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Alternative Splice Acceptor Utilization during Human Immunodeficiency Virus Type 1 Infection of Cultured Cells

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The utilization of alternative splice acceptors for excision of the 5′ major intron of human immunodeficiency virus type 1 RNA was observed after infection in vitro. Specific splice events were monitored by a cDNA-polymerase chain reaction. These splice events shared a common splice donor but utilized several alternative splice acceptors. In addition to identifying the previously documented splice acceptors for *tat* and *nef* (S. K. Arya, C. Guo, S. F. Josephs, and F. Wong-Staal, Science 229:69–73, 1985), nucleotide sequence analysis of cDNA-polymerase chain reaction fragments also revealed the following: (i) two splice acceptors 15 and 9 nucleotides upstream from the *rev* start codon, which are utilized to create transcripts suitable for specific *rev* expression; and (ii) use of the splice acceptor previously attributed to *nef* to generate a singly spliced, *env*-encoding transcript. Hybridization signals representing the *nef/env*, *tat*, and *rev* splice events increased in intensity between 6 and 12 h after infection of CEM cells with the LAV-1_{BRU} strain of human immunodeficiency virus type 1. In contrast, the signal for utilization of the *nef/env* splice acceptor for the singly spliced *env* transcript appeared first at 12 h and increased to maximum intensity by 24 h. The *nef/env* splice acceptor was dominant at all time points examined. We propose that this dominance ensures efficient downstream splicing proximal to the *env* initiation codon in singly spliced transcripts. However, early after infection, the dominance of the *nef/env* splice acceptor appears to divert primary transcripts away from *tat*- and *rev*-specific processing paths. The relative proportions of hybridization signals representing these alternative splice events remained constant throughout the viral replicative cycle. This result suggests that *trans*-acting factors that might influence splice choices are not induced during infection, but rather that *cis*-acting, sequence-specific splice preferences determine the relative efficiency of alternative acceptor utilization.

The genome of human immunodeficiency virus type 1 (HIV-1) encodes multiple structural and regulatory genes of diverse functions; however, viral DNA contains only one transcription initiation site. Differential gene expression must therefore be achieved by posttranscriptional functions: RNA processing, transport, or translational utilization. In this report we examine the possibility that differential gene expression is controlled at the level of gene-specific RNA splice events. The utilization of gene-specific splice acceptors during high-multiplicity HIV-1 infection of the T-lymphoblastoid cell line CEM (10) has been examined to determine the extent to which alternative splicing provides for temporally ordered gene expression.

Processed RNAs of HIV-1 are, in general, doubly or singly spliced (22). The major 5′ intron includes the *gag-pol* sequence and is deleted from singly spliced transcripts. These transcripts are believed to express the structural gene *env* as well as genes *vif*, *vpr*, and *vpu* (22, 23). The 3′ intron includes a large portion of the *env* coding region and is deleted from the doubly spliced transcripts believed to express the regulatory genes *tat*, *rev*, and *nef*. These regulatory gene transcripts accumulate before the appearance of singly spliced transcripts after transfection with biologically competent viral DNA (30) and after single-cycle infection of lymphoid cell lines (15). However, differentiation of these RNAs encoding the three regulatory genes has been difficult because of their similar sizes (1.8 to 2.0 kilobases) and shared nucleotide sequences.

The determination of a temporal order of regulatory gene expression is of interest because these gene products have disparate effects on viral replication. The *tat* gene product is a positive *trans* activator of viral long terminal repeat-directed transcription and enhances the translatability of such messages (2, 4, 7, 24). The *rev* gene product promotes the accumulation of singly spliced and unspliced RNAs while modestly depressing transcription (7, 8, 19). Both *tat* and *rev* genes are required for viral replication (5, 9, 28). The *nef* gene product has a controversial role as a repressor of transcription (1, 14, 16, 17).

We have exploited the fact that although transcripts encoding the regulatory genes *tat*, *rev*, and *nef* have identical 3′ introns, they differ in their utilization of splice acceptors for deletion of their 5′ introns (2, 25). At least three splice acceptors are present in a 200-nucleotide region located in the middle of genome. By utilizing primer-directed in vitro amplification (polymerase chain reaction [PCR]) (26), it is possible to generate short cDNA fragments that represent the alternative use of these splice acceptors. These fragments are easily resolved by electrophoresis and detected by using oligonucleotide probes, revealing a hierarchy of alternative splice acceptor utilization during HIV-1 infection.

