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Two Overlapping Transcription Units Which Extend across the L-S Junction of Herpes Simplex Virus Type 1

ROY A. BOHENZKY,[1] MICHAEL LAGUNOFF,[2] BERNARD ROIZMAN,[2]
EDWARD K. WAGNER,[3] AND SAUL SILVERSTEIN[1]*

Department of Microbiology, College of Physicians and Surgeons, Columbia University, New York, New York 10032[1]; The Marjorie B. Kovler Viral Oncology Laboratories, The University of Chicago, Chicago, Illinois 60637[2]; and Department of Molecular Biology and Biochemistry, University of California, Irvine, Irvine, California 92717[3]

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A region of the herpes simplex virus type 1 genome located upstream of the α0 promoter contains a promoter which regulates transcription in the opposite orientation to that driven by α0. Analyses of mutants from which this promoter, αX, was deleted and a mutant in which a fragment that serves as a transcription terminator and polyadenylation signal was inserted upstream of this promoter demonstrate that two distinct transcription units overlap this region of the genome and are transcribed in a direction antisense to the neurovirulence gene $\gamma_1$34.5. One unit, dependent on the αX promoter, is active when cells are infected in the presence of the protein synthesis inhibitor cycloheximide. The second unit, independent of αX, is active during the course of productive infection. This transcription unit originates from a promoter upstream of αX which is distinct from the latency-associated promoter (LAP). Two polyadenylated transcripts of 0.9 and 4.9 kb accumulate from this region of the genome during productive infection, but no mature transcripts accumulate in infected cells maintained in the presence of cycloheximide. Kinetic analyses demonstrate that the transcripts that accumulate during productive infection fall into the β class of herpes simplex virus type 1 genes.

The herpes simplex virus type 1 (HSV-1) genome is a double-stranded DNA genome of approximately 150 kb (23, 29–31) that contains at least 77 genes, 7 of which are repeated (reviewed in references 53 and 54). The transcription pattern of HSV is temporally regulated, consisting of at least three kinetic classes of genes termed immediate-early (α), delayed-early (β), and late (γ) whose expression is coordinately regulated (21, 22, 27, 48). Five α genes encoding the infected-cell proteins (ICP) 4, 0, 22, 27, and 47 have been described. Transcription of these genes can occur in HSV-infected cells in the absence of protein synthesis, and at least four of these gene products, ICP4, -0, -22, and -27, are regulators of subsequent HSV gene expression (11, 15, 28, 36, 40–43, 57). Furthermore, ICP4 and ICP27 can negatively regulate α genes (11, 15, 36, 39, 41–43, 49). The β genes are expressed prior to the onset of HSV DNA replication but require α-gene expression. These genes can be divided into two subclasses (β1 and β2) based on their time of expression (55). The γ genes are maximally expressed after the onset of viral DNA replication, although they also can be divided into two subclasses (γ1 and γ2) based on the obligatory requirement of DNA replication for their expression (50).

The genome contains two unique DNA sequences that differ in size and are designated the long unique (U_L) and short unique (U_S) regions (Fig. 1A) (44, 52). Flanking the unique sequences are the inverted repeats specific to the individual unique regions, and they are designated R_L and R_S. These repeats are composed of sequences designated *ab* and *ac*, respectively. The common *a* sequence flanks the entire genome and comprises the joint region where inverted copies of R_L and R_S meet. Circularization of the HSV genome during infection results in the generation of a second physically identical but inverted joint where the terminal repeats flanking U_L and U_S are juxtaposed and separated by a second set of *a* sequences.

Several transcription units have been mapped to the repeat region (Fig. 1A). Two of the α transcription units, α0 and α4, map to the repeats flanking the U_L and U_S regions, respectively. When these repeats are juxtaposed in the structure of the joint, the RNAs are transcribed in the same polarity. A transcription unit of the γ class, $\gamma_1$34.5, has been mapped between the α0 and α4 transcription units and is transcribed in the same polarity (5, 6). The only other transcription unit of that polarity within the repeat regions maps to a location upstream of the origin of DNA replication (ori_S) and extends through ori_S and coterminates with α4 (51).

The transcription map of the opposite strand of the repeat regions is less well characterized. The latency-associated transcripts (LATs) originate in the *b* sequence flanking U_L at a point about 1,600 bases downstream of the α0 transcription unit, extend through the α0 region and the joint region including the $\gamma_1$34.5 transcription unit, and terminate at a polyadenylation signal near the 3′ end of the α4 transcription unit (10, 12, 47, 62). Analysis of RNAs from this region demonstrates a complex pattern of transcripts during productive infection. These include the 8.5- to 9.0-kb primary LAT, its 2-kb stable LAT intron, and a number of other polyadenylated species. The family of transcripts identified in latently infected neurons is nearly as complex. It includes the 9.0-kb polyadenylated primary LAT, its 2-kb intron, one or more nonpolyadenylated spliced derivatives of the intron, and several smaller polyadenylated species. This last class of transcripts may depend on use of a second latency active promoter (LAP) which requires the major LAP for its activity (16, 34). Another transcription unit, α22 or α47 depending on the DNA isomer, maps downstream of LAT and is of the same polarity. These genes utilize a duplicated promoter that maps to *c*, the sequence flanking U_S. However, they are transcribed into opposite ends of U_S and therefore encode different structural genes (58). Recently,

* Corresponding author. Mailing address: Department of Microbiology, Columbia University, 701 W. 168th St., New York, NY 10032. Phone: (212) 305-8149. Fax: (212) 305-1468. Electronic mail address: sjs@cuccfa.ccc.columbia.edu.

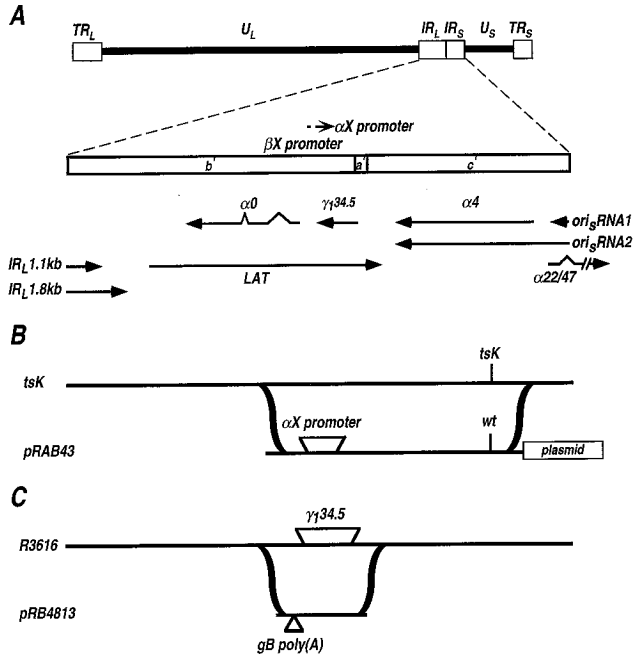

FIG. 1. Map of HSV-1 repeats and recombination schemes for construction of vRAB1a, vRAB1b, and R7508. (A) The genome of HSV-1 is schematically represented. The notations U, IR, and TR refer to the unique, internal repeat, and terminal repeat regions, respectively. The subscripts L and S refer to the long and short regions, respectively. An expanded region composed of IR_L and IR_S juxtaposed is shown. The solid arrows denote the known mRNAs. The dotted arrow indicates the position and polarity of the αX promoter. (B) Recombination scheme for the construction of vRAB1a and vRAB1b from pRAB43 and the virus *ts*K. (C) Recombination scheme for the construction of R7508 from pRB4813 and the virus R3616.

two transcripts of 1.1 and 1.8 kb mapping upstream of LAT have also been described (45).

