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Genomic Localization, Sequence Analysis, and Transcription of the Putative Human Cytomegalovirus DNA Polymerase Gene

REGINE HEILBRONN,[1]* GERHARD JAHN,[2] ALEXANDER BÜRKLE,[1] ULRICH-KARL FREESE,[1] BERNHARD FLECKENSTEIN,[2] AND HARALD ZUR HAUSEN[1]

Deutsches Krebsforschungszentrum, 6900 Heidelberg,[1] and Institut für Klinische Virologie der Universität Erlangen-Nürnberg, 8520 Erlangen,[2] Federal Republic of Germany

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The human cytomegalovirus (HCMV)-induced DNA polymerase has been well characterized biochemically and functionally, but its genomic location has not yet been assigned. To identify the coding sequence, cross-hybridization with the herpes simplex virus type 1 (HSV-1) polymerase gene was used, as suggested by the close similarity of the herpes group virus-induced DNA polymerases to the HCMV DNA polymerase. A cosmid and plasmid library of the entire HCMV genome was screened with the *Bam*HI Q fragment of HSV-1 at different stringency conditions. One *Pst*I-*Hinc*II restriction fragment of 850 base pairs mapping within the *Eco*RI M fragment of HCMV cross-hybridized at T_m − 25°C. Sequence analysis revealed one open reading frame spanning the entire sequence. The amino acid sequence showed a highly conserved domain of 133 amino acids shared with the HSV and putative Esptein-Barr virus polymerase sequences. This domain maps within the C-terminal part of the HSV polymerase gene, which has been suggested to contain part of the catalytic center of the enzyme. Transcription analysis revealed one 5.4-kilobase early transcript in the sense orientation with respect to the open reading frame identified. This transcript appears to code for the 140-kilodalton HCMV polymerase protein.

Human cytomegalovirus (HCMV), a member of the herpesvirus group, represents an important human pathogen. It not only is a leading cause of congenital malformations (36), but also establishes latency after initial infection with occasional reactivation (18, 35). Thus, it represents a major threat to patients immunocompromised as a consequence of allograft transplantation (32) or acquired immune deficiency syndrome (3).

Considerable effort has been put into the development of antiviral drugs which inhibit HCMV replication without affecting the host (23–25). Against herpes simplex virus (HSV) infections, several nucleoside analogs have been proven to be highly effective and selective. These drugs inhibit the HSV-encoded DNA polymerase after activation by the HSV-encoded thymidine kinase (9). Although the HCMV-induced DNA polymerase closely resembles the HSV-induced enzyme biochemically and functionally, the same drugs appear to be far less effective against the HCMV DNA polymerase, apparently due to the lack of an HCMV-specific thymidine kinase activity in infected cells (7, 10, 23, 40).

The HCMV-induced DNA polymerase was first described for HCMV-infected human fibroblasts by Huang in 1975 (15). It could be distinguished from host cell enzymes by chromatographic behavior, template primer specificity, and sedimentation behavior, but most notably by the requirement for 200 mM KCl or 100 mM ammonium sulfate for maximal activity (15, 28). The polymerase is inhibited by phosphonoacetic acid (16), aphidicolin, ara-ATP, and *N*-ethylmaleimide, but not by dideoxy-TTP. Thus, it behaves similarly to the HSV-1 and -2 DNA polymerases and cellular DNA polymerase alpha (28). On the other hand, the HCMV-induced enzyme is inhibited by various triphosphate nucleoside analogs to a greater extent than the cellular alpha polymerase (23, 24). Like the HSV-encoded enzyme, the HCMV polymerase has an associated 3′-to-5′ exonuclease activity (19, 28). The purified HCMV-induced enzyme consists of two polypeptides of 140 and 58 kilodaltons (kDa) in sodium dodecyl sulfate (SDS)-polyacrylamide gels, similar to the HSV enzyme, for which two proteins of 150 and 54 kDa copurify (24). For HSV-2 the polymerase activity has been assigned to the 150-kDa protein exclusively. The 58-kDa protein is encoded by a different gene (38).

Although the biochemical properties of the HCMV-induced polymerase have been characterized extensively, the genomic location has not yet been assigned. Our approach to identify the HCMV DNA polymerase gene made use of hybridization under reduced stringency with a probe of the HSV-1 polymerase gene, as suggested by the close similarity of the herpes group virus-induced enzymes to the HCMV-induced enzyme. With this approach, a region on the HCMV genome was identified whose amino acid sequence shows striking homology to the HSV DNA polymerase amino acid sequence. Transcription analysis revealed one 5.4-kilobase (kb) early transcript that appears to code for the 140-kDa polymerase protein.

MATERIALS AND METHODS

Recombinant DNA clones. Overlapping cosmid clones covering the entire HCMV (strain AD169) genome have been described before (11). Subclones of the *Eco*RI M fragment were cloned into pSP64/65 (26) by standard cloning procedures (6).

Radioactive labeling of DNA and RNA probes. Radioactive labeling of RNA probes was done with [α-^{32}P]GTP (Amersham and Buchler) as described previously (24). Nick translation of DNA with [α-^{32}P]dCTP was done as described

* Corresponding author.

