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Construction of poxviruses as cloning vectors: Insertion of the thymidine kinase gene from herpes simplex virus into the DNA of infectious vaccinia virus

(recombination *in vivo*/replica filter plating/recombinant DNA/eukaryotic virus vector)

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ABSTRACT **We have constructed recombinant vaccinia viruses containing the thymidine kinase gene from herpes simplex virus. The gene was inserted into the genome of a variant of vaccinia virus that had undergone spontaneous deletion as well as into the 120-megadalton genome of the large prototypic vaccinia variant. This was accomplished via *in vivo* recombination by cotransfection of eukaryotic tissue culture cells with cloned *Bam*HI-digested thymidine kinase gene from herpes simplex virus containing flanking vaccinia virus DNA sequences and infectious rescuing vaccinia virus. Pure populations of the recombinant viruses were obtained by replica filter techniques or by growth of the recombinant virus in biochemically selective medium. The herpes simplex virus thymidine kinase gene, as an insert in vaccinia virus, is transcribed *in vivo* and *in vitro*, and the fidelity of *in vivo* transcription into a functional gene product was detected by the phosphorylation of 5-[^{125}I]iodo-2′-deoxycytidine.**

Various methods for the introduction of defined foreign DNA sequences into eukaryotic cells are currently under investigation. These include microinjection of DNA (1), fusion of liposomes containing DNA (2, 3), erythrocyte-mediated gene transfer (4), direct introduction into cells of calcium orthophosphate-precipitated DNA (5–8), and eukaryotic viral vectors (9–18).

Recently, we demonstrated that endogenous subgenomic elements can be inserted into infectious progeny vaccinia virus via recombination *in vivo* (19). The ability to integrate vaccinia virus DNA sequences into infectious vaccinia virus progeny suggested the possibility for insertion of foreign genetic elements into infectious vaccinia virus via similar protocols. To test this, we chose the thymidine kinase (TK) gene of herpes simplex virus (HSV). The HSV *TK* gene is available as a clone, and its sequence has been determined and is known to contain the gene's own regulatory signals (20, 21). Vaccinia recombinant viruses containing the HSV *TK* gene can be detected by nucleic acid hybridization, by selection in appropriate methotrexate-containing medium, or by a specific enzymatic assay.

We were able to insert the HSV *TK* gene into a number of vaccinia virus preparations and to obtain pure cultures of recombinant vaccinia virus expressing the herpes virus gene. These studies are reported in this communication.

MATERIALS AND METHODS

Cells and Viruses. TK$^-$ human (line 143) cells derived by C. Croce and K. Huebner (Wistar Institute, Philadelphia, PA) were obtained from B. Moss (National Institutes of Health, Bethesda, MD). African green monkey kidney cells (CV-1) and baby hamster kidney cells (BHK-21) (C13) were maintained as monolayer cultures in Eagle's modified medium containing 10% fetal calf serum. The L and S variants of vaccinia virus have been described in detail (22). VTK$^-$11, a TK$^-$ mutant containing the L variant genome, was derived by R. Condit (State University of New York, Buffalo) and obtained through D. Hruby (University of Wisconsin, Madison). VTK$^-$79, a TK$^-$ mutant containing the S variant genome, was derived in our laboratory.

Construction of Plasmid Vectors. Plasmid pBR322 in which the *Bam*HI Q fragment of HSV DNA (which includes the *TK* gene) had been inserted into the *Bam*HI site (23) was provided by D. Hruby. The *Hin*dIII F fragment of vaccinia virus DNA was isolated from preparative agarose gels by binding to glass powder (24) as described (22). Approximately 200 ng of *Hin*dIII-cleaved pBR322 DNA was combined with 500 ng of isolated F fragment and ligated with phage T4 DNA ligase (New England BioLabs) in 50 mM Tris·HCl, pH 7.6/10 mM $MgCl_2$/10 mM dithiothreitol/1 mM ATP overnight at 10°C. *Escherichia coli* HB101 (25) cells were transformed (26) with the ligated DNA. Ampicillin-resistant tetracycline-sensitive recombinants were analyzed by restriction analysis by the method of Holmes and Quigley (27). A recombinant plasmid containing the *Hin*dIII F fragment was isolated and designated pDP3. Preparative amounts of pDP3 DNA were prepared by the cleared lysate procedure of Clewell and Helinski (28) after chloramphenicol amplification (29). Linearized pDP3 DNA from a partial *Bam*HI digest was ligated with purified *Bam*HI *TK* fragment and used to transform *E. coli* HB101. Transformants were screened for *Bam*HI-produced *TK* inserts by colony hybridization (30).