MATERIALS AND METHODS

Virus and infection. The LAV-1_{BRU} strain of HIV was propagated in the CD4-positive, lymphoblastoid cell line CEM (10). Viral stocks were prepared by freeze-thaw lysis followed by low-speed centrifugation to remove cellular debris (13). Titers of stocks were determined by a terminal-dilution assay on 96-well microdilution plates containing CEM cells. Infected wells were determined by p24 antigen

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assay (Abbott Laboratories, North Chicago, Ill.) of the culture supernatants at 9 days. For time-course experiments, CEM cells were exposed to a multiplicity of infection of 10 50% tissue culture infective doses per cell for 2 h at 4°C. Cells were warmed to 37°C for 30 min, washed, and then incubated at 37°C. At each time point, samples were taken for nucleic acid purification, determination of cell count and viability, and culture supernatant plus p24 antigen assay. The times indicated in the figures indicate the periods of incubation at 37°C after infection.

cDNA amplification and hybridization analysis. Total nucleic acids were prepared by cell lysis in 0.2% sodium dodecyl sulfate–150 mM NaCl–10 mM EDTA–20 mM Tris (pH 7.5)–200 μg of proteinase K per ml at 50°C for 45 min. Lysates were extracted twice with phenol-chloroform and once with chloroform, adjusted to 0.8 M LiCl, and precipitated with 3 volumes of ethanol. Pelleted nucleic acids were suspended briefly in 10 mM Tris (pH 8.3) and then made up to 100 μl in a solution containing 50 mM Tris (pH 8.3), 10 mM KCl, 2.5 mM $MgCl_2$, 10 μg of bovine serum albumin, 0.2 mM each deoxynucleoside triphosphate, and 0.25 μg of each oligonucleotide primer. Nucleic acids were warmed for 90 s at 65°C to denature any RNA secondary structure and then cooled to 42°C for 2 min to anneal primers to target RNAs. Murine leukemia virus reverse transcriptase (100 U; Bethesda Research Laboratories, Inc., Gaithersburg, Md.) was added, followed by incubation at 42°C for 30 min. The preparations were boiled for 2 min to denature RNA-DNA hybrids and then cooled to 55°C for 1 min before the addition of 2.5 U of *Taq* DNA polymerase (Perkin-Elmer Corp., Norwalk, Conn.). A 30-cycle PCR protocol (26) with primer extension at 72°C for 3 min, denaturation at 94°C for 1 min, and primer annealing at 55°C for 1 min was accomplished in a Perkin-Elmer thermal cycler. This protocol efficiently amplified targets at least 600 base pairs in length. Then 10 μl (10%) of each amplification reaction was applied to a 5% nondenaturing polyacrylamide gel. After electrophoresis, gels were blotted in TBE buffer (20) onto Zeta-Probe nylon membranes (Bio-Rad Laboratories, Richmond, Calif.), and nucleic acids were denatured on the membrane by treatment with 0.4 N NaOH for 30 min and then neutralized with 1 M Tris (pH 8) for 2 to 3 min. Hybridization for 1 h was performed at 55°C in 1% sodium dodecyl sulfate–5× SSPE (20)–0.5% bovine serum albumin–0.5% polyvinylpyrrolidone, including 25 ng of ^{32}P end-labeled oligonucleotide probe, followed by washing in 1% sodium dodecyl sulfate–1× SSPE at 55°C. Autoradiography was performed at −70°C with one intensifying screen for 1 to 2 h. Oligonucleotides were prepared with an Applied Biosystems synthesizer and purified with a C_{18} column by high-pressure liquid chromatography or were obtained after gel purification from Genetic Designs, Houston, Texas.