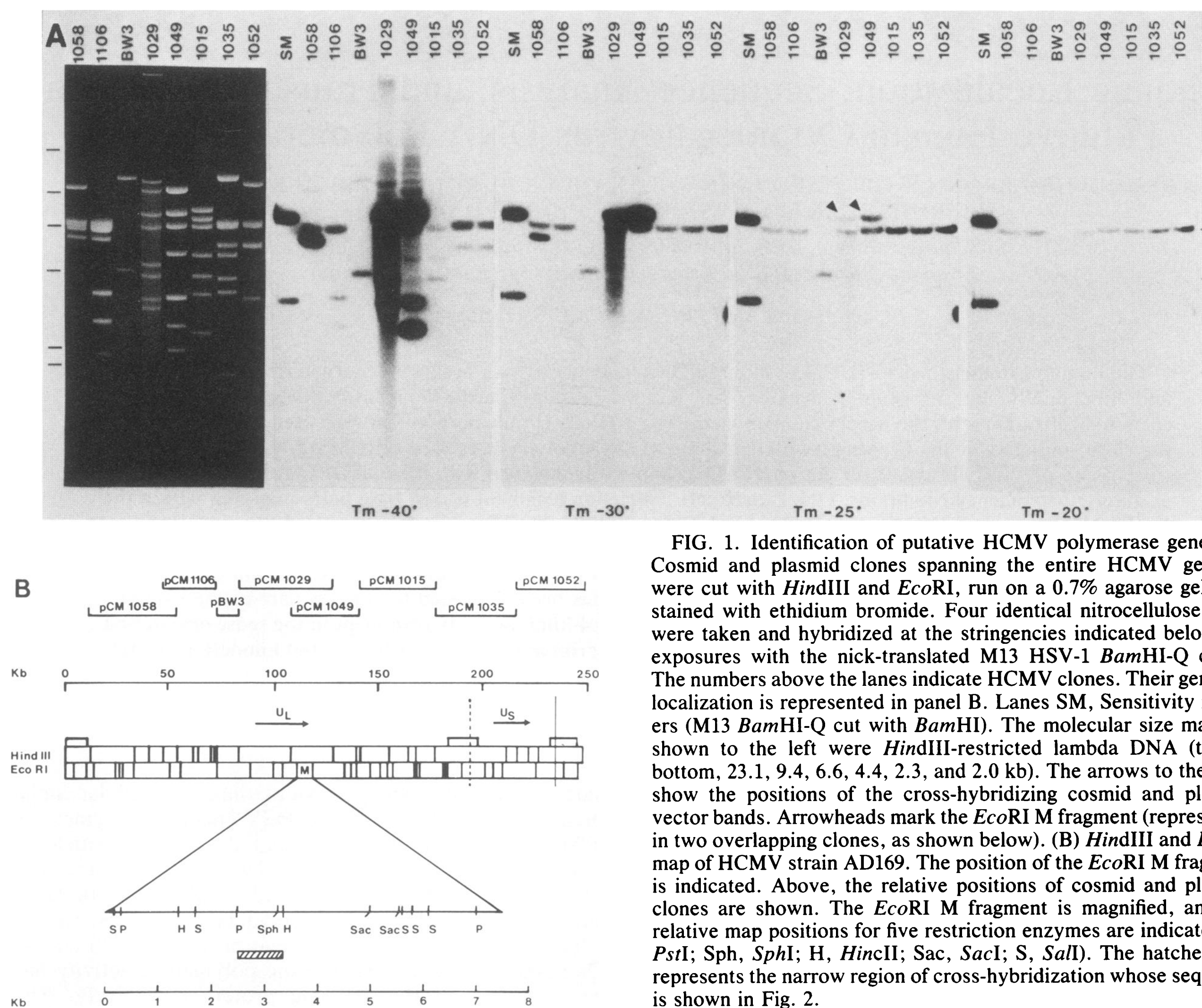

FIG. 1. Identification of putative HCMV polymerase gene. (A) Cosmid and plasmid clones spanning the entire HCMV genome were cut with *Hin*dIII and *Eco*RI, run on a 0.7% agarose gel, and stained with ethidium bromide. Four identical nitrocellulose blots were taken and hybridized at the stringencies indicated below the exposures with the nick-translated M13 HSV-1 *Bam*HI-Q clone. The numbers above the lanes indicate HCMV clones. Their genomic localization is represented in panel B. Lanes SM, Sensitivity markers (M13 *Bam*HI-Q cut with *Bam*HI). The molecular size markers shown to the left were *Hin*dIII-restricted lambda DNA (top to bottom, 23.1, 9.4, 6.6, 4.4, 2.3, and 2.0 kb). The arrows to the right show the positions of the cross-hybridizing cosmid and plasmid vector bands. Arrowheads mark the *Eco*RI M fragment (represented in two overlapping clones, as shown below). (B) *Hin*dIII and *Eco*RI map of HCMV strain AD169. The position of the *Eco*RI M fragment is indicated. Above, the relative positions of cosmid and plasmid clones are shown. The *Eco*RI M fragment is magnified, and the relative map positions for five restriction enzymes are indicated (P, *Pst*I; Sph, *Sph*I; H, *Hin*cII; Sac, *Sac*I; S, *Sal*I). The hatched box represents the narrow region of cross-hybridization whose sequence is shown in Fig. 2.

previously (33). The specific activity of the nick-translated probes was 10^8 cpm/μg of DNA.

