JOURNAL OF VIROLOGY, Mar. 1986, p. 802–808
0022-538X/86/030802-07$02.00/0
Copyright © 1986, American Society for Microbiology
Vol. 57, No. 3

Herpes Simplex Virus Specifies Two Subunits of Ribonucleotide Reductase Encoded by 3′-Coterminal Transcripts

MARGARET A. SWAIN AND DENISE A. GALLOWAY*
Fred Hutchinson Cancer Research Center, Seattle, Washington 98104

Received 25 September 1985/Accepted 5 December 1985

We have previously described a transcription unit located between map coordinates 0.558 and 0.595 on the herpes simplex virus type 2 strain 333 genome which encodes two mRNAs of 5.0 and 1.2 kilobases that share a common 3′ terminus, and we have determined the nucleotide sequence of a 38,000-dalton protein specified by the smaller RNA (D. A. Galloway and M. A. Swain, J. Virol. 49:724–730, 1984). The entire nucleotide sequence of the 140,000-dalton protein specified by a 3,432-base-pair open reading frame within the large mRNA is presented, as are transcriptional regulatory sequences upstream of the RNA. The 140,000-dalton protein shows strong homology with the large subunit of well-characterized ribonucleotide reductase enzymes from the mouse and from *Escherichia coli* and with an Epstein-Barr virus gene. The 38,000-dalton protein has been shown previously to have homology with the small subunit of these enzymes (B.-M. Sjoberg, H. Eklund, J. A. Fuchs, J. Carlson, N. M. Standart, J. V. Ruderman, S. J. Bray, and T. Hunt, FEBS Lett. 183:99–102, 1985). This is the first example of a herpesvirus transcriptional unit that encodes functionally related proteins.

The herpesviruses are large DNA viruses which encode a number of enzymes required for their biosynthesis, e.g., DNA polymerase and thymidine kinase. Upon infection, herpes simplex virus types 1 and 2 (HSV-1 [8] and HSV-2 [9]), equine herpesvirus type 1 (10), Epstein-Barr virus (EBV) (21), and pseudorabies virus (26) all induce a ribonucleotide reductase activity which is distinct from that of the cellular enzyme. Ribonucleotide reductase is an essential component of living cells, providing the precursors for DNA synthesis by catalyzing the reduction of all four ribonucleotides to their respective deoxyribonucleotides (38). In many cases the enzyme is encoded by two nonidentical subunits (33).

Evidence that ribonucleotide reductase activity is virally encoded has come from three sources. First, an HSV-1 strain 17 temperature-sensitive (*ts*) mutant failed to induce reductase activity at the nonpermissive temperature (12), and the mutation in *ts*1207 was mapped to sequences encoding a polypeptide referred to as Vmw136 (32). This protein, also referred to as ICP6 (22) and 140K (1) has been shown to be encoded by a 5.0-kilobase (kb) message located between map coordinates 0.558 and 0.595 (1, 28, 29). Analysis of the HSV-2 genome has revealed a colinear organization including a protein of 140,000 (140K) daltons (15) that is referred to in other studies as ICP10 (31) and 144K (23). A comparison of the DNA sequence encoding the carboxy termini of the HSV-1 and HSV-2 140K proteins has suggested that these proteins are homologous (17, 30).

The second indication that the reductase activity is virally encoded comes from studies which characterize the virally induced enzyme and use antibodies directed against viral proteins to inhibit enzymatic activity. Ribonucleotide reductase from HSV-2-infected cells was purified and used to produce antibodies which reacted primarily with a 144K protein and other minor components (24). By using monoclonal antibodies which had previously been shown to precipitate hybrid-selected, in vitro-translated proteins (15), it was possible to demonstrate that the 144K and 38K proteins associated with reductase activity were equivalent to the 140K and 38K proteins mapping to 0.558 to 0.595 on the HSV genome (4). By using monoclonal antibodies directed against Vmw136 and an oligopeptide serum directed against Vmw38, there is evidence that the HSV-1 proteins form a complex to function as the large and small subunits of the HSV-induced enzyme (13).

The evidence that the 38K protein is a subunit of ribonucleotide reductase comes from an analysis of the DNA sequence of the HSV-1 and HSV-2-encoded 38K proteins. Both the HSV-1 (11) and HSV-2 (17, 30) 38K proteins have been sequenced, and a comparison of the two sequences has revealed that the carboxy-terminal 307 amino acids are highly homologous and only the amino-terminal 30 amino acids show significant intertypic diversity. Recently, the small subunit of *Escherichia coli* reductase was compared with the HSV-2 38K protein, and they were found to be distantly related (16%) when comparing identical amino acids (36). This analysis revealed a related protein in EBV and in the clam *Spisula solidissima* and showed that in all four proteins a few specific regions, presumed to be of functional importance, showed striking similarities.

In this study we completed the nucleotide sequence of the region of HSV-2 encoding the 140K protein and its upstream regulatory sequences. By computer-assisted analysis we compared the amino acid sequence of the 140K protein with the large subunit of the *E. coli* (7) and mouse (6) ribonucleotide reductases and found significant homology among these proteins and with a gene from EBV. In addition we compared the HSV-2 140K protein with the sequence available (29) for the HSV-1 140K homolog and found that the carboxy terminus is far better conserved than the amino terminus.

MATERIALS AND METHODS

Construction of recombinant M13 bacteriophage. The pBR322 recombinant plasmid pBamE containing the *Bam*HI E fragment (0.532 to 0.583 map units) of HSV-2 strain 333 was digested with either *Sal*I or *Pst*I, or in one case with *Pst*I-*Eco*RI-*Hpa*I. Specific fragments were purified from low-melting-point agarose and were ligated to the appropri-

* Corresponding author.

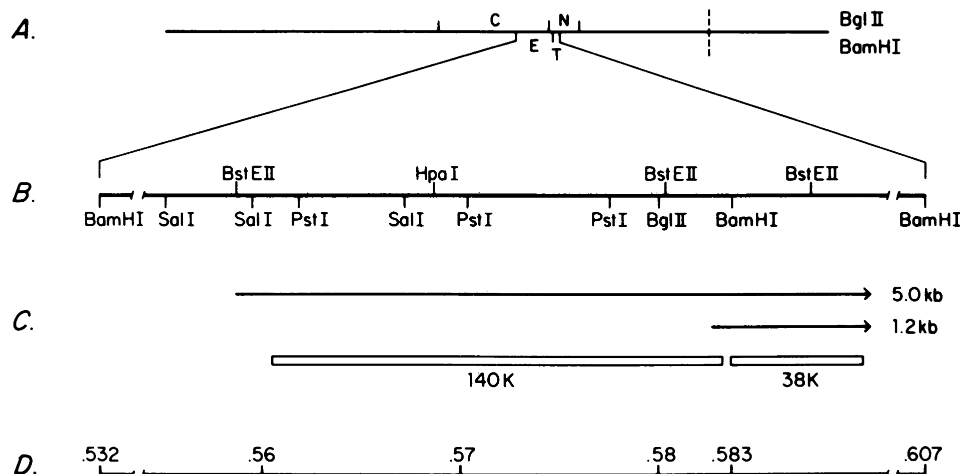

FIG. 1. Organization of the region of the HSV-2 genome encoding ribonucleotide reductase. (A) Location of these genes on the HSV-2 genome relative to the *BglII* C and N fragments and the *BamHI* E and T fragments. (B) An expansion of this region showing relevant restriction enzyme sites. (C) Positions of the mRNAs (arrows) and the proteins (boxes) they encode. (D) Map coordinants along the HSV-2 genome.