Nucleotide sequence analysis of cDNA fragments. Nucleotide sequences of specific amplification products were determined by either analysis of plasmid clones or direct analysis of gel-purified cDNA-PCR fragments. For plasmid cloning, primers encoding the identical sequences as A and B but containing 5′ extensions encoding *Bam*HI or *Hin*dIII sites plus 25 additional bases were used to amplify nucleic acids from 10^4 HIV-infected CEM cells. Total cDNA-PCR products from two reactions were pooled, digested overnight with *Bam*HI and *Hin*dIII, and then gel purified from a 2% agarose gel. DNA fragments of 100 to 150 base pairs were ligated to *Bam*HI-*Hin*dIII-cut, phosphatase-treated psp6$T_7$19 (Bethesda Research Laboratories). After transformation of *Escherichia coli* MC1061, miniculture plasmid DNA preparations were used as templates for α-^{35}S incorporation, dideoxy-sequencing reactions (Sequenase protocol; U.S. Biochemical Corp., Cleveland, Ohio). For direct sequencing of cDNA-PCR fragments, 25-μl (25%) samples of the amplification products were electrophoresed on 5% polyacrylamide gels and stained with ethidium bromide, and the specific bands were excised. DNA was passively eluted into TE (20), ethanol precipitated, and then suspended in 25 μl of TE. The concentration of the DNA was estimated by comparative ethidium intensity on agarose gels. Approximately 0.01 fmol of fragment was amplified by using asymmetric PCR (12) to generate single strands as described above, except that 0.5 μg of 5′ primer (A) and 0.005 μg of 3′ primer (B or D) was used in a 35-cycle protocol using 5 U of *Taq* polymerase, 72°C primer extension was for 2 min, denaturation was at 94°C for 30 s, and annealing was at 55°C for 30 s. This protocol generates an excess of single-strand, plus-sense DNA due to the limiting amount of 3′ antisense primer. The products of the asymmetric PCR were purified on Nensorb columns (Dupont, NEN Research Products, Boston, Mass.) and suspended in 15 μl of TE, and 7 μl was used in dideoxy-sequencing reactions as above, except that 1 pmol of sequencing primer was used. Fragments F290, F285, and F100 were sequenced with antisense primer C; F290 was additionally sequenced with an antisense primer (5′ GCAATGAAAGCAACACTTTTTACAATA 3′) that is complementary to a region 5′ of the *rev* splice.

RESULTS

Characterization of cDNA fragments representing alternative splice acceptor utilization for deletion of the 5′ intron. CEM cells were infected at a low multiplicity and harvested 14 days after infection, when maximal activity reverse transcriptase in the culture supernatants was attained (10). Nucleic acids isolated from such late harvests were expected to include mRNAs for all regulatory and structural genes. When cDNA sequences from such cells were amplified by using oligonucleotide primers corresponding to sequences on opposites sides of the 5′ splice junctions used to generate processed HIV-1 RNAs, several HIV-specific fragments resulted (Fig. 1). In this experiment, primer A corresponded to the sequence immediately 5′ of the splice donor (nucleotide 287); primer B was complementary to the sequence at the 3′ end of the first coding exon of the *tat* and *rev* genes. Four cDNA-PCR fragments were characterized; all fragments contained consensus splice junctions upon nucleotide sequence analysis, predicting introns ending in the dinucleotide AG (Table 1). Fragment F290 reflected a splice junction between nucleotides 287 and 5356, identical to that contained in cDNA clone pCV1 (2). Such cDNAs are known to express *tat* and, less efficiently, *rev* (24). Fragments F118 and F112 reflected splice junctions between nucleotides 287 and 5533 and 5539, respectively. These splice acceptors, located 15 and 9 nucleotides upstream from the *rev* start codon, were suitably positioned to generate processed transcripts capable of specifically expressing the *rev* gene while excluding expression of *tat*. Indeed, site-specific mutagenesis of this acceptor region has resulted in a *rev*-deficient phenotype (25). Interestingly, the splice junction sequences depicted by F118 and F112 differed by three nucleotides from that obtained by S_1 nuclease protection mapping (25); however, F118 and F112 sequences appeared to resemble more closely the splice junction consensus (21), predicting introns ending in the dinucleotide AG. Fragment F100 reflected a splice junction between nucleotides 287 and 5555,