Hybridization conditions for Southern blots. Southern blots were hybridized with nick-translated probes under conditions of various stringency as calculated from the formula given in Beltz et al. (2), assuming an average G+C content of 57% for HCMV (31). Filters were hybridized at 42°C overnight in solutions containing 0.1% Denhardt solution, 50 mM sodium phosphate buffer, pH 6.5, 100 μg of yeast tRNA per ml, 1 μg of poly(C) per ml, 0.1% SDS, and 52% formamide–3× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) (T_m − 20°C), 50% formamide–5× SSC (T_m − 25°C), 40% formamide–5× SSC (T_m − 30°C), or 30% formamide–3× SSC (T_m − 40°C). Filters were washed four times for 20 min each in 1× SSC–0.1% SDS at 72°C (T_m − 20°C), 2× SSC–0.1% SDS at 72°C (T_m − 25°C), 1× SSC–0.1% SDS at 62°C (T_m − 30°C), or 1× SSC–0.1% SDS at 52°C (T_m − 40°C).

DNA sequencing. DNA fragments were subcloned into M13mp8 and M13mp9 (27) and sequenced by the chain termination method (34).

RNA analysis. Propagation of HCMV (strain AD169) in human foreskin fibroblasts and the isolation of HCMV immediate-early, late, and noninfected control RNA have been described before (17). For the isolation of early RNA, cells were maintained in the presence of 100 μg of phosphonoformic acid per ml and harvested 24 h after infection. Polyadenylated [poly(A)$^+$]RNA selection was done by using poly(U)-coated messenger affinity paper (Medac, Hamburg, Federal Republic of Germany) as described previously (39). Samples (1 μg) of poly(A)$^+$ selected RNA were run in 1% formaldehyde–agarose gels and blotted onto nitrocellulose filters (Schleicher & Schuell, Dassel, Federal Republic of Germany or GeneScreen Plus (New England Nuclear Corp., Boston, Mass.). Northern blots were exclusively hybridized with SP6-transcribed RNA probes at 68°C in the presence of 50% formamide–5× SSC–0.1% Denhardt solution–50 mM sodium phosphate buffer (pH 6.5)–100 μg of yeast tRNA per ml–1 μg of poly(C) per ml and either 0.1% SDS for nitrocellulose blots or 1% SDS for GeneScreen Plus membranes.

RESULTS

Identification of the putative HCMV DNA polymerase gene. To identify the HCMV DNA polymerase gene, we searched the HCMV genome for cross-hybridization with a DNA probe from the HSV-1 DNA polymerase gene.

A

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  1 CTGCAGGACGCCGTATTGGTGCGCGATCTGTTCAACACCATTAATTTTCACTACGAGGCCGGGGCATCGCGCGGCTGGCTAAAATTCCGTTGCGGCGTGT
    LeuGlnAspAlaValLeuValArgAspLeuPheAsnThrIleAsnPheHisTyrGluAlaGlyAlaSerArgGlyTrpLeuLysPheArgCysGlyValSer

101 CATCTTTGACGGACAGCAGATCCGTATCTACACCTCGCTGCTGGACGAGTGCGCCTGCCGCGATTTTATCCTGCCCAACCACTACAGCAAAGGTACGACG
      SerLeuThrAspSerArgSerValSerThrProArgCysTrpThrSerAlaProAlaAlaIleLeuSerCysProThrThrThrAlaLysValArgArg

201 GTGCCCGAAACGAATAGCGTTGCTGTGTCACCTAACGCTGCTATCATCTCTACCGCCCGTGTGCCCGGCGACGCGGGTTCTGTGGCGGCTATGTTTCAGA
     CysProLysArgIleAlaLeuLeuCysHisLeuThrLeuLeuSerSerLeuProProValCysProAlaThrArgValLeuTrpArgLeuCysPheArg

301 TGTCGCCGCCCTTGCAATCTGCCGTCCAGTCAGGAGCCGGTTTCACCCGGCTCCGGCAGTAACAGTAGTAGCAGCGTCGGCGTTTTCAGCGTCGGCTCCG
    CysArgArgProCysAsnLeuProSerSerGlnGluProValSerProGlySerGlySerAsnSerSerSerSerValGlyValPheSerValGlySerGly

401 GCAGTAGTGGCGGCGTCGGCGTTTCCAACGACAATCACGGCGCCGGCGGTACTGCGGCGGTTTCGTACCAGGGCGCCACGGTGTTTGAGCCCGAGGTGGG
      SerSerGlyGlyValGlyValSerAsnAspAsnHisGlyAlaGlyGlyThrAlaAlaValSerTyrGlnGlyAlaThrValPheGluProGluValGly

501 TTACTACAACGACCCCGTGGCCGTGTTCGACTTTGCCAGCCTCTACCCTTCCATCATCATGGCCCACAACCTCTGCTACCTCACCCTGCTGGTGCCGGGT
     TyrTyrAsnAspProValAlaValPheAspPheAlaSerLeuTyrProSerIleIleMetAlaHisAsnLeuCysTyrLeuThrLeuLeuValProGly

601 GGCGAGTACCCTGTGGACCCCGCCGACGTATACAGCGTCACGCTAGAGAACGGCGTGACCCACCGCTTTGTGCGTGCTTCGGTGCGCGTCTCGGTGCTCT
    GlyGluTyrProValAspProAlaAspValTyrSerValThrLeuGluAsnGlyValThrHisArgPheValArgAlaSerValArgValSerValLeuSer