ately cleaved vectors M13mp11 or mp19. The M13 recombinant phage constructed in this way contained a 2.75-kb *SalI* fragment (0.567 map units to the *SalI* site in the pBR322 portion of pBamE), a 1.15-kb *SalI* fragment (0.559 to 0.567 map units), a 0.65-kb *SalI* fragment (0.555 to 0.559 map units), a 1.29-kb *PstI* fragment (0.562 to 0.57 map units), and a 1.3-kb *PstI* fragment (0.553 to 0.562 map units). The identities and orientations of these clones were confirmed by sizing and restriction analysis of M13 replicative form DNA on agarose gels and by hybridization of M13 single-strand DNAs to each other to establish pairs. The replicative form DNAs of M13 recombinant phages containing HSV-2 DNA were linearized with pairs of enzymes (*BamHI-SstI* or *BamHI-KpnI* or *XbaI-SstI*) and deleted subclones obtained by the method of Henikoff (20) as previously described (37). Selection and growth of recombinant phage and preparation of phage DNA were done by standard techniques (35). Recombinants were characterized by T-track analysis (2) before running full sequencing reactions.

DNA sequencing methods and analysis. Dideoxynucleoside triphosphate chain termination sequencing methods (34) were performed with M13 recombinant phage DNA templates as previously described (37). DNA sequence data were obtained from both strands of a 4.0-kb region and were compiled and analyzed on a DEC Rainbow computer with the Genepro software written by J. Brown and J. Wallace. Homology searches on DNA and amino acid sequences used the method of Wilbur and Lipman (40).

RESULTS

To obtain the complete nucleotide sequence of the segment of DNA encoding the HSV-2 140K protein, we sequenced the region from the *SalI* site (map coordinate 0.555) to the *BamHI* site of the *BamHI* E-T border (map coordinate 0.583). The overall strategy was to clone specific restriction fragments into appropriate M13 vectors. In all cases, two sets of fragments were used to obtain data spanning each restriction site. To provide templates of suitable length for sequencing, the replicative form of the recombinant M13 phage was purified, cleaved at two sites within the polylinker to leave a 5′ extension proximal to the insert and a distal 3′ extension, treated with exonuclease III for various intervals, and treated with S1 nuclease, Klenow polymerase, and T4 DNA ligase by the method of Henikoff (20). The details of the recombinant plasmids used and the construction of the M13 phage is given in Materials and Methods. We had previously sequenced the segment from the *BglII* (0.58) to the *BamHI* (0.583) site which contains the start of translation of the 38K protein and the start of transcription of the 1.2-kb message, and we tentatively identified the termination of translation and the carboxy-terminal 163 amino acids of the 140K protein (17). This region, including the relevant restriction enzyme sites, the location of the 5.0- and 1.2-kb RNAs, and the location of the coding sequences for the 140K and 38K proteins, is summarized in Fig. 1.

The nucleotide sequence of the noncoding strand of the HSV-2 140K gene is shown in Fig. 2. An open reading frame of 3,432 bp is seen (from nucleotide 419 to 3850) which gives rise to a protein of 1,144 amino acids. The translated protein predicted from the DNA sequence is shown above the appropriate nucleotides. The open reading frame is consistent with our previously published assignment of the carboxy terminus of the protein (17) and confirms an interesting organization of the sequences encoding the 38K and 140K proteins. The start of transcription for the 1.2-kb message has been mapped to nucleotide 3769 (30) which would place it and 80 nucleotides of the 1.2-kb RNA leader sequence within the translated portion of the 140K protein.