Construction, Screening, and Recovery of Recombinant Vaccinia Viruses. Insertion of the HSV *TK* gene into infectious vaccinia virus followed the general protocol for the rescue of endogenous genetic markers described by Nakano *et al.* (19). Five to 10 μg of *Hin*dIII-cleaved pDP132 or pDP137 DNA was coprecipitated with 2–10 μg of vaccinia virus carrier DNA. The calcium orthophosphate-precipitated DNA (0.5 ml) was flooded onto either BHK 21 or CV-1 monolayers previously infected (1 hr) with infectious vaccinia virus. The cells were harvested after 24 hr, lysed by three cycles of freezing and thawing, and screened for recombinant vaccinia viruses. Three approaches were utilized for screening and isolating recombinant vaccinia viruses containing the HSV *TK* gene: (*i*) Infected cell monolayers on which the plaques were visualized by neutral red staining were imprinted onto nitrocellulose filters according to Villarreal and Berg (31) as described (19). A mirror image replica was imprinted onto a second nitrocellulose filter by contact with the primary filter. The primary nitrocellulose filter was used for *in situ* hybridization (31), using ^{32}P-labeled nick-translated (32) *Bam*HI HSV *TK* DNA fragment as probe to detect viral plaques containing the HSV *TK* insert. Areas of the secondary nitrocellulose replica filter corresponding to those plaques showing

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Abbreviations: TK, thymidine kinase; HSV, herpes simplex virus; MDal, megadalton(s).

positive hybridization were punched out and infectious recombinant viruses were recovered by inoculating monolayer cultures. (*ii*) Recombinant vaccinia virus expressing HSV TK activity on monolayers of TK$^-$ human (line 143) cells were selected in the presence of methotrexate-containing medium (33) by using a modification of the procedure of Campione-Piccardo *et al.* (34). This cell line is viable in the presence of methotrexate-containing medium for up to 48 hr, long enough to allow detection of vaccinia virus plaques. (*iii*) Vaccinia virus recombinants containing functional HSV TK were detected by the phosphorylation of [^{125}I]iododeoxycytidine. Vaccinia virus was plated on monolayers of CV-1, and after 48 hr, 5 μCi (1 Ci = 3.7×10^{10} becquerels) of 5-[^{125}I]iodo-2′-deoxycytidine (New England Nuclear) was added in 1.5 ml of medium for overnight labeling. After staining with neutral red to visualize the plaques, the monolayers were washed three times with phosphate-buffered saline and imprinted onto nitrocellulose filters, and plaques expressing HSV TK were localized by radioautography. Infectious recombinant virus was recovered from punched-out areas of the nitrocellulose filter. All recombinant viruses were further purified by two consecutive cycles of plaque isolation.

DNA Restriction Analysis of Recombinant Vaccinia Virus. DNA from recombinant vaccinia virus-infected CV-1 monolayers was extracted by the procedure of Esposito *et al.* (35) or from virus purified from infected HeLa cells according to Joklik (36) as described (19, 22). *Hin*dIII-, *Bam*HI-, or *Sst* I-digested DNA was analyzed on agarose gels and transferred to nitrocellulose (Schleicher and Schuell, BA85) (37) for hybridization with ^{32}P-labeled nick-translated HSV *TK* probe as described (22, 38).

Analysis of RNA Transcripts. ^{32}P-Labeled recombinant vaccinia virus RNA was synthesized *in vitro* by using [α-^{32}P]GTP as described (22). The RNA was selected by hybridization and eluted from cloned HSV *TK* DNA bound to diazobenzyloxymethyl-paper as described (39). The selected RNA was hybridized to Southern blots of pDP137 digested with *Sst* I or with *Hin*dIII/*Bam*HI as described (22).

