

A net +1 frameshift permits synthesis of thymidine kinase from a drug-resistant herpes simplex virus mutant

(clinical isolate/pathogenesis/latency/acyclovir/*in vitro* transcription–translation)

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ABSTRACT Clinical resistance to antiviral drugs requires that a virus evade drug therapy yet retain pathogenicity. Thymidine kinase (TK)-negative mutants of herpes simplex virus are resistant to the drug, acyclovir, but are attenuated for pathogenicity in animal models. However, numerous cases of clinical resistance to acyclovir have been associated with viruses that were reported to express no TK activity. We studied an acyclovir-resistant clinical mutant that contains a single-base insertion in its *tk* gene, predicting the synthesis of a truncated TK polypeptide with no TK activity. Nevertheless, the mutant retained some TK activity and the ability to reactivate from latent infections of mouse trigeminal ganglia. The mutant expressed both the predicted truncated polypeptide and a low level of a polypeptide that comigrated with full-length TK on polyacrylamide gels and reacted with anti-TK antiserum, providing evidence for a frameshifting mechanism. *In vitro* transcription and translation of mutant *tk* genes, including constructs in which reporter epitopes could be expressed only if frameshifting occurred, also gave rise to truncated and full-length polypeptides. Reverse transcriptase–polymerase chain reaction analysis coupled with open reading frame cloning failed to detect alterations in *tk* transcripts that could account for the synthesis of full-length polypeptide. Thus, synthesis of full-length TK was due to an unusual net +1 frameshift during translation, a phenomenon hitherto confined in eukaryotic cells to certain RNA viruses and retrotransposons. Utilization of cellular frameshifting mechanisms may permit an otherwise TK-negative virus to exhibit clinical acyclovir resistance.

Herpes simplex virus (HSV) is an important human pathogen, especially in patients with AIDS. A major advance in antiviral therapy has been the use of acyclovir to treat HSV infections, but acyclovir resistance is a problem of increasing clinical significance in immunocompromised patients (1). Clinical resistance implies that HSV can mutate to evade drug therapy yet retain pathogenicity. Because the sensitivity of HSV to acyclovir is due largely to the viral thymidine kinase (TK), which activates the drug (2), HSV *tk* mutations can confer acyclovir resistance (3). Indeed, there have been numerous cases of patients who suffered severe HSV disease despite acyclovir therapy and shed acyclovir-resistant viruses that were reported to express no detectable TK activity and/or full-length TK polypeptides (4–13).

Although TK is not essential for viral replication in cell culture, it is important for viral pathogenesis in animal models. In particular, TK⁻ mutants fail to reactivate from latent infections of mouse sensory ganglia (14–16), due to the *tk* mutation (16). Thus, the association of virus that appears TK⁻ with severe HSV disease has been puzzling. One possible resolution of this paradox is that the clinical isolates

expressed low levels of TK that were not detected. There are HSV mutants that are severely impaired for TK activity and thus acyclovir-resistant, yet express low levels of TK that are sufficient for certain pathogenic phenotypes, including the ability to reactivate (17–20).

We report here a clinical acyclovir-resistant isolate that encodes a gene predicted to express an inactive truncated TK polypeptide due to a single-base insertion mutation. Despite this, the virus retains a low level of TK activity and the ability to reactivate from latency and expresses both the predicted truncated polypeptide and low levels of full-length TK polypeptide. We show that full-length TK expression is due to a net +1 frameshift during translation. The results provide a potential mechanism for clinical acyclovir resistance and have uncovered an unusual example of frameshifting that may have unique mechanistic features.

MATERIALS AND METHODS

Cells and Viruses. Vero cells were propagated and maintained as described (21). Plaque-purified HSV-1 clinical isolates 294.1 and 615.9 and the *tk* deletion mutant *dlsptk* have been described (14, 22). Mutant K5DG1 was isolated from the wild-type laboratory strain KOS by selection with 40 μM ganciclovir (C.B.C.H., unpublished results) and plaque-purified three times. Viruses were grown and titrated as described (23).

