JOURNAL OF VIROLOGY, May 1995, p. 3161–3166 Vol. 69, No. 5
0022-538X/95/$04.00+0

Immediate-Early Transcription from the Channel Catfish Virus Genome: Characterization of Two Immediate-Early Transcripts†

PETER S. SILVERSTEIN, R. CURTIS BIRD,* VICKY L. VAN SANTEN, AND KENNETH E. NUSBAUM

Department of Pathobiology, College of Veterinary Medicine, Auburn University, Auburn, Alabama 36849-5519

Received 5 October 1994/Accepted 19 January 1995

With cDNA probes and by Northern (RNA) blot analysis, a region containing immediate-early (IE) genes in the channel catfish virus (CCV) genome was identified. IE transcription in CCV-infected cells appears to be restricted to the terminal repeat region, suggesting that CCV is most closely related to the alpha subfamily of herpesviruses. CCV DNA fragments from this region encoding IE transcripts were cloned. Northern analysis with one of these cloned fragments, a 3,927-bp *Eco*RI-*Xba*I fragment, indicates that it encodes two IE transcripts. Both transcripts (*ie1* and *ie2*) were characterized by S1 nuclease analysis, primer extension analysis, and analysis of cDNAs. The *ie2* transcript is a 1.3-kb bicistronic mRNA containing open reading frame (ORF) 8a and ORF 9. ORF 8a is a 5′-truncated version of ORF 8 which, along with ORF 9, was previously identified (A. J. Davison, Virology 186:9–14, 1992). The *ie1* transcript is 0.6 kb in size, contains only ORF 9, and is expressed at a level approximately six times that of *ie2* in cycloheximide-treated cells. The putative product of ORF 9 is predicted to have a basic pI and contains a potential zinc-binding domain, making it a probable transcription factor. ORF 8a encodes a putative product which is very hydrophobic, an unusual characteristic for an IE protein.

Channel catfish virus (CCV; ictalurid herpesvirus 1) is a pathogen of channel catfish (*Ictalurus punctatus*). CCV was first isolated by Fijan (11) and was classified as a herpesvirus on the basis of morphology. The viral genome is 134 kb in size, with 97 kb of unique sequence flanked by direct terminal repeats of 18.5 kb. CCV was classified as an alphaherpesvirus on the basis of biological characteristics such as a short life cycle and rapid destruction of tissue culture cells (25). The complete nucleotide sequence of the viral genome was determined (8, 9). Comparison of CCV open reading frames (ORFs) with other herpesvirus ORFs (8), as well as analysis of relative dinucleotide abundance (13), suggests that CCV is not closely related to any of the well-characterized herpesviruses.

Immediate-early (IE) genes are essential for the replication of herpesviruses (35). For example, IE175 of herpes simplex virus type 1 is necessary for the expression of early and late viral transcripts (36). Transactivation by IE genes has been well studied in other herpesviruses (reviewed in references 15, 26, and 31). IE genes also play a role in the establishment and reactivation of latent infections (19).

Although there have been some molecular investigations of this virus (4, 5), nothing is known about the structure, function, or regulation of expression of IE genes of CCV. To explore CCV gene regulation and to gain insight into its relationship with other herpesviruses, we identified and characterized IE genes of CCV.

To identify regions of the CCV genome encoding IE RNAs, a cDNA probe that would hybridize only to those regions of the CCV genome that encode IE transcripts was synthesized. The probe was synthesized from IE RNA isolated from channel catfish ovary (CCO) cells infected with 5 50% tissue culture infective doses (20) of CCV per cell in the presence of 100 μg of cycloheximide per ml. After infection (3 to 4 h), growth medium was removed and total cellular RNA was prepared (34). Poly(A)$^+$ RNA was isolated with the PolyATract system (Promega, Madison, Wis.) and was used to generate a randomly primed cDNA probe as previously described (34). The cDNA probe was then hybridized to a Southern blot of CCV DNA fragments. Initial experiments with a field isolate of CCV (Auburn-2) revealed that a 3.9-kb *Eco*RI-*Xba*I fragment hybridized to the cDNA probe (data not shown). This fragment was homologous to the 3,927-bp *Eco*RI-*Xba*I fragment from the Auburn-1 strain of CCV, as determined by analysis of restriction sites.

To identify other IE genes in the Auburn-1 strain of CCV, viral DNA from this strain was digested with *Eco*RI and *Xba*I and further restricted with a third enzyme (*Pst*I, *Bam*HI, *Sal*I, *Bgl*II, or *Hin*dIII). Enzymes were chosen on the bases of the number of sites present in the CCV genome and the presence in the polylinker of pTZ19U to facilitate cloning. Although several fragments hybridized to an IE cDNA probe (Fig. 1A), these fragments represented only a subset of the fragments generated in the restriction digests. All of the CCV DNA fragments that hybridized could be identified on the basis of the published genomic sequence (8). The size of fragments that hybridized to the IE cDNA probe changed in the different digests exactly as predicted by the sequence data. For example, the 3,046-bp *Eco*RI fragment identified by the IE cDNA probe in the *Eco*RI-*Xba*I digests was not cut by *Hin*dIII, *Bam*HI, *Sal*I, or *Pst*I but was reduced to 2,807 bp when the *Eco*RI-*Xba*I digest was subsequently cleaved with *Bgl*II (Fig. 1A). In some digests, small fragments derived from the terminal repeat (e.g., subfragments of the 7,707-bp *Eco*RI-*Xba*I fragment) were not

* Corresponding author. Phone: (334) 844-2707. Fax: (334) 844-2652.

