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Location and Cloning of the Herpes Simplex Virus Type 2 Thymidine Kinase Gene

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The herpes simplex virus type 2 thymidine kinase gene has been mapped to a position colinear with the herpes simplex virus type 1 thymidine kinase gene and cloned within a 4.0-kilobase fragment in pBR 322.

The transfer of thymidine kinase (TK) genes from restriction endonuclease fragments of herpes simplex virus types 1 (HSV-1) and 2 (HSV-2) DNA to TK^- deficient mammalian cells has been demonstrated in a number of laboratories (4, 6, 13, 14). The HSV-1 TK gene has been unambiguously mapped to a segment of the genome mapping between 0.28 and 0.32 by genetic (7) and biochemical transfer (10) methods. The HSV-1 *Bam*HI fragment (3.4 kilobases [kb]) containing the coding region for viral TK has been cloned in the *Escherichia coli* plasmid pBR 322 (1, 2; C. Weisman, N. Nantei, N. Boll, R. F. Weaver, N. Wilkie, B. Clements, T. Taniguchi, A. Van Ooyen, J. Van Den Berg, M. Fried, and K. Murray, *in* D. W. Ribbons, J. F. Noessher, and J. Schultz, Expression of cloned viral and chromosomal plasmid-linked DNA in cognate host cells, in press) and shown to have biological activity by transformation of TK^- cells to a TK^+ phenotype (1; Weisman et al., in press). Further cleavage of the cloned fragment with restriction endonuclease *Pvu*II followed by recloning in pBR 322, produced a 2-kb fragment which transforms with high efficiency (1).

The HSV-2 TK gene has been mapped by genetic methods between positions 0.27 and 0.35 (7), but the initial data from biochemical transformation experiments placed the TK gene sequence between 0.53 and 0.65 on the HSV-2 genome (4). Subsequent reassociation kinetic analysis of these transformed cells showed that a more extensive set of viral sequences was present than that represented within these map positions (Galloway and McDougall, unpublished data). Further experiments to resolve the discrepancy between the genetic and biochemical data and to clone the HSV-2 TK gene in *E. coli* are reported here.

HSV-2 DNA (strain 333) was cleaved with restriction endonucleases *Eco*RI, *Bgl*II, *Bam*HI, *Kpn*I, *Hpa*I, *Hin*dIII, and *Xba*I. The total digests were used to transfect mouse cells deficient in TK activity (LMTK$^-$ clone B82) which were then maintained in Dulbecco-modified Eagle medium with 10% calf serum and supplemented with hypoxanthine (10^{-4} M), aminopterin (4×10^{-7} M), and thymidine (1.6×10^{-4} M) (HAT medium) and observed for the appearance of TK^+ colonies. The method used for transfection was the calcium phosphate precipitation technique of Graham and Van der Eb (3) with modifications as previously described (14). Transfection with HSV-2 DNA cleaved by *Eco*RI, *Kpn*I, or *Bam*HI did not result in the appearance of TK^+ colonies during an 8-week observation period. Cleavage by *Xba*I, *Hpa*I, *Bgl*II, and *Hin*dIII provided DNA fragments capable of transforming B82 cells to a TK^+ phenotype. Individual fragments from a *Hin*dIII digest of HSV-2 DNA separated by electrophoresis in 0.4% agarose were recovered by elution and further purified by phenol extraction and ethanol precipitation. The purified fragments were used to transfect TK^- cells, and only the *Hin*dIII-H fragment (0.299–0.419) converted cells to a TK^+ phenotype.

In each case the TK^+ activity in the transformed mouse cells was shown to be virus specific by the criteria of thermal inactivation (Fig. 1) and electrophoretic mobility (Fig. 2). The R_f value for BHK cell TK in this gel system is identical to that of mouse cytosol TK (8) and is well separated from virus-specific TK and mitochondrial TK (8). The radioactivity detected below the virus-specific TK activity is consistent with the presence of mouse mitochondrial TK, which has been observed in both TK^+ and TK^- L cell lines (8). Reversion of the B82 (TK^-) mouse L cell line has never been observed in these experiments, consistent with the observation that the reversion frequency of TK^- L cells is less than 10^{-8} (9).

