Herpes Simplex Virus Causes Amplification of Recombinant Plasmids Containing Simian Virus 40 Sequences

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SUMMARY

Simian virus 40 (SV40) DNA, inserted into a plasmid vector, does not replicate when transfected into baby hamster kidney cells. However, when the recipient cells are superinfected with herpes simplex virus type 1 (HSV-1), extensive amplification of the introduced plasmid occurs. Deletion of the late SV40 region or part of the coding sequences of the small tumour (t) antigen has no effect on the efficiency of amplification, whereas manipulations affecting either the SV40 origin of replication or the integrity of large tumour (T) antigen substantially decrease HSV-induced amplification. Phosphonoacetic acid, an inhibitor of HSV DNA polymerase, strongly inhibits plasmid replication. Also, an HSV-1 mutant with a temperature-sensitive defect in the DNA polymerase gene (tsH) is unable to carry out amplification of test plasmids at the non-permissive temperature. On the other hand, a further mutant (tsS) causes SV40-plasmid amplification independent of the temperature, but this mutant fails to amplify a plasmid with an HSV origin at the non-permissive temperature. Thus, HSV-induced amplification of heterologous DNA is possible in the absence of HSV DNA replication. Since tsS putatively has a defect in the gene coding for an HSV origin-binding protein (UL9), this observation appears plausible. The implications for interaction between herpesviral replication functions and heterologous (possibly cellular) DNA sequences are discussed.

INTRODUCTION

Infection with herpes simplex virus (HSV) turns off the DNA replication of the host cell (Fenwick & Walker, 1978). It is, therefore, surprising that in several simian virus 40 (SV40)-transformed cell lines, SV40 DNA sequences are over-replicated upon HSV infection (Schlehofer et al., 1983, 1986; Matz et al., 1984, 1985). This specific DNA amplification has been shown to depend on the herpesviral DNA polymerase (Matz et al., 1984). The structure of amplified SV40 DNA in the Syrian hamster cell line Elona has been analysed and found to consist of large concatemeric molecules (Matz, 1987). The structural similarity of the amplified DNA species with herpesviral DNA from defective interfering particles (Frenkel, 1981) supported the assumption that this illegitimate over-replication occurs under the direction of herpesviral DNA replication functions. Analysis of the amplified SV40 DNA revealed minor differences from standard SV40 DNA and failed to identify any sequence element that resembled a herpesvirus-specific origin of replication (Matz, 1987). In this report the question was addressed as to whether amplified DNA, isolated from the HSV-infected Elona cell line, or simply standard SV40 DNA can substitute for a herpesviral origin of replication in a functional assay previously designed for the identification of HSV replication origins (Stow, 1982).

METHODS

Cells and virus. Baby hamster kidney cells (Macpherson & Stoker, 1962) clone 13 and Vero cells (Macfarlane & Sommerville, 1969) were grown in Eagle's MEM supplemented with 5% foetal calf serum (FCS), amino acids, vitamins, and antibiotics. Herpes simplex virus type 1 (HSV-1) wild-type strain 17syn*, the phosphonoacetic acid...
(PAA)-resistant mutant IP2, and temperature-sensitive (ts) HSV-1 mutants tsH and tsS were kindly provided by Drs H. S. Marsden and J. H. Subak-Sharpe. Viruses were propagated on Vero cells and quantified as described (Brown et al., 1973).

Plasmids. All of the recombinant plasmids used in this study were based upon the vector pSPT18 (Pharmacia) and were propagated in Escherichia coli DH1 (Hanahan, 1983). Preparation of plasmid DNA was carried out according to Birnboim & Doly (1979) followed by banding in CsCl density gradients containing ethidium bromide. Some plasmid DNA preparations were alternatively purified by chromatography on Qiagen (Diagen) instead of banding in CsCl gradients.

The plasmid pFR106, containing an HSV-1-specific origin of replication (oriK) was isolated by randomly cloning unseparated Asp718 fragments (Asp718 is a isoschizomer of KpnI) of viral genomic DNA (prepared according to Wilkie, 1973) into Asp718-restricted and phosphatase-treated pSPT18. The identity of the inserted fragment (KpnIa) was tested physically by comparison with the restriction maps of genomic HSV-1 DNA (Wilkie, 1976; Preston et al., 1978) and was confirmed functionally as outlined in Results.

Three recombinants (pFR125, pFR160, pFR187) were derived from total DNA of the SV40-transformed hamster cell line Elona that had been infected with HSV-1. The amplified SV40-specific DNA consisting of large concatemeric molecules was partially purified, cleaved with Asp718 and cloned in pSPT18. The procedure has been described earlier (Matz, 1987). The plasmid clone pFR125 contains one copy of the tandemly repeated SV40-like multimers. Cleavage of pFR125 with HindIII and religation resulted in pFR160, which retains the sequences between nucleotide 517 and the KpnI (Asp718) site at position 294. Plasmid pFR187 was constructed by cutting pFR160 with Asp718 and NcoI, filling in the sticky ends with Klenow DNA polymerase and blunt end religation. The structures of insertions relative to the SV40 genome are illustrated in Fig. 1 (b) and Fig. 2 (a).