† Publication 2493 of the College of Veterinary Medicine, Auburn University, Auburn, Ala.

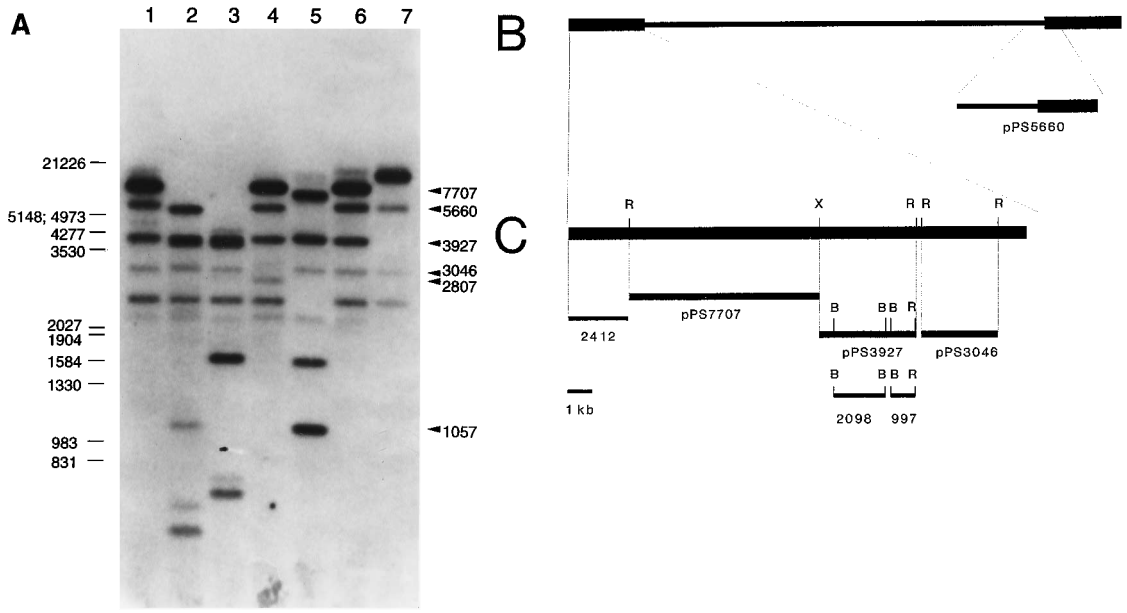


FIG. 1. Identification of CCV DNA fragments that encode IE RNA. A ^{32}P-labeled cDNA probe was synthesized with poly(A)$^+$ RNA isolated from CCO cells infected with CCV in the presence of cycloheximide and hybridized to a Southern blot of CCV DNA digested with restriction enzymes as listed below. (A) Autoradiogram of Southern blot of CCV DNA digests after hybridization to an IE cDNA probe. Lanes: 1, *Eco*RI-*Xba*I-*Hin*dIII; 2, *Eco*RI-*Xba*I-*Bam*HI; 3, *Eco*RI-*Xba*I-*Sal*I; 4, *Eco*RI-*Xba*I-*Bgl*II; 5, *Eco*RI-*Xba*I-*Pst*I; 6, *Eco*RI-*Xba*I; 7, *Eco*RI. Positions and sizes (in base pairs) of fragments from a *Hin*dIII-*Eco*RI digest of λ phage DNA are noted on the left. Positions and sizes (in base pairs) of CCV DNA fragments noted in the text are indicated on the right. (B) Representation of the CCV genome. Thick lines represent direct terminal repeats, and the thinner line represents unique sequence. The location from which clone pPS5660 was derived is shown. (C) Map of one of the terminal repeats of the CCV genome. CCV DNA fragments represented by clones pPS7707, pPS3927, and pPS3046 are indicated. Letters above the lines represent relevant restriction sites (X, *Xba*I; R, *Eco*RI; B, *Bst*EII). Subfragments labeled 2098 and 997 were derived from pPS3927. The numbers in each construct label represent the size (in base pairs) of the CCV genomic fragment.

identified by the cDNA probe. Further analysis demonstrated that these fragments do not encode IE transcripts.

