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The Open Reading Frame ORF S3 of Equine Infectious Anemia Virus Is Expressed during the Viral Life Cycle

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The genome of equine infectious anemia virus (EIAV) contains several small open reading frames (ORFs), the importance of which in the development of the virus is not clear. We investigated the possibility that the largest of these ORFs (ORF S3) is expressed during the course of the viral infection. The ORF S3 information was expressed in *Escherichia coli*, and the antigen was used to raise monospecific antiserum. A 20-kDa protein expressed in cells producing EIAV was identified as the gene product of ORF S3. Furthermore, sera from EIAV-infected animals specifically recognized this protein, indicating that the ORF S3 antigen is expressed in vivo as well. A model for the expression of this new viral antigen is presented. The proposed splicing pattern is similar to that of the VEP-1 protein of maedi-visna virus, which tempts us to speculate that ORF S3 defines the second exon of the EIAV Rev protein.

Like other members of the lentivirus subfamily, equine infectious anemia virus (EIAV) contains in addition to the structural retroviral genes *gag*, *pol*, and *env* (3, 28) several small open reading frames (ORFs), the expression of which has not been demonstrated directly (14, 23). For the human immunodeficiency virus (HIV), it has been demonstrated that several small regulatory proteins involved in the control of the viral life cycle are encoded in small ORFs and are expressed from multiple spliced messenger RNAs (9, 26). One of these regulatory proteins is the HIV transactivator of transcription (Tat), the first coding exon of which is situated in front of the envelope gene and the second coding exon of which is localized in the carboxy-terminal part of the envelope transmembrane region and read in a different reading frame (9). Another HIV-regulatory protein (Rev) is also expressed from two coding exons, both localized in the same areas as the Tat exons (26).

Recently, it has been demonstrated that a region downstream of *pol* and overlapping the start of the *env* gene of EIAV and containing the small ORFs ORF S1 and ORF S2 is necessary for transactivation of the autologous promoter (7, 25). Furthermore, inspection of the published sequence data on the EIAV proviral genome allows the identification of a third ORF (ORF S3) capable of encoding 135 amino acids (14, 23). This coding sequence is completely contained within the gene encoding the transmembrane protein of EIAV. This localization is similar to the one described for the second coding exons of both *tat* and *rev* genes of HIV type 1 (HIV-1) and HIV-2.

The HIV-1 *nef* gene, on the other hand, is localized beyond the envelope-coding region, whereas for HIV-2, the *nef* equivalent overlaps the carboxy terminus of the transmembrane protein and extends into the 3′ long terminal repeat (12, 29).

In this study, we expressed the ORF S3 information of EIAV in *Escherichia coli*, and antibodies were raised to the purified protein. These reagents allowed us to demonstrate that the ORF S3-encoded information is expressed in EIAV-infected cell cultures to yield a protein of about 20 kDa. Furthermore, we show that 15% of the serum samples from EIAV-infected animals specifically recognized this protein.

Expression of ORF S3 in *E. coli*. The genetic information encoding the ORF S3 antigen was isolated from the proviral molecular clone 1369 of EIAV, which was kindly provided by S. Aaronson (14). An *Apa*I-*Xba*I fragment, containing almost all the ORF S3 information (Fig. 1A), was subcloned into the bacterial expression vector pEX (27). The pEX vectors are designed to allow the cloned information to be fused carboxy terminal to β-galactosidase to yield a recombinant fusion protein that can be easily detected in Coomassie blue-stained gels after electrophoretic separation of the total cellular extract. Expression of the cloned information is controlled by the lambda rightward promoter. Transcription can be regulated by the *c*I857 repressor, provided from a compatible plasmid (21). This repressor *ts* mutant is active at 28°C but can be inactivated by shifting the culture to 42°C (induction). All inductions were done over a period of 3 h, and culture aliquots (1 ml) were removed every hour.

Cells were pelleted and lysed by boiling in sodium dodecyl sulfate-containing sample buffer (50 mM Tris hydrochloride [pH 7.5], 2% sodium dodecyl sulfate, 5% β-mercaptoethanol, 10% glycerol, 0.01% bromophenol blue), and the supernatant was fractionated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

The gels were either stained for protein or used for Western immunoblot transfer by using the conditions described by Dunn (8). From these preliminary experiments, it was already clear that some sera from EIAV-infected horses react with the β-galactosidase fusion protein, whereas no reaction was obtained with β-galactosidase (not shown).