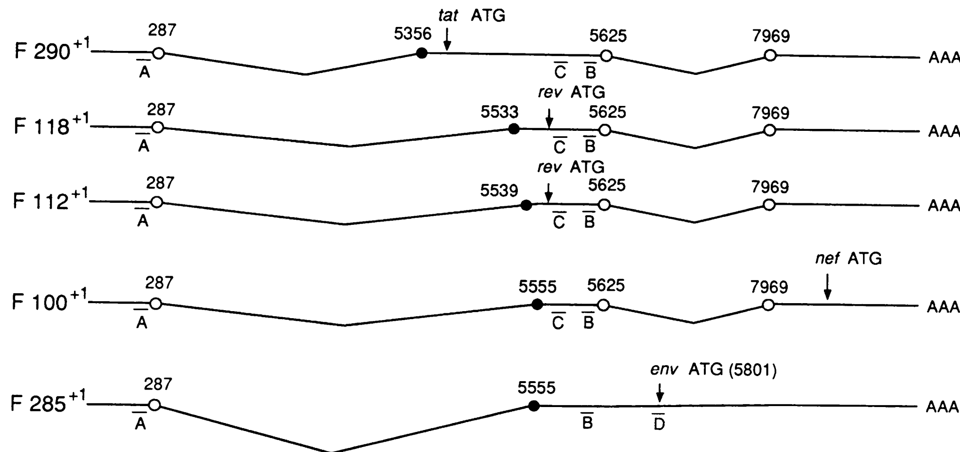

FIG. 1. Strategy and results of alternative splice acceptor detection. Line drawings represent target mRNAs: +1, cap site; ○, splice donor; ●, splice acceptor. F290, F118, F112, and F100 represent DNA fragments resulting from cDNA-PCR reactions with primers A and B. The splice junctions contained in each fragment were determined by nucleotide sequence analysis. F290 resulted from use of an acceptor at nucleotide 5356; F118 used an acceptor at 5533, F112 used an acceptor at 5539, and F100 used an acceptor at 5555. Oligonucleotide C is a probe for a sequence common to F290, F118, F112, and F100. F285 represents a DNA fragment resulting from cDNA-PCR functions with primers A and D. F285 used the acceptor at nucleotide 5555. The oligonucleotide sequences used were as follows: A, 5′ ACGGCAAGAG GCGAGGGGAGGCGACTG 3′; B, 5′ CTTTGATAGAGAAACTTGATGAGTCTG 3′, C, 5′ CGGAGACAGCGACGAAGACCTCCTCAA GGC 3′; D, 5′ CTTCACTCTCATTGCCACTGTCTTCTGC 3′.

identical to that contained in clone pCV3 (2). Doubly spliced transcripts utilizing this splice acceptor are believed to express the *nef* gene, since the *nef* open reading frame is the only gene fully encoded by this sequence (2).

Identification of the major splice acceptor utilized for singly spliced transcripts. The analysis described above reflected the use of alternative splice acceptors suitably positioned to result in differential expression of the *tat*, *rev*, and *nef* genes; however, the 3′ ends of the RNAs that yielded these amplification products were not determined. These splice acceptors could potentially be used to generate either doubly or singly spliced transcripts. To define the major splice event leading to singly spliced transcripts, cDNA sequences from HIV-1-infected, late-harvested CEM cells were amplified by using primers A and D (Fig. 1). Primer D spans the *env* start codon, located in the 3′ intron of doubly spliced transcripts, and can only give rise to amplification products representing singly spliced sequences. The nucleotide sequence of the major fragment, F285, contained a splice junction between nucleotides 287 and 5555, identical to that contained in F100 and, as noted above, previously ascribed to *nef*-expressing transcripts (Fig. 1, Table 1). The major splice acceptor utilized for singly spliced RNAs, therefore, is identical to the previously proposed *nef* splice acceptor. Singly spliced RNA utilizing this acceptor would encode *vpu* and *env* coding regions. This acceptor is hereafter referred to as *nef/env*.

Temporal order of alternative splice events during HIV-1 replication. To determine whether these characterized alternative splice events occurred in an ordered fashion during the HIV-1 replicative cycle, CEM cultures were examined sequentially after infection with the HIV-1_{BRU} strain. High multiplicities of infection were used to obtain a synchronous infection. At a multiplicity of infection of 10 50% tissue culture infective doses per cell, cDNA amplification with primers A and C (Fig. 2A) revealed the presence of sequences containing the *nef/env* (F100), *tat* (F290), and *rev* (F118/F112) splice junctions at 1 h postinfection. The *nef/env* splice acceptor was predominantly utilized at this and all subsequent time points. Hybridization signals representing all three splice junctions began to increase between 6 and 8 h and reached maximum intensity at 12 h postinfection. cDNA-PCR with primers A and D (Fig. 2B) revealed initial