All fragments hybridizing to the cDNA probe were confined to the terminal repeat region, or an adjacent region, of the CCV genome. Four fragments (7,707-bp *Eco*RI-*Xba*I, 5,660-bp *Eco*RI, 3,927-bp *Eco*RI-*Xba*I, and 3,046-bp *Eco*RI) that hybridized to the IE cDNA probe were isolated and cloned into pTZ19U, creating clones pPS7707, pPS5660, pPS3927, and pPS3046, respectively (Fig. 1C). All of these fragments, except for the 5,660-bp fragment, were derived entirely from the terminal repeat region. The 5,660-bp fragment contained 2,412 bp from the right terminal repeat and 3,248 bp from the adjacent unique sequence (Fig. 1B). Previous sequence analysis demonstrated that the terminal repeats are identical (8). Thus, the 2,412-bp portion of the right terminal repeat present in pPS5660 is identical to the 2,412-bp fragment that exhibits strong hybridization to the cDNA probe and is derived from the extreme left portion of the left terminal repeat (Fig. 1).

To precisely localize IE transcripts, each cloned DNA fragment was digested with several restriction enzymes, transferred to a nylon membrane, and probed with an IE cDNA probe. In all digests, some fragments hybridized to the probe and others did not. Hybridization to pPS5660 was restricted to the portion derived from the terminal repeat (data not shown). All of the other CCV DNA fragments that hybridized to the cDNA probe were derived from the terminal repeat, demonstrating that all CCV IE transcription originates from the terminal repeat. Although minor amounts of IE gene transcription from the unique sequence cannot be ruled out, the vast majority of IE transcription originates from the terminal repeat region.

Although the position of the IE genes is not generally considered a unique feature of herpesvirus subfamilies, interesting patterns emerge when the position of these genes in different subfamilies is examined. In both betaherpesvirus and gammaherpesvirus subfamilies, IE genes are located in unique regions. This location has been demonstrated in genomes of the gammaherpesviruses Epstein-Barr virus (15), herpesvirus saimiri (23), and bovine herpesvirus 4 (34) and in the betaherpesviruses human herpesvirus 6 (22, 28), murine cytomegalovirus (14), and guinea pig cytomegalovirus genomes (38). In human cytomegalovirus the major IE transcripts are encoded in the unique region of the genome (31, 32); however, a minor IE transcript, *trs1*, is encoded in the repeated region (30). In contrast, in the alphaherpesviruses, IE genes are located within repeated regions of the genome or in unique sequences adjacent to the repeats. IE genes have been mapped to repeat regions in the alphaherpesviruses herpes simplex virus type 1, bovine herpesvirus 1, varicella-zoster virus, equine herpesvirus 1, and pseudorabies virus (2, 6, 7, 12, 16, 26, 37). The position of the IE genes in the CCV genome, along with the short replication cycle and rapid destruction of cells in tissue culture (25), suggests that CCV is more closely related to the alphaherpesviruses than to other subfamilies of herpesviruses. Although CCV shares little homology with other alphaherpesviruses at the level of protein or nucleic acid sequence, perhaps the IE gene cluster has been conserved in the repeated sequences of the alphaherpesviruses. Positional conservation may result from a selective advantage conferred by the possession of two copies of the IE genes. Such a gene cluster might persist even though significant sequence homology is no longer detectable.

Sequence data indicated the 3,927-bp fragment contained several ORFs, including ORF 9, which encodes a potential zinc-binding domain (8). Hybridization of the 3,927-bp fragment to Northern (RNA) blots of poly(A)$^+$ IE RNA revealed

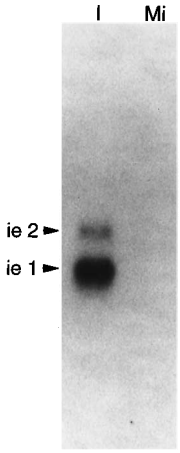


FIG. 2. Identification of IE transcripts encoded by the 3,927-bp *Eco*RI-*Xba*I fragment. A Northern blot, prepared with RNA isolated from CCO cells infected with CCV in the presence of cycloheximide, was probed with the cloned 3,927-bp CCV DNA fragment isolated from pPS3927. Lanes: I, poly(A)$^+$ RNA isolated from CCO cells infected in the presence of cycloheximide; Mi, poly(A)$^+$ RNA isolated from mock-infected cells. Arrowheads labeled ie 1 and ie 2 indicate IE RNAs of 0.6 and 1.3 kb, respectively. Size estimates were derived from three separate determinations with either poly(A)$^+$ RNA or total RNA and 0.16 to 1.77 kb and 0.24 to 9.5 kb RNA ladders (Life Technologies) as size standards.

the presence of two transcripts, one of approximately 0.6 kb (*ie1*) and another of approximately 1.3 kb (*ie2*) (Fig. 2). A 997-bp *Bst*EII-*Eco*RI subfragment of pPS3927, containing all of ORF 9, also hybridized to both transcripts (data not shown). Densitometry showed that *ie1* was consistently expressed at a level approximately six times that of *ie2* in CCO cells infected with the Auburn-1 strain of CCV in the presence of cycloheximide. Because of the abundance of *ie1* and the presence of the potential zinc-binding domain, this region was chosen for further characterization. Northern blots probed with three other cloned CCV DNA fragments (pPS7707, pPS5660, and pPS3046) revealed additional IE transcripts which are being characterized.