To investigate this in more detail and to eliminate all cross-reaction with the β-galactosidase moiety, we expressed the ORF S3 protein in a vector of the pIGAL series (Fig. 1B). These vectors have transcription and translation control signals analogous to the pEX vectors. However, the fusion proteins synthesized contain only about 50 amino acids of Cro-LacI information at the amino terminus. Moreover, all fusion proteins produced in these vectors can be detected with a monoclonal antibody directed to the Cro-LacI sequences (monoclonal antibody CL). The ORF S3

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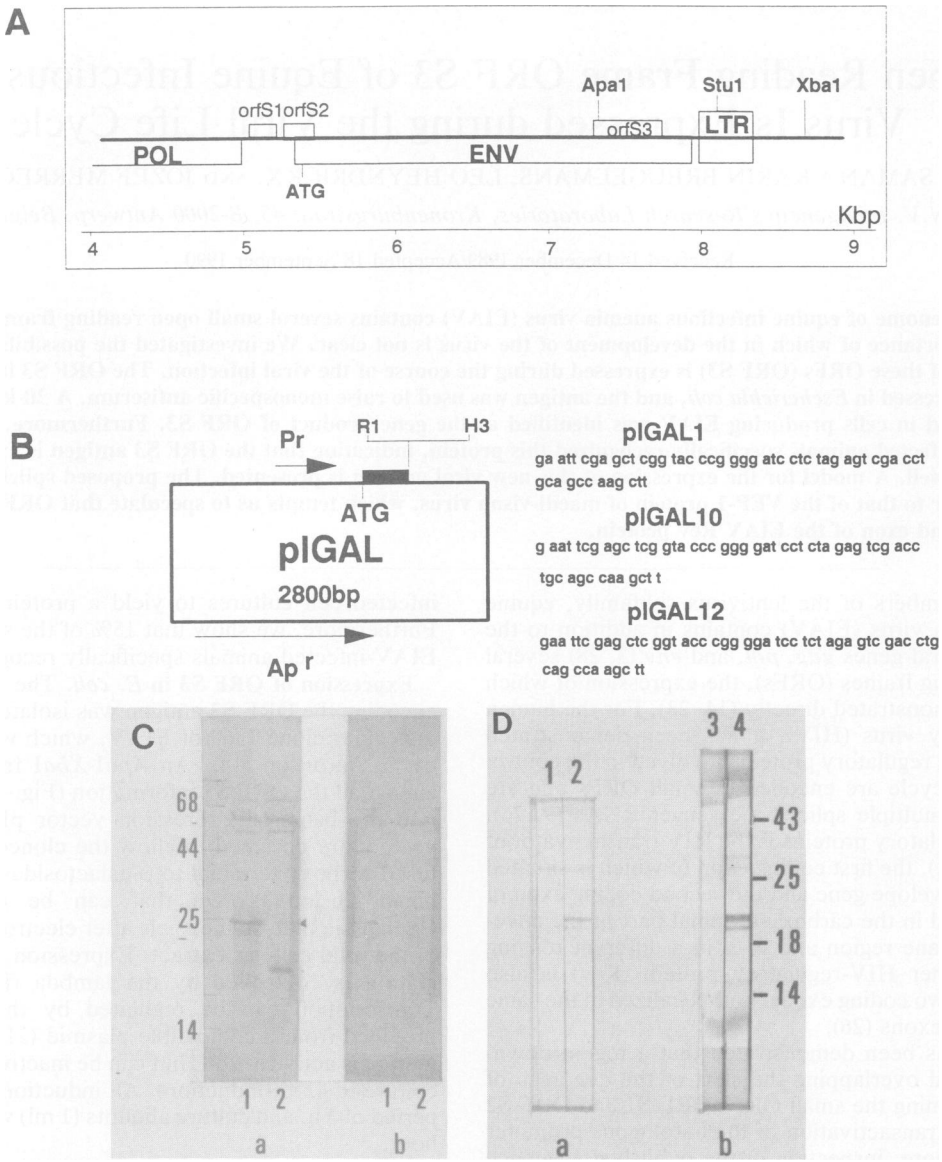

