

Mapping of the Varicella Zoster Virus Deoxyprimidine Kinase Gene and Preliminary Identification of Its Transcript

MARK H. SAWYER, JEFFREY M. OSTROVE,¹ JAMES M. FELSER,
AND STEPHEN E. STRAUS

Laboratory of Clinical Investigation, Medical Virology Section, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20892

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The varicella-zoster virus (VZV) deoxyprimidine kinase (dPK) gene was mapped by transfection of cloned viral DNA fragments into thymidine kinase-deficient mouse L (LTK⁻) cells and subsequent biochemical transformation of these cells to the LTK⁺ phenotype. Such transforming activity was limited to the *Bam*HI-H and *Eco*RI-D fragments of the VZV genome, which overlap by 2.2 kb between map units 0.50 and 0.52. Biochemically transformed cells were shown to contain a high copy number of viral DNA sequences that had integrated into the cellular DNA. Extracts of these cells showed a higher level of dPK activity than did extracts of parental LTK⁻ cells. With the use of Northern hybridization analysis of transformed and VZV-infected cell RNAs, it was possible to tentatively assign a 1.8-kb transcript to the VZV dPK. © 1986 Academic Press, Inc.

INTRODUCTION

Three human herpes viruses direct the synthesis of deoxyprimidine kinases (dPK) during their replicative cycles. The enzymes encoded by herpes simplex virus types 1 and 2 (HSV-1, HSV-2) and varicella-zoster virus (VZV) have been characterized extensively. The initial mapping of the HSV thymidine kinase (TK) genes and much of our understanding of them were facilitated by the biochemical transformation to LTK⁺ of mouse L cells deficient in thymidine kinase (LTK⁻) by inactivated virions or purified HSV DNA sequences (Munyon *et al.*, 1971; Bacchetti and Graham, 1977; Wigler *et al.*, 1977, 1978; Maitland and McDougall, 1977; Pellicer *et al.*, 1978; McDougall *et al.*, 1980; Reyes *et al.*, 1982). Through a judicious selection of sequentially smaller viral DNA fragments for such transformation experiments, it was possible to localize the HSV-1 and

HSV-2 TK genes with some precision. DNA sequence analysis has demonstrated that each of the genes encoding the HSV-1 and HSV-2 TKs consists of an open reading frame of 1128 nucleotides capable of encoding a protein of 376 amino acids (McKnight, 1980; Wagner *et al.*, 1981; Swain and Galloway, 1983). Three polypeptide products of the HSV-1 gene with molecular weights (MW) of 38,000, 39,000, and 43,000 have been identified by *in vitro* and *in vivo* pulse-chase experiments (Marsden *et al.*, 1983).

VZV is also known to induce an enzyme with dPK activity in infected cells (Dobersten *et al.*, 1976; Ogino *et al.*, 1977; Hackstadt and Mallavia, 1978; Cheng *et al.*, 1979), and cell-associated VZV has been used to biochemically transform LTK⁻ cells (Yamanishi *et al.*, 1981). Studies of dPK activity in VZV-infected cells suggest that this activity is associated with a 35,000 MW polypeptide that may exist as a 70,000 MW protein composed of nearly identical subunits (Lopetegui *et al.*, 1983; Shiraki *et al.*, 1985).

In this report, we describe the stable transfer of VZV dPK activity to LTK⁻ cells by transfection of cloned viral DNA re-

¹ Author to whom requests for reprints should be addressed at: Medical Virology Section, Laboratory of Clinical Investigation, NIAID, NIH, Building 10, Room 11N-113, Bethesda, Md. 20892.

striction fragments. This process has allowed the mapping of the VZV dPK gene, the preliminary enzymatic characterization of its product, and the tentative identification of its primary transcript.

MATERIALS AND METHODS

Cell culture. The LTK⁻ cells (a gift from M. Rao and R. Klausner) were propagated in Eagle's minimum essential medium (EMEM) (Quality Biological, Inc., Gaithersburg, Md.) supplemented with 10% fetal calf serum, penicillin G at 100 units/ml, streptomycin at 100 µg/ml, and bromodeoxyuridine (BUdR) (Sigma Biochemicals, St. Louis, Mo.) at 20 µg/ml. Prior to transfection, the cells were passed at least twice in the absence of BUdR. LTK⁺ transformants were selected and grown in EMEM supplemented with serum, antibiotics, and HAT (hypoxanthine, 15 µg/ml; aminopterin, 1 µg/ml; and thymidine, 5 µg/ml) (Littlefield, 1963). Vero cells (African green monkey kidney cells) were grown in a mixture of EMEM and medium 199 (1:1) (Quality Biological, Inc.) also supplemented with 10% fetal calf serum and antibiotics.