The start of transcription of the HSV-1 5.0-kb RNA has been mapped to nucleotide 176 (14). Upstream (nucleotides 148 through 153) is the canonical TATA homology ATAAAA. A comparison of these transcriptional regulatory sequences with those of HSV-1 strain KOS (11) show both regions of strong homology and regions with little or no homology. Of the 50 nucleotides surrounding TATA (123 through 173), 42 are identical whereas the next 36 nucleotides upstream (87 through 122) show no intertypic homology. This is followed by a 22-bp sequence (66 through 87) in which 20 nucleotides are identical between HSV-1 and HSV-2. The 22-bp conserved sequence is A-C rich, a feature shared with many other HSV early promoters (39). In the untranslated leader sequence there are regions of

```
              10         20         30         40         50         60         70         80         90
   1  GTGTGTTTGGCGTGTGTCTCTGAAATGGCGGAAACCGACATGCAAATGGGATTCATGGACACGTTACACCCCCCTGACTCAGGAGATAGGCATATCCTCC

 101  TTAGATTGACTCAGCACACGATCGCACCCCACCCCTGTGTGCCGGGGATAAAAGCCAACGCGGGCGGTCTGGGTTACCACAACAGGTGGGTGCTTCGGGG

 201  ACTTGACGGTCGCCACTCTCCTGCGAGCCCTCACGTCTTCGCCCACCGATTCCTGTTGCGTTCCTGTCGGCCGGTGCTGTCCTGTCGACAGATTGTTGGC

 301  GACTGCCCGGGTGATTCGTCGGCCGGTGCGTCCTTTCGGTCGTACCGCCCACCCCGCCTCCCACGGGCCCGCCGCTGTTTCCGTTCATCGCGTCCGAGCC

                         MetAlaAsnArgProAlaAlaSerAlaLeuAlaGlyAlaArgSerProSerGluArgGlnGluProArgGluProGluValA
 401  ACCGTCACCTTGGTTCCAATGGCCAACCGCCCTGCCGCATCCGCCCTCGCCGGAGCGCGGTCTCCGTCCGAACGACAGGAACCCCGGGAGCCCGAGGTCG

      laProProGlyGlyAspHisValPheCysArgLysValSerGlyValMetValLeuSerSerAspProProGlyProAlaAlaTyrArgIluSerAspSe
 501  CCCCCCCTGGCGGCGACCACGTGTTTTGCAGGAAAGTCAGCGGCGTGATGGTGCTTTCCAGCGATCCCCCCGGCCCCGCGGCCTACCGCATTAGCGACAG

      rSerPheValGlnCysGlySerAsnCysSerMetIluIluAspGlyAspValAlaArgGlyHisLeuArgAspLeuGluGlyAlaThrSerThrGlyAla
 601  CAGCTTTGTTCAATGCGGCTCCAACTGCAGTATGATAATCGACGGAGACGTGGCGCGCGGTCATTTGCGTGACCTCGAGGGCGCTACGTCCACCGGCGCC

      PheValAlaIluSerAsnValAlaAlaGlyGlyAspGlyArgThrAlaValValAlaLeuGlyGlyThrSerGlyProSerAlaThrThrSerValGlyT
 701  TTCGTCGCGATCTCAAACGTCGCAGCCGGCGGGGATGGCCGAACCGCCGTCGTGGCGCTCGGCGGAACCTCGGGCCCGTCCGCGACTACATCCGTGGGGA

      hrGlnThrSerGlyGluPheLeuHisGlyAsnProArgThrProGluProGlnGlyProGlnAlaValProProProProProProProPheProTrpGl
 801  CCCAGACGTCCGGGGAGTTCCTCCACGGGAACCCAAGGACCCCCGAACCCCAAGGACCCCAGGCTGTCCCCCCGCCCCCTCCTCCCCCCTTTCCATGGGG

      yHisGluCysCysAlaArgArgAspAlaArgGlyGlyAlaGluLysAspValGlyAlaAlaGluSerTrpSerAspGlyProSerSerAspSerGluThr
 901  CCACGAGTGCTGCGCCCGTCGCGATGCCAGGGGCGGCGCCGAGAAGGACGTCGGGGCCGCGGAGTCATGGTCAGACGGCCCGTCGTCCGACTCCGAAACG

      GluAspSerAspSerSerAspGluAspThrGlySerGlySerGluThrLeuSerArgSerSerSerIluTrpAlaAlaGlyAlaThrAspAspAspAspS
1001  GAGGACTCGGACTCCTCGGACGAGGATACGGGCTCGGGTTCGGAGACGCTGTCTCGATCCTCTTCGATCTGGGCCGCAGGGGCGACTGACGACGATGACA

      erAspSerAspSerArgSerAspAspSerValGlnProAspValValValArgArgArgTrpSerAspGlyProAlaProValAlaPheProLysProAr
1101  GCGACTCCGACTCGCGGTCGGACGACTCCGTGCAGCCCGACGTTGTCGTTCGTCGCAGATGGAGCGACGGCCCTGCCCCCGTGGCCTTTCCCAAGCCCCG

      gArgProGlyAspSerProGlyAsnProGlyLeuGlyAlaGlyThrGlyProGlySerAlaThrAspProArgAlaSerAlaAspSerAspSerAlaAla
1201  GCGCCCCGGCGACTCCCCCGGAAACCCCGGCCTGGGCGCCGGCACCGGGCCGGGCTCCGCGACGGACCCGCGCGCGTCGGCCGACTCCGATTCCGCGGCC

      HisAlaAlaAlaProGlnAlaAspValAlaProValLeuAspSerGlnProThrValGlyThrAspProGlyTyrProValProLeuGluLeuThrProG
1301  CACGCCGCCGCACCCCAGGCGGACGTGGCGCCGGTTCTGGACAGCCAGCCCACTGTGGGAACGGACCCCGGCTACCCAGTCCCCCTAGAACTCACGCCCG

      luAsnAlaGluAlaValAlaArgPheLeuGlyAspAlaValAspArgGluProAlaLeuMetLeuGluTyrPheCysArgCysAlaArgGluGluSerLy
1401  AGAACGCGGAGGCGGTGGCGCGGTTTCTGGGGGACGCCGTCGACCGCGAGCCCGCGCTCATGCTGGAGTACTTCTGTCGGTGCGCCCGCGAGGAGAGCAA

      sArgValProProArgThrPheGlySerAlaProArgLeuThrGluAspAspPheGlyLeuLeuAsnTyrAlaLeuAlaGluMetArgArgLeuCysLeu
1501  GCGCGTGCCCCCACGAACCTTCGGCAGCGCCCCCCGCCTCACGGAGGACGACTTTGGGCTCCTGAACTACGCGCTCGCTGAGATGCGACGCCTGTGCCTG

      AspLeuProProValProProAsnAlaTyrThrProTyrHisLeuArgGluTyrAlaThrArgLeuValAsnGlyPheLysProLeuValArgArgSerA
1601  GACCTTCCCCCGGTCCCCCCCAACGCATACACGCCCTATCATCTGAGGGAGTATGCGACGCGGCTGGTTAACGGGTTCAAACCCCTGGTGCGGCGGTCCG

      laArgLeuTyrArgIluLeuGlyIluLeuValHisLeuArgIluArgThrArgGluAlaSerPheGluGluTrpMetArgSerLysGluValAspLeuAs
1701  CCCGCCTGTATCGCATCCTGGGGATTCTGGTTCACCTGCGCATCCGTACCCGGGAGGCCTCCTTTGAGGAATGGATGCGCTCCAAGGAGGTGGACCTGGA

      pPheGlyLeuThrGluArgLeuArgGluHisGluAlaGlnLeuMetIluLeuAlaGlnAlaLeuAsnProTyrAspCysLeuIluHisSerThrProAsn
1801  CTTCGGGCTGACGGAAAGGCTTCGCGAACACGAGGCCCAGCTAATGATCCTGGCCCAGGCCCTGAACCCCTACGACTGTCTGATCCACAGCACCCCGAAC

