are shown) or a 6 week old adult (ad). The upper panel shows different autoradiographic exposures of the individual lanes. The same blot was sequentially hybridized with murine IVSlc, Cβ exon, and CHO-A cDNA probes. The CHO-A probe detects a ubiquitously expressed housekeeping gene (13,26) which serves as a control for RNA loading. C. Blot of total cellular (10µg) or poly(A)+ RNA from human Jurkat cells hybridized with a human IVSlc probe. The blot shown provides results with the 3' probe; the 5' probe also hybridized with the 0.4kb transcript in Jurkat cells (see materials and methods).

displays different autoradiographic exposures for each developmental stage to allow visualization of the 0.5kb IVSlc transcript at each stage, whilst the second panel displays a single autoradiographic exposure to show relative levels of expression at different stages of thymic development. The results indicate that the spliced IVSlc intron exhibits a pattern of expression during fetal ontogeny that is different than other TCR-β transcripts. Pre-mRNAs (1.5-2.5kb and 5-7kb transcripts migrating similar to 18S and 28S rRNA transcripts, respectively) and mature mRNAs (1.0 and 1.3kb) increased in levels 15-20 fold between day 16 of fetal ontogeny and the adult stage, whilst 0.5kb IVSlc transcripts increased >50 fold over the same time interval (Fig. 1B). This difference suggests that the level of the free intron which accumulates is not entirely dependent on the rate of transcription, but may also reflect alterations in the rate of RNA splicing or RNA degradation.

We tested other murine TCR-β introns to determine whether they also accumulate in T cells. Northern blot analysis indicated that a probe to the first intron of the Cβ gene (IVSlc) also hybridized with an abundant 0.5kb transcript present in SL12.4 nuclear RNA (data not shown). In contrast, murine probes specific for IVS2-β, IVS2β, IVS3-β, IVS3β, and IVS β did not detect transcripts of a size consistent with free introns in either nuclear SL12.4 RNA or total adult thymus RNA. These intron probes did hybridize with TCR-β pre-mRNAs, showing their efficacy as hybridization probes (data not shown). Thus, IVSl from both the Cβ and Cβ loci display a unique stability that is not reflected in other TCR-β introns. If stability is an important characteristic of IVS1, one may expect this feature to be conserved in animal species besides mice. Hybridization analysis of total cellular RNA from human Jurkat T-leukaemia cells showed the presence of an IVSlc transcript (Fig. 1C) of the size predicted for the free intron (0.4kb) based on sequence analysis (20,21). This 0.4kb transcript was present in the nucleus of Jurkat cells (data not shown). Since human IVSlc does not contain internal polyadenylate tracts (20,21), it was not detectable in poly(A)+ RNA, even when the autoradiogram was sufficiently exposed to observe the larger IVS1-β-containing pre-mRNAs (Fig. 1C).

Figure 2. Nucleotide sequence of murine IVS1. Underlined sequences indicate the position of complementary oligonucleotides used for RNase H analysis and PCR amplification. Lines drawn above the sequences indicate the conserved dinucleotides at the termini of the intron, and a conserved motif also present in mouse IVS1 (15,19) and human IVS1 (20,21). The partial sequence analysis of murine IVS1 which was previously published (15) is in agreement with the full sequence reported here except that these authors show a 'T' at position 12.

migrating similar to 18S and 28S rRNA transcripts, respectively) and mature mRNAs (1.0 and 1.3kb) increased in levels 15-20 fold between day 16 of fetal ontogeny and the adult stage, whilst 0.5kb IVSlc transcripts increased >50 fold over the same time interval (Fig. 1B). This difference suggests that the level of the free intron which accumulates is not entirely dependent on the rate of transcription, but may also reflect alterations in the rate of RNA splicing or RNA degradation.

We tested other murine TCR-β introns to determine whether they also accumulate in T cells. Northern blot analysis indicated that a probe to the first intron of the Cβ gene (IVSlc) also hybridized with an abundant 0.5kb transcript present in SL12.4 nuclear RNA (data not shown). In contrast, murine probes specific for IVS2-β, IVS2β, IVS3-β, IVS3β, and IVS β did not detect transcripts of a size consistent with free introns in either nuclear SL12.4 RNA or total adult thymus RNA. These intron probes did hybridize with TCR-β pre-mRNAs, showing their efficacy as hybridization probes (data not shown). Thus, IVSl from both the Cβ and Cβ loci display a unique stability that is not reflected in other TCR-β introns. If stability is an important characteristic of IVS1, one may expect this feature to be conserved in animal species besides mice. Hybridization analysis of total cellular RNA from human Jurkat T-leukaemia cells showed the presence of an IVSlc transcript (Fig. 1C) of the size predicted for the free intron (0.4kb) based on sequence analysis (20,21). This 0.4kb transcript was present in the nucleus of Jurkat cells (data not shown). Since human IVSlc does not contain internal polyadenylate tracts (20,21), it was not detectable in poly(A)+ RNA, even when the autoradiogram was sufficiently exposed to observe the larger IVS1-β-containing pre-mRNAs (Fig. 1C).