We have previously reported the existence of a novel promoter mapping to the *b* sequence repeat that flanks U_L (Fig. 1) (3). This promoter drives transcription in the same polarity as that of LAT and could generate transcripts that would overlap the 8.3-kb LAT and the $\gamma_1$34.5 and α4 transcripts in the opposite polarity. Nascent transcripts which map downstream from this promoter demonstrated characteristics of two different kinetic classes. During the course of productive viral infection, these transcripts appeared with β kinetics but demonstrated a high constitutive level of synthesis when cells were infected and maintained in the presence of cycloheximide, which is indicative of α kinetics. This promoter contained a high-affinity DNA binding site for the ICP4 gene product and was negatively regulated by ICP4 in transient expression assays. Recently, Yeh and Schaffer have reported the existence of a set of polyadenylated transcripts mapping downstream of this promoter which are seen exclusively in cells infected with defective α4 mutants (61). The 5′ end of these transcripts was mapped to a point within the previously described high-affinity ICP4-binding site, suggesting initiation of transcription from this promoter.

Here, we report that the RNAs we detect downstream from this partially characterized promoter belong to two overlapping transcription units. One transcription unit, αX, is negatively regulated by ICP4 and transcribed in the presence of cycloheximide. However, transcripts seen during the course of productive viral infection are derived from another transcription unit (βX) and their promoter maps upstream of the αX promoter. Furthermore, we demonstrate that this promoter is distinct from the LAT promoter. Finally, we demonstrate the existence of two polyadenylated transcripts of 0.9 and 4.0 kb which map downstream of the αX promoter and are detected during productive infection.

MATERIALS AND METHODS

Cell lines and viruses. Monolayer cultures of Vero cells were maintained in Dulbecco's modified Eagle's medium supplemented with 5% calf serum and incubated at 37°C. A derivative of Vero cells containing the U_L52 gene from HSV-1, 2D6 (generously provided by Sandra Weller, University of Connecticut), was maintained under the same conditions except the medium was further supplemented with 250 μg of geneticin per ml.

Mutant and wild-type stocks of HSV-1 were propagated in Vero cells and assayed by plaque formation as previously described (35). Nucleocapsids were prepared according to the method of Wagner and Summers (56). The LAT promoter disruption mutant FLA5 has been described previously (14). A mutant in which the U_L52 gene was deleted, *hr*114, was kindly provided by Sandra Weller, University of Connecticut.

Plasmid and phage constructs. Restriction endonucleases and DNA-modifying enzymes were purchased from New England Biolabs Inc.; International Biotechnologies, Inc.; or Boehringer Mannheim Biochemicals, and reactions were carried out according to the suppliers' instructions. Ligation mixtures were transfected into *Escherichia coli* DH5α according to the method of Hanahan (18). Colony hybridizations were performed according to the method of Grunstein and Hogness (17), and small-scale DNA preparations were performed as described by Holmes and Quigley (20). Large-scale plasmid purification was performed by the alkaline lysis technique of Birnboim and Doly (1) as described by Maniatis et al. (26). DNA blots were performed according to a variation of the method of Southern (46) by using Nytran (Schleicher & Schuell) membranes.

The riboprobe construct pRAB1 and the phage constructs ϕRAB1, -2, -23, -24, α0-11, and α0-18 have been described previously. The M13mp19 thymidine kinase (TK) phage construct contained an 840-bp *Pst*I fragment that overlapped the 5′ end of the TK open reading frame (ORF). The M13mp18 gC phage construct contained a 983-bp *Eco*RI-to-*Pvu*I fragment from the 5′ end of the gC ORF. The plasmid used to create the αX promoter deletion mutants, pRAB43, was constructed as follows. The vector pKS8 was derived from pBR322 by digestion with *Dra*I and isolation of the 3650-bp fragment followed by ligation of a *Xho*I linker, transformation, and selection by tetracycline. The 8,240-bp *Xho*I-*Sal*I fragment spanning the joint region of HSV-1(17) was cloned into the *Xho*I site of pKS8 to create pRAB30. This plasmid was digested with *Bst*XI and *Dra*I to yield a 10,575-bp fragment, which was ligated to *Bgl*II linkers and recircularized (pRAB37), and a 1,315-bp fragment, which was ligated to *Bam*HI linkers and inserted into the *Bam*HI site of pBR322 (pRAB38). Digestion of pRAB38 with *Stu*I and *Sac*I followed by ligation to an *Asp* 718 linker and recircularization resulted in pRAB40, where the αX promoter is deleted and substituted with the *Asp* 718 linker as a physical marker. This fragment was built back into the *Xho*I-*Sal*I fragment by insertion of the *Bam*HI fragment of pRAB40 with *Bgl*II-linearized pRAB37 to create the recombination substrate pRAB43 shown in Fig. 1B. The recombination substrate used to create the polyadenylation insertion mutant upstream of the αX promoter, pRB4813, was constructed by the insertion of a 500-bp *Nco*I fragment from the HSV-1(F) gB gene from pRB2108 into the *Bst*XI site in pRB143.

Nuclear run-on transcription assays. Nuclear run-on transcription assays were performed by a variation of the technique of Weinheimer and McKnight (59). The differences were that the cells were washed three times in ice-cold phosphate-buffered saline before being resuspended in reticulocyte standard buffer containing 0.1% Nonidet P-40 and 20 U of RNase inhibitor (Boehringer Mannheim Biochemicals) per ml. Reactions were performed at 30°C instead of 27°C and utilized 100 μCi of [α-^{32}P]GTP per mixture. Equal counts per minute from each reaction were hybridized to GeneScreen Plus filters (NEN) containing specific single-stranded DNA probes. Hybridizations were carried out in 750 mM NaCl–150 mM Tris-HCl (pH 7.0)–18 mM NaH_2PO_4–28 mM Na_2HPO_4–0.08% polyvinylpyrrolidone–0.08% Ficoll 400–0.08% bovine serum albumin (fraction V)–2% sodium dodecyl sulfate (SDS)–50% formamide supplemented with 100 μg of sonicated denatured calf thymus DNA per ml. Hybridization was at 47°C for 40 h. The filters were washed twice with 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) at 25°C for 15 min, twice with 0.1× SSC–0.5% SDS at 68°C for 30 min, and once in 0.1× SSC at 68°C for 30 min. Following autoradiography, the filters were subjected to two-dimensional β-emission spectroscopy and the counts were determined for specific bands.

RNA extraction and analysis. Total cellular RNA was isolated by extraction with guanidinium isothiocyanate (GuSCN) by a modification of the procedure of Chirgwin et al. (4, 15). Cytoplasmic RNA was isolated by pretreatment of the cells with 0.5% Nonidet P-40 and centrifugation at 3,500 × *g* to pellet nuclei before extraction with GuSCN. Alternatively, cytoplasmic RNA was extracted from cells by using RNA STAT-60 (Tel-Test, Inc.) by a modification of the manufacturer's instructions whereby cytoplasmic extracts were diluted 1:8 in

RNA STAT-60. Polyadenylated RNA was purified by using the poly(A) tract system (Promega).

Northern (RNA) blot analyses were performed by electrophoresis of RNA through 1% agarose–6% formaldehyde gels according to the method of Lehrach et al. (25) as described by Maniatis et al. (26). Gels were blotted by capillary action to GeneScreen Plus membranes (New England Nuclear) in 10× SSC (pH 7.0). Blots were hybridized to ^{32}P-labeled DNA probes prepared by random priming (13) for α4, to ^{32}P-labeled riboprobes prepared according to the method of Melton et al. (33) for pRAB1, or to ^{32}P-labeled strand-specific DNA probes made from templates cloned into M13 vectors as described elsewhere (26) for TK and gC.

RESULTS

Construction of HSV-1 mutants with an αX promoter deletion. The plasmid, pRAB43, which was used to delete the αX promoter contains a synthetic *Asp* 718 linker inserted in place of a 253-bp fragment extending from a *Stu*I site upstream of the αX TATA box through the two *Sac*I sites downstream (see Materials and Methods). The HSV-1(17) sequence within pRAB43 extended 6,177 bp downstream of the *Asp* 718 marker substitution to a *Sal*I site located within the α4 gene. The clone overlaps the region of the α4 gene containing the A-to-V mutation at codon 474 which is responsible for the phenotype of the mutant *ts*K (9, 38). There is another 1,544 bp of overlap upstream (relative to α4) of the *ts*K allele and 1,805 bp upstream (relative to αX) of the αX promoter deletion at the other end. This construct was used in a recombination scheme (Fig. 1B) in which recombinants were first screened by rescue of the *ts*K allele and subsequently screened for the *Asp* 718 site substitution by Southern blot.