701 CGGAACTGCTCAACAAGTGGGTTTCGCAGCGGCGTGCCGTGCGCGAATGCATGCGCGAGTGTCAAGACCCTGTGCGCCGTATGCTGCTCGACAAGGAACA
      GluLeuLeuAsnLysTrpValSerGlnArgArgAlaValArgGluCysMetArgGluCysGlnAspProValArgArgMetLeuLeuAspLysGluGln

801 GATGGCGCTCAAAGTAACGTGCAACGCTTTCTACGGTTTTACCGGCGTGGTCGAC
     MetAlaLeuLysValThrCysAsnAlaPheTyrGlyPheThrGlyValValAsp
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B

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 1 VSYQGATVFEPEVGYYNDPVAVFDFASLYPSIIMAHNLCYLTLLVPGGEYPV-D--PADVYSVTLENGVT HCMV
 1 DGYQGATVIQPLSGFYNSPVLVVDFASLYPSIIQAHNLCYSTMITPGEEHRLAGLRPGEDYESFRLTGGV EBV
 1 VGYQGARVLDPTSGFHVNPVVVFDFASLYPSIIQAHNLCFSTLSLRADA--VAHLEAGKDYLEIEVGGRR HSV1
   ..****.*   *  .*...  ** *.**********.*****..*.    ..  .   ..  .  .. .*      *
   VGYQGATV   P  SGFYN  PV VFDFASLYPSIIQAHNLCYSTL    PG  E   VA  L  PG  DY      G    CONSENSUS

68 HRFVRASVRVSVLSELLNKWVSQRRAVRECMRECQDPVRRMLLDKEQMALKVTCNAFYGFTGV     HCMV
71 YHFVKKHVHESFLASLLTSWLAKRKAIKKLLAACEDPRQRTILDKQQLAIKCTCNAVYGFTGV     EBV
69 LFFVKAHVRESLLSILLRDWLAMRKQIRSRIPQS-SPEEAVLLDKQQAAIKVVCNSVYGFTGV     HSV1
     **...*..*  *.  **   *..  *....       .  .*   .  .***.*  *.*..**..******
     FVKAHVRES  LS  LL   WLA  RKAIR       C  DP   R  LLDKQQ  AIKVTCNAVYGFTGV     CONSENSUS
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FIG. 2. Sequence analysis of the HCMV fragment cross-hybridizing with the HSV-1 *Bam*HI-Q probe. (A) Nucleotide sequence of the 850-bp *Pst*I-*Hin*cII cross-hybridizing fragment (Fig. 1, hatched box) was determined by the dideoxy chain termination method in the two strands independently. The orientation is the same as in Fig. 1. The amino acid sequence of the only continuous open reading frame is indicated below the nucleotide sequence. (B) The amino acid sequence was compared with those coded by the EBV and HSV-1 polymerase sequences, and a stretch of about 130 highly conserved amino acids is shown (positions 460 to 855 on the HCMV sequence shown in panel A). Asterisks mark amino acids identical in all three sequences, and dots mark amino acids identical in two out of three, thus defining a consensus sequence for all three sequences.

A set of cosmid and plasmid clones spanning the entire genome of HCMV strain AD169 (11) was hybridized under conditions of different stringency with a 3.5-kb DNA fragment from the DNA polymerase gene of HSV-1 (*Bam*HI-Q, strain Angelotti; kindly provided by K.-W. Knopf) cloned into M13mp8. The HCMV cosmid and plasmid clones were cleaved with *Hin*dIII and *Eco*RI and run on a 0.7% agarose gel. Four identical blots on nitrocellulose filters were taken from the same gel. These blots were hybridized with the nick-translated M13 *Bam*HI-Q DNA at T_m − 20°C, − 25°C, − 30°C, and − 40°C. These hybridizations were done in the presence of poly(C) (1 μg/ml), as previous experiments had shown that nonspecific cross-hybridization at conditions of lower stringency, possibly due to the high G+C content of herpesviruses, could be significantly reduced by the hybridization competitor poly(C) (data not shown). The ethidium bromide-stained gel and the Southern blots hybridized at increasing stringency (T_m − 40°C to T_m − 20°C) are shown in Fig. 1A. The relative intensities of the bands at different stringencies reflect their relatedness to the hybridization probe (M13 *Bam*HI-Q), as the sensitivity marker, M13 *Bam*HI-Q cut with *Bam*HI, showed the same intensity in all exposures. The bands indicated by arrows on the right-hand side represent slight hybridization between the M13 probe and the cosmid and plasmid vector bands. The two bands indicated by arrowheads in the blot hybridized at T_m − 25°C