RESULTS

Strategy for the Construction of Recombinant Vaccinia Virus. The noninfectious nature of purified vaccinia virus DNA, its cytoplasmic site of replication, and its large genome present unique problems to approaches of genetic manipulation of the virus. A significant breakthrough demonstrating the feasibility of marker rescue experiments using defined DNA fragments has recently been reported (19, 40). The insertion of foreign DNA into infectious progeny vaccinia virus involves the formation of recombinant DNA containing the foreign gene flanked by contiguous vaccinia virus genomic sequences. This chimeric DNA is introduced into cells as a calcium orthophosphate precipitate. The cells are additionally infected with vaccinia virus. *In vivo* recombination occurs between the flanking vaccinia virus DNA sequences and homologous sequences present in the replicating vaccinia virus genome. The recombined DNA containing the foreign genetic insert replicates and is subsequently packaged into infectious virus.

Construction of Chimeric Plasmid Vectors. Insertion of foreign genes into vaccinia virus must occur at loci that do not disrupt essential gene functions. An obvious locus of nonessential DNA resides within the 5% of the genome deleted from the L variant vaccinia virus previously described (22). As part of another project to identify nonessential genetic loci, the single *Bam*HI site localized within the internal *Hin*dIII F fragment (22) was found not to disrupt essential genetic information and was utilized for the insertion of the HSV *TK* fragment. The construction of the recombinant DNA plasmids utilized in this study is outlined in Fig. 1. The 2.8-megadalton (MDal) pBR322 digested with *Hin*dIII was ligated with purified 8.6-MDal vaccinia virus *Hin*dIII F fragment. Full-length linearized plasmid from a partial *Bam*HI digest of the recombinant (11.3-MDal) plasmid pDP3 was isolated and ligated with the 2.3-MDal HSV *Bam*HI *TK* fragment. Plasmids containing the HSV *TK* insert were identified by colony hybridization using ^{32}P-labeled nick-translated HSV *TK* DNA probe. Two of these plasmids, pDP132 (13.6 MDal) and pDP137 (15.9 MDal), which contain the HSV *TK* gene inserted into the proper *Bam*HI site, were selected for further studies after analysis with *Hin*dIII and *Sst* I (*Sac* I). The presence of a single, asymmetric *Sst* I restriction site in both the *Hin*dIII F fragment and the *HSV TK* was used to determine the orientation and copy number of the HSV *TK* insert. *Sst* I digestion of pDP132 gave two fragments of 10.1 and 3.5 MDal, whereas *Sst* I digestion of pDP137 generated three fragments of 10.8, 2.8, and 2.3 MDal. These data demonstrated that the insertion of the HSV *TK* in pDP132 was in opposite orientation from the HSV *TK* insert in pDP137 and that two copies of the *TK* gene were present in pDP137 as a "head-to-tail" tandem. This was confirmed by additional digestion with *Bam*HI and densitometric analysis.