TABLE 1. Nucleotide sequences of cDNA fragments and splice acceptors[a]

Fragment	Sequence
F290 (*tat*)	5′ GGGGAGGCGACTG^AATTGGGTGTCGACATAGCAGAATAGGCGTTACTCGACAGAGGAGAGAGCAAGAAATGG 3′
F285 (*env*)	5′ GGGGAGGCGACTG^GAAGAAGCGGAGAACACAGC 3′
F118 (*rev*)	5′ GGGGAGGCGACTG^CCTTAGGCATCTCCTATGG 3′
F112 (*rev*)	5′ GGGGAGGCGACTG^GCATCTCCTATGG 3′
F100 (*nef/env*)	5′ GGGGAGGCGACTG^GAAGAAGCGGAGACATCGAC 3′
Splice acceptor sequences	YnNYAG^G
tat	TTTATCCATTTCAG^AA
rev	TTCACAACGAAAAG^CC and AACGAAAGCCTTAG^GC
nef/env	ATCTCCTATGGCAG^GA

[a] Portions of the nucleotide sequences of characterized cDNA fragments. The sequences of F290, F285, and F100 were obtained by using asymetric PCR to generate single-strand copies of the original gel-purified fragments. The sequences of F118 and F112 were obtained from plasmid clones. Splice junctions are indicated by "^". Initiation codons are underlined. The intron-exon boundaries for the *tat*, *rev*, and *nef/env* splice acceptors are shown with the splice acceptor consensus (21). Y indicates pyrimidine, and N indicates any base. The intron sequences for the *rev* and *nef/env* acceptors were obtained in this study and conform to published lymphadenopathy-associated virus sequence (29); the intron sequence for the *tat* acceptor is from the literature (29).

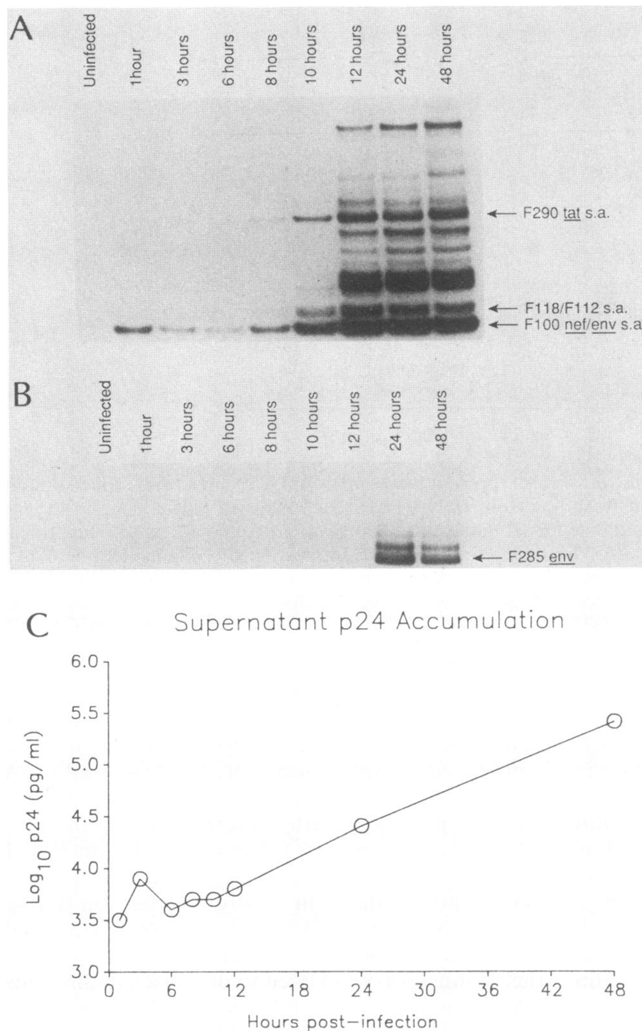

FIG. 2. Kinetics of spliced sequence accumulation and p24 antigen during high-multiplicity infection. CEM cells were infected at a multiplicity of infection of 10 50% tissue culture infective doses per cell. At the indicated times, cells were harvested, and nucleic acids were isolated. (A) cDNA-PCR amplification with primers A and B; nucleic acids from 10^4 cells were amplified in each reaction, and 10% of the amplification products were applied to a 5% acrylamide gel. After blotting, membranes were hybridized to oligonucleotide C and exposed at −70°C for 1 h. (B) cDNA-PCR reactions with primers A and D. Equal amounts of the nucleic acid extracts analyzed in panel A were used. Hybridization was to oligonucleotide B, and exposure time was 2 h. (C) Concentrations of p24 antigen in the supernatants of this culture.

appearance of the *nef/env* splice junction in singly spliced transcripts at 12 h, followed by an increase to maximum intensity by 24 h. The onset of an exponential increase in p24 antigen in the culture supernatant correlated with the appearance of the structural *env* transcript (Fig. 2C).