FIG. 1. (A) Schematic representation of a part of the EIAV proviral genome. The various relevant ORFs as well as the restriction sites used for the construction of expression plasmids are shown. The *Apa*I site is located 13 bp downstream from the start of ORF S3 (position 7245, as defined by Kawakami et al. [14]). (B) pIGAL vectors used for expression of EIAV proteins. The rightward lambda promoter (p_r), the Cro-LacI peptide (■), and the polylinker are indicated. The pIGAL vectors 1, 10, and 12 differ in reading frame relative to the Cro-LacI peptide. R1, *Eco*RI; H3, *Hin*dIII. Sequences of the three different pIGAL linkers and the respective reading frames are shown. (C) Immunoblot of ORF S35 and EIAV-TM1. *E. coli* cell lysates were analyzed for the presence of EIAV-TM1 (lanes 1) or ORF S35 (lanes 2) fusion proteins. The blots were incubated with an anti-EIAV horse serum (a) or a control horse serum (b). (D) Reaction of ORF S35 expression product with rabbit antiserum raised to the ORF S3-derived synthetic peptide (a) or to the ORF S3 protein A fusion protein (b). Western blot strips were incubated with the preimmune sera (lanes 1 and 3) or with the antisera (lanes 2 and 4) and developed with an anti-rabbit alkaline phosphatase conjugate. Calibration of both gels is indicated in kilodaltons.

information was transferred from the pEX vector to the pIGAL-10 vector as an *Eco*RI-*Stu*I fragment. This construction should produce a fusion protein of 21,214 Da, referred to as ORF S35. To verify the reading frame fusion, the nucleotide sequence of the ORF S35 expression plasmid was determined in the gene fusion region by using the dideoxy chain termination method. The sequence was found to be in complete agreement with the one predicted to yield a Cro-LacI-ORF S3 fusion protein. Since the ORF S3 is completely contained within the gene fragment coding for the carboxy-terminal part of the transmembrane protein, expression of the overlapping part of the envelope information was achieved by introducing the same gene fragment into the appropriate reading frame of the pIGAL vector (pIGAL1). The resulting plasmid was termed pEIAV-TM1.

Both plasmids were induced for expression in *E. coli* SG4044 (10), and cell extracts were analyzed by Western blotting (Fig. 1C). Detection of horse antibodies was performed with anti-horse immunoglobulin G coupled with alkaline phosphatase (Jackson Immunoresearch) and 5-

bromo-4-chloro-3-indolylphosphate combined with Nitro Blue Tetrazolium as substrate. The reaction was terminated by washing with water.

This experiment shows the expression of a 25-kDa protein from pEIAV-TM1 (Fig. 1C, lanes 1) and a 20-kDa protein from pORF S35 (Fig. 1C, lanes 2), both of which are reactive with serum from an EIAV-infected horse. The experimentally determined values are in good agreement with the calculated molecular masses of the respective fusion proteins. These results confirm that the ORF S35 construct does produce the protein encoded by the small ORF S3.

For immunization purposes, the EIAV ORF S3 information was introduced into the protein A fusion vector pRIT2T (19), the transcription of which is controlled by the lambda p_L promoter. Upon induction, the production of a 50-kDa protein A fusion protein could be demonstrated by Western blot analysis of lysed cells and by using a rabbit antiserum (data not shown). The fusion protein was purified from cleared lysate by affinity chromatography on human immunoglobulin G coupled to Sepharose (Pharmacia) (20). From a 500-ml culture, about 0.5 mg of purified protein was obtained and used to immunize a rabbit by subcutaneous injection of 100 to 150 μg in complete Freund adjuvant. The animal was then boosted with antigen in incomplete Freund adjuvant at 2-week intervals over a period of 2 months. The serum was subsequently tested for reactivity with the ORF S35 expression product. At the same time, serum from a rabbit immunized with a synthetic peptide derived from the ORF S3 sequence (see Fig. 4, amino acids 15 to 28) was also investigated.

From Fig. 1D it is obvious that the sera obtained from both rabbits recognize the ORF S35 protein in the Western blot, whereas the preimmune serum does not. The ORF S35 protein is often revealed as a doublet (see also Fig. 3); the faster migrating band probably represents a degradation product, as is often seen in crude *E. coli* preparations. Internal initiation can be ruled out, since no internal methionine codons appear in the sequence.