IVS1 exists in the form of multiple lariat molecules
Stable introns accumulate in the form of lariats in yeast mutant strains that lack intron debranching activity (6). Hence, we considered the possibility that IVSlc may remain in a lariat
conformation, permitting it to be resistant to exonuclease attack. Lariats are known to migrate slower than linear molecules of equivalent size in acrylamide gels (2–4,6,7,9). To test whether IVSI_C^1 is a lariat molecule, total cellular RNA from adult thymus was electrophoresed in a denaturing polyacrylamide gel, electroblotted, and hybridized with the IVSI_C^1 probe. This probe failed to detect any transcripts migrating at 0.5kb (Fig. 3A, lane 2), indicating that the free IVSI_C^1 intron is not a linear molecule. Instead, the probe hybridized with three discrete transcripts that migrated in a 5% polyacrylamide gel to a linear size equivalent of 0.9–1.2kb (Fig. 3A, lane 2), well above the linear IVSI_C^1 DNA standard that hybridized with the probe (Fig. 3A, lane 1). The thymic transcripts larger than 1.3kb detected by the IVSI_C^1 probe (Fig. 3A, lane 2) are IVSI_C^1-containing pre-mRNAs, based on their ability to hybridize with the C^1 exon probe (data not shown).
Spliced IVS1 from the Cε2 gene (IVS1<sub>Cε2</sub>) was also a non-linear molecule since it migrated as a single dominant transcript to a linear size equivalent of over 1kb (Fig. 3A, lane 4), even though its actual length is 0.5kb (19). The IVS1<sub>Cε2</sub> probe did not cross-hybridize with IVS1<sub>Cε1</sub> sequences, as judged by the distinct pattern of RNA transcripts which hybridize to the probe, and because this probe failed to detectably hybridize with IVS1<sub>Cε1</sub> DNA (Fig. 3A, lane 5). Thus, neither IVS1<sub>Cε1</sub> nor IVS1<sub>Cε2</sub> transcripts appear to exist as linear molecules in the thymus.

We also tested whether IVS1<sub>Cε1</sub> is a non-linear molecule in the SL12.4 T cell line. To conclusively demonstrate that the free 0.5kb intron rather than the larger intron-containing-pre-mRNAs is responsible for the hybridization signal, the 0.5kb fraction of SL12.4 poly (A)<sup>+</sup> RNA was purified in an agarose gel, and then run on polyacrylamide gels for blot analysis. On a 5% polyacrylamide gel, purified free IVS1<sub>Cε1</sub> migrated as three discrete species (Fig. 3B, left panel) to positions equivalent to that observed for the thymus RNA sample (Fig. 3A, lane 2). The 0.5kb IVS1<sub>Cε1</sub> transcripts migrated even slower in a 7% polyacrylamide gel (Fig. 3B, right panel), as expected for non-linear lariat molecules (9). We conclude that free IVS1<sub>Cε1</sub> is not a linear molecule, but instead is likely to be a set of lariat molecules.

We further characterized the multiple species of spliced IVS1<sub>Cε1</sub> molecules by incubation with oligonucleotides (oligos) complementary to different regions of this intron (see Figs. 2 & 3C), followed by RNase H digestion, and electrophoretic analysis. The RNA used for the analysis was free 0.5kb IVS1<sub>Cε1</sub> RNA purified in an agarose gel (Fig. 3C, lane labeled ‘none’; note that the two major IVS1<sub>Cε1</sub> transcripts appear as a single band in the 6% polyacrylamide gel shown). Oligo D, an oligonucleotide complementary with the 3' terminus of IVS1<sub>Cε1</sub>, was used to determine if some of the putative lariat molecules still possess a 3' tail. Oligo D treatment increased the migration of the two largest IVS1<sub>Cε1</sub> transcripts. This result indicates that both of these larger transcripts contain a 3' tail, and that they may differ in migration due to different tail lengths. Incubation with an oligonucleotide complementary with the 5' terminus of IVS1<sub>Cε1</sub>, oligo A, generated ~0.5kb transcripts (Fig. 3C). This is consistent with the conversion of the lariat molecules to linear molecules. We presume that the inefficient cleavage mediated by oligo A is due to steric hindrance from the branchpoint region of the intron. Incubation with both oligos A and D generated a single ~0.5kb linear transcript with slightly lower migration than that generated by oligo A treatment alone, as expected if cleavage occurred at both ends of the intron. Oligo B released two intermediate size transcripts; this is consistent with the generation of two Y-shaped molecules (9) from the two major transcripts that possess different length tails. The third minor band of ~0.5kb resulting from oligo B treatment is likely to be generated from the low abundance IVS1<sub>Cε1</sub> lariat molecule. The migration of this smaller cleavage product implies that its parental lariat molecule has little or no 3' tail.