To provide further confirmation of the map position of HSV-2 TK, we used a series of recombinant plasmids derived from ligation of either *Bgl*II-digested HSV-2 DNA or *Bgl*II/*Hin*dIII-digested HSV-2 DNA with cleaved pBR 322. The plasmids, which were grown in *E. coli* K-12, were analyzed for content and purity of virus

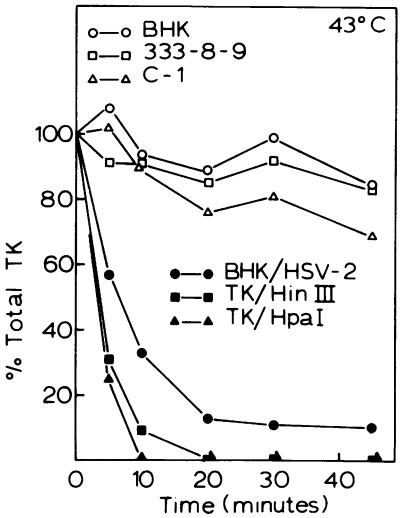

FIG. 1. *Heat stability of TK in HSV-2 transformants. Cell pellets, dispersed in a buffer containing 100 mM Tris, 1 mM ATP, 1 mM $MgCl_2$, 200 mM NaF, 14 mM 2-mercaptoethanol, and 1% Nonidet P-40, were centrifuged for 1 min at 1,500 × g at 0°C. Volumes (100 μl) of the supernatant were incubated at 43°C, and at time intervals shown 50 μl was mixed with an equal volume of assay mix containing 0.2 M Tris, 0.1 M KCl, 5 mM $MgCl_2$, 5 mM ATP, 14 mM 2-mercaptoethanol, and 0.4 mM [^{3}H]thymidine (5 Ci/mmol, Amersham) and incubated at 37°C for 10 min. Samples (50 μl) were spotted onto DE-81 paper which was dried and then washed three times in 95% ethanol, dried, and counted. The results are expressed as a percentage of total TK at time zero. Closed symbols show heat lability of viral TK.*

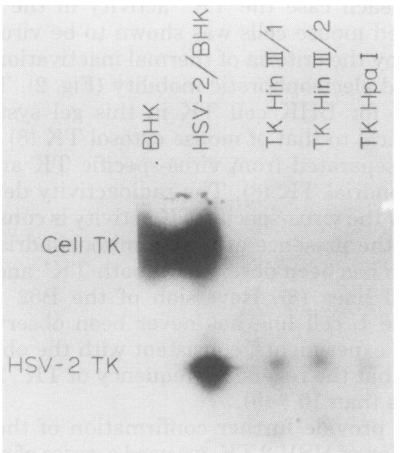

FIG. 2. *Electrophoretic mobility of viral TK. Cell pellets prepared as described for Fig. 1 were loaded in 100-μl volumes onto polyacrylamide slab gels as described (4). Electrophoresis was carried out at 10 mA (50 V). Localization of TK activity was detected as previously described (4). HSV-2/BHK, Hamster kidney cells infected with HSV-2 and expressing both cell and viral forms of TK. The three HSV-2 transformants of LMTK$^-$ (B82) cells only express the viral form.*

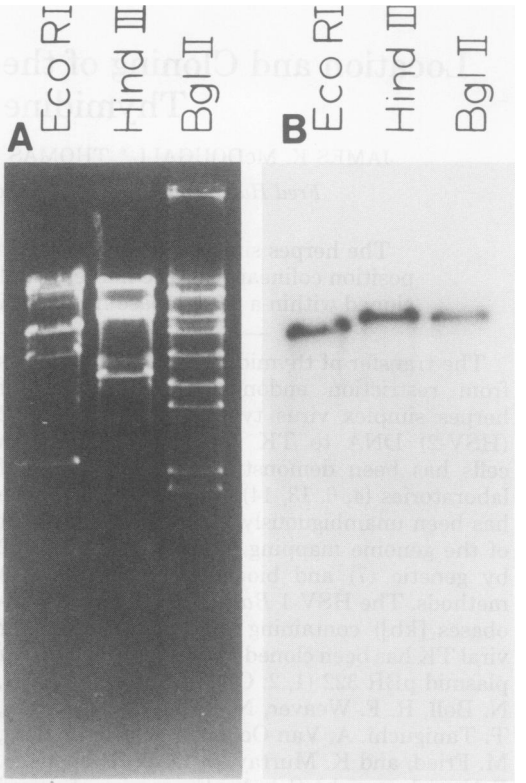

FIG. 3. *Identification of HSV-2 DNA sequences in pDG 504. HSV-2 DNA (1 μg/slot) was digested with restriction endonucleases EcoRI, HindIII, and BglII, fractionated by electrophoresis through 0.4% agarose, and stained with ethidium bromide (A). The DNA fragments were transferred to a nitrocellulose sheet (12) which was then hybridized to ^{32}P-labeled nick-translated (5) pDG 504 DNA (specific activity, 10^8 cpm/μg). The resulting autoradiograph (B) shows three radioactive bands corresponding to fragments EcoRI-I, HindIII-H, and BglII-G. This localizes the viral sequences contained in pDG 504 to a HindIII/BglII fragment mapping at 0.299–0.326 on the HSV-2 genome.*