Ten plates of Vero cells were cotransfected with nucleocapsids from *ts*K and pRAB43 DNA which was linearized at the *Xho*I site 1,805 bp upstream of the αX promoter deletion. The plates were incubated at the permissive temperature (31.5°C) and harvested at 3 days postinfection. The virus yield from each plate was plated and incubated at the nonpermissive temperature (39.5°C) to select for recombinants. Four plaques were picked from each plate and amplified, and infected-cell DNA was extracted. The DNA was digested with *Asp* 718 and subjected to Southern blot analysis (data not shown). Three of the 40 plaques screened positive for the substitution but were heterogeneous, probably because only one of the two copies was mutated. These isolates were subjected to three further rounds of plaque purification and screening. One of the isolates reverted to the wild type, but the other two homogenized to the mutant form and were designated vRAB1a and vRAB1b.

The structure of the mutant genotypes was determined by Southern blot hybridization (Fig. 2A). When *Asp* 718 digests of DNA extracted from infected cells were blotted and probed with a 515-bp *Bst*XI-*Dra*III fragment, two sets of bands were seen in the digests of wild-type and *ts*K DNA. A doublet of 10.5 and 12 kb results from digestion of a joint fragment. This species is a doublet because of the two orientations of U_S. The 3.7-kb band represents the terminal fragment. The deletion of the αX promoter and introduction of the new *Asp* 718 site resulted in the digestion of the 10.5-kb–12-kb joint doublet to 7.2-kb–9.7-kb and 2.1-kb fragments. The 7.2-kb fragment was not detected in the vRAB1b digest. This may result from another mutation within the *a* repeats that prevents isomerization of the viral genome, such as the mutations described by Poffenberger et al. (37). The 3.2-kb terminal fragment was digested to a 2.1-kb fragment and a residual 1.3-kb fragment.

To verify that both copies of the *ts*K allele were rescued, we probed for a restriction site polymorphism which was created by the *ts*K mutation. The C-to-T transition at nucleotide +1717 results in the formation of an *Mae*II site (9). Therefore,

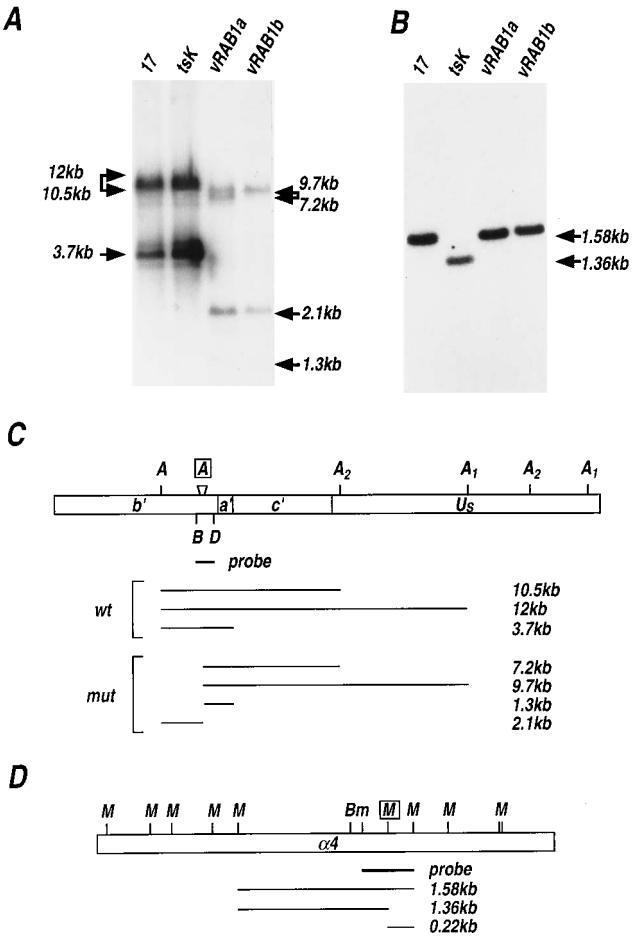

FIG. 2. Southern blots of vRAB1a and vRAB1b. (A) *Asp* 718 digest of HSV-1(17), *ts*K, vRAB1a, and vRAB1b electrophoresed on a 1% agarose gel, transferred to Nytran membrane, and hybridized to the *Bst*XI-*Dra*III probe shown in panel C below. (B) *Mae*II digest of HSV-1(17), *ts*K, vRAB1a, and vRAB1b electrophoresed on a 1% agarose gel, transferred to Nytran membrane, and hybridized to the *Bam*HI-*Mae*II probe shown in panel D below. (C) Map of relevant restriction endonuclease sites for the autoradiogram depicted in panel A. The probe and specific fragments are shown below the map, and the fragment sizes are noted at the right. The boxed site is present only in the mutant. *A*, *Asp* 718; *B*, *Bst*XI; *D*, *Dra*III. The subscripts denote the positions of the *Asp* 718 sites in either genomic orientation. wt, wild type; mut, mutant. (D) Map of relevant restriction endonuclease sites for the autoradiogram depicted in panel B. The probe and specific fragments are shown below the map, and the fragment sizes are noted at the right. The boxed site is present only in the mutant. *M*, *Mae*II; *Bm*, *Bam*HI.

infected-cell DNA was digested with *Mae*II, blotted, and hybridized to a 368-bp *Mae*II-*Bam*HI probe located within the wild-type fragment. As shown in Fig. 2B, the 1.58-kb *Mae*II wild-type DNA fragment is reduced to 1.36 kb in *ts*K. The residual 222-bp fragment cannot be seen on this gel. The two mutants contain only the 1.58-kb fragment indicative of homogeneous diploid wild-type α4.

Construction of an HSV-1 mutant with a polyadenylation site upstream of the αX promoter. As described in Materials and Methods, the plasmid pRB4813 contains a 500-bp fragment including the polyadenylation signal of the glycoprotein B gene, which is inserted into the *Bst*XI site located 138 bp upstream of the *Stu*I site used to define the αX promoter deletion in the previous mutants. This construct was used in a recombination scheme (Fig. 1C) in which recombinants were selected on the basis of the rescue of a 1-kb deletion in the

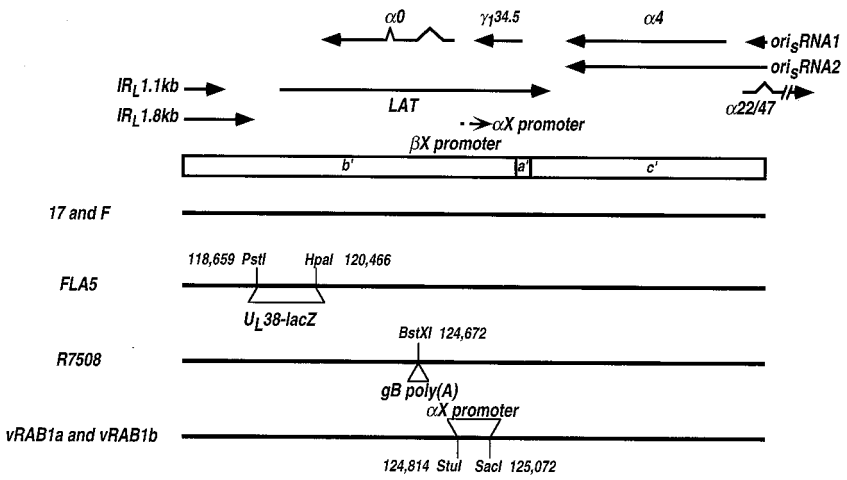

FIG. 3. Structures of mutants used in this study.

$\gamma_1$34.5 gene in the mutant R3616. The plasmid was cotransfected with R3616 DNA into rabbit skin cells, and the resulting virus yield was used to infect mice by the corneal route (32). Because of the deletion in $\gamma_1$34.5, R3616 is unable to replicate in sensory ganglia (5, 60). Therefore, the viruses harvested from the ganglia 3 days postinoculation and plaque purified on Vero cells were recombinants in which $\gamma_1$34.5 was rescued. These mutants were then screened for the presence of the polyadenylation site in both copies of the repeat flanking U_L (data not shown), and the mutant R7508 was isolated.