We also used RNase H analysis to investigate the approximate position of the branchpoint in this intron. Intron branchpoints typically occur at an adenylate residue just upstream of the polypyrimidine tract present at the 3' termini of introns (1). Treatment with oligo C, which is complementary to such a region in IVS1<sub>Cε1</sub>, generated a ~0.5kb linear molecule, implying that this region does indeed contain a branchpoint. In order to precisely determine the branchpoint sequence(s), we have attempted primer extension experiments using primer D and the gel purified IVS1<sub>Cε1</sub> lariat. These experiments have not yielded specific sized products, perhaps because of the close proximity of the 3' end of the intron to the putative branchpoint.

Taken together, the results indicate that IVS1<sub>Cε1</sub> exists as three discrete lariat molecules that possess different length tails. The heterogeneity of tail length may be due to exonuclease cleavage of the tail in vivo to preferred sites, or alternatively, the use of more than one branchpoint site. A tail has also been detected on a β-globin intron lariat in vivo, but this tailed lariat may simply be a short-lived intermediate since the debranched linear form of the intron is also observed (9). In yeast, tailed intron lariats have been detected in wild type cells, but in mutants where debranching is inhibited, only tailless lariats accumulate, presumably due to exonuclease degradation (5,6). Since it appears that IVS1<sub>Cε1</sub> may be blocked in debranching, it is curious that it exists primarily in a tailed lariat form. Perhaps, IVS1<sub>Cε1</sub> is sequestered in a niche in the nuclear environment that protects its tail from degradation.

The accumulation of IVS1<sub>Cε1</sub> in transfected non-lymphoid cells

We sought to determine if accumulation of IVS1<sub>Cε1</sub> lariat structures is a peculiarity of T cells, or if this intron is also stable in non-lymphoid cells. HeLa cells (of epithelial origin) stably transfected with a construct containing IVS1<sub>Cε1</sub> (including donor and acceptor splice junctions) accumulated free IVS1<sub>Cε1</sub> (0.5kb) which co-migrated with the endogenous IVS1<sub>Cε1</sub> transcript from SL12.4 T-lymphoma cells on agarose gels (Fig. 3D). As with T cells, transfected HeLa cells accumulated the free intron in the nuclear compartment (Fig. 3D). Similarly, transfected rat1 cells (of mesenchymal origin) accumulated the 0.5kb IVS1<sub>Cε1</sub> transcript (Fig. 3D). The hybridization of the IVS1<sub>Cε1</sub> probe to the 0.5kb transcript in transfected cells was specific since RNA from control cells (that were not transfected) failed to give rise to a detectable hybridization signal (Fig. 3D and data not shown). To determine if free IVS1<sub>Cε1</sub> exists in lariat form in transfected HeLa cells, the free intron (0.5kb fraction) was purified on an agarose gel, followed by blot analysis on a polyacrylamide gel. Transfected HeLa cells accumulated three lariat species with identical migration and relative abundance as the endogenous transcripts in the SL12.4 T cell clone (Fig. 3E).

Thus, IVS1<sub>Cε1</sub> can accumulate as nuclear lariat molecules in cell types derived from multiple lineages. This implies that the stability of IVS1<sub>Cε1</sub> may be an intrinsic property that is independent of cell type. IVS1<sub>Cε1</sub> may adopt a secondary or tertiary structure that is inherently resistant to debranching. Alternatively, the stability of IVS1<sub>Cε1</sub> may be mediated by a general factor(s) that is present in many different cell lineages. Since stable IVS1<sub>Cε1</sub> exists in lariat form, one could envisage that a stabilizing protein might act by preventing debranching. One hypothesis is that IVS1<sub>Cε1</sub> remains associated with splicesomes following its excision from pre-mRNAs. However, IVS1<sub>Cε1</sub> does not have an intrinsic affinity for snRNPs since antibodies against these molecules failed to co-immunoprecipitate free IVS1<sub>Cε1</sub> (M.W.; unpublished observations).