Immunoprecipitation of the ORF S3 protein from EIAV-infected cells. To identify the ORF S3 antigen in EIAV-infected cells, immunoprecipitation and blotting techniques were performed with the ORF S3 monospecific antiserum. Therefore, canine fetal thymus cells (Cf2Th) persistently infected with the cell-adapted EIAV Wyoming strain (16) (kindly provided by J. Dahlberg) were grown in Dulbecco modified Eagle medium supplemented with 10% fetal calf serum (GIBCO). Subconfluent cultures of EIAV-infected and noninfected controls were washed with methionine-free Dulbecco modified Eagle medium and labeled for 2 h with 3 ml of the same medium containing 100 μCi (3.7 MBq) each of [^{35}S]methionine and [^{35}S]cysteine per ml (Amersham; 37 TBq/mmol) at 37°C and 5% CO_2. Subsequently, the cells were harvested and lysed in 1 ml of 10 mM sodium phosphate buffer, pH 7.5, containing 100 mM NaCl, 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate, 0.05% aprotinin, and 0.2 mM phenylmethylsulfonyl fluoride. The cleared lysate (0.2 ml) was then used for immunoprecipitation with the appropriate sera (5 μl), and the immune complexes were collected with protein A-Sepharose 4B beads (Pharmacia) (13) and separated on a 15% sodium dodecyl sulfate-polyacrylamide gel (15). Gels were subsequently processed for fluorography (Amplify; Amersham) and exposed at −70°C (Fuji X-ray film) for 24 h or longer.

Alternatively, persistently infected cells and uninfected control Cf2Th cells were grown to confluency, collected, and lysed in Laemmli sample buffer. The soluble fraction was then analyzed by polyacrylamide gel electrophoresis and Western blotting. Immunodetection was performed with the rabbit anti-ORF S3 serum (serum 71) and an anti-rabbit immunoglobulin G conjugated with alkaline phosphatase (Dakopatts, Glostrup, Denmark), as described above.

The results shown in Fig. 2 indicate that in the immunoprecipitation reaction (Fig. 2A and B) as well as in the Western blot analysis (Fig. 2C), a protein of 18 to 20 kDa (depending on the way it is determined) is revealed in infected cells but not in control cells. This indicates that ORF S3 protein is expressed in these EIAV-infected cells.

Analysis of sera from EIAV-infected animals. To evaluate whether the ORF S3 protein is antigenic in vivo, several serum samples from EIAV-infected animals and normal control sera were tested for reactivity with the *E. coli*-produced protein. All sera were preincubated with extract made from induced *E. coli* SG4044 cells containing a plasmid vector without any EIAV information. The cells were concentrated from the induced culture by centrifugation, resuspended in 1/10 of the original volume, and lysed by sonication. The soluble fraction was used for incubation with an equal volume of each serum at room temperature for 1 h.

The mixture was then further diluted 50-fold in TBS (10 mM Tris hydrochloride, pH 7.5, containing 150 mM NaCl and 0.1% bovine serum albumin), and Western blot strips containing extract from the ORF S35-producing bacteria were incubated with this reagent for 2 h at room temperature with constant agitation.

The strips were washed with TBS (3 times for 10 min each) and incubated with an anti-horse immunoglobulin G conjugated to alkaline phosphatase (Jackson Immunoresearch). After three more washings, the strips were developed as described above.

Figure 3 shows some representative strips developed with EIAV-positive horse sera. Of the 40 serum samples from EIAV-infected horses tested, 6 (15%) were found to react with the ORF S3 protein, whereas 29 (72%) were positive for the EIAV Gag precursor (p55) expressed in the same configuration (data not shown). The typical ORF S35 reaction was not seen when extracts from noninduced *E. coli* cultures or when extracts from induced cultures not containing EIAV information were used. Sera from noninfected animals did not react with the ORF S35 fusion protein either (11 serum samples; data not shown). All sera that reacted with ORF S35 also reacted with the EIAV Gag precursor. These results show that in some animals, an immune response toward the ORF S3 protein is mounted upon infection with EIAV.

The data indicate that this protein is produced in infected animals but that not all animals have detectable levels of antibodies to it. This is comparable with data obtained with some nonstructural HIV antigens, for which antibodies can be detected only in some patients with the acquired immune deficiency syndrome (1, 2).

The data presented in this paper demonstrate that the ORF S3 represents a novel retroviral gene of EIAV which is expressed in infected animals as well as in persistently infected cells. Since ORF S3 does not contain a regular initiation codon, it is most probable that expression is achieved from a spliced mRNA, although initiation at other codons has also been described (4).