Plaque Autoradiography. [¹²⁵I]Iododeoxycytidine plaque autoradiography was performed as described (24), except that a methylcellulose overlay (0.5%) was used instead of human immune serum globulin.

Reactivation from Latency. Seven-week-old CD-1 mice were inoculated with 2 × 10⁶ plaque-forming units of virus via the cornea as described (20). Thirty days after inoculation, trigeminal ganglia were excised and tested for the presence of reactivatable virus by a dissociation protocol (25), modified as described (16).

PCR and Conventional Sequencing. Virion “mini-prep” DNA was prepared (26). The DNA was ethanol-precipitated and the *tk* gene was amplified by PCR as described (27) except that annealing was at 60°C and oligonucleotide primers corresponding to nt –220 to –203 and nt 1348 to 1327 of the KOS *tk* gene (28) were used. These PCR products, processed as described (29), or cloned plasmid DNAs were

Abbreviations: HSV, herpes simplex virus; TK, thymidine kinase; RT, reverse transcriptase.

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sequenced using Sequenase (United States Biochemical) according to the manufacturer's instructions with the two primers listed above and primers from the *tk* gene corresponding to nt 29–50, 90–68, 330–350 (TK9), 451–428, 490–509, 587–607 (TK16), 655–635 (TK5), 841–858, 1037–1018, and 1045–1065.

Plasmids. The *tk* genes of 294.1 and 615.9 were amplified by PCR as described above using the primer corresponding to nt –220 to –203 and a primer corresponding to nt 45,564–45,582 of the HSV-1 strain 17 sequence (30), which lie downstream of the *EcoRI* site in the glycoprotein H gene. The amplified DNAs were digested with *Bgl* II and *EcoRI* and inserted into *Bam*HI/*EcoRI*-digested pGEM-7ZF(+) (Promega) to yield plasmids pBH15 and pBH13, respectively. The inserts were sequenced to ensure that secondary mutations were not present. To construct plasmids pBX1 and pBX22, we started with pTKWT, which contains the 1.9-kbp *EcoRI*–*Pvu* II *tk* fragment from KOS inserted into the *EcoRI* and *Sma* I sites of pGEM-3Zf(+) (Promega) and pTKN20, which contains the *Kpn* I–*Bam*HI 3.4-kbp *tk* fragment from K5DG1 in pGEM-7Zf(+). Plasmid pSVTK1 was then constructed by inserting the *EcoRI*–*Xba* I fragment from pTKWT into pSVK3 (Pharmacia). Plasmid pSVTKN20 was constructed by inserting the *EcoRI*–*Xba* I fragment from pTKN20 into pSVK3. Plasmids pBX1 and pBX22 entailed the insertion of the *Bgl* II–*Xba* I fragment from pSVTK1 and pSVTKN20, respectively, into pBlueScript II KS(+) (Stratagene) at the *Bam*HI and *Xba* I sites. pBX22 was constructed by replacing the *Sac* I fragment of pBX2 downstream of the K5DG1 mutation with that of pBX1. Plasmid pBX15 was constructed by replacing the *Sry* I–*Xba* I fragment of pBX1 with the *Nco* I–*Xba* I fragment from HSV *pol* plasmid pDP1 (31). Sequencing confirmed that the junction of *tk* and *pol* sequences was in-frame. The mutant version (pBX16) of this fusion construct was constructed by exchanging the *Sac* I fragment of pBX22 into pBX15. Plasmids pBH3 and pBH4 were constructed in several steps. First, the fragments between the *Sac* I sites in the *tk* gene and the vector in plasmids pBX1 and pBX22 were deleted to yield plasmids pBS1 and pBS2, respectively. The *EcoRV* fragments including the 5' ends of the *tk* genes from these plasmids were then replaced with a blunt-ended 1.1-kb *Sal* I fragment that encodes the N-terminal portion of HSV Pol from plasmid p911 (32) to form pBH1 and pBH2, respectively. These were cut with *Sac* I and blunt-ended, and the 2.2-kb *Pvu* II fragment from p911 that encodes the C-terminal portion of HSV Pol was inserted to yield pBH3 and pBH4, respectively.