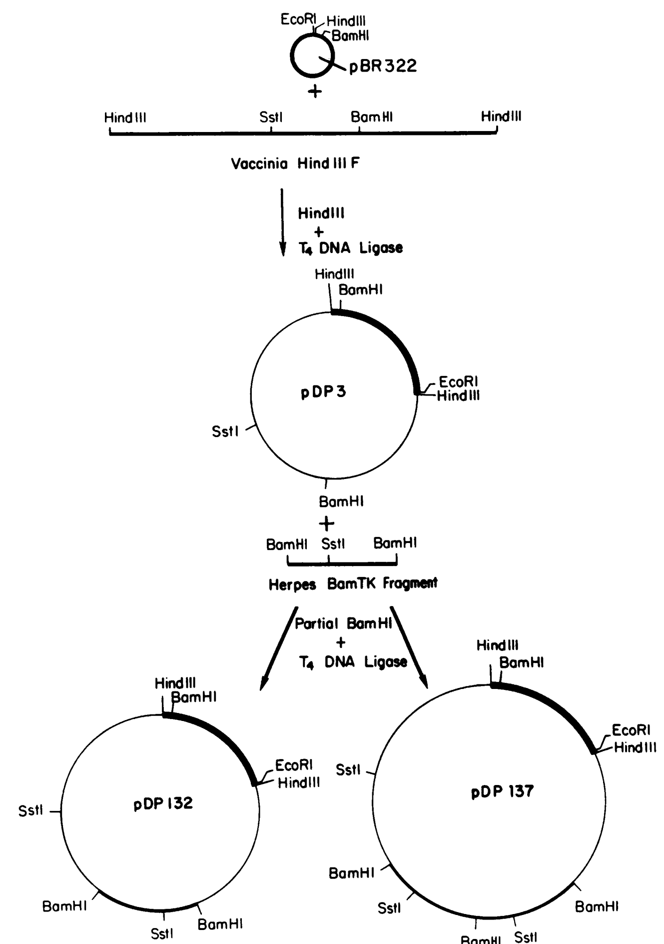

FIG. 1. Construction of chimeric vaccinia virus–herpesvirus plasmids. The purified 8.6-MDal vaccinia *Hin*dIII F fragment was combined with the *Hin*dIII-digested 2.8-MDal pBR322 plasmid and ligated with T4 DNA ligase. A partial *Bam*HI digest of the derivative pDP3 (11.3 MDal) plasmid was combined with the (2.3-MDal) HSV *Bam*HI *TK* fragment and ligated with T4 DNA ligase. After detection of colonies containing the HSV *TK* gene by colony hybridization and further additional restriction analysis, two plasmids, pDP132 (13.6 MDal) and pDP137 (15.9 MDal), were selected. pDP137 contains a head-to-tail tandem of the HSV *TK* in opposite orientation from the single-copy HSV *TK* gene in pDP132. *Bam*HI, *Sst* I, *Hin*dIII, and *Eco*RI sites are mapped as indicated and the plasmids are drawn essentially to scale.

Construction and Recovery of Recombinant Virus Containing the HSV *TK* gene. The construction and analysis of six recombinant vaccinia virus populations, vP1–6, containing the HSV *TK* gene in the single *Bam*HI site of the vaccinia virus *Hin*dIII F fragment will be described. The vaccinia virus recombinants vP1 and vP2 were constructed by employing the strategy of *in vivo* recombination outlined above. Donor DNA consisted of a calcium orthophosphate precipitate of pDP132 for vP1 or pDP137 for vP2 and purified S variant vaccinia virus DNA as carrier. The recipient was infectious S variant vaccinia virus. *In vivo* recombination and subsequent screening for vaccinia virus progeny containing the HSV *TK* insert were performed on CV-1 cells with two approaches. The first consisted of screening replica nitrocellulose filters. Approximately 0.4% of the plaques generated from the progeny of *in vivo* recombination contained the HSV *TK* insert, as detected by *in situ* hybridization. The second approach took advantage of the observation of Cooper (41) that the substrate specificity of the HSV TK allows phosphorylation of halogenated pyrimidine nucleosides, whereas cellular TK does not. This observation has been exploited by Summers and Summers (42), who used [^{125}I]-iododeoxycytidine in combination with radioautography as a sensitive and specific assay for HSV TK. This assay can be used to detect vaccinia virus recombinants containing and expressing the HVS *TK* even if vaccinia virus carries its own TK activity, because the vaccinia virus TK fails to phosphorylate iododeoxycytidine. With this assay, no plaques equivalent to vP1 generated with pDP132 donor DNA were detected, but a number of plaques equivalent to vP2 generated with pDP137 donor DNA were detected, suggesting that the HSV *TK* was expressed in the vaccinia virus recombinant vP2 but not in recombinant vP1. The ability of recombinant vaccinia virus containing the HSV *TK* to phosphorylate [^{125}I]iododeoxycytidine is shown in Fig. 2. Only recombinant vaccinia virus vP2, containing the HSV *TK* gene insert, utilized [^{125}I]iododeoxycytidine. Uninfected, vaccinia virus-infected, or recombinant vaccinia virus vP1-infected CV-1 monolayers did not. The only difference between vP1 and vP2 is the orientation of HSV *TK* in the donor DNA.