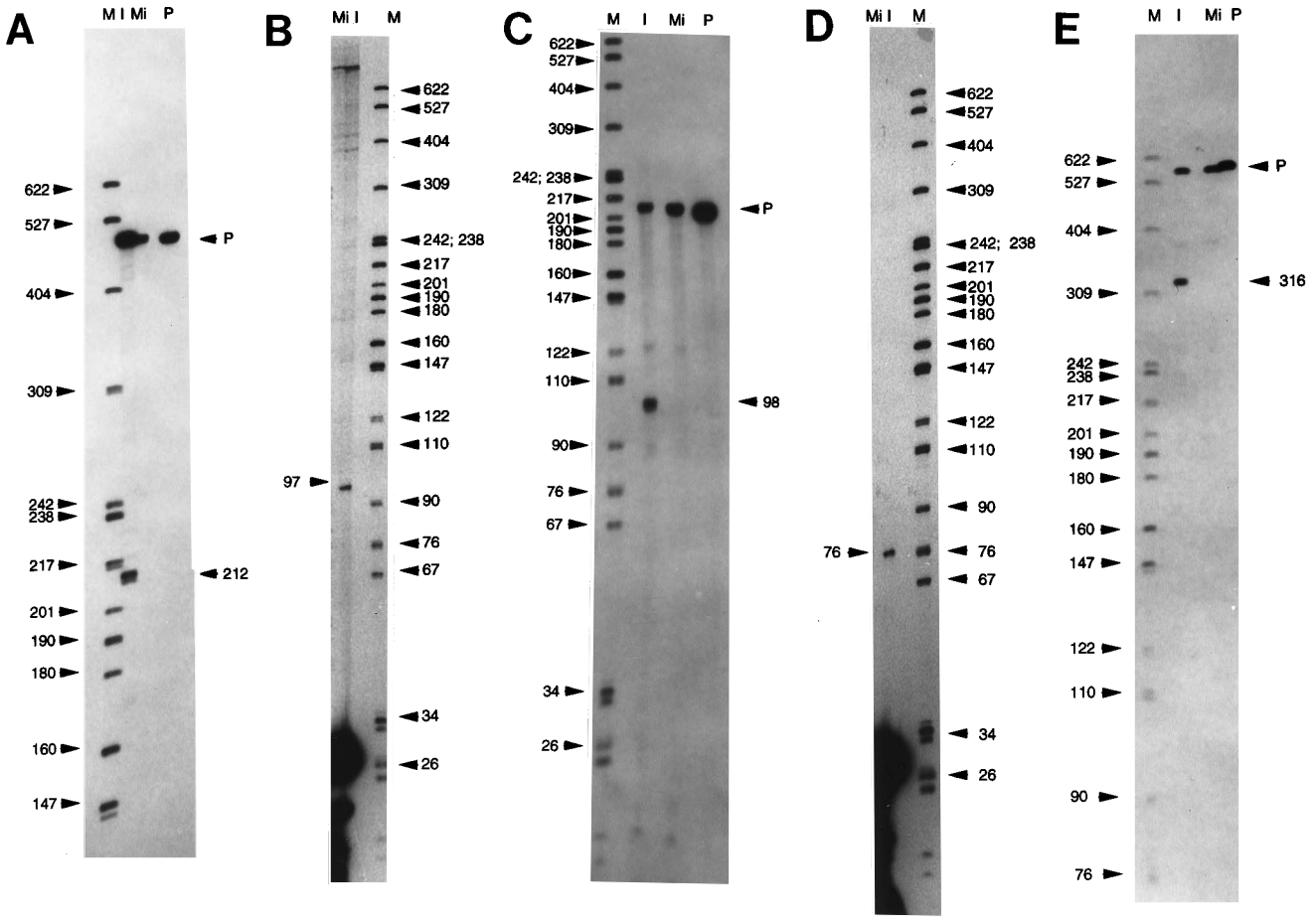
Because of the number of transcripts and possible complexity of the IE region, transcripts were analyzed by S1 nuclease analysis, primer extension analysis, and analysis of cDNAs. To construct a cDNA library enriched in cDNAs representing IE transcripts, IE RNA was prepared as described above. Poly(A)$^+$ RNA was prepared by two rounds of selection, and the cDNA library was prepared with a Lambda Zap II cDNA synthesis kit (Stratagene, La Jolla, Calif.). The library was amplified and screened by hybridization with a cloned CCV DNA fragment isolated from pPS3927 and radiolabeled with ^{32}P by random primer extension (Dupont-New England Nuclear). After obtaining pure plaques, plasmids containing cDNAs were obtained from recombinant lambda phage by in vivo excision (Stratagene).