Viral and cellular DNA purifications. *EcoRI* and *BamHI* digests of whole VZV DNA were cloned into λgtWES·λB as previously described (Straus *et al.*, 1982). The digests were further subcloned into pBR 322, pBR 325, and PUC8 and then grown in LE392, HB101, and JM83. Plasmid DNAs were prepared by the method of Birnboim and Doly (1979) and further purified with two isopycnic bandings in cesium chloride followed by ethanol precipitation. A DNA probe prepared for use in Southern and Northern blot analyses consisted of the 2.2-kb overlapping fragment between *BamHI*-H and *EcoRI*-D. Twenty micrograms of *BamHI*-H DNA in pBR 322 was digested with *BamHI* and *EcoRI*, and the bands were resolved on a 0.8% agarose gel. The 2.2-kb fragment was electro-eluted from the gel and purified through a Nacs-Prepac column [Bethesda Research Laboratories (BRL), Gaithersburg, Md.].

Cellular DNA was prepared from frozen cell pellets by overnight digestion with proteinase K (200 µg/ml) (Sigma) in the

presence of 1% sodium dodecyl sulfate, 50 mM ethylenediamine tetraacetic acid (EDTA), and 10 mM Tris-HCl (pH 7.4) at 37°. This mixture was extracted twice with phenol and twice with chloroform; it was then precipitated overnight at -20° in 0.4 M sodium acetate with 70% ethanol. The pellet was resuspended in TE buffer (10 mM Tris-HCl and 1 mM EDTA, at pH 7.4) and digested with a RNase A (20 µg/ml) (Worthington, Freehold, N. J.) at 37° for 2 hr. The DNA was reextracted with phenol and chloroform, reprecipitated in ethanol, and dialyzed overnight against TE buffer. Following an additional ethanol precipitation, the final DNA pellet was resuspended in TE buffer.

DNA transfection. Plasmid DNA fragments (0.5-10 µg) were transfected into LTK⁻ cells in 60-mm dishes (containing approximately 1 × 10⁶ cells) using the calcium phosphate technique of Graham and van der Eb (1973) as modified by Wigler *et al.* (1978). Calf thymus DNA was used as carrier to achieve a final DNA concentration of 20 µg/ml. The cell monolayers were exposed to the calcium phosphate-DNA mixture (0.5 ml/dish) for 30 min at room temperature before fresh medium was added. The cultures were fed 24 hr later with HAT-containing selective media. Control dishes treated with either carrier DNA alone or no DNA were included with each experiment. As a positive control for TK transformation, we used the HSV-1 TK gene cloned into pBR 322 (HSV-106) (BRL). Colonies were isolated with the use of 5-mm filter paper discs (Whatman, No. 1) that had previously been soaked in 0.05% trypsin with 0.1% EDTA (Quality Biological). The discs were placed over the colonies for 5 min and then transferred into dishes containing fresh HAT medium. The dishes were fed with fresh HAT medium every 4 to 5 days.

DNA hybridization. Cellular DNA was digested with *BamHI*, *EcoRI*, *SmaI*, *PstI*, *PvuII*, *SstI*, *HindIII*, or *XbaI* restriction endonucleases (BRL) according to the manufacturer's instructions. The cellular DNA was electrophoresed through 1% agarose horizontal slab gels, denatured *in situ*, and transferred to nitrocellulose paper

(Southern, 1975). Cloned VZV DNA fragments were labeled *in vitro* with [α - 32 P]deoxycytidine triphosphate (dCTP) (sp act, >3000 Ci/mmol) (Amersham, Arlington Heights, Ill.) to sp act of 0.5 - 3.0×10^8 cpm/ μ g using Nick Translation Kits (BRL). Autoradiography was accomplished at -70° with Kodak XAR-5 film in cassettes fitted with Dupont Lightning-Plus intensifying screens for 2 to 16 hr. Relative DNA concentrations were estimated by densitometry. DNA fragment sizes were determined by comparison to bacteriophage lambda DNA digested with *Hind*III (BRL).