Transcription of HSV-1 mutants downstream of the αX promoter. In addition to the HSV-1(17) αX promoter deletion mutants, vRAB1a and vRAB1b, and the mutant in which a fragment containing the transcription termination and polyadenylation site of the gB gene was inserted at the *Bst*XI site upstream of the αX promoter in HSV-1(F), R7508, one other mutant was obtained for transcription analysis. In this mutant, FLA5, both LAT promoters in HSV-1(17) were deleted and a fragment containing the *lacZ* gene under the control of the U_L38 promoter was substituted (Fig. 3) (14).

To ensure that the replication kinetics of the αX promoter deletion mutants and the polyadenylation insertion mutant were equivalent to those of wild-type virus, single-step growth curve experiments were performed. Vero cells were infected with virus at a multiplicity of infection of 5 PFU per cell, and the yields were titrated at regular intervals postinfection (Fig. 4). Neither the deletion of the αX promoter nor the insertion of the polyadenylation signal upstream of the αX promoter had any effect on the replication of the mutants relative to that of wild-type virus under these conditions. The LAT promoter deletion mutant also grew like wild-type virus (data not shown).

Transcription downstream of the αX promoter was measured by nuclear run-on assays. The high level of transcription detected by the ϕRAB1 probe in extracts from cells infected and maintained in the presence of cycloheximide with wild-type virus also occurred with FLA5 and R7508. However, very little transcription was detected from the vRAB1 viruses with this probe. The low level detected was equivalent to the level detected by the upstream ϕRAB23 probe. This amount of transcription was not seen in cells infected with the LAT promoter deletion virus, FLA5, suggesting that it was derived from transcription of LAT. Therefore, high-level transcription in the presence of cycloheximide required the αX promoter and was independent of some upstream promoter (Fig. 5).

Interestingly, nascent transcripts from the opposite strand, as detected by ϕRAB2, were more abundant in cells infected by the vRAB1 mutants. This would suggest that deletion of the αX promoter results in the loss of some regulatory element which normally inhibits transcription from the opposite strand. The significance of this is unclear, as no immediate-early transcription from this locus has been detected with any wild-type virus.

Different results were obtained, however, in assays performed with nuclei from cells infected with virus in the absence of inhibitor and harvested at 8 h postinfection, the peak period of transcription of sequences downstream of the αX promoter (Fig. 6) (3). Under these conditions, transcription detected downstream of the αX promoter by ϕRAB1 was similar in cells infected with vRAB1a and vRAB1b to that seen in cells infected with wild-type virus. However, in cells infected with R7508 transcription from this region was decreased. In contrast, transcription of the α0 and LAT genes, as detected by the α0-11 and α0-18 probes, respectively, was unchanged. This suggests that the transcripts seen during productive infection originate from a promoter located upstream of the αX promoter. The pattern of transcription from FLA5 is equivalent to that from the wild type, demonstrating that this upstream promoter is not either of the two previously characterized LAT

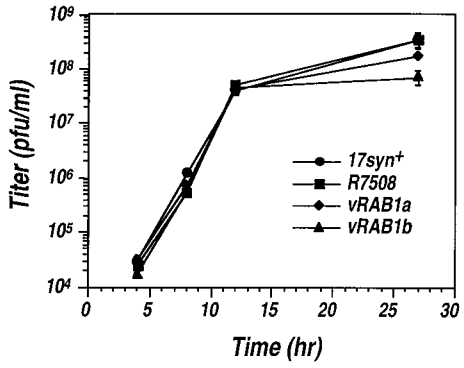

FIG. 4. Single-step growth curve of mutants. Vero cells were infected with virus at a multiplicity of infection of 5 in triplicate and harvested at 4, 8, 12, and 27 h postinfection. Titers and yields were determined for each time point and are expressed as means. The error bars indicate standard deviations.

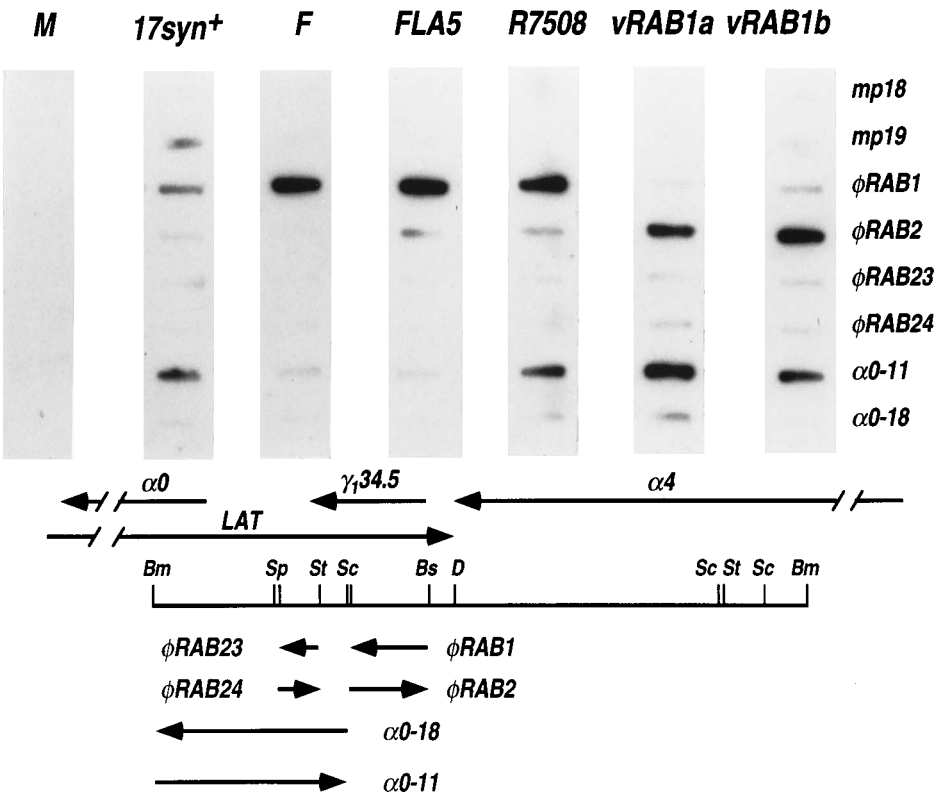

FIG. 5. Transcription of mutants in the presence of cycloheximide. Autoradiograms of nuclear run-on transcription assays are shown. The specific probes used are noted at the right, and their positions are depicted at the bottom. The virus strain or mutant used is noted at the top of each column. All reactions were performed with nuclei prepared 6 h postinfection and were carried out in the presence of cycloheximide. M, mock infected cells. *Bm*, *Bam*HI; *Sp*, *Sph*I; *St*, *Stu*I; *Sc*, *Sac*I; *Bs*, *Bst*XI; *D*, *Dra*III.

promoters but represents transcription from a previously undescribed promoter.

Preliminary characterization of stable transcripts mapping downstream of the αX promoter. The existence of two overlapping transcription units downstream of the previously characterized αX promoter which are expressed under different conditions suggested the possibility of several novel stable RNA species mapping to this region of the HSV-1 genome. To examine this possibility, Vero cells were infected with HSV-1(F) and harvested at 2, 6, or 16 h postinfection. Steady-state levels of polyadenylated RNA from cytoplasmic extracts of these cells were measured by Northern blot analysis. A strand-specific riboprobe transcribed from the *Sac*I-*Bst*EII fragment (pRAB1) detected two transcripts of 0.9 and 4.0 kb at all three time points utilized. In the experiment shown, they were most abundant at 6 h (Fig. 7). More detailed analysis demonstrated that accumulation was greatest at 10 h postinfection (data not shown). A third stable RNA, approximately 5.4 kb in size, was detected at the 2-h time point in this experiment, but it was not seen in other experiments.

In addition, some cells were infected in the presence of 100 μg of cycloheximide per ml and incubated for 6 h prior to harvest. A subset of these cells were washed at 5 h postinfection and incubated in the absence of cycloheximide for 1 h. In the presence of cycloheximide, no RNA homologous to the pRAB1 probe was detected. However, after reversal of the cycloheximide treatment transcripts corresponding to those detected at 10 h postinfection accumulated at a level comparable to that for the early time point. Thus, although αX nascent transcription occurs in the presence of cycloheximide, no stable transcripts accumulated.