In Vitro Transcription and Translation. *In vitro* transcription and translation were performed as described (33).

Protein Analysis. Vero cells in 60-mm dishes were mock-infected or infected at a multiplicity of 3 and incubated at 37°C. Five hours after infection, [³⁵S]methionine (DuPont/NEN) was added to 100 μCi/ml (1 Ci = 37 GBq). Two and a half hours later, the medium was removed, and the monolayers were washed twice with phosphate-buffered saline (PBS) and then scraped into 1 ml of PBS. The cells were pelleted by low-speed centrifugation at 4°C and resuspended in 1 ml of IP buffer (33). These lysates were then incubated with rabbit preimmune sera and formalin-fixed *Staphylococcus* A (GIBCO/BRL) overnight at 4°C and microcentrifuged. The resulting supernatants or reticulocyte lysates were subjected to immunoprecipitation as described (33) using antisera specifically reacting with HSV-1 TK (kindly provided by W. C. Summers, Yale University, New Haven) or the C-terminal portion of HSV-1 Pol (EX3; kindly provided by K. Weisshart and C. Knopf, Deutsches Krebsforschungszentrum, Heidelberg; ref. 34). ³⁵S-labeled polypeptides were separated on SDS/polyacrylamide gels and visualized by fluorography with Amplify (Amersham) by using preflashed film. The ratio of full-length to truncated products was quan-

tified by densitometry using a Microtek scanner and normalized for the number of methionine residues in each product.

Reverse Transcriptase (RT)–PCR and Open Reading Frame Cloning. After transcription of pBX1 and pBX22, nucleic acids were treated for 1 h at 37°C with 10 units of RNase-free DNase I (Promega). Reaction products were phenol/chloroform-extracted and ethanol-precipitated, and the RNA was used as a template for cDNA synthesis with the TK5 primer and avian myeloblastosis virus RT (Promega) by following the manufacturer's directions. One-twentieth of the resulting cDNA was then diluted in PCR amplification buffer (27) and amplified by PCR using TK9 and TK5 as described for PCR sequencing above. The resulting PCR products were cleaved with *Rsa* I and *Sac* I and treated with T4 DNA polymerase in the presence of all four dNTPs to remove the overhanging 3' ends at the *Sac* I site, and the relevant *Rsa* I–*Sac* I fragment was isolated and ligated into *Sma* I-digested phosphatase-treated pMR100 (ref. 35; plasmid kindly provided by M. Gray, Tufts University, Boston). The resulting ligation mixture was transformed into *Escherichia coli* JM109, plated, and scored on MacConkey agar as described (35, 36).

RESULTS

A Clinical HSV Mutant with a *tk* Insertion Mutation. We studied a clinical acyclovir-resistant *tk* mutant of HSV, 615.9, that was plaque-purified from virus isolated from a patient who suffered severe HSV esophagitis despite acyclovir therapy (22). Mutant 615.9 is genetically related to strain 294.1, which was plaque-purified from a drug-sensitive pretreatment isolate from the same patient (22). We sequenced the 615.9 and 294.1 *tk* genes. The only difference between the 615.9 and 294.1 *tk* genes was the insertion of a guanosine into a run of seven guanosines that begins at nt 540 relative to the transcriptional start site (37), thereby creating a run of eight guanosines. The insertion mutation shifts the *tk* mRNA sequence out of frame so that translation would be predicted to stop at a UGA codon, yielding a truncated polypeptide of 227 aa (Fig. 1). Moreover, as the shift in frame is upstream of residues reported to be critical for TK activity (38–40), the predicted truncated polypeptide would be expected to lack TK activity.