Twelve cDNAs were isolated after four rounds of screening. On the basis of length and hybridization to the 997-bp *Eco*RI-*Bst*EII and 2,098-bp *Bst*EII subfragments of pPS3927 (Fig. 1C), six cDNAs were chosen for further analysis. Approximately 150 bp were sequenced from each end of the six cDNAs. Two cDNAs, one 1,070 bp and the other 598 bp, were chosen for further sequence analysis because their lengths approximated those of transcripts identified by CCV genomic clone pPS3927. In addition, both cDNAs strongly hybridized to the 997-bp probe (data not shown). The longer cDNA, pC3927L, extends from nucleotide (nt) 12625 to 13695, while the shorter cDNA, pC3927S, extends from nt 13195 to 13795. Both cDNAs were completely sequenced, and comparison with the genomic sequence indicated that both represent unspliced mRNAs. pC3927L represents a bicistronic mRNA containing ORFs 8 and 9, while pC3927S contains only ORF 9. It should be noted that the 3′ termini of the other four cDNAs were also located at nt 13795, suggesting that all mRNAs identified are 3′ coterminal (see below).

Using information provided by analysis of the cDNAs, S1 probes were designed to define the 3′ and 5′ ends of the mRNAs. To determine the 5′ end of the shorter mRNA, a 478-bp *Acc*III-*Pvu*II (nt 12915 to 13396) fragment was 5′ end labeled with γ-^{32}P and T4 polynucleotide kinase (U.S. Biochemicals). Conditions for hybridization to poly(A)$^+$ IE RNA and S1 nuclease digestion were as previously described (34). A 212-bp fragment was protected, indicating that the 5′ end (or a splice site) is located at nt 13184 (Fig. 3A and F). To confirm that the fragment produced by protection of 5′-end-labeled probe actually represented the 5′ end of the RNA rather than a splice site, primer extension analysis also was employed to determine the 5′ end of the RNA. An oligonucleotide, PSS 1 (5′-GAAGATCACCCTCTTGATCCTATCCACCTT-3′, nt 13251 to 13280), was labeled at the 5′ end with γ-^{32}P and T4 polynucleotide kinase and hybridized to poly(A)$^+$ IE RNA. Conditions for hybridization and primer extension were as previously described (27). Extension of PSS 1 produced a 97-nt product, placing the start site of *ie1* at nt 13185 (Fig. 3B and F). To determine the 5′ end of the longer RNA, a 208-bp fragment from a *Cla*I digest of pPSS3927 (nt 12383 to 12593) was 5′ end labeled and used for S1 nuclease analysis. A 98-nt fragment was protected, indicating that the 5′ end of the RNA (or a 3′ splice acceptor) is located at nt 12494 (Fig. 3C and F). Primer extension was performed to confirm that the 5′ end of the RNA, as determined by S1 analysis, was the actual 5′ end and not a splice site. Extension of an oligonucleotide, PSS 2 (5′-GAGAGATTGGGCCAGTGAGACCGTGATACA-3′, nt 12541 to 12570), produced a 76-nt product, placing the start site of *ie2* at nt 12494 (Fig. 3D and F). Lengths of the primer extension products indicated that the 5′ ends of both mRNAs corresponded with the 5′ ends determined by S1 nuclease analysis.

All of the cDNAs isolated from the cDNA library, except for one, had identical 3′ ends. The 3′ end of pC3927L was located at nt 13695 in a region where there are 10 consecutive A residues, whereas the 3′ ends of all of the other cDNAs were located at nt 13795. Several lines of evidence lead us to believe that the terminus of pC3927L at nt 13695 is an artifact and that all of the RNAs represented by these cDNAs are 3′ coterminal at nt 13795. For example, four other cDNAs, three of which were longer than the short RNA (*ie1*), had their 3′ termini located at nt 13795 (data not shown). Furthermore, there are polyadenylation signals only at nt 13776 and at nt 11499, the latter being upstream of the 5′ end of the mRNA. To confirm that the transcripts represented by these cDNAs are 3′ coterminal, the 569-bp *Eco*RI-*Mlu*I (nt 14046 to 13477) fragment was isolated from pPS3927, and the *Mlu*I site was 3′ end labeled with Klenow polymerase (3). Only the *Mlu*I site was labeled by the addition of dGTP and [α-^{32}P]dCTP (3,000 Ci/mmol). IE RNA protected only one fragment (316 nt) of this probe, indicating that both IE mRNAs shared the same 3′ terminus (Fig. 3E and F). The apparent 3′ terminus predicted by cDNA pC3927L at nt 13695 was most likely caused by hybridization of the oligo(dT) primer/adapter to an AT-rich region upstream from the 3′ end of the RNA as discussed above. If the nt 13695 site were the 3′ end of the RNA, a 218-nt fragment would have been protected. This was not the case.