In two control groups, cells were either mock infected or infected with HSV-2(G). These cells were incubated for 2 or 6 h prior to harvest. In each instance, polyadenylated cytoplasmic RNA was purified and subjected to Northern blot analysis and no stable mRNAs were seen. This confirmed that the transcripts detected were of viral origin and not cellular transcripts which were constitutively expressed, or, for the HSV-2(G) infection, induced by the virus. To control for differential RNA degradation, a similar blot was made and probed for the α4 transcript by using a ^{32}P-labeled *Bam*HI Y fragment. The kinetics of accumulation of this transcript resembled those of other α RNAs and were distinct from those of the RNAs transcribed through the junction between the L and S components of HSV DNA.

To determine whether the transcription seen during productive infection occurs with β or γ kinetics, we obtained a mutant virus, *hr*114, in which one of the subunits of the helicase-primase complex, U_L52, was deleted. This gene is required for viral DNA replication, and infection of noncomplementing Vero cells with *hr*114 results in a block of the viral life cycle so that late gene products are not detectable by Western blot (immunoblot) analysis (data not shown). Northern blot analysis was performed with cytoplasmic RNAs extracted at 10 h from cells infected with wild-type or mutant viruses under conditions in which DNA replication was blocked either genetically (for *hr*114) or by the use of 300 μg of phosphonacetic acid per ml (a concentration that inhibits virus DNA replica-

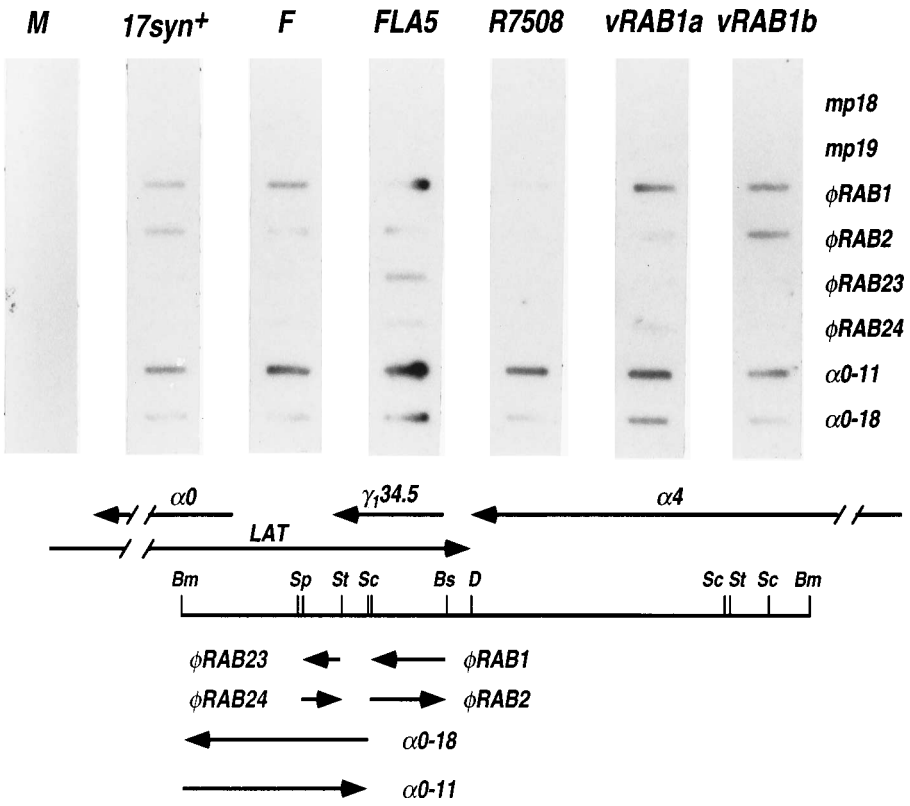

FIG. 6. Transcription of mutants during productive infection. Autoradiograms of nuclear run-on transcription assays are shown. The specific probes used are noted at the right, and their positions are depicted at the bottom. The virus strain or mutant used is noted at the top of each column. All reactions were performed with nuclei prepared 8 h postinfection. M, mock infected cells. *Bm*, *Bam*HI; *Sp*, *Sph*I; *St*, *Stu*I; *Sc*, *Sac*I; *Bs*, *Bst*XI; *D*, *Dra*III.

tion). The results of this experiment demonstrated that stable RNAs (4.0 and 0.9 kb) homologous to a probe downstream of the αX promoter accumulated independently of DNA replication. These RNAs are therefore derived from a β gene (Fig. 8). Similarly, RNAs derived from this region of the genome were readily detected when *hr*114 was used to infect the complementing cell line 2D6 and when wild-type viruses KOS (the parent of *hr*114) and 17 infected Vero cells in the presence or absence of phosphonacetic acid. The defect was due solely to the loss of the U_L52 gene product and not to a secondary mutation within the βX promoter, as accumulation of these transcripts in 2D6 cells was normal. In contrast, no RNA derived from the gC gene, a true γ gene, was detected after infection of Vero cells by *hr*114 or by either of the wild-type viruses in the absence of DNA replication.

It is interesting that in this experiment, the relative abundance of the 0.9- and 4.0-kb transcripts was reversed compared with what was seen in the experiment shown in Fig. 7. The explanation for this discrepancy is not clear, but we note that the RNAs shown in Fig. 7 were poly(A) selected whereas those displayed in Fig. 8 were unselected total cytoplasmic RNAs.

DISCUSSION

Existence of two overlapping transcription units independent of the LAT promoter. We previously reported the existence of the αX promoter, which was located upstream of and in opposite orientation to the α0 promoter within the HSV-1 TR_L/IR_L sequences (3). Nascent transcription downstream of this promoter was detected under two conditions. During productive infection, maximum transcription occurs at 8 h postinfection, with little or no nascent RNA detected at early time points. Paradoxically, high levels of transcription were detected when infections were performed in the presence of cycloheximide. Although transcription in the presence of cycloheximide was consistent with the existence of the high-affinity ICP4-binding site at the RNA start site and the repression of the αX promoter by ICP4 in transient expression assays, it was difficult to reconcile the shutoff of αX very early in infection with the apparent resumption of transcription at later times.

The evidence presented here suggests that transcription detected downstream of the αX promoter arises from two independent transcription units which are distinguished by the phenotypes of mutants with different mutations at or near the αX promoter. Deletion of the promoter results in the loss of nascent transcripts during infection of cells in the presence of cycloheximide but does not affect transcription detected during productive infection. In contrast, insertion of a transcription termination and polyadenylation site upstream of the promoter results in the loss of transcription during productive infection while leaving transcription in the presence of cycloheximide unaffected. From these data we conclude that this promoter is active only under conditions in which there is no active ICP4 to bind to its cognate high-affinity site and repress transcription. During productive infection when ICP4 is present, this promoter is inactivated and the transcription detected under these conditions arises from another promoter upstream of it. These transcripts are terminated by the insertion of a gB polyadenylation signal just upstream of αX. The presence of nascent transcripts from a mutant in which both LAT promoters were deleted demonstrates that the promoter