Mutant 615.9 Retains Low TK Activity. These sequencing results were surprising because mutant 615.9 had previously been reported to exhibit some TK activity (22). To exclude the possibility that the TK activity was due to a low level of TK⁺ virus in the 615.9 stock, we performed [¹²⁵I]iododeoxycytidine plaque autoradiography. This technique can distinguish TK⁺ from TK-deficient virus by the relative intensity of labeling of plaques. Drug-sensitive TK⁺ strain 294.1 formed heavily labeled plaques (Fig. 2A). A *tk* deletion mutant *dlsptk*, which is completely TK[–] (14), failed to form labeled plaques (Fig. 2C). Mutant 615.9 anabolized the nucleoside less efficiently than 294.1, but more efficiently than *dlsptk* giving rise to less-intense plaques that were nevertheless clearly labeled along their rims (Fig. 2B). Similar intensities were observed among >200 plaques. Reconstruction experiments (data not shown) showed that the technique can readily detect a TK⁺ virus in a background of TK-deficient virus. We conclude that mutant 615.9 consists of a homogeneous population of virus that expresses a low level of TK.

Mutant 615.9 Can Reactivate from Latency in Mouse Trigeminal Ganglia. We tested the ability of mutant 615.9 to reactivate from latency in mouse trigeminal ganglia after corneal inoculation. In the mouse, truly TK[–] mutants are completely deficient for reactivation (14–16); for example, TK[–] mutant *dlsactk* has failed to reactivate from any of 37 ganglia (14, 16). In contrast, 615.9 reactivated from 3 of 12 ganglia upon explant and dissociation in the presence of Vero

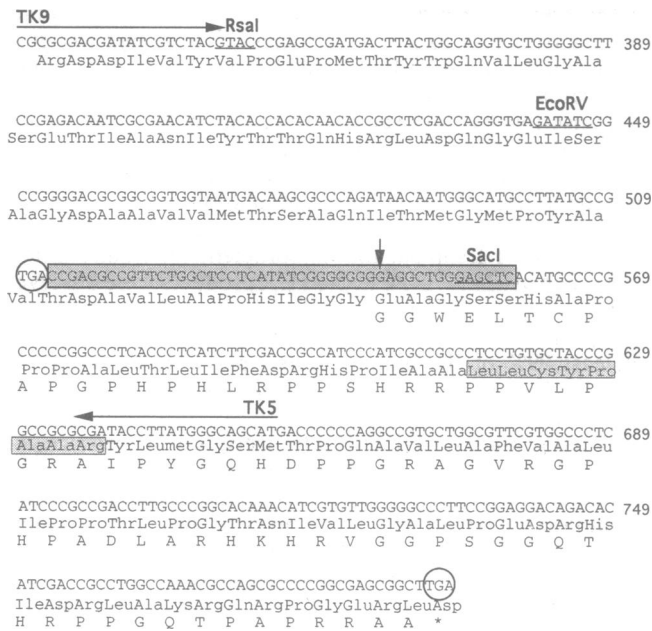


FIG. 1. Relevant sequences of the HSV 294 and 615.9 *tk* genes. For each line, the DNA sequence is presented above (starting at residue 330 from the transcriptional start site) and the predicted amino acid sequence is below with the normal TK reading frame in the three-letter code and the -1 reading frame in the single-letter code after the single guanosine insertion in mutants 615.9 and K5DG1, which shifts translation to the -1 reading frame (vertical arrow). A stop codon that closes this frame is shown with a star. The locations of primers TK9 and TK5 (horizontal arrows) and restriction sites *Rsa* I, *Eco*RV, and *Sac* I are indicated. The location of a stop codon within the +1 frame is circled. The sequence between this codon and the *Sac* I site is shaded, as a +1 frameshift event should begin downstream of the stop codon and the signals responsible for frameshifting lie upstream of the *Sac* I site. A putative nucleoside binding region (aa 168–176) (38, 40) is indicated within a lightly shaded box.

cells. In plaque autoradiography experiments similar to those in Fig. 2, the reactivated viruses were indistinguishable from the inoculated virus, 615.9 (E.P. and D.M.C., unpublished results). The results indicate that 615.9 expresses sufficient TK activity for reactivation from latency.