Since the size of the protein immunoprecipitated from the EIAV-infected cells is comparable to the calculated size for the ORF S3 protein, we hypothesize that only a small part of the protein would be coded for by the putative first exon. Similar situations exist for HIV-1 and HIV-2, in which the

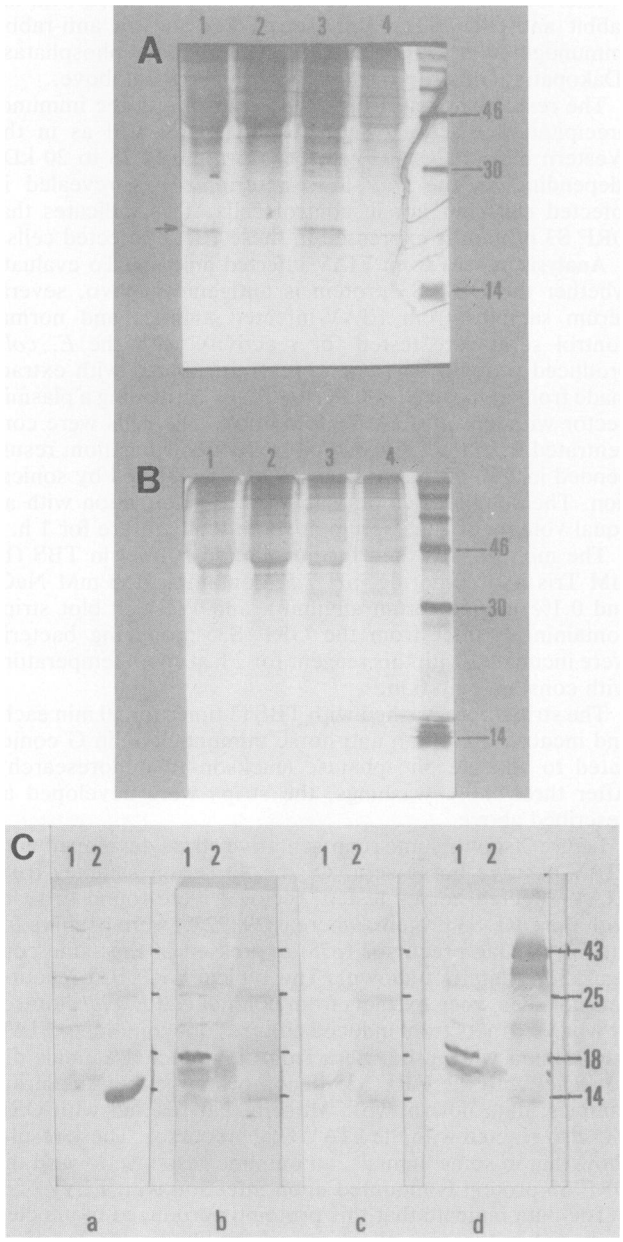

FIG. 2. Analysis of EIAV-infected cells by immunoprecipitation and Western blot. (A) Extracts from metabolically labeled EIAV-infected Cf2Th cells were prepared, and aliquots were used for immunoprecipitation with rabbit 71 serum (lane 1), the corresponding preimmune serum (lane 2), the peptide antiserum (ORF S3 amino acids 15 to 28) (lane 3), and the preimmune control (lane 4). The right lane shows ^{14}C marker proteins (Amersham). (B) Extract from noninfected Cf2Th control cells, labeled and processed as described for panel A. (C) Extract from EIAV-infected Cf2Th cells (lanes 1) or control cells (lanes 2) analyzed by immunoblotting. Strips were incubated with rabbit 71 preimmune (a) and ORF S3 immune (b) serum or antipeptide preimmune (c) and antipeptide (d) antiserum. Development was as described in the legend to Fig. 1. Calibration of all gels is in kilodaltons.

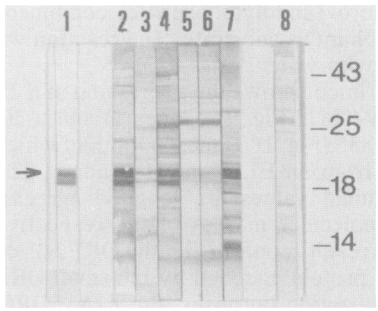

FIG. 3. Sera from experimentally infected ponies (lanes 2 and 7) or from naturally infected horses (lanes 3, 4, 5, and 6) were analyzed for their reactivity with the ORF S35 protein expressed in *E. coli*. Total *E. coli* lysate was used to prepare the Western blot strips. Reaction of the ORF S35 protein with the CL monoclonal antibody (lane 1) and reaction with a serum sample from a noninfected animal (lane 8) are also shown. The ORF S35 protein is indicated (→). Calibration is in kilodaltons.