      ThrLeuValGluArgGlyLeuGlnSerAlaLeuLysTyrGluGluPheTyrLeuLysArgPheGlyGlyHisTyrMetGluSerValPheGlnMetTyrT
1901  ACGCTCGTCGAGCGGGGGCTGCAGTCGGCGCTGAAGTACGAAGAGTTTTACCTCAAGCGCTTCGGCGGGCACTACATGGAGTCCGTCTTCCAGATGTACA

      hrArgIluAlaGlyPheLeuAlaCysArgAlaThrArgGlyMetArgHisIluAlaLeuGlyArgGlnGlySerTrpTrpGluMetPheLysPhePhePh
2001  CCCGCATCGCCGGGTTCCTGGCGTGCCGGGCGACCCGCGGCATGCGCCACATCGCCCTGGGGCGACAGGGGTCGTGGTGGGAAATGTTCAAGTTCTTTTT

      eHisArgLeuTyrAspHisGlnIluValProSerThrProAlaMetLeuAsnLeuGlyThrArgAsnTyrTyrThrSerSerCysTyrLeuValAsnPro
2101  CCACCGCCTCTACGACCACCAGATCGTGCCGTCCACCCCCGCCATGCTGAACCTCGGAACCCGCAACTACTACACGTCCAGCTGCTACCTGGTAAACCCC

      GlnAlaThrThrAsnGlnAlaThrLeuArgAlaIluThrGlyAsnValSerAlaIluLeuAlaArgAsnGlyGlyIluGlyLeuCysMetGlnAlaPheA
2201  CAGGCCACCACTAACCAGGCCACCCTCCGGGCCATCACCGGCAACGTGAGCGCCATCCTCGCCCGCAACGGGGGCATCGGGCTGTGCATGCAGGCGTTCA

      snAspAlaSerProGlyThrAlaSerIluMetProAlaLeuLysValLeuAspSerLeuValAlaAlaHisAsnLysGlnSerThrArgProThrGlyAl
2301  ACGACGCCAGCCCCGGCACCGCCAGCATCATGCCGGCCCTGAAGGTCCTGGACTCCCTGGTGGCGGCGCACAACAAACAGAGCACGCGCCCCACCGGGGC

      aCysValTyrLeuGluProTrpHisSerAspValArgAlaValLeuArgMetLysGlyValLeuAlaGlyGluGluAlaGlnArgCysAspAsnIluPhe
2401  GTGCGTGTACCTGGAACCCTGGCACAGCGACGTTCGGGCCGTGCTCAGAATGAAGGGCGTCCTCGCCGGCGAGGAGGCCCAGCGCTGCGACAACATCTTC

      SerAlaLeuTrpMetProAspLeuPhePheLysArgLeuIluArgHisLeuAspGlyGluLysAsnValThrTrpSerLeuPheAspArgAspThrSerM
2501  AGCGCCCTCTGGATGCCGGACCTGTTCTTCAAGCGCCTGATCCGCCACCTCGACGGCGAGAAAAACGTCACCTGGTCCCTGTTCGACCGGGACACCAGCA

      etSerLeuAlaAspPheHisGlyGluGluPheGluLysLeuTyrGluHisLeuGluAlaMetGlyPheGlyGluThrIluProIluGlnAspLeuAlaTy
2601  TGTCGCTCGCCGACTTTCACGGCGAGGAGTTCGAGAAGCTGTACGAGCACCTCGAGGCCATGGGGTTCGGCGAAACGATCCCCATCCAGGACCTGGCGTA

      rAlaIluValArgSerAlaAlaThrThrGlySerProPheIluMetPheLysAspAlaValAsnArgHisTyrIluTyrAspThrGlnGlyAlaAlaIlu
2701  CGCCATCGTGCGCAGCGCGGCCACCACCGGAAGCCCCTTCATCATGTTTAAGGACGCGGTAAACCGCCACTACATCTACGACACGCAAGGGGCGGCCATT

      AlaGlySerAsnLeuCysThrGluIluValHisProSerSerLysArgSerSerGlyValCysAsnLeuGlySerValAsnLeuAlaArgCysValSerA
2801  GCCGGCTCCAACCTCTGCACGGAGATCGTCCACCCGTCCTCCAAACGCTCCAGCGGGGTCTGCAACCTGGGCAGCGTGAATCTGGCCCGATGCGTCTCCC

      rgArgThrPheAspPheGlyMetLeuArgAspAlaValGlnAlaCysValLeuMetValAsnIluMetIluAspSerThrLeuGlnProThrProGlnCy
2901  GGCGGACGTTCGATTTTGGCATGCTCCGCGACGCCGTGCAGGCGTGCGTGCTAATGGTTAATATCATGATAGACAGCACGCTGCAGCCGACGCCCCAGTC

      sAlaArgGlyHisAspAsnLeuArgSerMetGlyIluGlyMetGlnGlyLeuHisThrAlaCysLeuLysMetGlyLeuAspLeuGluSerAlaGluPhe
3001  CGCCCGCGGCCACGACAACCTGCGGTCCATGGGCATTGGCATGCAGGGCCTGCACACGGCGTGCCTGAAGATGGGCCTGGATCTGGAGTCGGCCGAGTTC

      ArgAspLeuAsnThrHisIluAlaGluValMetLeuLeuAlaAlaMetLysThrSerAsnAlaLeuCysValArgGlyAlaArgProPheSerHisPheL
3101  CGGGACCTGAACACACACATCGCCGAGCTGATGCTGCTCGCGGCCATGAAGACCAGTAACGCGCTGTGCGTTCGCGGGGCGCGTCCCTTCAGCCACTTTA

      ysArgSerMetTyrArgAlaGlyArgPheHisTrpGluArgPheSerAsnAlaSerProArgTyrGluGlyGluTrpGluMetLeuArgGlnSerMetMe
3201  AGCGCAGCATGTACCGGGCCGGCCGCTTTCACTGGGAGCGCTTTTCGAACGCCAGCCCGCGGTACGAGGGCGAGTGGGAGATGCTACGCCAGAGCATGAT

      tLysHisGlyLeuArgAsnSerGlnPheIluAlaLeuMetProThrAlaAlaSerAlaGlnIluSerAspValSerGluGlyPheAlaProLeuPheThr
3301  GAAACACGGCCTGCGCAACAGCCAGTTCATCGCGCTCATGCCCACCGCCGCCTCGGCCCAGATCTCGGACGTCAGCGAGGGCTTTGCCCCCCTGTTCACC

      AsnLeuPheSerLysValThrArgAspGlyGluThrLeuArgProAsnThrLeuLeuLeuLysGluLeuGluArgThrPheGlyGlyLysArgLeuLeuA
3401  AACCTGTTCAGCAAGGTGACCAGGGACGGCGAGACGCTGCGCCCCAACACGCTCTTGCTGAAGGAACTCGAGCGCACGTTCGGCGGGAAGCGGCTCCTGG

      spAlaMetAspGlyLeuGluAlaLysGlnTrpSerValAlaGlnAlaLeuProCysLeuAspProAlaHisProLeuArgArgPheLysThrAlaPheAs
3501  ACGCGATGGACGGGCTCGAGGCCAAGCAGTGGTCTGTGGCCCAGGCCCTGCCTTGCCTGGACCCCGCCCACCCCCTCCGGCGGTTCAAGACGGCCTTCGA

      pTyrAspGlnGluLeuLeuIluAspLeuCysAlaAspArgAlaProTyrValAspHisSerGlnSerMetThrLeuTyrValThrGluLysAlaAspGly
3601  CTACGACCAGGAACTGCTGATCGACCTGTGTGCAGACCGCGCCCCCTATGTTGATCACAGCCAATCCATGACTCTGTATGTCACAGAGAAGGCGGACGGG

      ThrLeuProAlaSerThrLeuValArgLeuLeuValHisAlaTyrLysArgGlyLeuLysThrGlyMetTyrTyrCysLysValArgLysAlaThrAsnS
3701  ACGCTCCCCGCCTCCACCCTGGTCCGCCTTCTCGTCCACGCATATAAGCGCGGCCTGAAGACGGGGATGTACTACTGCAAGGTTCGCAAGGCGACCAACA

      erGlyValPheAlaGlyAspAspAsnIluValCysThrSerCysAlaLeu
3801  GCGGGGTGTTCGCCGGCGACGACAACATCGTCTGCACAAGCTGCGCGCTGTAAGCAACAGCGCTCCGATCGGGGTCAGGCGTCGCTCTCGGTCCCGCATA

           MetAspProAlaValSerProAlaSerThrAspProLeuAspThrHisAlaSerGlyAlaGlyAlaAlaProIluProValCysProThrPro
3901  TCGCCATGGATCCCGCCGTCTCCCCCGCGAGCACCGACCCCCTAGATACCCACGCGTCGGGGGCCGGGGCGGCCCCGATTCCGGTGTGCCCCACCCCC
```