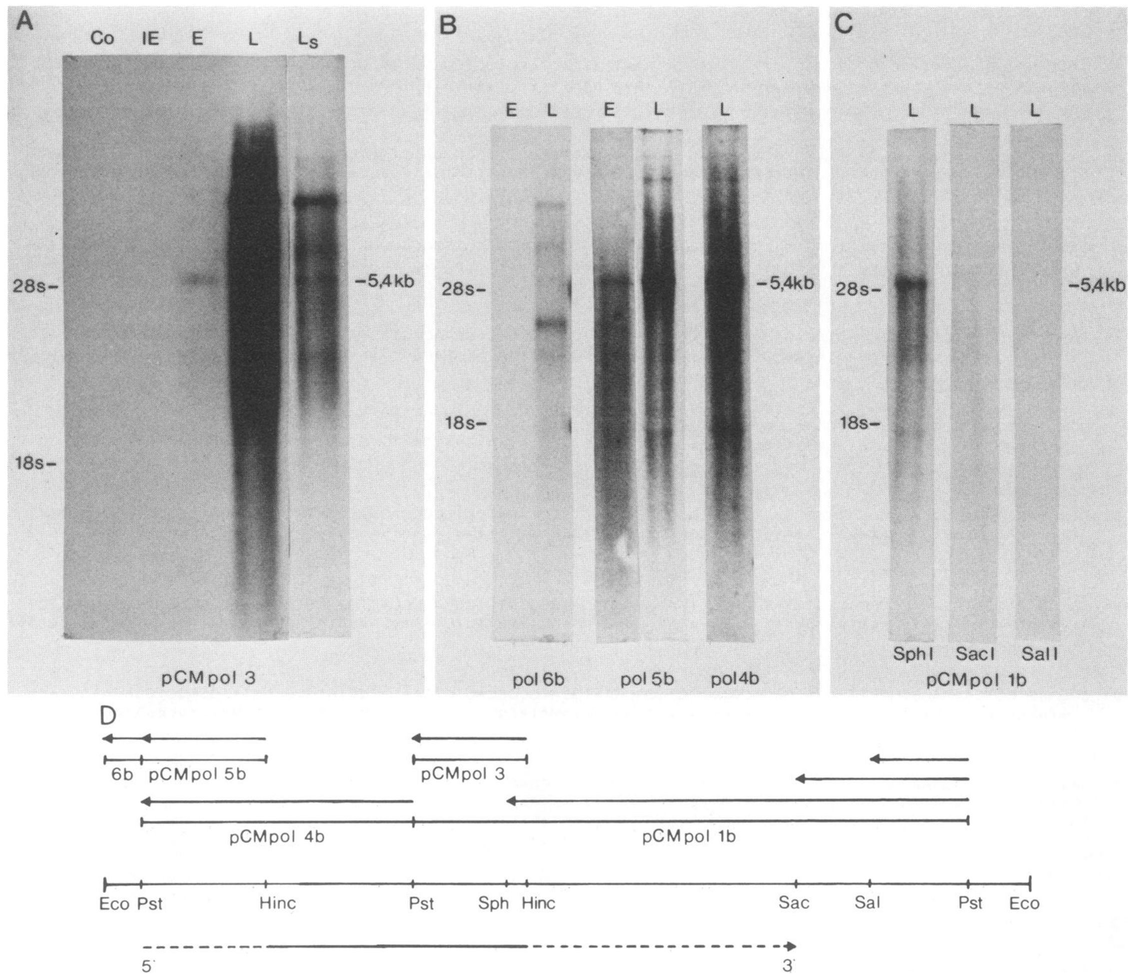

FIG. 3. Transcription analysis. Samples (1 μg) of poly(A)$^+$ RNA at different times after infection were run on 1% agarose–formaldehyde gels, blotted onto nitrocellulose filters or GeneScreen Plus, and hybridized with single-stranded RNA probes. The probes used are indicated below the lanes. (A) Identification of the 5.4-kb transcript in early RNA (probe: pCMpol3 antisense to the open reading frame identified in Fig. 2A). Lanes: Co, uninfected control RNA; IE, immediate-early RNA; E, early RNA; L, late RNA; L_S, same as L, but a shorter exposure. (B) Identification of the 5′ region of the 5.4-kb transcript (probes: pol6b, pCMpol6b; pol5b, pCMpol5b; pol4b, pCMpol4b). Lanes: E, early RNA; L, late RNA. (C) Identification of the 3′ region of the 5.4-kb transcript (probe: pCMpol1b linearized with either *Sal*I, *Sac*I, or *Sph*I). Lanes: L, late RNA. (D) Location of probes within the *Eco*RI M fragment. The SP6 clones are marked by vertical bars, and the transcripts generated from them are shown as arrows. Below the *Eco*RI M fragment, the polymerase transcript is shown; the 5′ and 3′ ends lie within the dashed areas.

mark the only HCMV fragment (*Eco*RI-M) that hybridized with the HSV-1 *Bam*HI-Q probe under relatively stringent conditions (the *Eco*RI M fragment is represented in two overlapping cosmid clones, as indicated in Fig. 1B). Even on the blot hybridized at T_m − 20°C this fragment can be seen faintly when overexposed. This indicates that the HSV-1 *Bam*HI Q fragment, derived from the DNA polymerase gene, and the HCMV *Eco*RI M fragment have considerable DNA sequence homology.

To further substantiate the significance of that cross-hybridization, we wanted to find out whether the HCMV *Eco*RI M fragment also hybridized with a DNA probe derived from the putative Epstein-Barr virus (EBV) polymerase gene (1). A subclone of the EBV DNA polymerase open reading frame comprising the *Bgl*II Q fragment (29) was used as the hybridization probe for a blot with clones spanning the HCMV genome. At T_m − 30°C, this EBV subclone reacted with the *Eco*RI M fragment exclusively (data not shown).

To characterize the cross-hybridizing region on the HCMV genome in more detail, the minimal hybridizing fragment was determined. The *Eco*RI M fragment was mapped with *Sal*I, *Hinc*II, *Sph*I, and *Sac*I (Fig. 1B). The *Pst*I sites are taken from the literature (13). Restriction digests of the *Eco*RI M fragment were hybridized with the HSV *Bam*HI-Q fragment at T_m − 25°C. The cross-hybridizing region of the HCMV genome could thus be reduced to an 850-base-pair (bp) *Pst*I-*Hinc*II fragment, indicated as a hatched box in Fig. 1B. The right-flanking fragment also showed some minor hybridization (data not shown).