Mutant 615.9 Expresses Both Truncated and Full-Length TK Polypeptides. To account for the TK activity and reactivation from latency of 615.9, we hypothesized that this mutant might synthesize active full-length TK via translational frameshifting (41, 42), thereby compensating for the insertion mutation. We favored this possibility over other mechanisms such as transcriptional errors because pretranslational gene expression is generally highly faithful, whereas translational frameshifting has many precedents (42). To begin to examine this possibility, we immunoprecipitated radiolabeled TK from lysates of radiolabeled 615.9-, 294.1-, and mock-infected cells by using anti-TK antiserum (Fig. 3).

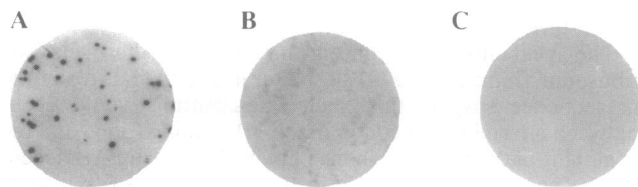


FIG. 2. TK activities of HSV strains. Vero-cell monolayers were infected with 50–250 plaque forming units (confirmed by crystal violet staining) of HSV wild-type isolate 294.1 (A), mutant 615.9 (B), or *tk* deletion mutant *dsptk* (C) and analyzed by the [¹²⁵I]iododeoxycytidine plaque autoradiography.

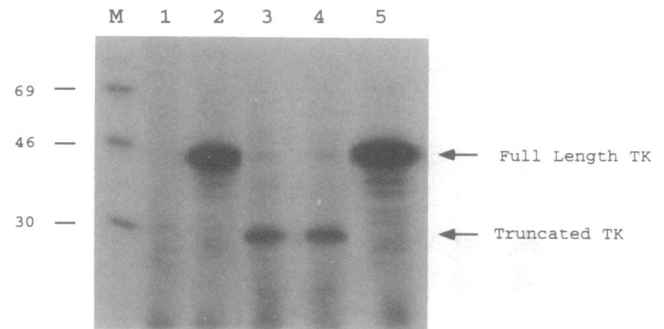


FIG. 3. Immunoprecipitation of TK from infected cell lysates. Vero cells were mock-infected (lane 1) or infected with wild-type 294.1 (lane 2), mutant 615.9 (lane 3), mutant K5DG1 (lane 4), or wild-type KOS (lane 5) and radiolabeled with [³⁵S]methionine. Cell lysates were prepared, immunoprecipitated with anti-TK serum, and analyzed on an SDS/12.5% polyacrylamide gel. The positions of molecular mass markers (lane M) are indicated on the left in kDa and the positions of full-length and truncated TK polypeptides are on the right.

The major polypeptide immunoprecipitated from 615.9-infected lysates corresponded in size (≈ 25 kDa) to that predicted from the insertion mutation (lane 3). However, small amounts of a species that comigrated with full-length TK were also observed (lane 3), consistent with our hypothesis of translational frameshifting.

Frameshifting *in Vitro*. To confirm the findings in infected cells and to test the frameshifting hypothesis further, we sought to reconstitute frameshifting *in vitro*, adopting the approach first used to examine translational frameshifting of Rous sarcoma virus (41). We cloned the 294.1 and 615.9 *tk* genes into a vector to permit *in vitro* transcription by bacteriophage SP6 RNA polymerase. We similarly cloned the *tk* genes from the wild-type laboratory strain KOS and the acyclovir-resistant mutant strain K5DG1, which arose spontaneously from KOS. The K5DG1 *tk* gene differs from the KOS *tk* gene (28) by the same single-base insertion found in 615.9 (Fig. 1). The two mutant *tk* genes also differed from each other at several nucleotides, as did their parental drug-sensitive strains, demonstrating the independence of the mutant isolates. Similar to 615.9, K5DG1 expressed small amounts of full-length TK in infected cells (Fig. 3, lane 4).