Both of the IE transcripts characterized were unspliced and 3′ coterminal. This was determined by two independent meth-



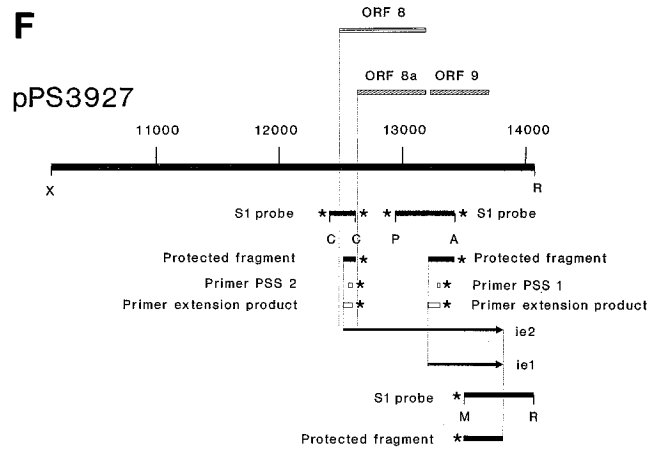


FIG. 3. Determination of the 5′ and 3′ ends of *ie1* and *ie2* RNA with S1 nuclease analysis and primer extension analysis. The lanes in all panels are labeled as follows: Mi, poly(A)$^+$ RNA isolated from mock-infected CCO cells; I, poly(A)$^+$ RNA isolated from CCO cells infected in the presence of cycloheximide; M, size marker; P, probe. (A) S1 analysis of the 5′ end of *ie1* with an *Acc*III-*Pvu*II fragment (nt 12915 to 13396). Arrowheads on the left indicate the size and position of fragments in the marker (*Msp*I digest of pBR322), and arrowheads on the right indicate the probe (P) and the protected fragment of 212 nt. (B) Primer extension analysis of *ie1* with primer PSS1. The arrowhead on the left indicates the primer extension product of 97 nt, and arrowheads on the right indicate fragments in the size marker (*Msp*I digest of pBR322). (C) S1 analysis of the 5′ end of *ie2* with a *Cla*I fragment (nt 12384 to 12593). Arrowheads on the left indicate sizes and positions of fragments in the marker (*Msp*I digest of pBR322). Arrowheads on the right indicate the probe (P) and the protected fragment of 98 nt. (D) Primer extension analysis of *ie2* with the PSS2 primer. The arrowhead on the left indicates the primer extension product of 76 nt, and arrowheads on the right indicate positions of fragments in the size marker. (E) S1 nuclease analysis of the 3′ ends of *ie1* and *ie2*. The probe was an *Eco*RI-*Mlu*I fragment (nt 14046 to 13477) radiolabeled at the 3′ end at the *Mlu*I site. Arrowheads on the left indicate fragments in the size marker (*Msp*I digest of pBR322), and arrowheads on the right indicate position of the probe (P), as well as the protected fragment (316 nt). (F) Map of pPS3927 indicating positions of relevant landmarks. The numbers above the fragment indicate nucleotide numbers in the CCV genome from which the fragment was derived. ORFs 8 and 9 were identified by Davison (8), and ORF 8a was identified in this work. The letters P, A, C, M, and R indicate the *Pvu*II, *Acc*III, *Cla*I, *Mlu*I, and *Eco*RI sites, respectively, on the S1 probes. Asterisks indicate the labeled end of the probe or protected fragment for S1 experiments and the labeled end of the oligonucleotide or extension products for primer extension.

ods: first, cDNAs representing each of the transcripts were sequenced and the sequence was compared to the published genomic sequence (8), and second, S1 nuclease analysis encompassing the entire transcription unit was performed. Previous studies of IE genes from other herpesviruses have revealed both spliced and unspliced IE RNAs (6, 39). Although many herpesvirus genes have their own polyadenylation signals, it is not uncommon for transcripts to share 3′ termini (21). In the case of CCV, this may be due to the relative paucity of polyadenylation signals in the viral genome (8).

The 5′ end of the longer IE transcript (*ie2*) is nt 12494, and the 3′ end of the transcript is nt 13795. Davison's analysis (8)

predicted an ORF, ORF 8, extending from nt 12462 to 13160. Because the predicted ORF 8 starts upstream of the 5′ end of the *ie2* transcript, it cannot be encoded in this transcript. However, our work does not preclude the possibility that an mRNA containing ORF 8 is transcribed at early or late times. Another ORF is contained within the *ie2* transcript from nt 12609 to 13157 which we refer to as ORF 8a. This ORF is in the same reading frame as ORF 8 and thus represents a 5′-truncated version. Unlike ORF 8, the initiation codon of ORF 8a conforms to the Kozak consensus sequence at positions −3 and +4 (18).

ORF 8 was originally predicted (8) to encode a membrane-associated protein because of the presence of substantial hydrophobic regions. The difference between ORF 8 and ORF 8a is the deletion of a hydrophilic region of 49 amino acids at the amino terminus. Therefore, ORF 8a contains the hydrophobic region and could also encode a membrane-associated protein. However, the protein encoded by ORF 8a (pI = 5.9) is predicted to be substantially more acidic than that predicted for ORF 8 (pI = 6.9), because the truncation eliminates 11 basic amino acids and only 6 acidic amino acids. The putative product of ORF 8a does not appear to be significantly related to other proteins when analyzed with FastA (24) or BLASTP (1) algorithms and the GenBank database.