Sequence analysis of the HCMV fragment cross-hybridizing with the HSV-1 *Bam*HI-Q probe. The cross-hybridizing 850-bp *Pst*I-*Hinc*II fragment was subcloned into M13mp8 and M13mp9 and sequenced by the dideoxy chain termination method (34). To confirm the sequence in the complementary strand, convenient subclones were sequenced as well so that the entire 850 bp were sequenced in both strands separately. The DNA sequence is shown in Fig. 2A. The orientation is the same as in Fig. 1, from the *Pst*I site to the

*Hinc*II site. There is one continuous open reading frame spanning the entire sequence. The amino acid sequence derived from it could be aligned to the published sequences of HSV-1 DNA polymerase (12, 30) and the putative EBV DNA polymerase (1) open reading frames with good homology and showed one region of about 130 amino acids highly conserved among the three viruses (Fig. 2B).

This highly conserved region corresponds to the part within the HSV polymerase gene where most of the drug resistance mutations have been mapped (12, 20). Thus, the structural similarity appears to reflect a conserved functional domain of the polymerase enzyme. This in turn makes it very likely that the HCMV fragment encodes part of the so far unidentified DNA polymerase gene.

Transcription analysis of the HCMV polymerase gene. The sequence analysis of the 850-bp *Pst*I-*Hinc*II fragment (Fig. 1) revealed one open reading frame spanning the entire sequence. To analyze the transcription of this and adjacent regions, fragments of the *Eco*RI M fragment were cloned into the SP6 transcription vectors pSP64 and pSP65 for the preparation of highly sensitive ^{32}P-labeled single-stranded RNA probes. The subclones used are shown in Fig. 3D, and the ^{32}P-labeled RNA transcripts are indicated above the clones as arrows. The transcription of pCMpol3 (linearized with *Pst*I) generated an antisense RNA with respect to the open reading frame shown in Fig. 2A. As shown in Fig. 3A, this probe recognized a single transcript of 5.4 kb in a Northern analysis of 1 μg of poly(A)$^+$ HCMV early RNA. Immediate-early RNA and RNA from noninfected cells did not shown any hybridization. At late times after infection, the respective transcript was still visible, apparently at a relatively high level. It could only been distinguished from the background in a short exposure. There were additional transcripts in this region at late times after infection whose functions are unknown.

To characterize the 5′ and 3′ regions of the transcript, Northern blots of early and late RNAs were hybridized with SP6-transcribed antisense probes from neighboring clones. In Fig. 3B, the 5′ region was characterized by hydridizations of parallel blots with [^{32}P]RNA probes generated from pCMpol6b, -5b, and -4b. The latter two recognized a 5.4-kb transcript, whereas pCMpol6b did not. Therefore, the 5′ end of the transcript must lie within the 1.1-kb *Pst*I-*Hinc*II fragment contained in pCMpol5b. Splicing to regions outside the *Eco*RI M fragment cannot be excluded. Figure 3C shows the characterization of the region where the 3′ end of the transcript mapped. One single Northern blot was hybridized first with pCMpol1b (linearized with *Sal*I), then with pCMpol1b (linearized with *Sac*I), and last with pCMpol1b (linearized with *Sph*I). Only then was the 5.4-kb transcript found. Thus, the 3′ end of the transcript maps 5′ to the *Sac*I site, provided that splicing to regions beyond the *Eco*RI M fragment does not take place.

DISCUSSION

The *Eco*RI M fragment has been identified by hybridization with the *Bam*HI Q fragment derived from the HSV-1 DNA polymerase gene at $T_m - 25°C$ as a candidate for the HCMV DNA polymerase gene. Sequence analysis of an 850-bp subfragment to which the cross-hybridization could be confined revealed one continuous open reading frame. The amino acid sequence shows a highly conserved domain of 133 amino acids shared with the HSV and putative EBV polymerase sequences. The domain lies within the 3′ half of the HSV polymerase gene. This is the region where most of the temperature-sensitive and drug resistance mutations of HSV map (4, 5, 12, 20). The C-terminal portion of the HSV polymerase has been proposed to contain the deoxynucleoside triphosphate-, PP$_i$-, and aphidicolin-binding sites, thus probably representing part of the polymerase catalytic site (12, 20). Exactly the same region showed the most pronounced homology with the amino acid sequences of the putative EBV and HCMV polymerases. Since both enzymes are sensitive to phosphonoacetic acid, aphidicolin, and the triphosphate derivative of the nucleoside analog acycloguanosine (8, 16, 22, 23, 25), as is the HSV-encoded enzyme (20, 37), it seems reasonable to correlate their similarity in drug sensitivity to their conserved structure.

In the course of sequencing the entire HCMV genome, a 3.7-kb open reading frame within the *Eco*RI M fragment has been identified that can be aligned to the HSV-1 and putative EBV polymerase sequences (21). The 850-bp sequence described here is located in the 3′ half of the 3.7-kb open reading frame and is identical to the sequence described by Kouzarides et al. (21). The size of 3.7 kb corresponds to that of the HSV-1 and putative EBV polymerase open reading frames.