The splice junctions detected between 1 and 10 h after infection (Fig. 2A) are likely to represent sequences contained in doubly spliced RNAs. This inference is based on the later appearance of singly spliced sequences depicted in Fig. 2B. The detection of spliced sequences at 1 h after infection appeared to be due to their presence in the virus inoculum. Although spliced RNAs should theoretically be absent from the inoculum, low levels of *tat*, *rev*, and *nef/env* splice junction sequences were detected in the cell-free viral preparation (data not shown). Furthermore, pretreatment of cells with azidothymidine failed to block consistently the appearance of spliced sequences at 1 h after infection (data not shown). Whether these spliced sequences (Fig. 2A) are present as free RNA or are packaged at low efficiency is not clear.

DISCUSSION

The temporal use of alternate splice acceptors for viral RNA processing during HIV-1 replication has been analyzed. This analysis addresses the question of whether gene-specific alternative splicing defines an order of viral gene expression. To observe closely related alternative splice events occurring in a small number of cells, an assay based upon PCR has been developed by positioning primers for in vitro amplification of cDNAs to encompass alternative splice sites. Each splice event yielded a DNA fragment of a specific size. Several features of this approach merit emphasis. Sequences representing alternative splice events were amplified by using the same primers in the same reaction and were hybridized with a probe that was specific for a common sequence (Fig. 1). Thus, it is unlikely that the different signal intensities for the alternatively spliced sequences are attributable to differential amplification or hybridization efficiencies.

HIV-1 pre-mRNA splicing is complicated by the use of multiple splice donors and acceptors (2, 22, 23). The use of these sites generates both multiple transcripts capable of expressing the same gene and polycistronic transcripts theoretically capable of expressing several genes. By restricting our analysis to the utilization of alternative 5′ splice acceptors, however, the interpretation of the data derived by cDNA-PCR can be simplified.

The *tat* gene, for example, can be expressed from doubly spliced transcripts such as those represented by clone pCV_1 (2), from singly spliced transcripts producing a truncated but functional protein due to an in-frame termination codon (18), or from a transcript containing an additional exon encoding part of the *env* gene sequence (*tnv* gene) (27). Yet all these transcripts utilize the splice acceptor at nucleotide 5356 and are represented by fragment F290 (Fig. 1). The importance of this splice site was also noted when this acceptor region was deleted from a genomic viral DNA clone; the clone did not support *trans* activation and supported only greatly reduced viral production (9).

In the case of *rev*, expression could conceivably result from translation of a polycistronic transcript utilizing the acceptor at nucleotide 5356 (Fig. 1). However, a point mutation 5 nucleotides downstream from the splice acceptor contained in F118 (Fig. 1) has been shown previously to eliminate full-length viral RNA production and require a *rev* expression vector for complementation; S1 nuclease analysis predicted a splice acceptor 3 nucleotides downstream of the F118 acceptor (25). It is of interest that the mutation causing partial *rev* deficiency involved an AG dinucleotide 6 base pairs 3′ of the AG reported for F118. This is the AG that defines the 3′ end of the intron predicted by F112 (Table 1). Careful examination of F118/F112 band reveals that it comprises a doublet of bands of similar intensity (data not shown). It is likely that both acceptors are used for *rev* RNAs, perhaps explaining the incomplete *rev* deficiency obtained by mutation at the more 3′ AG alone.

The finding that the splice acceptor at nucleotide 5555 (*nef/env*) was the dominant acceptor used for singly spliced

transcripts (Fig. 2B) is in agreement with the reported S1 analysis of a mutant genomic clone that expressed only 1.8- and 4.3-kilobase RNA (25). The presence of amplification products larger than F285 indicates that acceptors further 5′ are used as well (Fig. 2B).