Both mRNAs characterized in this report also contain ORF 9 (8). *ie1* mRNA contains only ORF 9 and is approximately sixfold more abundant than the bicistronic *ie2* mRNA in cycloheximide-treated cells. The putative protein encoded by ORF 9 has a predicted pI of 11.3 and contains a potential zinc-binding domain. Several herpesvirus IE proteins encode zinc-binding domains, the best-characterized example being IE110 of HSV-1 (10). The combination of a zinc-binding domain and a high pI of the predicted ORF 9 product makes this a possible candidate for a transcription factor.

The presence of a putative zinc-binding domain and the high pI suggest that ORF 9 fits the classic paradigm of an IE gene. In contrast, the predicted characteristics of the protein encoded by ORF 8a (i.e., possible membrane association and high levels of hydrophobicity) would make this an unusual IE gene. One possible explanation for the appearance of the bicistronic ORF 8a/9 mRNA is that ORF 8a is really an early gene expressed at low levels because inhibition of protein synthesis by cycloheximide was not complete. However, this is not likely, because in experiments with a different strain of virus (Auburn-2), the longer RNA, presumably containing ORF 8a and ORF 9, was more abundant than the RNA containing ORF 9 alone (29). Although it may be unusual for an IE transcript to encode a membrane-associated protein, it is not without precedent. The unique long region of the HCMV genome encodes an IE transcript whose predicted product is a potential membrane glycoprotein (17, 33). Therefore, it is likely that ORF 8a is an IE gene, but one which is different from most IE genes described for other herpesviruses.

We thank Allen Screws for technical assistance and Ralph Paxton, Bernhard Kaltenboeck, and Stuart Price for critical reviews of the manuscript.

This work was supported by USDA grant 92-03847. We also thank Lauren Wolfe for generous financial support.