A

```
HSV-2  MANRPAASALAGARSPSERQEPREPEVAPPGGD---------HVFCRKVSGVMVLSSDPPGPAAYRISDSSFVQCGSNCS   70
       ** *****    ** *   **   *  *  *           ** *  * ******   ** * ****** ***** **
HSV-1  MASRPAASSPVEARAPVGGQEAGGPSAATQGEAAGAPLAHGHHVYCQRVNGVMVLSDKTPGSASYRISDSNFVQCGFNCT   80

HSV-2  MIIDGD-VARGHLRDLEGATSTGAFVAISNV   101
       *****   **
HSV-1  MIIDGRRGARAPQTRGPAGIPRSLRCCDKHR   111
```

B

```
HSV-2  NLFSKVTRDGETLRPNTLLLKELERTFGGKRLLDAMDGLEAKQWSVAQALPCLDPAHPLRRFKTAFDYDQELLIDLCADR   1074
       *************************** *****  ** * ************* * ************** *********
HSV-1  NLFSKVTRDGETLRPNTLLLKELERTFSGKRLLEVMDSLDAKQWSVAQALPCLEPTHPLRRFKTAFDYDQKLLIDLCADR

HSV-2  APYVDHSQSMTLYVTEKADGTLPASTLVRLLVHAYKRGLKTGMYYCKVRKATNSGVFAGDDNIVCTSCAL   1144
       ********** ** ******************************************* ******* ****
HSV-1  APYVDHSQSMHLYDTEKADGTLPASTLVRLLVHAYKRGLKTGMYYCKVRKATNSGVFGGDDNIVCMSCAL
```

FIG. 3. Comparison of the amino and carboxy termini of the HSV-2 and HSV-1 140K proteins. The amino acid sequence of the HSV-2 protein predicted from our sequence and for the HSV-1 protein predicted from the sequence of McLaughlin and Clements (29) is shown for the amino termini (A) and carboxy termini (B) of the proteins. The numbers at the right are the numbers of amino acids (no numbers are given for the HSV-1 C terminus because the complete sequence is not known). The single letter amino acid code is used.

strong intertypic homology (e.g., nucleotides 179 through 228), moderate homology (e.g., nucleotides 256 through 283), and little or no homology preceding the start of translation (e.g., nucleotides 354 through 418).

Only a portion of the sequences encoding the HSV-1 140K protein are available, encompassing 111 residues of the amino terminus and 150 residues of the carboxy terminus (29). A comparison of the termini of the HSV-1 strain 17 and HSV-2 strain 333 140K protein is shown (Fig. 3). The carboxy termini of the proteins are highly conserved (92%), whereas the amino termini are much less similar. The HSV-1 protein has a segment of nine amino acids which are not present in the HSV-2 protein, and residues 80 through 101 of the HSV-2 protein show no homology to residues 91 through 111 of the HSV-1 protein. Until the complete sequence of the HSV-1 gene is available it is impossible to determine whether this difference represents an insertion or just a region of nonhomology, and it is not possible to establish where the protein conservation will begin. Of the remaining 80 amino acids of the HSV-2 amino terminus, there is 60% homology, indicating the overall functional relatedness of the HSV-1 and HSV-2 140K proteins.

It was recently demonstrated that the EBV genome encoded a protein which shows significant homology to the HSV-1 and HSV-2 38K proteins (18) and that these proteins are homologous to the small subunit of the well-characterized ribonucleotide reductase of *E. coli* (36). The DNA sequence encoding the large subunit of the *E. coli* reductase, designated B1, has been determined (7) as has the sequence of the large subunit of the mouse reductase, designated M1 (6). In addition the entire sequence of the EBV genome has been completed (5).

A computer-assisted analysis of a comparison of the sequence of the HSV-2 140K protein with those of the three other genes was done (Fig. 4). The HSV-2 protein is the largest, having 1,144 amino acids. The amino terminus of the HSV-2 protein contains a domain of 350 through 400 residues which is not shared with the other reductases, suggesting an additional function for this segment of the protein. By secondary structure analysis (data not shown) this region is extremely rich in hydrophilic residues and appears structurally distinct from the rest of the protein. At least a portion of this domain is present in the HSV-1 protein, although there is less conservation than in the carboxy terminus of the 140K protein. Perhaps this domain is required for binding to the respective 38K proteins or alternatively to other enzymes in an HSV replication complex. The three other reductases show a longer carboxy terminus than does HSV-2. The figure highlights the homologous regions by showing in reverse print residues which are identical or which show conservative changes in at least three of four proteins when all four sequences are aligned or in all of the proteins when either two or three sequences are available. By these criteria nearly 40% of the shared amino acids are conserved. This is in close agreement with the overall relatedness (38%) of the *E. coli*, clam, EBV, and HSV-2 small subunits when comparing conserved residues (data not shown). The HSV-2 gene is more closely related to the EBV gene than to that of *E. coli* or the mouse, nevertheless, blocks of homology are common to all four proteins. Biochemical analysis of the large subunit of other reductases has indicated the importance of two thiols that are oxidized to a disulfide during the enzymatic reaction (38) and has shown that the reaction is catalyzed by either thioredoxin or glutaredoxin. The active site of thioredoxin or glutaredoxin contains two cysteines separated by two amino acids, which allows the formation of a stable 15-member ring structure containing the disulfide. This structure should be present on the large subunit, and this sequence was found only once in the *E. coli* protein (7) at residues 667 and 670 (see Fig. 4). In the HSV-2 protein this sequence is also present only once at residues 1139 and 1142, two amino acids away from the carboxy terminus. Interest-