Northern blot analysis. Total cellular RNA was isolated from VZV-infected Flow 5000 cells (Flow Laboratories, McLean, Va.), LTK⁻ cells, or VZV-dPK transformants. Briefly, each cell line was grown to confluence in two 150-cm² tissue culture flasks, washed three times in ice cold phosphate-buffered saline (PBS) (pH 7.4), and lysed *in situ* with 4 M guanidinium thiocyanate. The lysate was layered onto a 5.7 M cesium chloride cushion and centrifuged in an SW41 rotor at 33,000 rpm for 25 hr (Chirgwin *et al.*, 1979; Glisen *et al.*, 1974). The supernatant was decanted, and the RNA pellet was further purified as previously described (Ostrove *et al.*, 1985). Aliquots of cellular RNA were heated in 50% formamide, 6% formaldehyde, and 1X electrophoresis buffer (20 mM sodium [3-N-morpholino-propane-sulfonic acid [MOPS], 5 mM sodium acetate, and 1 mM EDTA at pH 7.0) for 10 min. RNA samples (4-10 μ g/lane) were electrophoresed at 35 mA for 16 hr through 6% formaldehyde/1.5% agarose horizontal slab gels (Rave *et al.*, 1979). The transfer of RNA to nitrocellulose and the hybridizations were carried out as previously described (Ostrove *et al.*, 1985). RNA sizes were determined by comparison to single-stranded DNA markers.

dPK assay. The various cell lines to be used for both the TK and the deoxycytidine kinase activity studies were grown to confluence in 60-mm dishes, washed three times in PBS (pH 7.4), and scraped from the dishes. The cells were pelleted at 1500 rpm for 10 min in microcentrifuge tubes, and the supernatant was completely re-

moved. The cell pellets were stored at -70° . Pellets of each cell type to be tested were thawed on ice, and 100 μ l of lysis buffer was added (10 mM Tris-HCl at pH 8.0, 160 mM KCl, 250 mM sucrose, 1.5 mM MgCl₂, 0.5% NP-40, and 3 mM 2-mercaptoethanol). The samples were vortexed twice (2 sec each) and incubated on ice for 10 min. The samples were then vortexed vigorously for 5 sec and spun for 10 min in a microcentrifuge (13,800 *g*). The supernatants were tested for enzyme activity (modification of Kit *et al.*, 1973; Kit and Leung, 1974). For the dPK assays, 100- μ l reaction mixtures were prepared containing varying amounts of cell extract (5-20 μ l), 50 mM Tris-HCl (pH 8.0), 5 mM MgCl₂, 5 mM adenosine triphosphate, 10 mM NaF, 50 μ M cold thymidine or deoxycytidine, and 2 μ Ci of [3 H]thymidine or [3 H]deoxycytidine (New England Nuclear, Waltham, Mass.) (modification of Yamanishi *et al.*, 1981). Fifty-microliter samples of the reaction mixtures were spotted onto DE81 filters (Whatman, 2.4 cm), given two 60-min washes in distilled water, dipped five times in 95% ethanol, and dried. The filters were placed in Aquafluor (New England Nuclear, Boston, Mass.) and counted in a liquid scintillation counter. Enzyme activities were corrected for the amount of protein in the extracts. Protein concentrations were determined by the method of Bradford (1976).

RESULTS

Sixteen recombinant VZV DNA *Bam*HI and *Eco*RI fragments representing 75% of the viral genome (map units 0.18-0.66 and 0.73-1.00, except for about 500 bp of the unique short segment (U_S) were transfected into LTK⁻ cells. The induction of stable biochemical transformants expressing TK activity was determined by formation of LTK⁺ clones under HAT selection. This activity was occasionally observed with large amounts of calf thymus DNA and was seen with the HSV TK-containing recombinant (HSV-106) and with two fragments of VZV DNA: *Bam*HI-H (map units 0.47-0.52) and *Eco*RI-D (map units 0.50-0.62). These fragments are

known to overlap and share a 2.2-kb region of the genome (map units 0.50–0.52) (Straus *et al.*, 1982) (Fig. 1).