The mRNA coding for an HCMV polymerase should meet certain requirements: (i) minimal size to encode a 140-kDa protein (approximately 4 kb); (ii) expression before virus replication at early times after infection, as it has been shown that the HCMV-induced DNA polymerase is essential for virus replication; and (iii) sense orientation of the transcript with respect to the open reading frame that is highly homologous to that of the HSV-1 and putative EBV polymerase open reading frames.

The 4.2- and 4.3-kb early transcripts within the polymerase gene of HSV-1 (14) meet these requirements. We report here the identification of a 5.4-kb HCMV early transcript in sense orientation with respect to the 3.7-kb HCMV open reading frame. The 5′ and 3′ ends of the transcript map beyond the limits of this open reading frame, although splicing to sequences outside the *Eco*RI M fragment cannot be excluded. Constitutive expression of the open reading frame will make possible functional testing of the sequence identified.

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LITERATURE CITED

1. **Baer, R., A. T. Bankier, M. D. Biggin, P. L. Deininger, P. J. Farrell, T. J. Gibson, G. Hatfull, G. S. Hudson, S. C. Satchwell, C. Séguin, P. S. Tuffnell, and B. G. Barrell.** 1984. DNA sequence and expression of the B95-8 Epstein-Barr virus genome. Nature (London) **310:**207–211.
2. **Beltz, G. A., K. A. Jacobs, T. H. Eickbush, P. T. Cherbas, and F. C. Kafatos.** 1983. Isolation of multigene families and determination of homologies by filter hybridization methods. Methods Enzymol. **100:**266–285.
3. **Centers for Disease Control.** 1986. Update: acquired immunodeficiency syndrome—United States. Morbid. Mortal. Weekly Rep. **35:**17–21.