After *in vitro* transcription, the resulting synthetic mRNAs were translated in rabbit reticulocyte lysates (Fig. 4A). Plasmids containing the 615.9 (pBH13) or K5DG1 (pBX22) *tk* gene directed the synthesis of both truncated and full-length TK polypeptides (lanes 2 and 3), consistent with the results from infected cells. Both the truncated and full-length polypeptides could be specifically immunoprecipitated with anti-TK antiserum (data not shown). Densitometric scanning showed that the full-length products were at $\approx 2\%$ relative to truncated products.

To demonstrate frameshifting more rigorously, we then constructed plasmids pBX15 and pBX16 in which the C-terminal portion of the HSV *pol* gene was fused in-frame to the KOS and K5DG1 *tk* genes, respectively, at a *Sty* I site downstream of the TGA codon that terminates the truncated TK polypeptide translated from the mutant *tk* gene. Any bona fide full-length product specified by pBX16 would thus be recognized by anti-Pol antiserum EX3 (31), which does not recognize *pol* translation products that are out-of-frame (data not shown). As expected, the fusion protein directed by wild-type plasmid pBX15 reacted with both anti-Pol and anti-TK antisera (Fig. 4B, lanes 2 and 3). Mutant plasmid pBX16 directed the synthesis of a truncated product that was recognized by anti-TK antiserum but not by anti-Pol antiserum and low levels of a full-length product that was immunoprecipitated by both antisera (lanes 5 and 6). These results