FIG. 2. Nucleotide sequence of the noncoding strand of the region encoding the 140K protein. The signals for the initiation (nucleotides 419 through 421) and termination (3851 through 3853) of translation of the 140K protein are shown in reverse print, and the translated amino acid sequence is shown above the nucleotide sequence. The amino terminus of the 38K protein is shown beginning with its initiation codon (3906 through 3908). The TATA homologies for the 5.0-kb message (148 through 153) and the 1.2-kb message (3742 through 3747) are also shown in reverse print.

```
HSV-2    MANRPAASALAGARSPSERQEPREPEVAPPGGDHVFCRKVSGVMVLSSDPPGPAAYRISDSSFVQCGSNCSMIIDGDVARGHLRDLEGATSTGAFVAISN    100

HSV-2    VAAGGDGRTAVVALGGTSGPSATTSVGTQTSGEFLHGNPRTPEPQGPQAVPPPPPPPFPWGHECCARRDARGGAEKDVGAAESWSDGPSSDSETEDSDSS    200

HSV-2    DEDTGSGSETLSRSSSIWAAGATDDDDSDSDSRSDDSVQPDVVVRRRWSDGPAPVAFPKPRRPGDSPGNPGLGAGTGPGSATDPRASADSDSAAHAAAPQ    300

E. COLI                                                                MNQNLLVTKRDGSTERINLDKIHRVLDWAAEGLHNVSISQVELRS     45
MOUSE                                                                      MHVIKRDGRQERVMFDKITSRIQKLCYGLNMDFVDPAQITM     41
EBV
HSV-2    ADVAPVLDSQPTVGTDPGYPVPLELTPENAEAVARFLGDAVDREPALMLEYFCRCAREESKRVPPRTFGSAPRLTEDDFGLLNYALAEMRRLCLDLPPVP    400

E. COLI  H-IQF-YDGIKTSDIHETIIKAAADLISRDAPDYQYLAARLAIFHLRKKATASLRPPA--LYDHVVKMVEMGKYDNH--LLEDYTEEEFKQMDTFIDHDR    139
MOUSE    KVIQGLYSGVTTVELDTLAAETAATL-TTKHPDYAILAARIAVSNLHKETKKVFSDVMEDLYNYINPHNGRHSPMVASSTL-DIVMANKDRLNSAIIYDR    139
EBV            MATTSHVEH---ELLSKLI-DELKVKANSDPEADVLAGRLLHRLKAESVTHTVAEYLEVFSDKFYDEEFFQYHRDELETRVSAFAQSPAYER     88
HSV-2    PNAYTPY-HLREYATRL---VNGFKPL-VRRSARLYRILGILVHLRIRTREASFEEWMRSKEVDLDFGLTERLREHEAQLMILAQALNPYDCLIHSTPNT    495

E. COLI  DMTFSYAAVKQLEGKYLVQNRVTGEIYESAQFLYILVAACLFSNYPREGLQYVKRFYDAVSTFKISLPTPIM--SGVRTPTRQFSSCVLIECGDSLDSIN    237
MOUSE    DFSYNYFGFKTLERSYLLK--INGKVAERPQHMIMRVSVGIHKEDIDAAIET----YNLLSEKWFTHASPTLFNAGTNRP--QLSSCFLLSMKDDSIEGI    231
EBV      IVSSGYLSALRYYDTYLYV-GRSGK-QESVQHFYMRLAGFCASTTCLYAGLRA----ALQRARPEIESDMEVFDYYFEHLTSQTVCCSTPFVRFAGVENS    182
HSV-2    LVERGLQSALKYEEFYLKRFG--GHYMESVFQMYTRIAGFLA-CRATRGMRHI----ALGRQGSWWEMFKFFFHRLYDH---QIV-PSTPAMLNLGTRNY    584

E. COLI  ATSSAIVKYVSQRAGIASTPGVFV-RWVARFAVVKRSIPAAFRS-------------TTFPDSGES--CSRRCAGRRCGNVFYPM-WHLEVESLLVLKNN    320
MOUSE    YDTLKQCALISKSAGGIGVAVSCI-RATGSYIAGTNGNSNGLVP-----------MLRVYNNTARYV--DQGGNKRPGAFAIYLEPWHLDIFEFLDLKKN    317
EBV      --TLASCILTTPDLSSEWDVTQALYRHLGRYLF-QRAGVGVGVTGAGQDGKHISLLMRMINSHVEY---HNYGCKRPVSVAAYMEPWHSQIFKFLETKLP    276
HSV-2    Y-T-SSCYLVNPQATTNQATLRAITGNVSAILA-RNGGIGLCVQAFNDASPGTASIMPALKVLDSLVAAHNKQSTRPTGACVYLEPWHSDVRAVLRMKGV    681

E. COLI  R-GVEGNRVRHMDYGVQINKLMYT---RLLKGEDITLFSPSDVPGLYDAFFADQEEFERLYTKYEKDDSIRKQRVKAVEL-FSLMMQERASTGRIYIQNV    415
MOUSE    T-GKEEQRARDLFFALWIPDLFMK---RVETNQDWSLMQPNECPGL-DEVWG--EEFEKLYESYEKQGRVRKV-VKAQQLWYAIIES-QTETGTPYMLYK    408
EBV      E-NHE--RCPGIFTGLFVPELFFK-LFRDTPWSDWYLFDPKDAGDL-ERLYG--EEFEREYYRLVTAGKFCG-RVSIKSLMFSIVNC-AVKAGSPFILLK    367
HSV-2    LAGEEAQRCDNIFSALWMPDLFFKRLIRHLDGEKNVTWSLFDRDTSMSLADFHGEEFEKLYEHLEAMGFGET--IPIQDLAYAIVRS-AATTGSPFIMFK    778

E. COLI  DHCITMSPFDPAIAPVRQSNLCLEIALPT-KPLNDVNDENGEIALCTLSAFNLGAINNLDELEELAILAVRALDALLDYQDYPIPAAKRGAMGRRT----    511
MOUSE    DSCNRKSNQQNLGT-IKCSNLCTEIVEYTSKDEVAVCNLASL-ALNMYVTPEHTYDF--EKLAEVTKVIVRNLNKIIDINYYPIPEAHLSNKRHRP----    501
EBV      EACNAHFWRDLQGEAVNAANLCAEVLQPSRK-SVATCNLANICLPRCLVNAPLAVR---AQRADTQGDELLLALPRLSVTL-PGEGAVGDGFSLARLRDA    462
HSV-2    DAVNRHYIYDTQGAAIAGSNLCTEIVHPSSKRSSGVCNLGSVNLARCVSRRTFDFG-MLRDAVQACVLMVNIMI--DS-TLQPTPQCARGHDNLR-----    871

E. COLI  ---------------------------LGIGVINFAYYLAKHGKRYSDGSANNLTHKTFEAIQYYLLKASNELA-KEQGACPWFNETTYAKGILPI-DTYK    583
MOUSE    ---------------------------IGIGVQGLADAFILMRYPFESPEAQLLNKQIFETIYYGALEASCELA-KEYGPYETYEGSPVSKGILQY-DMWN    573
EBV      TQCATFVVACSILQGSPTYDSRDMASMGLGVQGLADVFADLGWQYTDPPSRSLNKEIFEHMYFTALCTSSLIGLHTRKIFPGFKQSKYAGGWFHWHDWAG    562
HSV-2    --------------------------SMGIGMQGLHTACLKMGLDLESAEFRDLNTHIAEVMLLAAMKTSNALCVRGARPFSHFKRSMYRAGRFHW-ERFS    944

E. COLI  KIWIPSLMSRCITTGXLCVSQSKRTVCVTPTLSALMPSETSSQIS-N-------ATNGIERRAVTSASKRRKTVFCA-------R--WCRTTSTCTT---    665
MOUSE    VAPTD-LWDWKPLKEKIAKY-GIRN---SL-LIAPMPTASTAQILGNNESIEPYTSNIYTRR-VLSGEFQIVNPH--LLKDLTER------GLWNEE---    657
EBV      TDLSIPREIWSRLSERIVRD-GLFN---SQ-FIALMPTSGCAQVTGCSDAFYPFYANASTK--V-TNKEEALRPN----------RSFWRHVRLDDREALN    645
HSV-2    NASPRYEGEWEMLRQSMMKH-GLRN---SQ-FIALMPTAASAQISDVSEGFAPLFTNLFSK--V-TRDGETLRPNTLLLKEL-ERTFGGKRLLDAMDGLE   1034

E. COLI  PMSCCGKGRVTMVICNWWVSCRN---LSI--SRSLPTPTT-IRHASRQEKCPMQ-QLLKQLLT------------------AYKFGVKT-LYYQTPVTAV    736
MOUSE    MKNQIIACNGSIQSIPEIPDDLKQ--L--YKTVWEISQKTVLKMAAERGAFIDQSQSLNIHIAEPNYGK--LTSMHFYGWKQ---GLKTGMYYLRTRPAA    745
EBV      LVGGRVSQ---------LPEALRQRYL-RFQTAFDYNQEDLIQMSRDRAPFVDQSQSHSLFLREEDAAR--ASTLANLLVRSYELGLKTIMYYCRIEKAA    733
HSV-2    AKQWSVAQ--------ALPCLDPAHPLRRFKTAFDYDQELLIDLCADRAPYVDHSQSMTLYVTEKADGTLPASTLVRLLVHAYKRGLKTGMYYCKVRKAT   1196

E. COLI  KDAQDDLGAVNPGRWLETAHVRSDIEMRIGKRLIPVRLGL                                                               776
MOUSE    NPIQFTLNKEKLKDKEKALKEEEEKERNTAAMVCSLENREECLMCGS                                                        792
EBV      DLGVMECKASA------ALSVPREEQNERSPAEQMPPRPMEPAQVAGPVDIMSKGPGEGPGGWCVPGGLEVCYKYRQLFSEDDLLETDGFTERACESCQ    826
HSV-2    NSGVFAGDDNIVCTSC-AL                                                                                   1144
```

FIG. 4. Comparison of the HSV-2 140K protein sequence with those of the ribonucleotide reductases of *E. coli*, mouse, and EBV. The sequence of the *E. coli* B1 subunit was taken from reference 7, the mouse M1 subunit was from reference 6, and the EBV sequence was from reference 5. The sequences were aligned with that of the HSV-2 140K protein to maximize homology. Reverse print is used at positions where three of four, four of four, three of three, or two of two proteins have identical residues or show conservative changes of amino acids. Substitutions between chemically similar residues that were allowed were: ILMVA, RKH, YFW, DE, TS, GA, QN. No substitutions were allowed for P and C. The numbers to the right indicate the position of the amino acids.

ingly, in both the EBV and mouse proteins the same structure was present only once close to the C terminus (residues 822 and 825 in EBV and 787 and 790 in mouse proteins).