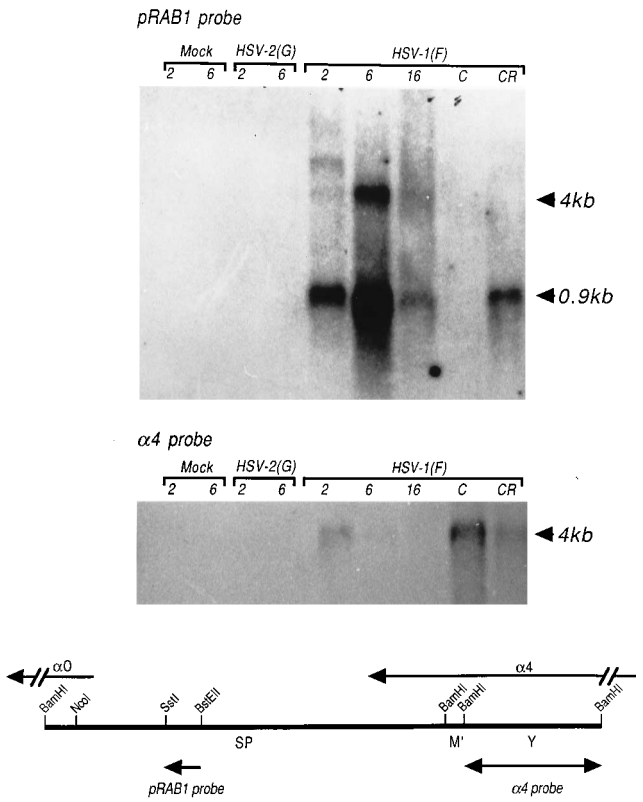

FIG. 7. Detection of transcripts downstream of the αX promoter by Northern blot analysis. Cytoplasmic polyadenylated RNA from mock-infected, HSV-2(G)-infected, or HSV-1(F)-infected cells was subjected to Northern blot analysis. The numbers over the lanes denote the times postinfection of harvest and RNA extraction. The lanes designated C contain RNA from cells infected with HSV-1(F) in the presence of cycloheximide and incubated with the inhibitor for 6 h. The lanes designated CR contain RNA from cells for which the cycloheximide treatment was reversed by washing at 5 h postinfection and which were then incubated for 1 h in the absence of cycloheximide. The blots were hybridized to either a ^{32}P-labeled riboprobe specific for the novel RNAs (upper gel) or a ^{32}P-labeled *Bam*HI Y restriction fragment specific for α4 RNA (lower gel). The locations of the probes are depicted at the bottom.

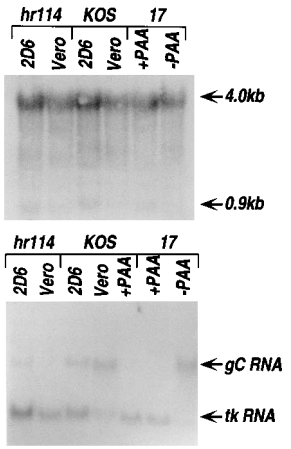

FIG. 8. Transcription kinetics of βX. Vero or 2D6 cells were infected with the virus strain or mutant noted at the top of each lane in the absence or presence of 300 μg of phosphonacetic acid per ml. After 10 h, cytoplasmic RNAs were extracted from each infected-cell culture and electrophoresed on formaldehyde gels. The RNAs were analyzed for the presence of transcripts downstream of the αX promoter (A) and for TK and gC RNAs (B) by hybridization with riboprobes or single-stranded DNA probes specific for each of the above-mentioned sequences.

used during productive infection is unique and has yet to be identified.

The existence of two separate transcription units active under different conditions can be verified by studies of mature transcripts from this region, but verification is complicated by the use of the unusual conditions needed to detect the stable αX RNAs. Infection in the presence of cycloheximide does not allow accumulation of mature transcripts, as measured by Northern blots using the same sequence to probe as was used to detect nascent transcription in nuclear run-on assays. It was reported that mature transcripts can be detected when cells are infected with HSV-1 mutants altered in the gene encoding ICP4 (61). In that study, the two most prominent RNA species migrated at 2.3 and 8.5 kb and their 5′ ends mapped to a point 30 bp downstream from the TATA box in the αX promoter. Presumably all other α functions remained active during these infections. This suggests that although transcription from the αX promoter does not require any α gene products, accumulation of αX mRNAs to levels detectable by Northern blot hybridization does require an α function other than α4.

Mature RNAs resulting from transcription during productive infection were also seen. Two mRNAs of 0.9 and 4.0 kb were detected by Northern blot analysis. These transcripts were not present in cells infected with HSV-2(G); therefore, they are unlikely to be cell transcripts induced by HSV infection. A time course experiment revealed that these transcripts accumulated maximally at 10 h postinfection (data not shown). This corresponds with the kinetics of nascent transcription as previously noted (3). Both of these transcripts are polyadenylated and transported to the cytoplasm (Fig. 7).

The existence of a 0.9-kb transcript from this region was reported previously (19). In that study, a double-stranded DNA probe, the *Bam*HI SP fragment, was used to monitor the kinetics of α4 mRNA accumulation. This probe also detected the 0.9-kb transcript. Presumably, that probe, which overlaps the pRAB1 probe used in this study, would also have hybridized to the 4.0-kb transcript. However, such hybridization would have been masked by the presence of the α4 transcript of the same size but opposite polarity. Our use of a strand-specific riboprobe revealed that there are two 4.0-kb species transcribed from this region of the genome, each from a different strand. The strand specificity of the probe also explains why the $\gamma_1$34.5 mRNA, which maps to this region of the genome, was not detected.

Potential role of the αX transcription unit. The existence of a polyadenylated transcript mapping downstream of the αX promoter suggests the possibility of a protein encoded by this region of the genome. Analysis of the DNA sequences of both HSV-1(17) (29, 30) and HSV-1(F) (6, 7) shows an ORF beginning 141 bp downstream of the RNA start site determined by Yeh and Schaffer (61) that encodes a potential polypeptide of 233 amino acids in strain 17 or 248 amino acids in strain F (Fig. 9). The difference in size reflects a difference in the number of repeats corresponding to the *a* regions between the two strains, resulting in a variable number of VAG repeats within the potential protein. Otherwise this reading frame is highly conserved between the two strains, showing only five other amino acid changes.

Two of us utilized the technique of epitope tagging to identify the ORFs within this region which are translated (24). The particular ORF described above, which was designated ORF P, was tagged and recombined into the HSV-1(F) genome. No tagged protein was detected from this virus during productive

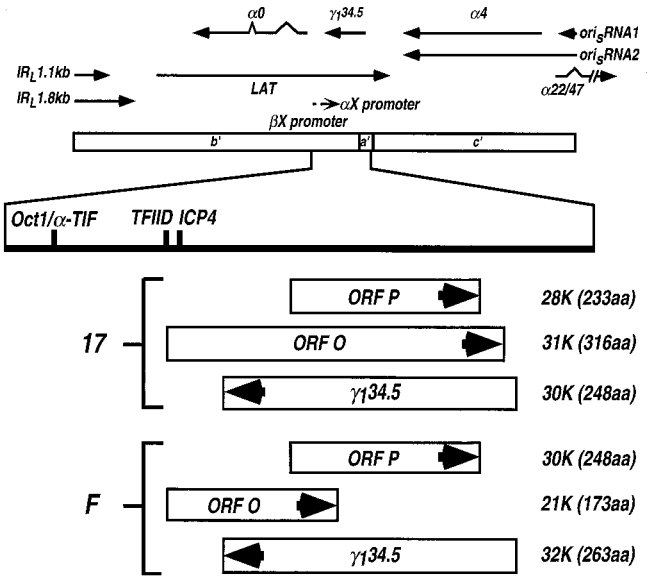

FIG. 9. Map of ORFs. The transcription map of the repeat regions of HSV-1 is shown at the top. The region surrounding the αX promoter is expanded, and the ORFs for both strain 17 and strain F are depicted at the bottom. The arrows denote the polarities of the ORFs. The predicted sizes of the encoded proteins are listed at the right. aa, amino acids.

infection. Strain F has a temperature-sensitive α4 gene. When cells were infected with strain F and maintained at 39.5°C, a protein with an M_r of approximately 26,000 was detected. Furthermore, this protein could be detected in cells infected and maintained in the presence of cycloheximide, which was subsequently washed out in the presence of the transcription inhibitor actinomycin D (24a). Under these conditions only α genes are transcribed, suggesting that enough stable RNA accumulated in the presence of cycloheximide to be translated, although it could not be detected by Northern blot. That detectable levels of mRNA accumulate in cells infected with α4 mutants (61) suggests that other α-gene products enhance its accumulation and that this RNA may be a pre-α transcript.

At this time, any biological role for αX remains highly speculative. Consideration must be given to the unusual circumstances under which expression is seen. This gene is expressed only at late times during an abortive infection when α4 is not active. It is completely shut off during productive infection, as if its expression would be antithetical to virus growth. This mode of expression is consistent with a model in which αX is a repressor required in some aspect of latency. No such HSV function has been described, and although no evidence is presented here to directly address this issue, it provides an interesting problem for further investigation.