DNA sequences (D. A. Galloway and M. Swain, submitted for publication). The analysis and map positions of the cloned DNAs used in this study are shown in Fig. 3 and 4. pDG 304 is derived from a *Bgl*II digest and contains only sequences within the *Bgl*II J fragment (0.326–0.402); pDG 305, derived similarly, contains the *Bgl*II region (0.29–0.326). pDG 504, derived from a *Hin*dIII/*Bgl*II double digest, contains a 4-kb

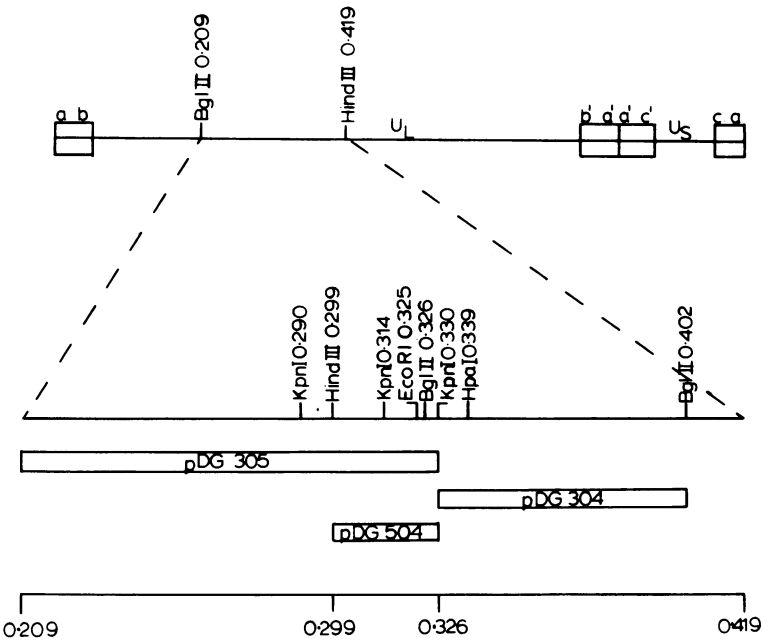

FIG. 4. *Localization of HSV-2 TK gene. The map positions of the viral sequences cloned in pDG 304, 305, and 504 are shown in relation to the KpnI, HindIII, EcoRI, HpaI, and BglII cleavage sites within map positions 0.209-0.419 in the unique long (U_L) region of HSV-2 strain 333 (11). TK is encoded by sequences within pDG 504 and is inactivated by cleavage at map positions 0.314 (KpnI) and 0.325 (EcoRI).*

TABLE 1. *Transfection of B82 cells by cloned HSV-2 DNA*

DNA	No. of TK^+ colonies/no. of dishes transfected	Efficiency of transformation	
		Per μg of HSV-2 DNA	Per μg of 4-kb fragment
pDG 504	110/10	27	27
pDG 305	41/10	6	24
pDG 304	0/10	0	0
pBR 322	0/10	0	0
*Hin*dIII digest	7/10	23	92
*Hin*dIII H fragment	53/10	11	46

subset of the sequences present in pDG 305, mapping between positions 0.299 and 0.326 on the HSV-2 (strain 333) genome. Hybridization of ^{32}P-labeled nick-translated pDG 504 to restriction endonuclease-cleaved HSV-2 DNA (Fig. 3) results in hybridization to fragments representing only one location on the genome. Transfection of mouse TK^- B82 cells with DNA from these plasmids resulted in conversion to TK^+ phenotype with pDG 305 and pDG 504 but not with pDG 304 (Table 1).

These results place the HSV-2 TK gene within map positions 0.299 and 0.326 in the unique L region and are consistent with the reported genetic data and show that the HSV-2 TK gene is colinear with the TK coding sequences on the HSV-1 genome. The only tenable explanation for the previously reported result (4), which suggested that the HSV-2 TK gene mapped between 0.53 and 0.65 on the genome, is that the gels contained partial digestion products and did not provide clean separation of fragments. The use of better-defined gels and cloned viral DNA fragments has corrected the assignment. Further definition of the arrangement of the viral sequences within the HSV TK-containing plasmids is in progress.

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