DISCUSSION

The sequence of the HSV-2 140K protein is an important step in the long-term goal of understanding the catalytic mechanism of ribonucleotide reductase. By site-directed mutagenesis it will be possible to determine the functional domains of this enzyme. Data obtained from studies of the mutant *ts*1207 suggest that a defect in ribonucleotide reductase has a lethal effect which strengthens the value of this gene as a target for antiviral therapy. The development of antiviral agents directed against the HSV ribonucleotide reductase will be aided by knowledge of the primary structure of this enzyme.

DNA sequence data has provided firm evidence that both the 140K and 38K proteins show significant homology with the large and small subunits of other ribonucleotide reductases. In the case of HSV-1 and HSV-2 these genes are encoded by two transcripts which share a 3′ terminus, providing the first example in the herpesvirus genome of functionally related genes sharing a transcriptional unit. In EBV the genes which encode the large and small subunits

are encoded by 93K and 34K proteins which are located in tandem and appear to be coded by distinct mRNAs (18). The genes, designated *nrd*A and *nrd*B, which encode the bacterial reductase genes are also adjacent to each other on the *E. coli* genome (7).

It is intriguing that many of the monoclonal antibodies which react with one of the components of the viral enzyme also react to some extent with the other component and frequently the antibody recognizes a type-specific epitope (4, 16, 19). There are two possible and nonexclusive explanations for this observation. The first is that the two proteins form a complex which can be immunoprecipitated. By using a monoclonal antibody directed against HSV-1 Vmw136, and an oligopeptide serum directed against the carboxy terminus of Vmw38, Frame et al. (13) present good evidence that coprecipitation with these antibodies is caused by the formation of a complex. Another possibility which could explain the coprecipitation is that the HSV-2 140K and 38K proteins contain related epitopes. We have suggested that this could be a contributory factor based on two lines of evidence. First, the monoclonal antibodies 6A6 and 6H11 react with both the 140K and 38K proteins, not only in immunoprecipitation reactions but also in immunoblot reactions (19). Also, K. Shriver and L. Goldstein (unpublished data) have isolated a 6A6 monoclonal antibody-resistant mutant of HSV-2 which does not immunoprecipitate the 140K protein but still retains the same degree of reactivity with the 38K protein as does wild-type virus. These data support the notion that certain antigenic sites (or a site) may be related. By computer-assisted analysis we looked for antigenic sites shared by the 140K and 38K proteins, and the results were largely inconclusive. We found 3 examples where 4 of 4 amino acids were identical, 10 in which 4 of 5 matched, 1 in which 5 of 6 matched, etc. One example which was of particular interest is the sequence Pro-Ala-Ser-Thr which is located at position 7 through 10 in the HSV-2 38K protein and at position 1097 through 1100 in the HSV-2 140K protein. Based on predicted secondary structures (data not shown) this sequence is in a potentially very antigenic site of the amino terminus of the 38K protein which differs in sequence from the type 1 equivalent. The same sequence is present in the HSV-1 140K protein. It is interesting to speculate that this could be the epitope recognized by the antibody 6H11. The antibody recognizes predominantly the HSV-2 38K protein and to a lesser extent the HSV-2 140K protein, a difference in avidity which could be attributable to less accessibility because of secondary structure of the epitope in the 140K protein. Reactivity to the HSV-1 140K protein at high antibody concentrations, but not to the HSV-1 38K protein, has been reported (4). Experiments with tryptic and synthetic peptides to prove which epitopes are recognized by the antibodies 6A6 and 6H11 will be needed to determine whether shared antigenic sites play a role in the coprecipitation of the subunits of HSV-2 ribonucleotide reductase.

It has been suggested that the HSV ribonucleotide reductase genes may play a role in morphological transformation (23) and in cervical carcinoma (3). Experiments with deletions of the *Bgl*II N fragment have shown that the region with transforming activity maps to around position 0.60, outside of the sequences encoding 38K (16), thus excluding a role for the small subunit of ribonucleotide reductase in morphological transformation. In experiments in which tumorigenic transformation was achieved by continuous passage of cells exposed to the *Bgl*II C-*Hpa*I fragment (See Fig. 1), the 140K protein was detected in the transformed cells as judged by a complement fixation assay (25). From the DNA sequence data we now know that such a fragment only encodes approximately one-third of the large subunit of ribonucleotide reductase and apparently not the domain encoding the catalytic site, so it appears unlikely that either subunit of ribonucleotide reductase is involved in morphological transformation. There is contradictory evidence as to whether the 140K protein is expressed on the plasma membrane of cervical carcinoma cells, with positive results in some cases (3) and negative results in a study with the monoclonal antibodies 6A6 and 6H11 (27). At this time it is clear that both HSV-1 and HSV-2 encode two subunits of ribonucleotide reductase, that the activity of the enzyme complex is required for viral replication, and that there is little reason to believe these genes are required for morphological transformation.

ACKNOWLEDGMENTS

We thank Jim Wallace for help with the computer analysis and Toni Higgs for preparing the manuscript.

This work was supported by Public Health Service grant CA 35568 from the National Cancer Institute.