Tat and Rev proteins are also produced from spliced mRNAs, with the first coding exon situated in front of the *env* ORF and the second coding exon in an alternative reading frame in the transmembrane coding region of the envelope protein (2, 26). Moreover, it was shown recently that in maedi-visna virus, an early viral protein (VEP-1) is produced from a spliced messenger composed of two coding exons, one of which is situated in front of the *env* gene and the other of which is located in the transmembrane protein coding sequence but read in a different frame (5, 17, 24).

By analogy with these other lentivirus examples, it is tempting to speculate that the ORF S3 of EIAV is spliced to the small ORF (ORF S2) which has been described previously (23) and which is located in front of the envelope gene (Fig. 1A). Since the ORF S2 contains an initiation codon (second codon) and a consensus splice donor site (CCCAG/GGGAAT, bp 5341 to 5351), it can be spliced to a splice acceptor site, situated in the ORF S3 sequence (TCCTC AG/G, bp 7235 to 7242) (18). Base pair positions are defined as described previously (14).

This would give rise to a protein of 152 amino acids (17,657 Da), in reasonable agreement with the value estimated from our polyacrylamide gel electrophoresis analysis (Fig. 2).

The hydrophilicity profile of the ORF S3 protein does not contain marked hydrophobic regions (Hopp and Woods algorithm) (Fig. 4). This is analogous to what has been described for the VEP-1 protein of maedi-visna virus (17) and is in contrast to the profile for the Nef protein of HIV-1 obtained by using the same algorithm.

Moreover, the ORF S3 protein does contain basic stretches of sequences that might function as nuclear location signals (amino acids 129 to 133, KRRRK, and amino acids 46 to 49, RRDR), as has been described for the visna virus VEP-1 protein (17) and for the HIV-1 Tat protein (11, 22), as well as for the simian immunodeficiency virus Gag precursor (6).

In conclusion, we have shown that EIAV encodes a novel retroviral protein which is expressed during the viral life cycle in vivo as well as in infected cell cultures. The calculated molecular mass of the deduced protein (Fig. 4) approximates the 18 to 20 kDa that was found experimentally, and the amino acid sequence does not show significant homology to any protein present in the NBRF and Swiss-Prot data banks. In some features, the protein resembles the VEP-1 protein described for visna virus, and in that case it was shown that the protein could be involved in *trans-*

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                                                  10                  20
M  G  L  F  G  K  G  V  T  W  S  A  S  H  S  M  G  G  S  Q
                                                  30                  40
G  P  L  E  S  D  Q  W  C  R  V  L  R  Q  S  L  P  E  E  K
                                                  50                  60
I  P  S  Q  T  C  I  A  R  R  H  L  G  P  G  P  T  Q  H  T
                                                  70                  80
P  S  R  R  D  R  W  I  R  G  Q  I  L  Q  A  E  V  L  Q  E
                                                  90                  100
R  L  E  W  R  I  R  G  V  Q  Q  A  A  K  E  L  G  E  V  N
                                                  110                 120
R  G  I  W  R  E  L  Y  F  R  E  D  Q  R  G  D  F  S  A  W
                                                  130                 140
G  G  Y  Q  R  A  Q  E  R  L  W  G  E  Q  S  S  P  R  V  L
                                                  150
R  P  G  D  S  K  R  R  R  K  H  L
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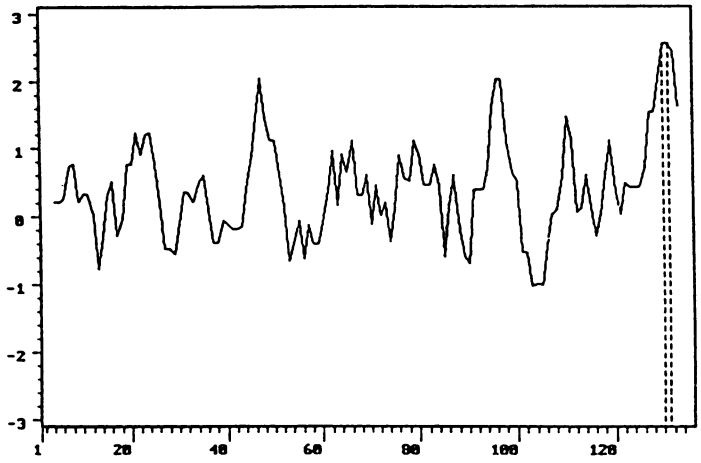