The cis-acting sequences that are important in dictating the stability of IVS1<sub>Cε1</sub> are not known. Examination of the IVS1<sub>Cε1</sub> sequence (Fig. 2) and the adjacent exon sequences (15) indicates that it possesses canonical RNA splicing motifs. The 5' splice junction displays perfect complementarity with the portion of U1 snRNA known to base pair with this region (24). Just upstream
of the ‘AG’ at the 3’ splice junction, it has a long tract of polypyrimidine residues (a homogeneous stretch of 35 polypyrimidines), as is typical of mammalian introns (24). Two potential branchpoint adenylate residues are found immediately upstream of the polypyrimidine stretch; the sequences surrounding these residues both display a 5/7 match with the mammalian branchpoint consensus sequence: YNYURAY (25). Since the debranching of IVS1Gβ1 appears to be inefficient, it may be that RNA sequences nearby the branchpoint mediate this effect, either because the sequences generate a conformation that is inherently resistant to debranching, or because the sequences bind to a regulatory protein that inhibits the debranching reaction. Sequence comparison of the putative branchpoint region of murine IVS1Gβ1 with the corresponding region of the two other stable introns that we identified (murine IVS1Gβ2 and human IVS1Gβ1) reveals the existence of a precisely conserved 6 nucleotide motif, AUUUUC (Fig. 2), that is not present in murine TCR-β introns that do not display stability (15,19). This sequence may be important in preventing de-branching, as well as promoting stability and nuclear retention. In addition, this sequence motif could be responsible for the inefficient splicing of IVS1Gβ1 from TCR-β pre-mRNAs. However, it should be noted that IVS2Gβ1 and IVS3Gβ1 lack this sequence motif but are also inefficiently spliced in T cells (Qian et al., submitted).

CONCLUDING REMARKS
A few other mammalian introns have been observed following their excision from precursor transcripts, including β-globin (9) and immunoglobulin-x introns (10). It is not clear if these spliced introns are selectively stable or, instead, if they are transient species which were detected simply because they are derived from highly abundant transcripts. In this report, we demonstrate that the IVS1Gβ1 and IVS1Gβ2 lariats are selectively stable; that other Gβ introns do not detectably accumulate. The evidence that spliced IVS1Gβ1 molecules exist exclusively as a set of lariat molecules in vivo is based on RNase H mapping analysis. Independent evidence that spliced IVS1Gβ1 transcripts in SL12.4 cells are lariats comes from experiments with HeLa debranching extracts (nuclear or S100) where we have reproducibly shown that IVS1Gβ1 lariat transcripts are converted to a linear sized transcripts (M.W.; unpublished observations).

The observation that the property of stability has been retained in the human and mouse versions of this intron suggests that IVS1Gβ1 may perform a function. Since this intron is confined to the nucleus, an attractive possibility is that IVS1Gβ1 participates in the regulation of splicing. Portions of murine IVS1Gβ1 are self-complementary (based on computer analysis; L.Q., unpublished observations), suggesting that the free intron may hybridize with IVS1Gβ1-containing pre-mRNAs in vivo and thereby play an autoregulatory role.

Alternatively, the free intron may compete with pre-RNAs for binding to proteins that specifically regulate TCR-β RNA splicing. It is possible that an unstable protein(s) regulates the splicing of TCR-β transcripts since protein synthesis inhibitors appear to dramatically augment the splicing of Gβ1 pre-mRNAs in SL12.4 cells (13; Qian et al., submitted). The stable intron and cell lines described in this report should provide a system suitable for an analysis of factors that are important in the regulation of RNA splicing, intron debranching, and RNA stability.

ACKNOWLEDGEMENTS
We are grateful to the following individuals for providing cloned TCR-β DNA: S.Hedrick (U.C.S.D., San Diego, CA) for Gβ1 genomic DNA (5C.C7), E.Palmer (National Jewish Center, Denver, CO) for the Gβ1 3’ untranslated region, M.Blackman, P.Marrack, and J.Kappler (National Jewish Center) for Gβ2 genomic DNA (a Vβ8.1Dβ2Jβ2,3Gβ2 construct), and K.Wang and L.Hood (California Institute of Technology, Pasadena, CA) for human Gβ1 genomic DNA. We would also like to thank N.Gascoigne (Scripps Clinic, San Diego, CA) for providing us with unpublished murine IVS1Gβ1 sequences and A.Krainer (Cold Spring Harbor, N.Y., N.Y.) for helpful advice during the course of the project.

REFERENCES