REFERENCES

1. **Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman.** 1990. Basic local alignment search tool. J. Mol. Biol. **215:**403–410.
2. **Anderson, K. P., R. H. Costa, L. E. Holland, and E. K. Wagner.** 1980. Characterization of herpes simplex virus type 1 RNA present in the absence of de novo protein synthesis. J. Virol. **34:**9–27.
3. **Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl (ed.).** 1989. Current protocols in molecular biology. John Wiley & Sons, Inc., New York.
4. **Bird, R. C., K. E. Nusbaum, E. A. Screws, R. R. Young-White, J. M. Grizzle, and M. Toivio-Kinnucan.** 1988. Molecular cloning of fragments of the channel catfish virus genome and expression of the encoded mRNA during infection. Am. J. Vet. Res. **49:**1850–1865.
5. **Boyle, J., and J. Blackwell.** 1991. Use of polymerase chain reaction to detect latent channel catfish virus. Am. J. Vet. Res. **52:**1965–1968.
6. **Cheung, A. K.** 1988. Fine mapping of the immediate-early gene of the Indiana-Funkhauser strain of pseudorabies virus. J. Virol. **62:**4763–4766.
7. **Davison, A. J.** 1991. Varicella-zoster virus. J. Gen. Virol. **72:**475–486.
8. **Davison, A. J.** 1992. Channel catfish virus: a new type of herpesvirus. Virology **186:**9–14.
9. **Davison, A. J.** 1994. Fish herpesviruses, p. 470–474. *In* R. G. Webster and A. Granoff (ed.), Encyclopedia of virology. Academic Press, Inc., New York.
10. **Everett, R. D., P. Barlow, A. Milner, B. Luisi, A. Orr, G. Hope, and D. Lyon.** 1993. A novel arrangement of zinc-binding residues and secondary structure in the C_3HC_4 motif of an alpha herpes virus protein family. J. Mol. Biol. **234:**1038–1047.
11. **Fijan, N. N.** 1968. Progress report on acute mortality of channel catfish fingerlings caused by a virus. Off. Int. Epizoot. Bull. **69:**1167–1168.
12. **Grundy, F. J., R. P. Baumann, and D. J. O'Callaghan.** 1989. DNA sequence and comparative analyses of the equine herpesvirus type 1 immediate early gene. Virology **172:**223–236.
13. **Karlin, S., E. S. Mocarski, and G. A. Schachtel.** 1994. Molecular evolution of herpesviruses: genomic and protein sequence comparisons. J. Virol. **68:** 1886–1902.
14. **Keil, G. M., A. Ebeling-Keil, and U. H. Koszinowski.** 1987. Immediate-early genes of murine cytomegalovirus: location, transcripts, and translation products. J. Virol. **61:**526–533.
15. **Kieff, E., and D. Liebowitz.** 1990. Epstein-Barr virus and its replication, p. 1889–1920. *In* B. N. Fields et al. (ed.), Virology, 2nd ed. Raven Press, Ltd., New York.
16. **Kinchington, P. R., J.-P. Vergnes, P. Defechereux, J. Piette, and S. E. Turse.** 1994. Transcriptional mapping of the varicella-zoster virus regulatory genes encoding open reading frames 4 and 63. J. Virol. **68:**3570–3581.
17. **Kouzarides, T., A. T. Bankier, S. C. Satchwell, E. Preddy, and B. G. Barrell.** 1988. An immediate early gene of human cytomegalovirus encodes a potential membrane glycoprotein. Virology **165:**151–164.
18. **Kozak, M.** 1991. An analysis of vertebrate mRNA sequences: intimations of translational control. J. Cell Biol. **115:**887–903.
19. **Leib, D. A., D. M. Coen, C. L. Bogard, K. A. Hicks, D. R. Yager, D. M. Knipe, K. L. Tyler, and P. A. Schaffer.** 1989. Immediate-early regulatory gene mutants define different stages in the establishment and reactivation of herpes simplex virus latency. J. Virol. **63:**759–768.
20. **Lennette, E. H., and N. J. Smith.** 1979. Diagnostic procedures for viral, rickettsial, and chlamydial infection, 5th ed., p. 34–35. American Public Health Association, Washington, D.C.
21. **McGeoch, D. J.** 1991. Correlation between HSV-1 DNA sequence and viral transcription maps, p. 29–47. *In* E. K. Wagner (ed.), Herpesvirus transcription and its regulation. CRC Press, Boston.
22. **Nicholas, J., and M. E. D. Martin.** 1994. Nucleotide sequence analysis of a 38.5-kilobase-pair region of the genome of human herpesvirus 6 encoding human cytomegalovirus immediate-early homologs and transactivating functions. J. Virol. **68:**597–610.
23. **Nicholas, J., E. P. Smith, L. Coles, and R. Honess.** 1990. Gene expression in cells infected with gammaherpesvirus saimiri: properties of transcripts from two immediate-early genes. Virology **199:**189–200.
24. **Pearson, W. R., and D. J. Lipman.** 1988. Improved tools for biological sequence comparison. Proc. Natl. Acad. Sci. USA **85:**2444–2448.
25. **Roizman, B.** 1990. Herpesviridae: a brief introduction, p. 841–847. *In* B. N. Fields et al. (ed.), Virology, 2nd ed. Raven Press, Ltd., New York.
26. **Roizman, B., and A. E. Sears.** 1991. Herpes simplex viruses and their replication, p. 849–894. *In* B. N. Fields and D. M. Knipe (ed.), Fundamental virology, 2nd ed. Raven Press, Ltd., New York.
27. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
28. **Schiewe, U., F. Neipel, D. Schreiner, and B. Fleckenstein.** 1994. Structure and transcription of an immediate-early region in the human herpesvirus 6 genome. J. Virol. **68:**2978–2985.
29. **Silverstein, P. S., R. C. Bird, V. L. van Santen, and K. E. Nusbaum.** Unpublished data.
30. **Stasiak, P. C., and E. S. Mocarski.** 1992. Transactivation of the cytomegalovirus ICP36 gene promoter requires the α gene product TRS1 in addition to IE1 and IE2. J. Virol. **66:**1050–1058.
31. **Stinski, M. F.** 1990. Cytomegalovirus and its replication, p. 1959–1980. *In* B. N. Fields et al. (ed.), Virology, 2nd ed. Raven Press, Ltd., New York.
32. **Stinski, M. F., D. R. Thomsen, R. M. Stenberg, and L. C. Goldstein.** 1983. Organization and expression of the immediate early genes of human cytomegalovirus. J. Virol. **46:**1–14.
33. **Tenney, D. J., and A. M. Colberg-Poley.** 1991. Expression of the human cytomegalovirus UL36-38 immediate early region during permissive infection. Virology **182:**199–210.

34. **van Santen, V. L.** 1991. Characterization of the bovine herpesvirus 4 major immediate-early transcript. J. Virol. **65:**5211–5224.
35. **Wagner, E. K.** 1991. Herpesvirus transcription-general aspects, p. 1–15. *In* E. K. Wagner (ed.), Herpesvirus transcription and its regulation. CRC Press, Boston.
36. **Watson, R. J., and J. B. Clements.** 1980. A herpes simplex virus type 1 function continuously required for early and late virus RNA synthesis. Nature (London) **285:**329–330.
37. **Wirth, U. V., B. Vogt, and M. Schwyzer.** 1991. The three major immediate-early transcripts of bovine herpesvirus 1 arise from two divergent and spliced transcription units. J. Virol. **65:**195–205.
38. **Yin, C.-Y., M. Gao, and H. C. Isom.** 1990. Guinea pig cytomegalovirus immediate-early transcription. J. Virol. **64:**1537–1548.
39. **Zhu, X., J. Chen, and S. Silverstein.** 1991. Isolation and characterization of a functional cDNA encoding ICP0 from herpes simplex virus type 1. J. Virol. **65:**957–960.