Additional vaccinia virus recombinants vP3 and vP4 were

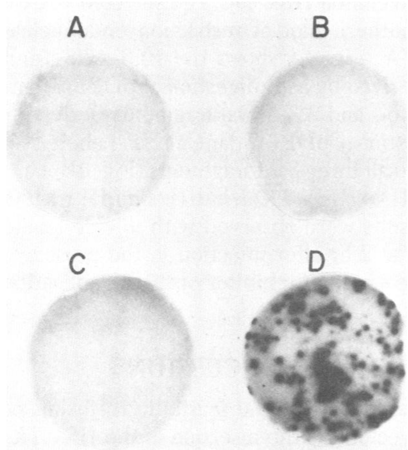

FIG. 2. [^{125}I]Iododeoxycytidine assay for the expression of HSV *TK* in recombinant vaccinia virus. Uninfected or 48-hr infected monolayers of CV-1 were incubated with 1.5 ml of liquid overlay medium containing 5 μCi of [^{125}I]iododeoxycytidine for 16 hr and washed three times with phosphate-buffered saline, and the neutral red-stained monolayers were imprinted onto nitrocellulose filters. A radioautogram of uninfected (*A*), S variant vaccinia virus-infected (*B*), recombinant vaccinia virus vP1-infected (*C*), or recombinant vaccinia virus vP2-infected (*D*) CV-1 monolayers is shown.

generated by *in vivo* recombination in BHK 21 cells by using pDP132 and pDP137 donor DNA, respectively. In this case, the carrier DNA used was purified from VTK$^-$79, an S variant vaccinia deficient in TK activity. VTK$^-$79 was also the rescuing virus. Because VTK$^-$79 lacks endogenous TK activity, successful insertion and expression of the inserted HSV *TK* gene was monitored in recombinant vaccinia virus progeny by growth on TK$^-$ human (line 143) cells in the presence of methotrexate-containing medium, using modifications of the procedure described by Campione-Piccardo *et al.* (34). Plaques were detected in neutral red-stained monolayers 48 hr after infection when pDP137 was used as donor DNA to generate recombinant vaccinia virus vP4, suggesting successful insertion and expression of the HSV *TK* gene into VTK$^-$79 vaccinia virus. No viral plaques were detected when pDP132 was used as donor DNA. That this latter result was due to a failure to express the HSV *TK* and not to a failure to insert the gene into the VTK$^-$79 vaccinia virus, was subsequently demonstrated by finding vP3 recombinant viruses containing the HSV *TK* insert by the *in situ* hybridization and replica filter method described above.

Two additional virus recombinants containing the HSV *TK* insert were derived by *in vivo* recombination in BHK monolayers. Donor DNA was either pDP132 or pDP137. Carrier DNA was derived from VTK$^-$11, an L variant of vaccinia virus deficient in TK activity. The rescuing infectious vaccinia virus was also VTK$^-$11. Two recombinants of VTK$^-$11, vP5 and vP6, were obtained by using pDP132 and pDP137, respectively. These two recombinants were identified by *in situ* hybridization for the presence of the HSV *TK* insert and infectious progeny derived from replica nitrocellulose filters. With the [^{125}I]iododeoxycytidine assay vP6 was positive for HSV *TK* expression but, as expected, vP5 was not.