Potential role for the βX transcription unit. As with the αX transcription unit, an analysis of HSV-1(F) and HSV-1(17) was performed to locate potential ORFs which might encode a corresponding protein from the βX transcription unit. This analysis was complicated by the fact that the locations of the βX promoter and transcription start site are unknown. The nearest upstream TATA box is located within IVS I of the α0 gene. ORF O, a potential ORF overlapping ORF P, begins within the TATA box of the αX promoter and extends for 316 codons in the HSV-1(17) sequence (Fig. 9). In the F strain, however, this ORF is terminated by an opal codon substituting for an R at position 173, which corresponds to position 158 in strain 17. The difference in codon numbers results from variation in the number of *a* sequence repeats. Other than the repeat variation and the difference in the stop codons, this reading frame is also highly conserved, with only three other amino acid differences.

The study by Lagunoff and Roizman (24) used the F strain. When recombinants in ORF O were tagged, no stable proteins were detected either during productive infection or at the nonpermissive temperature. The truncated nature of the ORF O polypeptide in the F strain, however, may lead to protein instability and an inability to detect the gene product in this strain.

The possible functions of βX are unknown. The fact that this transcription unit linearly overlaps with the $\gamma_1$34.5 unit transcribed from the opposite strand tempts speculation that some of the functions involving neurovirulence and programmed cell death attributed to $\gamma_1$34.5 (5, 8, 60) may actually be the result of βX activity. If, however, the βX gene product is indeed encoded by ORF O, which is truncated in strain F, all the previous experiments in which these activities were ascribed to $\gamma_1$34.5 were performed in a βX-defective background already. This would suggest that these functions were correctly attributed to $\gamma_1$34.5. A function for βX may be inferred from some difference already noted between the F and 17 strains. The 50% lethal doses of the two strains after intracranial inoculation differ by at least 2 orders of magnitude (2). It is possible that this may result from the expression of βX in the more virulent strain 17. Alternatively, the role of βX transcription may involve the regulation of $\gamma_1$34.5 expression through the production of antisense RNAs. In this context, it is useful to consider that expression of this transcription unit precedes that of $\gamma_1$34.5. Clearly, there is a need for further study of the nature of these gene products.

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REFERENCES

1. **Birnboim, H. C., and J. Doly.** 1979. A rapid extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Res. **7:**1513–1523.
2. **Bohenzky, R. A., E. Kern, and S. Silverstein.** Unpublished data.
3. **Bohenzky, R. A., A. G. Papavassiliou, I. H. Gelman, and S. Silverstein.** 1993. Identification of a promoter mapping within the reiterated sequences that flank the herpes simplex virus type 1 U_L region. J. Virol. **67:**632–642.
4. **Chirgwin, J. M., A. E. Przybla, R. J. MacDonald, and W. J. Rutter.** 1979. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. Biochemistry **18:**5294–5299.
5. **Chou, J., E. R. Kern, R. J. Whitley, and B. Roizman.** 1990. Mapping of herpes simplex virus-1 neurovirulence to gamma$_1$ 34.5, a gene nonessential for growth in culture. Science **250:**1262–1266.
6. **Chou, J., and B. Roizman.** 1985. The terminal *a* sequence of the herpes simplex virus genome contains the promoter of a gene located in the repeat sequences of the L component. J. Virol. **57:**629–637.
7. **Chou, J., and B. Roizman.** 1990. The herpes simplex virus gene for ICP34.5, which maps in inverted repeats, is conserved in several limited-passage isolates but not in strain 17syn$^+$. J. Virol. **64:**1014–1020.
8. **Chou, J., and B. Roizman.** 1992. The gamma$_1$ 34.5 gene of herpes simplex virus 1 precludes neuroblastoma cells from triggering total shutoff of protein synthesis characteristic of programmed cell death in neuronal cells. Proc. Natl. Acad. Sci. USA **89:**3266–3270.
9. **Davison, M. J., V. G. Preston, and D. J. McGeoch.** 1984. Determination of the sequence alteration in the DNA of the herpes simplex virus type 1 temperature-sensitive mutant tsK. J. Gen. Virol. **65:**859–863.
10. **Devi-Rao, G. B., S. A. Goodart, L. M. Hecht, R. Rochford, M. K. Rice, and E. K. Wagner.** 1991. Relationship between polyadenylated and nonpolyadenylated herpes simplex virus type 1 latency-associated transcripts. J. Virol. **65:**2179–2190.
11. **Dixon, R. F., and P. A. Schaffer.** 1980. Fine-structure mapping and functional analysis of temperature-sensitive mutants in the gene encoding the