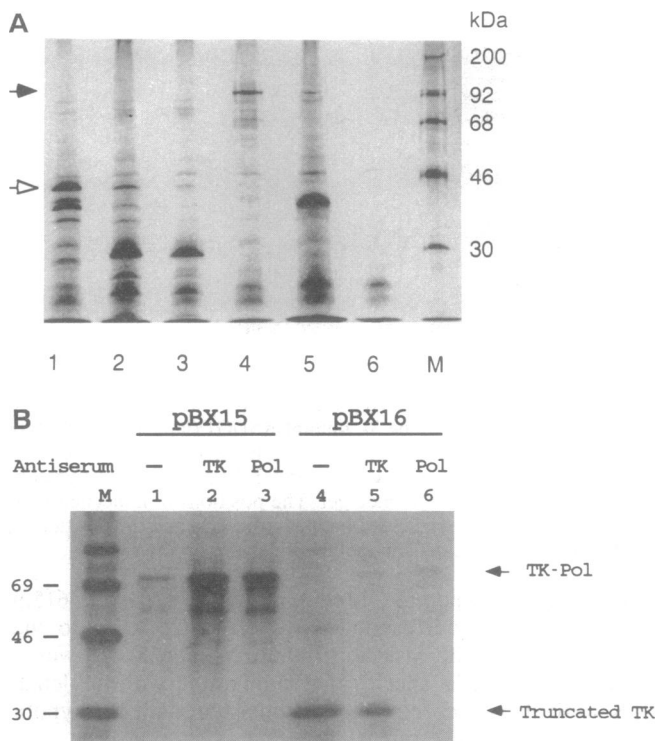


FIG. 4. Analysis of frameshifting *in vitro*. (**A**) Transcription and translation of *tk* genes. Wild-type *tk* plasmid pBH15 (lane 1), mutant *tk* plasmids pBH13 (lane 2) and pBX22 (lane 3), wild-type construct pBH3 (lane 4), mutant construct pBH4 (lane 5), and a water control (lane 6) were transcribed and translated *in vitro*, and the products were analyzed on an SDS/polyacrylamide gel alongside molecular mass markers (lane M). The positions of full-length TK and Pol-TK-Pol products are indicated with open and solid arrows, respectively. (**B**) Synthesis of fusion proteins in rabbit reticulocyte lysates. pBX15 and pBX16 were transcribed and translated *in vitro*, and the products were fractionated directly on an SDS/polyacrylamide gel (lanes 1 and 4) or immunoprecipitated with anti-TK antiserum (lanes 2 and 5) or anti-Pol antiserum (lanes 3 and 6) before fractionation. The positions of molecular mass markers (lane M) are shown at the left in kDa and the positions of full-length fusion polypeptides and truncated polypeptides are indicated at the right.

demonstrate that frameshifting occurs during the *in vitro* expression of the mutant *tk* gene.

Mutant *tk* Sequences Sufficient for Frameshifting. We sought to define a short mutant *tk* sequence that could mediate frameshifting. We reasoned that frameshifting must occur downstream of a termination codon in the +1 reading frame, which lies upstream of the insertion mutation (Fig. 1). Preliminary results (data not shown) further suggested that frameshifting did not require sequences beyond the *Sac* I site that lies just downstream of the insertion mutation (Fig. 1). We therefore subcloned the 111-bp *EcoRV-Sac* I fragment that encompasses this region (Fig. 1), which is identical in 615.9 and K5DG1, from either pBX1 or pBX22 into HSV *pol* sequences to create plasmids pBH3 and pBH4, respectively. Synthetic transcripts from these two plasmids were translated in rabbit reticulocyte lysates (Fig. 4A). The wild-type plasmid, pBH3, directed the synthesis of full-length product as expected (lane 4), whereas the mutant plasmid, pBH4, directed the synthesis of a truncated product of the expected size and a full-length product at $\approx 2\%$ of the truncated product (lane 5). The full-length polypeptide could be immunoprecipitated with antisera specific for the C-terminal region of Pol (data not shown). Therefore, the signals responsible for frameshifting are contained within the 111-bp fragment.

Failure to Detect Altered *tk* Transcripts. Although our results were consistent with a translational mechanism such

as ribosomal frameshifting (41, 42) and we considered other mechanisms less likely, nevertheless, we wished to determine whether frameshifting could have arisen from pretranslational mechanisms. These would entail changes in mutant *tk* transcripts by, for example, transcriptional stuttering (43) so that the wild-type reading frame was restored. Transcripts from mutant plasmid pBX22, which yielded $\approx 2\%$ frameshifting, or transcripts from wild-type plasmid pBX1 were isolated. Primers TK5 and TK9 (Fig. 1), which lie outside of the *EcoRV-Sac* I fragment that is sufficient for frameshifting, were then used in RT-PCR. No PCR products were observed in the absence of RT (data not shown). In a first experiment analyzing radiolabeled RT-PCR products (data not shown), at least 99% of the mutant transcripts were the expected length. However, this assay could not exclude the presence of $\approx 1\%$ altered transcripts.

To assay more sensitively for transcripts of altered size, we digested the PCR products with *Rsa* I and *Sac* I (Fig. 1), converted the 3' *Sac* I overhangs into blunt ends, and cloned them into the *Sma* I site of the open reading frame cloning vector, pMR100 (35). With this plasmid, insert DNA containing 3N - 1 bases and lacking termination codons in the reading frame set by the vector will yield active β -galactosidase, permitting formation of red colonies on MacConkey agar plates. If there were no alterations in the size of mutant transcripts, the blunt-ended mutant *Rsa* I-*Sac* I fragment would be expected to be 203 bp and, thus, yield red colonies. On the other hand, an alteration in transcripts such as the removal of one base (back to wild type) that would shift the reading frame would yield white colonies. As expected, plasmids containing the wild-type *Rsa* I-*Sac* I fragment yielded white colonies. Ligation of the mutant *Sac* I fragment yielded mostly red colonies. Of 742 colonies examined, 618 were red, indicating that there was no change in transcript length that changed reading frame. The 124 remaining white colonies were tested for the presence of *tk* inserts by colony hybridization with ^{32}P -labeled TK16 oligonucleotide. Only two were positive. Plasmid DNA prepared from these two colonies contained substantial deletions or other rearrangements based on restriction enzyme analysis, which explained the lack of β -galactosidase activity. Thus, $<0.2\%$ of the mutant transcripts contained alterations that could lead to synthesis of full-length TK. As this is much less than the frequency of frameshifting observed (2%), we infer that changes in transcripts do not account for frameshifting; i.e., frameshifting occurred during translation.