Restriction Analysis of Recombinant Vaccinia Virions Modified by the Insertion of the HSV *TK* Gene. DNA was extracted from S variant and from vP1 and vP2 recombinant vaccinia viruses, digested with *Hin*dIII, *Bam*HI, or *Sst* I, and analyzed on agarose gels. As indicated in Fig. 3*A*, *Hin*dIII digestion of vP1 (lane 2) and vP2 (lane 3) generated a new 10.9-MDal fragment not found in the S variant (lane 1). The 10.9-MDal fragment is composed of the 8.6-MDal internal vaccinia virus *Hin*dIII F fragment and the 2.3-MDal HSV *TK* insert. It should be noted that two moles of the *Hin*dIII F fragment of the S variant DNA are produced by digestion, one internal fragment and the left terminal fragment (22), but one mole of the F fragment is found in digests of vP1 and vP2, as expected from the insertion of the HSV *TK* into the internal F fragment. Digestion of vP1 (lane 5) and vP2 (lane 6) with *Bam*HI gives an additional one mole of 2.3-MDal fragment not found in the *Bam*HI digestion of S variant DNA (lane 4), consistent with the size of the HSV *TK* insert. Digestion of vP1 (lane 8) and vP2 (lane 9) with *Sst* I gives new fragments not found in the S variant DNA (lane 7). *Sst* I digestion of vP1 (lane 8) DNA gives fragments of 17.0 and 3.4 MDal while vP2 (lane 9) fragments of 17.7 and 2.7 MDal are detected. Because *Sst* I cuts the HSV *TK* gene once and asymmetrically, the digests of vP1 and vP2 confirm the insertion of the HSV *TK* into the *Hin*dIII F fragment in opposite orientation. Additionally, the absence of a 2.3-MDal band from vP2 (lane 9) confirms the presence of a single HSV *TK* insert in the recombinant virus. A Southern blot (37) of the digested DNA was prepared and ^{32}P-labeled nick-translated HSV *TK* DNA was hybridized under conditions previously described (22). As expected, the labeled probe hybridizes to the novel DNA fragments in vP1 and vP2 containing the HSV *TK* gene as described above (Fig. 3*B*, lanes 2, 3, 5, 6, 8, and 9). No hybridization of the probe to S variant digested DNA (lanes 1, 4, and 7) was detected. Reciprocal hybridizations using labeled vaccinia virus DNA as probe resulted in hybridization to all of the vaccinia