VZV-transformed LTK⁺ colonies developed 18 to 25 days after transfection and arose from a background of surviving cells. The majority of such surviving cells failed to proliferate into clones, perhaps demonstrating only transient expression of TK activity. No such background was seen in dishes transfected with carrier DNA alone. The VZV DNA-transformed cells resembled the parental LTK⁻ cells in terms of both morphology and growth rate. HSV-transformed colonies appeared more quickly (at approximately 14 days after transfection) and exhibited more rapid growth than VZV transformants. The transforming frequency of both *Bam*HI-H and *Eco*RI-D was 0.2–0.4 colonies/dish/ μ g of DNA transfected, which was approximately 100-fold lower than that seen with HSV TK genes under similar conditions.

No LTK⁺ revertants were seen in LTK⁻ cells not transfected with foreign DNA, consistent with the known reversion frequency of less than 10^{-9} for these cells (Wigler *et al.*, 1978).

Three stable clones resulting from the transfection of *Bam*HI-H (BH1 and BH2) and *Eco*RI-D (ED1) were established and have been maintained in HAT-containing media for more than 25 passages. Between passages 6 and 8, the DNAs from these biochemically transformed cell lines were purified, and the presence within them of viral or plasmid sequences was determined by Southern hybridizations with purified VZV *Bam*HI-H, HSV-106, or the 2.2-kb *Bam*HI-H/*Eco*RI-D overlap fragment as probes. All three VZV transformants, BH1, BH2, and ED1, were shown to contain viral and plasmid DNA sequences in high copy number (100–1000 copies per cell) (Figs. 2 and 3). Southern hybridization analysis of DNA from the VZV transformants ED1

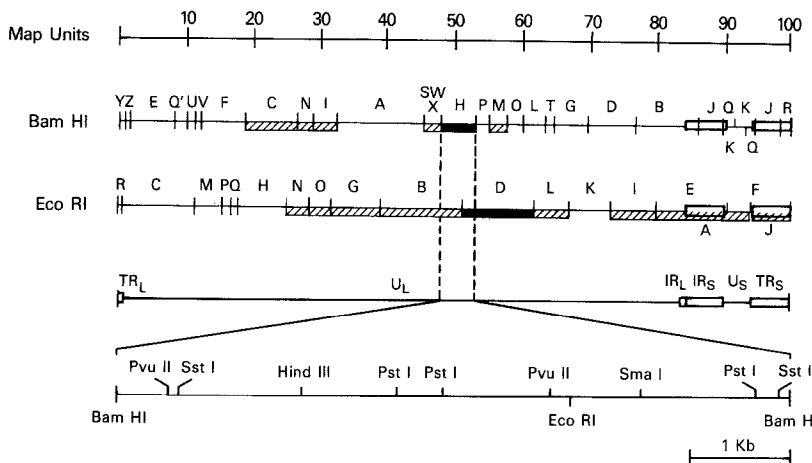


FIG. 1. Restriction endonuclease maps of the VZV genome, including the 6.7-kb *Bam*HI-H fragment. The genome can be conveniently divided into a unique long (U_L) segment bounded by small terminal and internal repeats (TR_L , IR_L), and a unique short (U_S) segment, bounded by large internal and terminal repeats (IR_S , TR_S). The hatched areas indicate those fragments used for the transfection studies; the solid areas indicate the fragments that led to stable HAT-resistant biochemical transformants. In the transfection experiments, 5 μ g per dish of *Bam*HI-H in pBR 322 yielded a total of 10 colonies in five dishes (0–3 per dish), for a transforming frequency of 0.4 colonies/ μ g of DNA per dish. *Eco*RI-D (7.5 μ g per dish) in pBR 322 yielded six colonies in five dishes (0–5 per dish), for a transforming frequency of 0.2 colonies/ μ g of DNA per dish. All other cloned fragments were tested in duplicate plates with 1.3–10 μ g of DNA per dish, and no colonies were observed. Control cultures transfected with 0.5–1.0 μ g per dish of HSV-106 DNA routinely yielded 25–50 colonies, for a transforming frequency of 50–100 colonies/ μ g of DNA per dish. The 1-kb scale marker refers to the detailed *Bam*HI-H map at the bottom of the figure.