FIG. 4. Amino acid sequence (single letter code) of the EIAV ORF S2-ORF S3 fusion protein resulting from the splicing pattern described in the text. The arrow indicates the splicing point. The basic amino acid stretches are underlined. The peptide used to raise antibodies is boxed. The hydropathic profile (Hopp and Woods) is also shown (average group length of 6 amino acids).

regulation of splicing (equivalent to HIV Rev) (24; R. Vigne, I. Gourdou, V. Mazarin, G. Querat, N. Sauze, G. Audoly, and P. Filippi, Proc. 21st Congr. Int. Assoc. Biol. Standardization, in press). If the splicing pattern described here for ORF S3 is correct, this might imply that the ORF S3 protein could encode the second exon of the EIAV Rev protein. Although other possibilities for the expression and function of ORF S3 remain, the present model warrants further investigation.

The presence of antibodies specific for the ORF S3 antigen in 15% of the sera tested demonstrates both its immunogenicity and its in vivo expression. It will be important to determine whether the in vivo expression affects the viral pathogenesis of EIAV infection and whether the presence of antibodies influences the clinical manifestation of the disease in horses.

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LITERATURE CITED

1. **Aldovin, A., C. Debouck, M. B. Feinberg, M. Rosenberg, S. K. Arya, and F. Wong-Staal.** 1986. Synthesis of the complete trans-activating gene product of human T-lymphotropic virus type III in Escherichia coli: demonstration of immunogenicity in vivo and expression in vitro. Proc. Natl. Acad. Sci. USA **83**:6672–6676.
2. **Arya, S. K., and R. C. Gallo.** 1986. Three novel genes of human T-lymphotropic virus type III: immune reactivity of their products with sera from acquired immune deficiency syndrome patients. Proc. Natl. Acad. Sci. USA **83**:2209–2213.
3. **Chiu, I. M., A. Yaniv, J. E. Dahlberg, A. Gazit, F. Skuntz, S. R. Tronick, and S. A. Aaronson.** 1985. Nucleotide sequence evidence for relationship of AIDS retrovirus to lentiviruses. Nature (London) **317**:366–368.
4. **Dasso, M. C., and R. J. Jackson.** 1989. Efficient initiation of mammalian mRNA translation at a CUG codon. Nucleic Acids Res. **17**:6485–6497.
5. **Davis, J. L., and J. E. Clements.** 1989. Characterization of a cDNA clone encoding the visna virus transactivating protein. Proc. Natl. Acad. Sci. USA **86**:414–418.
6. **Delchambre, M., D. Gheysen, D. Thines, C. Thiriart, E. Jacobs, E. Verdin, M. Horth, A. Burny, and F. Bex.** 1989. The Gag precursor of simian immunodeficiency virus assembles into virus-like particles. EMBO J. **8**:2653–2660.
7. **Dorn, P. L., and D. Derse.** 1988. *cis*- and *trans*-acting regulation of gene expression of equine infectious anemia virus. J. Virol. **62**:3522–3526.
8. **Dunn, S. D.** 1986. Effects of the modification of transfer buffer composition and the renaturation of proteins in gels on the recognition of proteins on Western blots by monoclonal antibodies. Anal. Biochem. **157**:144–153.
9. **Goh, W. C., C. Rosen, J. Sodroski, D. D. Ho, and W. A. Haseltine.** 1986. Identification of a protein encoded by the *trans* activator gene *tat*III of human T-cell lymphotropic retrovirus type III. J. Virol. **59**:181–184.
10. **Gottesman, S., M. Gottesman, E. Shaw, and M. L. Pearson.** 1981. Protein degradation in *E. coli*: the lon mutation and bacteriophage lambda N and CII protein stability. Cell **24**:225–233.
11. **Green, M., and P. M. Loewenstein.** 1988. Autonomous functional domains of chemically synthesized human immunodeficiency virus Tat trans-activator protein. Cell **55**:1179–1188.