herpes simplex virus type 1 immediate early protein VP175. J. Virol. **36:**189–203.
12. **Dobson, A. T., F. Sederati, G. Devi-Rao, W. M. Flanagan, M. J. Farvell, J. G. Stevens, E. K. Wagner, and L. T. Feldman.** 1989. Identification of the latency-associated transcript promoter by expression of rabbit beta-globin mRNA in mouse sensory nerve ganglia latently infected with a recombinant herpes simplex virus. J. Virol. **63:**3844–3851.
13. **Feinberg, A. P., and B. Vogelstein.** 1983. A technique for radiolabeling DNA restriction fragments to high specific activity. Anal. Biochem. **132:**6–13.
14. **Flanagan, W. M., A. G. Papavassiliou, M. Rice, L. B. Hecht, S. Silverstein, and E. K. Wagner.** 1991. Analysis of the herpes simplex virus type 1 promoter controlling the expression of U_L38, a true late gene involved in capsid assembly. J. Virol. **65:**769–786.
15. **Gelman, I. H., and S. Silverstein.** 1986. Coordinate regulation of herpes simplex virus gene expression is mediated by the functional interaction of two immediate early gene products. J. Mol. Biol. **191:**395–409.
16. **Goins, W. F., L. R. Sternberg, K. D. Croen, P. R. Krause, R. L. Hendricks, D. J. Fink, S. E. Straus, M. Levine, and J. C. Glorioso.** 1994. A novel latency-active promoter is contained within the herpes simplex virus type 1 U_L flanking repeats. J. Virol. **68:**2239–2252.
17. **Grunstein, M., and D. S. Hogness.** 1975. Colony hybridization: a method for the isolation of cloned DNAs that contain a specific gene. Proc. Natl. Acad. Sci. USA **72:**3961–3965.
18. **Hanahan, D.** 1983. Studies on transformation of Escherichia coli with plasmids. J. Mol. Biol. **166:**557–580.
19. **Harris-Hamilton, E., and S. L. Bachenheimer.** 1985. Accumulation of herpes simplex virus type 1 RNAs of different kinetic classes in the cytoplasm of infected cells. J. Virol. **53:**144–151.
20. **Holmes, D. S., and M. Quigley.** 1981. A rapid boiling method for the preparation of bacterial plasmids. Anal. Biochem. **114:**193–197.
21. **Honess, R. W., and B. Roizman.** 1974. Regulation of herpesvirus macromolecular synthesis. I. Cascade regulation of the synthesis of three groups of viral proteins. J. Virol. **14:**8–19.
22. **Honess, R. W., and B. Roizman.** 1975. Regulation of herpesvirus macromolecular synthesis: sequential transition of polypeptide synthesis requires functional viral polypeptides. Proc. Natl. Acad. Sci. USA **72:**1276–1280.
23. **Kieff, E., B. Hoyer, S. Bachenheimer, and B. Roizman.** 1972. Genetic relatedness of type 1 and type 2 herpes simplex viruses. J. Virol. **9:**738–745.
24. **Lagunoff, M., and B. Roizman.** 1994. Expression of a herpes simplex virus 1 open reading frame antisense to the $\gamma_1$34.5 gene and transcribed by an RNA 3′ coterminal with the unspliced latency-associated transcript. J. Virol. **68:**6021–6028.
24a. **Lagunoff, M., and B. Roizman.** Unpublished data.
25. **Lehrach, H. D., D. Diamond, J. M. Wozney, and H. Boedtker.** 1977. RNA molecular weight determinations by gel electrophoresis under denaturing conditions, a critical reexamination. Biochemistry **16:**4743–4751.
26. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
27. **Marsden, H. S., I. K. Crombie, and J. H. Subak-Sharpe.** 1976. Control of protein synthesis in herpesvirus-infected cells: analysis of the polypeptides induced by wild-type and sixteen temperature-sensitive mutants of HSV strain 17. J. Gen. Virol. **31:**347–372.
28. **McCarthy, A. M., L. McMahan, and P. A. Schaffer.** 1989. Herpes simplex virus type 1 ICP27 deletion mutants exhibit altered patterns of transcription and are DNA deficient. J. Virol. **63:**18–27.
29. **McGeoch, D. J., M. A. Dalrymple, A. J. Davison, A. Dolan, M. C. Frame, D. McNab, L. J. Perry, J. E. Scott, and P. Taylor.** 1988. The complete sequence of the long unique region in the genome of herpes simplex virus type 1. J. Gen. Virol. **69:**1531–1574.
30. **McGeoch, D. J., A. Dolan, S. Donald, and D. M. K. Brauer.** 1986. Complete DNA sequence of the short repeat region in the genome of herpes simplex virus type 1. Nucleic Acids Res. **14:**1727–1745.
31. **McGeoch, D. J., A. Dolan, S. Donald, and F. J. Rixon.** 1985. Sequence determination and genetic content of the short unique region in the genome of herpes simplex virus type 1. J. Mol. Biol. **181:**1–13.
32. **Meignier, B., B. Norrild, and B. Roizman.** 1983. Colonization of murine ganglia by a superinfecting strain of herpes simplex virus. Infect. Immun. **41:**702–708.
33. **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.
34. **Nicosia, M., S. L. Deshmane, J. M. Zabolotny, T. Valyi-Nagy, and N. W. Fraser.** 1993. Herpes simplex virus type 1 latency-associated transcript (LAT) promoter deletion mutants can express a 2-kilobase transcript mapping to the LAT region. J. Virol. **67:**7276–7283.
35. **Nishioka, Y., and S. Silverstein.** 1977. Degradation of cellular mRNA during infection by herpes simplex virus. Proc. Natl. Acad. Sci. USA **74:**2370–2374.
36. **O'Hare, P., and G. S. Hayward.** 1985. Three *trans*-acting regulatory proteins of herpes simplex virus modulate immediate-early gene expression in a pathway involving positive and negative feedback regulation. J. Virol. **56:**723–733.
37. **Poffenberger, K. L., E. Tabares, and B. Roizman.** 1983. Characterization of a viable, non-inverting herpes simplex virus 1 genome derived by insertion of sequences at the L-S junction. Proc. Natl. Acad. Sci. USA **80:**2690–2694.
38. **Preston, C. M.** 1979. Control of herpes simplex virus type 1 mRNA synthesis in cells infected with wild-type virus or the temperature-sensitive mutant *tsK*. J. Virol. **29:**275–284.
39. **Purves, F. C., W. O. Ogle, and B. Roizman.** 1993. Processing of the herpes simplex virus regulatory protein alpha22 mediated by the UL13 protein kinase determines the accumulation of a subset of alpha and gamma mRNAs and proteins in infected cells. Proc. Natl. Acad. Sci. USA **90:**6701–6705.
40. **Quinlan, M. P., and D. Knipe.** 1985. Stimulation of expression of a herpes simplex virus DNA-binding protein by two viral factors. Mol. Cell. Biol. **5:**957–963.
41. **Rice, S. A., and D. M. Knipe.** 1988. Gene-specific *trans*-activation by the herpes simplex virus type 1 alpha protein ICP27. J. Virol. **62:**3814–3823.
42. **Sacks, W. R., C. C. Greene, D. P. Aschman, and P. A. Schaffer.** 1985. Herpes simplex virus type 1 ICP27 is an essential regulatory protein. J. Virol. **55:**796–805.
43. **Sekulovich, R. E., K. Leary, and R. M. Sandri-Goldin.** 1988. The herpes simplex virus type 1 α protein ICP27 can act as a *trans*-repressor or a *trans*-activator in combination with ICP4 and ICP0. J. Virol. **62:**4510–4522.
44. **Sheldrick, P., and N. Berthelot.** 1974. Inverted repetitions in the chromosome of herpes simplex virus. Cold Spring Harbor Symp. Quant. Biol. **39:**667–678.
45. **Singh, J., and E. K. Wagner.** 1993. Transcriptional analysis of the herpes simplex virus type 1 region containing the TRL/UL junction. Virology **196:**220–231.
46. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98:**503–517.
47. **Stevens, J. G., E. K. Wagner, G. Devi-Rao, M. L. Cook, and L. T. Feldman.** 1987. RNA complementary to a herpesvirus alpha gene mRNA is prominent in latently infected neurons. Science **235:**1056–1059.
48. **Stringer, J. R., L. E. Holland, R. I. Swanstrom, K. Pivo, and E. K. Wagner.** 1977. Quantitation of herpes simplex virus type 1 RNA in infected HeLa cells. J. Virol. **21:**889–901.
49. **Su, L., and D. M. Knipe.** 1989. Herpes simplex virus α protein ICP27 can inhibit or augment viral gene transactivation. Virology **170:**496–504.
50. **Swanstrom, R. I., K. Pivo, and E. K. Wagner.** 1974. Restricted transcription of the herpes simplex virus genome occurring early after infection and in the presence of metabolic inhibitors. Virology **66:**140–150.
51. **Voss, J. H., and B. Roizman.** 1988. Properties of two 5′-coterminal RNAs transcribed part way and across the S component origin of DNA synthesis of the herpes simplex virus 1 genome. Proc. Natl. Acad. Sci. USA **85:**8454–8458.
52. **Wadsworth, S., R. J. Jacob, and B. Roizman.** 1975. Anatomy of herpes simplex virus DNA: II. Size, composition, and arrangement of inverted terminal repetitions. J. Virol. **15:**1487–1497.
53. **Wagner, E. K.** 1985. Individual HSV transcripts: characterization of specific genes, p. 45–104. *In* B. Roizman (ed.), The herpesviruses, vol. III. Plenum Press, New York.
54. **Wagner, E. K.** 1991. Herpesvirus transcription: general aspects, p. 1–15. *In* E. K. Wagner (ed.), Herpesvirus transcription and its regulation. CRC Press, Boca Raton, Fla.
55. **Wagner, E. K.** 1994. Herpes simplex viruses: molecular biology, p. 593–603. *In* R. G. Webster and A. Granoff (ed.), Encyclopedia of virology. Academic Press, San Diego, Calif.
56. **Wagner, M. M., and W. C. Summers.** 1978. Structure of the joint region and termini of the DNA of herpes simplex virus type 1. J. Virol. **27:**374–387.
57. **Watson, R. J., and J. B. Clements.** 1980. A herpes simplex type 1 function continuously required for early and late virus RNA synthesis. Nature (London) **285:**329–330.
58. **Watson, R. J., M. Sullivan, and G. F. Vande Woude.** 1981. Structures of two spliced herpes simplex virus type 1 immediate-early mRNA's which map at the junctions of the unique and reiterated regions of the virus DNA S component. J. Virol. **37:**431–444.
59. **Weinheimer, S. P., and S. L. McKnight.** 1987. Transcriptional and post-transcriptional controls establish the cascade of herpes simplex virus protein synthesis. J. Mol. Biol. **195:**819–833.
60. **Whitley, R. J., E. R. Kern, S. Chatterjee, J. Chou, and B. Roizman.** 1993. Replication, establishment of latency, and induced reactivation of herpes simplex virus gamma$_1$ 34.5 deletion mutants in rodent models. J. Clin. Invest. **91:**2837–2843.
61. **Yeh, L., and P. A. Schaffer.** 1993. A novel class of transcripts expressed with late kinetics in the absence of ICP4 spans the junction between the long and short segments of the herpes simplex virus type 1 genome. J. Virol. **67:**7373–7382.
62. **Zwaagstra, J. C., H. Ghiasi, S. M. Slanina, A. B. Nesburn, S. C. Wheatley, K. Lillycrop, J. Wood, D. S. Latchman, K. Patel, and S. L. Wechsler.** 1990. Activity of herpes simplex virus type 1 latency-associated transcript (LAT) promoter in neuron-derived cells: evidence for neuron specificity and for a large LAT transcript. J. Virol. **64:**5019–5028.