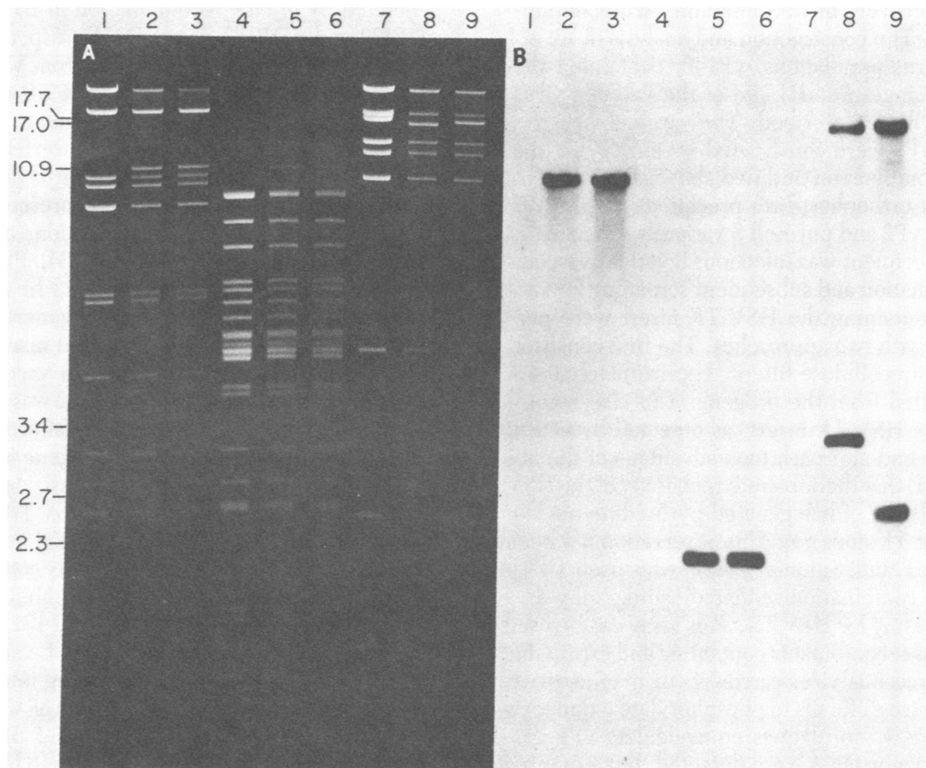

FIG. 3. Restriction analysis of recombinant vaccinia virus. DNAs of S variant and recombinant vP1 and vP2 vaccinia viruses were digested with *Hin*dIII, *Bam*HI, or *Sst* I and analyzed on an agarose gel. (*A*) *Hin*dIII digest of S variant (lane 1), vP1 (lane 2), and vP2 (lane 3); *Bam*HI digest of S variant (lane 4), vP1 (lane 5), and vP2 (lane 6); *Sst* I digest of S variant (lane 7), vP1 (lane 8), and vP2 (lane 9). (*B*) Radioautograph of the Southern blot to which ^{32}P-labeled nick-translated HSV *TK* DNA (1×10^5 cpm/ml) was hybridized. Hybridization to the 10.9-MDal *Hin*dIII fragment derived from vP1 (lane 2) and vP2 (lane 3), the 2.3-MDal *Bam*HI fragment derived from vP1 (lane 5) and vP2 (lane 6), and the 17.0- and 3.4-MDal fragments derived from vP1 (lane 8) and the 17.7- and 2.7-MDal fragments derived from vP2 (lane 9) by *Sst* I digestion are indicated. No hybridization to restricted S variant DNA (lanes 1, 4, and 7) was detected.

virus DNA-containing fragments but not to the *Bam*HI HSV *TK* fragment (data not shown).

Analysis of Transcription. Although regulatory signals are known to flank the body of the HSV *TK* gene (20, 21), it was not known whether the vaccinia virus transcriptional system would recognize these HSV *TK* regulatory signals. In order to gain information regarding the recognition of these signals by vaccinia virus, the recombinant vP2 was studied for transcription of the HSV *TK* gene, because this recombinant was shown to express HSV TK activity. ^{32}P-Labeled RNA was synthesized *in vitro* by using purified vP2 virus and was selected by hybridization to cloned HSV *TK*. The selected RNA was hybridized to a Southern blot of restriction endonuclease-digested pDP137 DNA. Fig. 4*A* shows the 10.8-, 2.8-, and 2.3-MDal fragments derived by *Sst* I digestion of pDP137 (lane 1) and the 5.4-, 3.1-, 2.6-, and 2.3-MDal fragments derived by *Hin*dIII/*Bam*HI digestion of pDP137 (lane 2). ^{32}P-Labeled selected RNA hybridized to all three *Sst* I fragments (Fig. 4B, lane 1) but only to the 2.3-MDal (HSV *TK*) *Hin*dIII/*Bam*HI fragment (lane 2). Identical results were observed with *in vivo* labeled RNA derived either at 2 hr after infection in the presence of cytosine arabinonucleoside, an inhibitor of DNA replication, or at 6 hr without metabolic inhibitors.

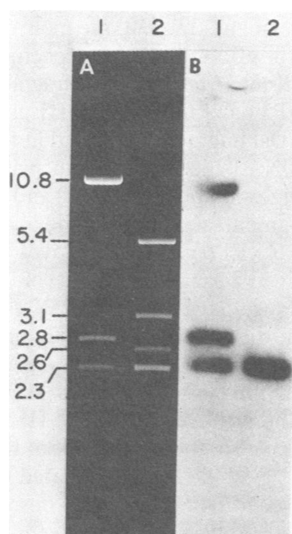

FIG. 4. Analysis of transcription of HSV *TK* in the vP2 vaccinia virus recombinant. (*A*) Agarose gel of *Sst* I- (lane 1) or *Hin*dIII/*Bam*HI- (lane 2) digested pDP137 DNA. (*B*) Radioautograph of hybridization of hybrid-selected ^{32}P-labeled RNA synthesized *in vitro* by using vP2 recombinant vaccinia virus.