4. **Chartrand, P., C. S. Crumpacker, P. A. Schaffer, and N. M. Wilkie.** 1980. Physical and genetic analysis of the herpes simplex virus DNA polymerase locus. Virology **103**:311–326.
5. **Chartrand, P., N. D. Stow, M. C. Timbury, and N. M. Wilkie.** 1979. Physical mapping of *paa*ʳ mutations of herpes simplex virus type 1 and type 2 by intertypic marker rescue. J. Virol. **31**:265–276.
6. **Crouse, G. F., A. Frischauf, and H. Lehrach.** 1983. An integrated and simplified approach to cloning into plasmids and single-stranded phages. Methods Enzymol. **101**:78–89.
7. **Crumpacker, C. S., L. E. Schnipper, J. A. Zaia, and M. J. Levin.** 1979. Growth inhibition by acyloguanosine of herpesviruses isolated from human infection. Antimicrob. Agents Chemother. **15**:642–645.
8. **Datta, A. K., J. Feighny, and J. S. Pagano.** 1980. Induction of Epstein-Barr virus associated DNA polymerase by 12-O-tetradecanoylphorbol-13-acetate. J. Biol. Chem. **255**:5120–5125.
9. **Elion, G. B., P. A. Furman, J. A. Fyfe, P. de Miranda, L. Beauchamp, and H. J. Schaeffer.** 1977. Selectivity of action of an antiherpetic agent, 9-(2-hydroxyethoxymethyl)guanine. Proc. Natl. Acad. Sci. USA **74**:5716–5720.
10. **Estes, J., and E.-S. Huang.** 1977. Stimulation of cellular thymidine kinase by human cytomegalovirus. J. Virol. **24**:13–21.
11. **Fleckenstein, B., I. Müller, and J. Collins.** 1982. Cloning of the complete human cytomegalovirus genome in cosmids. Gene **18**:39–46.
12. **Gibbs, J. S., H. C. Chion, J. D. Hall, D. W. Mount, M. J. Retondo, S. K. Weller, and D. M. Coen.** 1985. Sequence and mapping analyses of the herpes simplex virus DNA polymerase gene predict a C-terminal substrate binding domain. Proc. Natl. Acad. Sci. USA **82**:7969–7973.
13. **Greenaway, P. J., J. D. Oram, R. G. Downing, and K. Patel.** 1982. Human cytomegalovirus DNA: *Bam* HI, *Eco* RI and *Pst* I restriction cleavage maps. Gene **18**:355–360.
14. **Holland, L. E., R. M. Sandri-Goldin, A. L. Goldin, J. C. Glorioso, and M. Levine.** 1984. Transcriptional and genetic analyses of the herpes simplex virus type 1 genome: coordinates 0.29 to 0.45. J. Virol. **49**:947–959.
15. **Huang, E.-S.** 1975. Human cytomegalovirus. III. Virus-induced DNA polymerase. J. Virol. **16**:298–310.
16. **Huang, E.-S.** 1975. Human cytomegalovirus. IV. Specific inhibition of virus-induced DNA polymerase activity and viral DNA replication by phosphonoacetic acid. J. Virol. **16**:1560–1565.
17. **Jahn, G., E. Knust, H. Schmolla, T. Sarre, J. A. Nelson, J. McDougall, and B. Fleckenstein.** 1984. Predominant immediate-early transcripts of human cytomegalovirus AD169. J. Virol. **49**:363–370.
18. **Jordan, M. C.** 1983. Latent infection and the elusive cytomegalovirus. Rev. Infect. Dis. **5**:205–215.
19. **Knopf, K.-W.** 1979. Properties of the herpes simplex virus DNA polymerase and characterization of its associated exonuclease activity. Eur. J. Biochem. **98**:231–244.
20. **Knopf, K.-W., E. R. Kaufman, and C. Crumpacker.** 1981. Physical mapping of drug resistance mutations defines an active center of the herpes simplex virus DNA polymerase enzyme. J. Virol. **39**:746–757.
21. **Kouzarides, T., A. T. Bankier, S. C. Satchwell, K. Weston, P. Tomlinson, and B. G. Barrell.** 1987. Sequence and transcription analysis of the human cytomegalovirus DNA polymerase gene. J. Virol. **61**:125–133.
22. **Kullin, B., L. Sternas, A. K. Saemundssen, J. Luka, H. Jornvall, B. Erikkson, P.-Z. Tao, M. T. Nilsson, and G. Klein.** 1985. Purification of Epstein-Barr virus DNA polymerase from P3HR-1 cells. J. Virol. **54**:561–568.
23. **Mar, E.-C., J.-F. Chiou, Y.-J. Cheng, and E.-S. Huang.** 1985. Inhibition of cellular polymerase alpha and human cytomegalovirus-induced DNA polymerase by the triphosphates of 9-(2-hydroxyethoxymethyl)guanine and 9-(1,3-dihydroxy-2-propoxymethyl)guanine. J. Virol. **53**:776–780.
24. **Mar, E.-C., J.-F. Chiou, Y.-C. Cheng, and E.-S. Huang.** 1985. Human cytomegalovirus-induced DNA polymerase and its interaction with the triphosphates of 1-(2′-deoxy-2′-fluoro-β-D-arabinofuranosyl)-5-methyluracil, -5-iodocytosine, and -5-methylcytosine. J. Virol. **56**:846–851.
25. **Mar, E.-C., P. C. Patel, Y.-C. Cheng, J. J. Fox, K. A. Watanabe, and E.-S. Huang.** 1984. Effects of certain nucleoside analogues on human cytomegalovirus. J. Gen. Virol. **65**:47–53.
26. **Melton, D. A., P. A. Krieg, M. R. Rebagliati, T. Maniatis, K. Zinn, and M. R. Green.** 1984. Efficient in vitro synthesis of biologically active RNA and RNA hybridization probes from plasmids containing a bacteriophage SP6 promoter. Nucleic Acids Res. **12**:7035–7056.
27. **Messing, J., and J. Vieira.** 1982. A new pair of M13 vectors for selecting either DNA strand of double-digest restriction fragments. Gene **19**:269–276.
28. **Nishiyama, Y., M. Koichiro, and S. Yoshida.** 1983. Characterization of human cytomegalovirus-induced DNA polymerase and the associated 3′-to-5′ exonuclease. Virology **124**:221–231.
29. **Polack, A., G. Hartl, U. Zimber, U.-K. Freese, G. Laux, K. Takaki, B. Hohn, L. Gissmann, and G. W. Bornkamm.** 1984. A complete set of overlapping cosmid clones of M-ABA virus derived from nasopharyngeal carcinoma and its similarities to other Epstein-Barr virus isolates. Gene **27**:279–288.
30. **Quinn, J. P., and D. J. McGeoch.** 1985. DNA sequence of the region in the genome of herpes simplex virus type 1 containing the genes for DNA polymerase and the major DNA binding protein. Nucleic Acids Res. **13**:8143–8163.
31. **Rapp, F.** 1983. The biology of cytomegaloviruses, p. 1–53. *In* B. Roizman (ed.), The herpesviruses, vol. 2. Plenum Publishing Corp., New York.
32. **Richardson, W. P., R. B. Colvin, S. H. Cheeseman, N. E. Tolkoff-Rubin, J. T. Herrin, A. B. Cosimi, A. B. Collins, M. S. Hirsch, R. T. McCluskey, P. S. Russell, and R. H. Rubin.** 1981. Glomerulopathy associated with cytomegalovirus viremia in renal allografts. N. Engl. J. Med. **305**:57–62.
33. **Rigby, P. W. J., M. Dieckmann, C. Rhodes, and P. Berg.** 1977. Labelling of deoxyribonucleic acid to high specific activity *in vitro* by nick translation with DNA polymerase I. J. Mol. Biol. **113**:237–251.
34. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.
35. **Schrier, R. D., J. A. Nelson, and M. B. A. Oldstone.** 1985. Detection of human cytomegalovirus in peripheral blood lymphocytes in a natural infection. Science **230**:1048–1051.
36. **Stagno, S., R. F. Pass, M. E. Dworsky, R. E. Henderson, E. G. Moore, P. D. Walton, and C. A. Alford.** 1982. Congenital cytomegalovirus infection: the relative importance of primary and recurrent maternal infection. N. Engl. J. Med. **306**:945–949.
37. **St. Clair, M. H., P. A. Furman, C. M. Lubbers, and G. B. Elion.** 1980. Inhibition of cellular alpha and virally induced deoxyribonucleic acid polymerases by the triphosphate of acylovir. Antimicrob. Agents Chemother. **18**:741–745.
38. **Vaughan, P. J., D. P. J. Purifoy, and K. L. Powell.** 1985. DNA-binding protein associated with herpes simplex virus DNA polymerase. J. Virol. **53**:501–508.
39. **Werner, D., Y. Chemla, and M. Herzberg.** 1984. Isolation of poly(A)$^+$ RNA by paper affinity chromatography. Anal. Biochem. **141**:329–336.
40. **Zavada, V., V. Erban, D. Rezacova, and V. Vonka.** 1976. Thymidine kinase in cytomegalovirus infected cells. Arch. Virol. **52**:333–339.