DISCUSSION

Unusual Aspects of the Frameshift. The frameshift we have detected is unusual relative to those previously described in eukaryotic cells. Unlike other examples (for review, see ref. 42), it occurs in the expression of a protein that is not an RNA-dependent polymerase and the protein is encoded by a DNA virus not an RNA virus or retrotransposon. The frameshift could be a +1 slip, which has been described in yeast (44), but not higher eukaryotes, or it could be a -2 slip or larger "hop." For now, we refer to it as a net +1 frameshift.

The mechanism of frameshifting operating here may be ribosomal frameshifting (41, 42). Our data and features of the *tk* sequence suggest that such frameshifting would occur between a termination codon in the +1 reading frame and the beginning of the *Sac* I site in the mutant *tk* gene (Fig. 1). Recent results support this suggestion (B.H. and D.M.C., unpublished data). However, computer-aided analysis of this region did not reveal any of the known heptanucleotide "slippery sequences" that mediate ribosomal frameshifting (41, 42, 44, 45). Moreover, the analysis did not reveal elements known to facilitate translational pausing, thereby permitting easier ribo-

somal realignment, such as termination codons, pseudoknots, or "hungry" codons recognized by known rare tRNAs (42, 44, 45). Recent work in *E. coli* suggests (46), however, that codons recognized by abundant tRNAs can facilitate frameshifting if there is sufficient competition for these tRNAs by other codons. Perhaps, a similar mechanism aids ribosomal realignment on the mutant tk mRNA.

A second possible mechanism for the translation of full-length TK is frameshift suppression (47). There are several examples of frameshift suppression in which a tRNA decodes four bases rather than three (e.g., a run of four guanines for glycine), but such examples to date have entailed mutant tRNAs, rather than a naturally occurring tRNA (47). It should be interesting to determine the sequences and mechanisms that govern frameshifting in the mutant tk gene and whether such frameshifting occurs during translation of other DNA virus or cellular genes in higher eukaryotes.

Implications for Drug Resistance and Pathogenesis. Given the importance of TK for pathogenesis in animal models of HSV infection, it has been paradoxical that many viruses associated with severe herpetic disease in humans were reported to express no detectable TK (4–13). Several of these viruses, like mutant 615.9, were reported to express truncated TK polypeptides and/or contain frameshift mutations so that they would be expected to be completely TK⁻ (7, 13, 39). As described here for 615.9, acyclovir-resistant mutants that express rather low levels of TK can reactivate from latency and exhibit other pathogenic phenotypes (17–20). For several of these, it has often proven difficult to detect TK activity by standard enzyme assays (18, 20); this has also been true for mutant 615.9 (E.P. and D.M.C., unpublished data). Thus, our results raise the possibility that certain clinical isolates that have been reported to be TK⁻ might express low levels of TK due to frameshifting or other mechanisms. Continued molecular analyses of such mutants may illuminate mechanisms of clinical resistance and uncover interesting mechanisms of gene expression. We speculate that during the evolution of acyclovir-resistant virus in patients with serious HSV disease, there is selection for mutants such as 615.9 that are resistant to acyclovir yet express low levels of TK sufficient for pathogenesis. This may account for our having detected the unusual phenomenon of net +1 frameshifting in mammalian cells.

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