DISCUSSION

We have demonstrated the feasibility of using poxviruses as eukaryotic vectors by the insertion of the HSV *TK* into the genome of infectious vaccinia virus. Six unique recombinants have been constructed: vP1 and vP2 containing the HSV *TK* in wild-type S variant vaccinia virus; vP3 and vP4 containing the HSV *TK* in a TK$^-$ S variant vaccinia virus; and vP5 and vP6 containing the HSV *TK* in a TK$^-$ L variant genome. Expression of the HSV *TK* was observed in recombinants vP2, vP4, and vP6 but not in vP1, vP3, or vP5. Correct orientation appears to be essential for the expression of this gene at this site. Although the transcription analysis, indicating hybridization of *in vivo* and *in vitro* hybrid-selected RNA only to the HSV *TK* portion of the recom-

binant plasmid and not to the flanking vaccinia virus DNA sequences, might be interpreted as if the regulatory signals of the HSV *TK* gene are recognized, other (unpublished) data suggest that vaccinia signals may be operative for HSV *TK* expression.

The ability to insert foreign genetic material into the 120-MDal genome of the prototypic L variant vaccinia suggests that packaging of additional DNA is not prohibited, but the upper limit of packaged DNA remains to be determined. With the 6.3-MDal deletion of nonessential DNA found in the S variant (22) plus the additional 2.3-MDal HSV *TK* insert reported here in the L variant it appears that at least 8.6 MDal or approximately 2.5 simian virus 40 genome equivalents of foreign DNA can be readily handled by vaccinia virus. It is reasonable to assume that additional nonessential DNA can be deleted from the S variant, thus increasing the total amount of foreign DNA that can be inserted. This is one of the attractions of poxvirus vectors. In addition, unlike other established or potential eukaryotic viral vectors, such as simian virus 40, herpesvirus, adenovirus, retroviruses, or bovine papilloma virus, vaccinia virus is not considered to be oncogenic and therefore represents less of a risk at this level of consideration.

Although vaccinia virus recombinants vP2, vP4, and vP6 were constructed with the pDP137 recombinant plasmid containing a head-to-tail tandem of the HSV *TK* gene, no evidence for a tandem insertion was observed in these and two other additional vaccinia recombinants tested. The reason for this is not clear, but it may be loss of one copy by *in vivo* recombination.

We selected HSV *TK* for our first encounter with insertion of foreign DNA into vaccinia virus for a variety of reasons. The sequence of the gene is available and its regulatory signals are defined. The gene therefore offers a ready target for genetic manipulation. Vaccinia virus recombinants can be readily selected for TK expression and its unique substrate specificity, the ability to phosphorylate iododeoxycytidine, makes detection and assay simple. Splicing other foreign genes with nonbiochemically selectable characteristics to the HSV *TK* followed by insertion into the vaccinia virus genome would facilitate detection and selection of additional recombinant viruses. Alternatively, splicing other foreign genes into the body of the HSV *TK* would render the recombinant virus TK^- and therefore selectable by its resistance to growth in the presence of bromodeoxyuridine. Some of these advantages enumerated for the HSV *TK* gene will also be available by analogous manipulations of the endogenous vaccinia TK gene.

The observations reported here open the door for exploring the genetic regulation of vaccinia virus by programmed manipulations of foreign genes and the possible production of biological reagents and gene replacement therapy via poxvirus vectors. Perhaps the most interesting aspect because of its more immediate potential for realization is the use of vaccinia virus vectors for the production of live vaccines. These could be produced by the insertion and expression of appropriate genes encoding specific antigens into vaccinia virus recombinants. Although vaccinia virus as an immunizing agent is not without its risks, vaccination has been used for several hundred years since its introduction by Jenner, and the medical community is well versed in its use. The success of using vaccinia virus to immunize against smallpox has been noted by the recent declaration of the World Health Organization that the world is free of smallpox. It is conceivable that other human or veterinary disease processes may be controlled or eliminated by utilizing live vaccines produced by recombinant vaccinia viruses. The construction of recombinant vaccinia viruses containing genes for the expression of pertinent antigens and the use of these recombinants as live vaccines will be presented in future communications.

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