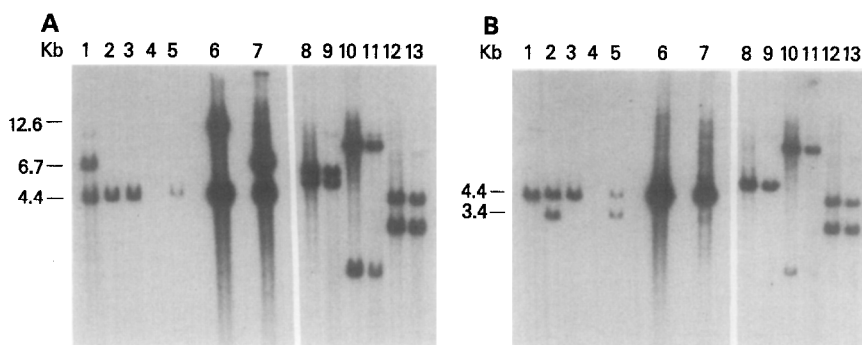


FIG. 2. Southern blot analysis of transformed cellular DNA. Two identical blots were probed with *in vitro*-labeled *Bam*HI-H in pBR 322 (A) and HSV-106 (B). In each panel, lanes 1-4 represent *Bam*HI-cleaved DNAs and comprise reconstruction experiments with 10 μ g of LTK⁻ cell DNA to which is added: in lane 1, 300 pg of purified *Bam*HI-H in pBR 322; in lane 2, 50 pg of HSV TK recombinant (HSV-106); in lane 3, 75 pg of pBR 322 DNA; and in lane 4, no addition. Lanes 5-7 contain transformed cell DNAs. These include 15 μ g of HSV-transformed DNA cleaved with *Bam*HI (lane 5), 10 μ g of ED1 cleaved with *Eco*RI (lane 6), and 10 μ g of BH2 cleaved with *Bam*HI (lane 7). Lanes 8-13 show paired samples of BH2 cellular DNA (450 ng/lane) and purified *Bam*HI-H in pBR 322 (300 pg/lane) to which was added sufficient LTK⁻ cellular carrier DNA to make the total DNA 10 μ g per lane. DNA was cut with *Sst*I (lanes 8, 9), *Hind*III (lanes 10, 11), and *Pvu*II (lanes 12, 13). (A) Shows hybridization with α -³²P *in vitro*-labeled *Bam*HI-H in pBR 322. (B) Shows the hybridization with *in vitro*-labeled HSV-106. Autoradiography was for 15 hr at -70°.

and BH2 following cleavage with *Bam*HI, *Eco*RI, *Sst*I, *Hind*III, *Sma*I, *Pst*I, or *Pvu*II revealed fragments of the sizes predicted from the restriction map (see Fig. 1). This suggests that most of the transfected DNAs were incorporated intact into the cells. Digestion of BH2 DNA with *Xba*I, an enzyme that does not cleave the VZV *Bam*HI-H fragment or within pBR 322, and subsequent hybridization with a homologous VZV DNA probe revealed a single band of very high molecular weight (>23 kb) (Fig. 3, lane 3). Further analysis of transformed cell DNAs with enzymes that cleave within the VZV inserts or pBR 322 revealed multiple bands of higher and lower molecular weights than those of the transfected DNA fragments. The combined data indicate that much of the transfected DNA is present as tandem repeats with integration into the cellular DNA in multiple sites.

The HSV DNA transformants contained a low copy number per cell of the HSV TK gene and its associated plasmid DNA. With the conditions employed, no cross-hybridization was seen between the HSV TK sequences and the VZV sequences. Transformants

that arose from transfections with calf thymus DNA alone contained no sequences homologous to VZV, HSV, or pBR 322 DNAs (data not shown).

Extracts from the transformed cell lines BH1, BH2, and ED1 as well as from a cell line transformed with the HSV-1 TK gene (HSV), LTK⁻ cells, and Vero cells were assayed for kinase activity. BH1, BH2, and ED1 cells demonstrated a 70- to 115-fold greater TK activity than the parental LTK⁻ cells (Table 1). The abilities of these cellular extracts to phosphorylate deoxycytidine were compared to their abilities to phosphorylate thymidine. It was observed that all cell lines transformed with VZV DNA showed greater deoxycytidine kinase activity than TK activity in accord with prior reports of the substrate preferences of purified VZV dPK (Dobersen *et al.*, 1976; Ogino *et al.*, 1977; Fyfe *et al.*, 1982) (Fig. 4). The opposite pattern was seen with extracts from cells transformed with HSV TK and Vero cells, also in accord with prior observations with HSV TK (Fyfe *et al.*, 1978).