12. **Guyader, M., M. Emerman, P. Sonigo, F. Clavel, L. Montagnier, and M. Alizon.** 1987. Genome organization and transactivation of the human immunodeficiency virus type 2. Nature (London) **326**:662–669.
13. **Huisman, H. G., I. N. Winkel, P. M. Lelie, M. Tersmette, M. Wester, F. Miedema, W. G. Van Aken, and J. Goudsmit.** 1987. Future of confirmatory testing: sensitivity of immunoblot analysis and radioimmunoprecipitation assay for the detection of antibodies to LAV/HTLV III, p. 199–216. *In* J. C. Petricciani, I. D. Gust, P. A. Hoppe, and H. W. Krijnen (ed.), AIDS, the safety of blood and blood products. John Wiley & Sons, Inc., New York.
14. **Kawakami, T., L. Sherman, J. Dahlberg, A. Gazit, A. Yaniv, S. R. Tronick, and S. A. Aaronson.** 1987. Nucleotide sequence analysis of equine infectious anemia virus proviral DNA. Virology **158**:300–312.
15. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
16. **Malmquist, W. A., D. Barnett, and C. S. Becvar.** 1973. Production of equine infectious anemia antigen in a persistently infected cell line. Arch. Virol. **42**:361–370.
17. **Mazarin, V., I. Gourdou, G. Quérat, N. Sauze, and R. Vigne.** 1988. Genetic structure and function of an early transcript of visna virus. J. Virol. **62**:4813–4818.
18. **Mount, S. M.** 1982. A catalogue of splice junction sequences. Nucleic Acids Res. **10**:459–472.
19. **Nilsson, B., L. Abrahmsen, and M. Uhlen.** 1985. Immobilization and purification of enzymes with staphylococcal protein A gene fusion vectors. EMBO J. **4**:1075–1080.
20. **Nilsson, B., T. Moks, B. Jansson, L. Abrahmsen, A. Elmblad, E. Holmgren, C. Henrichson, T. A. Jones, and M. Uhlen.** 1987. A synthetic IgG-binding domain based on staphylococcal protein A. Protein Eng. **1**:107–113.
21. **Remaut, E., P. Stanssens, and W. Fiers.** 1981. Plasmid vectors for high-efficiency expression, controlled by the PL promoter of coliphage lambda. Gene **15**:81–93.
22. **Ruben, S., A. Perkins, R. Purcell, K. Joung, R. Sia, R. Burghoff, W. A. Haseltine, and C. A. Rosen.** 1989. Structural and functional characterization of human immunodeficiency virus *tat* protein. J. Virol. **63**:1–8.
23. **Rushlow, K., K. Olsen, G. Stiegler, S. L. Payne, R. C. Montelaro, and C. J. Issel.** 1986. Lentivirus genomic organization: the complete nucleotide sequence of the *env* gene region of equine infectious anemia virus. Virology **155**:309–321.
24. **Sargan, D. R., and I. D. Bennet.** 1989. A transcriptional map of visna virus: definition of the second intron structure suggests a rev-like gene product. J. Gen. Virol. **70**:1995–2006.
25. **Sherman, L., A. Gazit, A. Yaniv, T. Kawakami, J. E. Dahlberg, and S. R. Tronick.** 1988. Localization of sequences responsible for *trans*-activation of the equine infectious anemia virus long terminal repeat. J. Virol. **62**:120–126.
26. **Sodroski, J., W. C. Goh, C. Rosen, A. Dayton, E. Terwilliger, and W. Haseltine.** 1986. A second post-transcriptional trans-activator gene required for HTLV-III replication. Nature (London) **321**:412–417.
27. **Stanley, K. K., and J. P. Luzio.** 1984. Construction of a new family of high efficiency bacterial expression vectors: identification of cDNA clones coding for human liver proteins. EMBO J. **3**:1429–1434.
28. **Stephens, R. M., J. W. Casey, and N. R. Rice.** 1986. Equine infectious anemia virus *gag* and *pol* genes: relatedness to VISNA and AIDS virus. Science **231**:589–594.
29. **Terwilliger, E., J. G. Sodroski, C. A. Rosen, and W. A. Haseltine.** 1986. Effects of mutations within the 3′ *orf* open reading frame region of human T-cell lymphotropic virus type III (HTLV-III/LAV) on replication and cytopathogenicity. J. Virol. **60**:754–760.