Alternative splice events have been reported in addition to those characterized in the present report (22, 23). The multitude of DNA fragments resulting from the cDNA-PCR analysis (Fig. 2A) also suggests that many additional 5′ splice acceptors are used. However, the data reviewed above support the contention that F290 represents *tat* RNA, F118/F112 *rev* RNA, and F285 *env* RNA. However, F100 present early after infection may not represent fully *nef* RNAs. Although it is likely that F100 signals appearing earlier than 24 h after infection originate from doubly spliced transcripts, a novel cDNA has recently been reported which represents a direct single splice from the 5′-most splice donor to the 3′-most splice acceptor in the viral genome (27). Such a transcript would also express *nef* and cannot be detected by the cDNA-PCR assays reported here.

The temporal study of alternative splicing during HIV-1 infection (Fig. 2) revealed low levels of *tat* (F290), *rev* (F118/F112), and *nef/env* (F100) splice junction sequences as early as 1 h after infection. These early signals representing spliced sequences were not sensitive to azidothymidine and were likely the result of residual sequences from the inoculum preparation. At 6 to 8 h after infection, the levels of these spliced sequences began to increase; this increase marked the onset of new viral RNA synthesis. The onset of accumulation of singly spliced structural gene (*env*) transcripts (F285) occurred an additional 6 h after the early RNAs, beginning at about 12 h after infection. This delay in the appearance of structural gene transcripts has been shown previously by Northern RNA blot analysis (15, 30). The accumulation of p24 antigen in the culture supernatant was coincident with the increase of *env* transcripts. These observations are consistent with the hypothesis that the appearance of singly spliced transcripts for structural polypeptides must follow the appearance of the *rev* protein.

In this CEM cell system and with the viral strain HIV-1_{BRU}, gene-specific splice acceptor utilization does not define a clear order of regulatory gene expression. Low but definite levels of each regulatory gene-specific splice were detected at the earliest assayed time point. Furthermore, the relative intensities of the hybridization signals for the characterized splice events remained fairly constant over time (Fig. 2A). This result implies that, rather than a switch over time from one splice site to another as might be regulated by cellular or viral *trans*-acting factors (3, 11), the relative amounts of alternatively spliced sequences are more likely determined by *cis*-acting, sequence-specific splice preferences (6).

A relative hierarchy of utilization of the *tat*, *rev*, and *nef/env* acceptors is apparent, however. The data clearly indicate that the *nef/env* splice acceptor is dominant compared with the acceptors for *tat* and *rev* (Fig. 2A). This dominance of the *nef/env* acceptor is seen with two distinct primer sets (AB and AD amplifications; Fig. 2A and B, respectively); this observation weighs against the possibility that this finding is an artifact of the amplification assay. Furthermore, the *nef/env* splice acceptor sequence seems to fit more closely the consensus sequence when compared with the *tat* and *rev* acceptors (Table 1) (21). In singly spliced transcripts, dominance of the *nef/env* acceptor would ensure relatively efficient downstream splicing more proximal to the *env* initiation codon. However, early after infection this dominance appears to favor *nef* splicing, diverting primary transcripts away from *tat* and *rev* expression. The viral genome appears to be organized such that expression of the essential positive regulatory genes *tat* and *rev* is dependent on utilization of relatively weak splice acceptors located 5′ of the more efficient *nef/env* splice acceptor. This arrangement may provide a mechanism for the virus to sense the host-cell transcriptional environment, delaying activation of its own positive regulatory mechanisms until this environment is optimal for replication.

The extension of the methods of analysis described here to other cells and viral isolates may reveal different alternative splice preferences. For example, we have found that primary human macrophages infected with the HTLV-$III_{Bal/85}$ strain of HIV-1 utilize the *tat* splice acceptor at nucleotide 5356 less efficiently than CEM cells infected with HIV-1_{BRU} (J. Guatelli and J. Munis, unpublished data). Such differences may be important in understanding the characteristics of HIV-1 replication in different host cell types. The present study serves as a base line for such comparisons.

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ADDENDUM IN PROOF

An extensive analysis of HIV-1 mRNA splicing has recently been reported which includes a description of the two *rev* splice acceptors described here (S. Schwartz, B. K. Felber, D. M. Benko, E. M. Fenyö, and G. N. Pavlakis, J. Virol. **64**:2519–2529, 1990).

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