Northern hybridization analysis of the cellular RNA extracted from BH1, BH2,

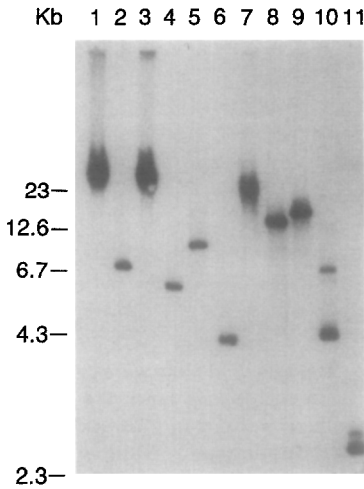


FIG. 3. Southern blot analysis of transformed cellular DNA probed with the 2.2-kb *Bam*HI-H/*Eco*RI-D overlapping fragment. BH2- and ED1-transformed cellular DNA was cleaved with several different restriction endonucleases and probed with the gel purified, *in vitro*-labeled, 2.2-kb overlap fragment. Lanes 1-6 show BH2 cellular DNA. Lanes 1 and 3 each show 2 μ g of BH2 DNA that is undigested (lane 1) or digested with *Xba*I (lane 3). Lanes 2, 4, 5, and 6 each show 300 ng of BH2 DNA that is digested with *Bam*HI (lane 2), *Sst*I (lane 4), *Hind*III (lane 5), and *Pvu*II (lane 6). Lanes 7-11 each show 150 ng of ED1 cellular DNA that is undigested (lane 7) or digested with *Eco*RI (lane 8), *Hind*III (lane 9), *Sma*I (lane 10), and *Pst*I (lane 11). Autoradiography was for 4 hr at -70° .

ED1, and LTK⁻ cells demonstrated the transcription of VZV-specific mRNAs in the BH1, BH2, and ED1 transformed cell lines. To identify the messages most likely to be associated with the transfected dPK activity, total cellular RNA from these cell lines were hybridized to the *in vitro* α -³²P-labeled, gel purified 2.2-kb DNA fragment representing the region of overlap between the *Bam*HI-H and *Eco*RI-D fragments. The VZV-specific messages from these transformed lines were then compared to those detected by the hybridization of VZV-infected Flow 5000 cell RNA using the same 2.2-kb overlap-fragment probe (Fig. 5). Two abundant messages of 1.8 and 2.5 kb were detected in VZV-infected cells. The BH1- and BH2-transformed cell lines contained an abundant message comigrating with the

TABLE 1

THYMIDINE KINASE ACTIVITIES OF EXTRACTS FROM VARIOUS CELL LINES

Cells	Counts per minute per microgram of protein
LTK ⁻	15
BH1	1041
BH2	1751
ED1	1043
HSV	1411
Vero	1653

1.8-kb message of infected cells. The ED1 line contained only one abundant message of about 2.2 kb in length. Other low-abundance transcripts were seen in common in the VZV dPK-transformed cells but did not comigrate with the transcripts seen in VZV-infected cells.

DISCUSSION

With the use of the well-established technique of transfection of viral DNA

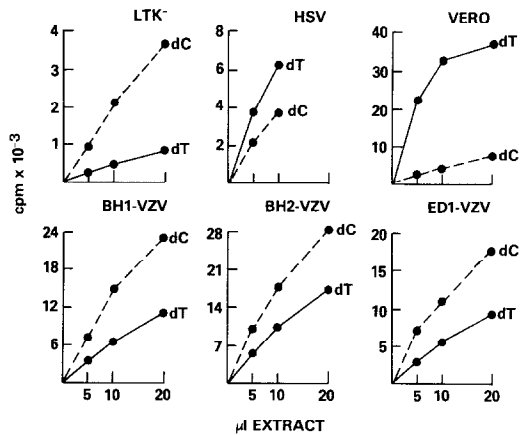


FIG. 4. Deoxypyrimidine kinase activities of cell lines. Deoxypyrimidine phosphorylation was determined for each cell line with three different amounts of cell extract: 5, 10, and 20 μ l. Reaction mixtures were run in parallel. BH1-VZV, BH2-VZV, and ED1-VZV indicate extracts from the VZV-transformed cell lines. LTK⁻ is the parental cell line. HSV is an extract from an HSV TK gene-transformed cell line.