LITERATURE CITED

1. **Anderson, K. P., R. J. Frink, G. B. Devi, B. H. Gaylord, R. H. Costa, and E. K. Wagner.** 1981. Detailed characterization of the mRNA mapping in the *Hin*dIII fragment K region of the herpes simplex virus type 1 genome. J. Virol. **37**:1011–1027.
2. **Anderson, S.** 1981. Shotgun DNA sequencing strategy using cloned DNase I-generated fragments. Nucleic Acids Res. **9**:3015–3027.
3. **Aurelian, L., I. I. Kessler, N. B. Rosenshein, and G. Barbour.** 1981. Viruses and gynecological cancers: herpesvirus protein (ICP10/AG4), a cervical tumor antigen that fulfills the criteria for a marker of carcinogenicity. Cancer **48**:455–471.
4. **Bacchetti, S., M. J. Evelegh, B. Muirhead, C. S. Sartori, and D. Huszar.** 1984. Immunological characterization of herpes simplex virus type 1 and 2 polypeptide(s) involved in viral ribonucleotide reductase activities. J. Virol. **49**:591–593.
5. **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. Sequin, 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.
6. **Caras, I. W., B. B. Levinson, M. Fabry, S. R. Williams, and D. W. Martin, Jr.** 1985. Cloned mouse ribonucleotide reductase subunit M1 cDNA reveals amino acid squence homology with *Escherichia coli* and herpesviruses ribonucleotide reductases. J. Biol. Chem. **260**:7015–7022.
7. **Carlson, J., J. A. Fuchs, and J. Messing.** 1984. Primary structure of the *Escherichia coli* ribonucleoside diphosphate reductase. Proc. Natl. Acad. Sci. USA **81**:4294–4297.
8. **Cohen, G. H.** 1972. Ribonucleotide reductase activity of synchronized KB cells infected with herpes simplex virus. J. Virol. **9**:408–418.
9. **Cohen, G. H., M. N. Factor, and M. Ponce de Leon.** 1974. Inhibition of herpes simplex virus type 2 replication by thymidine. J. Virol. **14**:20–25.
10. **Cohen, J. C., B. E. Henry, C. C. Randall, and D. O'Callaghan.** 1977. Ribonucleotide reductase activity in hydroxyurea resistant herpes virus replication. Proc. Soc. Exp. Biol Med. **155**:395–399.
11. **Draper, K. G., R. J. Frink, and E. K. Wagner.** 1982. Detailed characterization of an apparently unspliced β herpes simplex virus type 1 gene mapping in the interior of another. J. Virol. **43**:1123–1128.
12. **Dutia, B. M.** 1983. Ribonucleotide reductase induced by herpes simplex virus has a virus specified constituent. J. Gen. Virol. **64**:513–521.
13. **Frame, M. C., H. S. Marsden, and B. M. Dutia.** 1985. The

ribonucleotide reductase induced by herpes simplex virus type 1 involves minimally a complex of two polypeptides (136K and 38K). J. Gen. Virol. **66**:1581–1587.
14. **Frink, R. J., K. G. Draper, and E. K. Wagner.** 1981. Uninfected cell polymerase efficiently transcribes early but not late herpes simplex virus type 1 mRNA. Proc. Natl. Acad. Sci. USA **78**:6139–6143.
15. **Galloway, D. A., L. C. Goldstein, and J. B. Lewis.** 1982. Identification of proteins encoded by a fragment of herpes simplex virus type 2 DNA that has transforming activity. J. Virol. **42**:530–537.
16. **Galloway, D. A., J. A. Nelson, and J. K. McDougall.** 1984. Small fragments of herpesvirus DNA with transforming activity contain insertion sequence-like structures. Proc. Natl. Acad. Sci. USA **81**:4736–4740.
17. **Galloway, D. A., and M. A. Swain.** 1984. Organization of the left-hand end of the herpes simplex virus type 2 *Bgl*II N fragment. J. Virol. **49**:724–730.
18. **Gibson, T., P. Stockwell, M. Ginsburg, and B. Barrell.** 1984. Homology between two EBV early genes and HSV ribonucleotide reductase and 38K genes. Nucleic Acids Res. **12**:5087–5099.
19. **Goldstein, L. C., L. Corey, J. K. McDougall, E. Tolentino, and R. C. Nowinski.** 1983. Monoclonal antibodies to HSV: use in antigenic typing and rapid diagnosis. J. Infect. Dis. **147**:829–837.
20. **Henikoff, S.** 1984. Unidirectional digestion with exonuclease III creates targeted breakpoints for DNA sequencing. Gene **28**:351–359.
21. **Henry, B. E., R. Glaser, J. Hewetson, and D. J. O'Callaghan.** 1978. Expression of altered ribonucleotide reductase activity associated with the replication of the Epstein Barr virus. Virology **89**:262–271.
22. **Honess, R. W., and B. Roizman.** 1973. Proteins specified by herpes simplex virus. XI. Identification and relative molar rates of synthesis of structural and nonstructural herpes virus polypeptides in the infected cell. J. Virol. **12**:1347–1365.
23. **Huszar, D., and S. Bacchetti.** 1983. Is ribonucleotide reductase the transforming function of herpes simplex virus 2? Nature (London) **302**:76–79.
24. **Huszar, D., S. Beharry, and S. Bacchetti.** 1983. Herpes simplex virus-induced ribonucleotide reductase: development of antibodies specific for the enzyme. J. Gen. Virol. **64**:1327–1335.
25. **Jariwalla, R. J., L. Aurelian, and P. O. P. Ts'O.** 1980. Tumorigenic transformation induced by a specific fragment of DNA from herpes simplex virus type 2. Proc. Natl. Acad. Sci. USA **77**:2279–2283.
26. **Lankinen, H., A. Gräslund, and L. Thelander.** 1982. Induction of a new ribonucleotide reductase after infection of mouse L cells with pseudorabies virus. J. Virol. **41**:893–900.
27. **McDougall, J. K., C. P. Crum, C. M. Fenoglio, L. C. Goldstein, and D. A. Galloway.** 1982. Herpesvirus specific RNA and protein in carcinoma of the uterine cervix. Proc. Natl. Acad. Sci. USA **79**:3853–3857.
28. **McLaughlan, J., and J. B. Clements.** 1982. A 3′ co-terminus of two early herpes simplex virus type 1 RNAs. Nucleic Acids Res. **10**:501–502.
29. **McLaughlin, J., and J. B. Clements.** 1983. Organization of the herpes simplex virus type 1 transcription unit encoding two early proteins with molecular weights of 140,000 and 40,000. J. Gen. Virol. **64**:997–1006.
30. **McLaughlan, J., and J. B. Clements.** 1983. DNA sequence homology between two co-linear loci on the HSV genome which have different transforming abilities. EMBO J. **2**:1953–1961.
31. **Powell, K., and R. Courtney.** 1975. Polypeptides synthesized in herpes simplex virus type 2 infected HEp-2 cells. Virology **66**:217–224.
32. **Preston, V. G., J. W. Palfreyman, and B. M. Dutia.** 1984. Identification of a herpes simplex virus type 1 polypeptide which is a component of the virus-induced ribonucleotide reductase. J. Gen. Virol. **65**:1457–1466.
33. **Reichard, P., and A. Ehrenberg.** 1983. Ribonucleotide reductase: a radical enzyme. Science **221**:514–519.
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, P. H., and R. Cortese.** 1979. A fast and simple method for sequencing DNA cloned in the single stranded bacteriophage M13. J. Mol. Biol. **129**:169–172.
36. **Sjoberg, B.-M., H. Eklund, J. A. Fuchs, J. Carlson, N. M. Standart, J. V. Ruderman, S. J. Bray, and T. Hunt.** 1985. Identification of the stable free radical tyrosine residue in ribonucleotide reductase. FEBS Lett. **183**:99–102.
37. **Swain, M. A., R. W. Peet, and D. A. Galloway.** 1985. Characterization of the gene encoding herpes simplex virus type 2 glycoprotein C and comparison with the type 1 counterpart. J. Virol. **53**:561–569.
38. **Thelander, L., and P. Reichard.** 1979. Reduction of ribonucleotides. Annu. Rev. Biochem. **48**:133–158.
39. **Wagner, E. K.** 1985. Individual HSV transcripts: characterization of specific genes, p. 45–104. *In* B. Roizman (ed.), The herpesviruses, vol. III. Plenum Publishing Corp., New York.
40. **Wilbur, W. J., and D. J. Lipman.** 1983. Rapid similarity searches of nucleic acid and protein data banks. Proc. Natl. Acad. Sci. USA **80**:726–730.