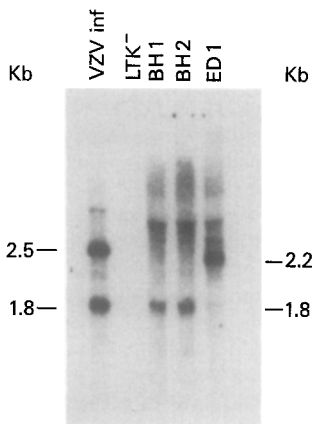


FIG. 5. Northern blot analysis of total cellular RNAs. Lane 1: 4 μ g of RNA from VZV-infected Flow 5000 cells. Lane 2: 10 μ g of RNA from LTK⁻ cells. Lanes 3-5 contain 10 μ g of RNA from transformed cell lines: BH1 (lane 3), BH2 (lane 4), and ED1 (lane 5). The *in vitro*-labeled 2.2-kb overlap fragment shared by *Bam*HI-H and *Eco*RI-D was used as the probe. Autoradiography was for 5 hr at -70° .

fragments into mouse LTK⁻ cells followed by selection in HAT medium, we were able to screen a large portion of the VZV genome for sequences capable of inducing biochemical transformation to the LTK⁺ phenotype. We identified two separate fragments—*Bam*HI-H and *Eco*RI-D—that exhibit transforming activity. These fragments share a 2.2-kb region of DNA, one that is large enough to encode a protein with a MW of greater than 35,000, the presumed size of the VZV dPK (Shiraki *et al.*, 1985), and is larger than the minimal corresponding fragment of HSV DNA needed to transfer TK activity in similar systems (Reyes *et al.*, 1982). Although LTK⁺ transformants were noted following transfections with calf thymus DNA alone, these cells exhibited very low TK activity and contained no viral RNA or DNA sequences.

The region of VZV DNA associated with TK transformation lies within the long unique (U_L) segment of the genome, as does the HSV TK gene in its genome. Its position, at map units 0.50-0.52, is 0.31 map units in from the U_L -internal repeat (IR_L) junction, and is similar to the distance of the HSV TK gene from the HSV terminal

repeat (TR_L) sequence. This finding is consistent with A. Davison's impression from cross-hybridization studies that the organization of the VZV genome is similar to that of HSV except for an inversion of part of the U_L sequence (personal communication).

The dPK activities of the VZV-transformed cell lines were markedly greater than that of the parental LTK⁻ cell line. Limited biochemical characterization of this activity showed that deoxycytidine was phosphorylated more rapidly than thymidine. Such findings are consistent with the reported behavior of the enzyme recovered from VZV-infected cells; however, they are the opposite of those reported for the HSV TK and those we observed in extracts prepared from HSV TK-transformed cells (Dobersen *et al.*, 1976; Ogino *et al.*, 1977; Fyfe *et al.*, 1978, 1982). The VZV-transformed cell lines contained extremely high copy numbers of the transfected DNA, yet produced dPK in amounts similar to that in the HSV-transformed cell line, where copy number is usually low (Pellicer *et al.*, 1978; Wigler *et al.*, 1978). Some possible explanations for these findings are that the VZV dPK promoter is a weak one or that additional VZV gene products are necessary for efficient VZV dPK gene expression.

We were further able to show that three independently derived VZV DNA-transformed cell lines expressed mRNAs homologous to the segment of VZV DNA associated with the dPK-transforming activity. Only the 1.8-kb message detected in the BH1 and BH2 transformants comigrated with an abundant message expressed from this region of the genome in infected cells. The ED1 line, however, contained an abundant message of 2.2 kb in length. The 2.2-kb transcript may originate from a fusion of the VZV dPK sequence with additional nonviral sequences and be initiated by a cellular or plasmid promoter. The 2.2-kb transcript contains pBR 322 sequences as determined by Northern blot analysis (data not shown). If the promoter for the VZV dPK gene were to lie to the left of the *Eco*RI site within the *Bam*HI-H fragment and the

body of the message (open reading frame) were to lie to the right of the *EcoRI* site, then expression of the dPK by the *EcoRI*-D fragment would require initiation by a nonviral promoter.

Consistent with these hypotheses are recent analyses of the VZV DNA sequence in this region of the genome. A gene with limited sequence homology to the HSV TK gene has been found in which the promoter is upstream and to the left of the adjacent *EcoRI* site (A. Davison, personal communication). Thus, we believe the VZV dPK gene lies between map units 0.50 and 0.52 in the U_L of the VZV genome and encodes a primary 1.8-kb transcript.

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