The SV40 variant strain E10 that had been used to establish the Elona cell line (Brandner et al., 1977) is the source of clones pFR165 and pFR176. Full-length genomic DNA was inserted, after Asp718 cleavage, into pSPT18 yielding pFR165, the derivative of which, pFR176, was constructed like pFR160 (Fig. 2 a). The late region present in pFR113 (Fig. 2a) was removed by BamHI cleavage and religation resulting in pFR190 (Fig. 4a). From pFR190, the 3' region of the large tumour (T) antigen gene was removed by PstI digestion and subsequent ligation, resulting in pFR214 (Fig. 4a). NcoI- and Asp718-cleaved pFR190 was treated with Klenow polymerase in the presence of dNTPs and blunt end religated to create pFR216 (Fig. 4a). pFR197 was
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constructed by cleaving pFR113 with SfiI, removing the 3' protruding ends with T4 DNA polymerase in the presence of dNTPs and blunt end religation (Fig. 4a). This procedure removed 3 bp (5239 to 5241) of the hairpin structure of the SV40 origin, destroying the SfiI site and creating a SacII site.

A clone with a deletion within the coding region of small turnout (t) antigen was constructed as follows, pFR190 was opened at the BstXI site (position 4759) in the 3' portion of the t antigen that lies within the intron sequence of the T antigen gene. Successive treatment with T4 DNA polymerase and S1 nuclease removed approx. 200 bp from that region. Klenow polymerase treatment in the presence of dNTPs and subsequent blunt end religation resulted in pFR235.

Plasmid replication assay. BHK cells were grown in plastic dishes (60 mm diameter) to near confluence. Fresh culture medium was added 2 to 4 h before DNA transfection (Graham & van der Eb, 1973; Stow, 1982). The transfection mixture was prepared as follows. Five µg of calf thymus DNA and 0-1 pmol of plasmid DNA were adjusted with sterile water to a volume of 188 µl; 62 µl of 1 M-CaCl₂ was added and the solution was slowly mixed into 250 µl of twice concentrated HEPES-buffered saline, HBS (2 × HBS is 50 mM-HEPES, 280 mM-NaCl, 2.8 mM-Na₂HPO₄ adjusted to pH 7.12 with NaOH). After 15 to 20 min at room temperature, the precipitate was added to the culture medium of the cells. In some experiments, 3 to 4 h after DNA addition the medium was removed and 0-5 ml of 1 × PBS containing 15% (v/v) glycerol was added for 2 to 3 min. After washing of the cells twice with MEM, incubation was continued with MEM-5% FCS for 6 to 16 h, then cells were infected with HSV at a multiplicity of 5 to 10 p.f.u./cell, or mock-infected. At 24 to 36 h post-infection, total DNA was isolated from cells by lysis in DNA mixture (10 mM-sodium EDTA pH 8.0, 0.5% SDS, 100 µg/ml proteinase K) at 50 °C for 1 to 3 h and subsequent phenol and chloroform/isooamyl alcohol (24 : 1 v/v) extractions followed by ethanol precipitation.

RESULTS

Earlier work has shown that HSV infection generates large tandemly reiterated SV40 DNA molecules in the transformed hamster cell line Elona (Matz, 1987). Isolation of this aberrantly amplified DNA species, cloning in a plasmid vector, and sequence analysis revealed two differences when compared to wild-type SV40 DNA: deletion of one copy of the transcriptional enhancers and a base exchange (C to T) corresponding to position 5209 on the standard viral genome (van Heuverswyn & Fiers, 1979). One of the questions raised in the present work is whether or not any of these alterations are important for HSV-mediated amplification.

Amplification of plasmids in BHK cells infected with HSV

Several plasmids containing DNA from the standard SV40 strain 776, DNA from the SV40 Elo isolate and DNA isolated from large concatemeric SV40 molecules in HSV-infected Elona cells were introduced by transfection into BHK cells, which are non-permissive for SV40 replication. As a negative control, the bacterial plasmid pSPT18 was used; an HSV-1 DNA fragment with the herpesviral orig, cloned into pSPT18, served as a positive control in transfection experiments. The cells were either superinfected with HSV-1 or mock-infected, and total cellular DNA was analysed by Southern hybridization for amplification of the introduced DNA species by probing with radioactively labelled vector plasmid DNA. It was found that plasmids with the SV40 core origin (pFR183 and pFR187) behaved like the negative control plasmid pSPT18 upon HSV superinfection (Fig. 2b). A similar result was obtained (Fig. 2b) when the extended regulatory region, as shown in Fig. 2(a), was present (pFR160, pFR176, pFR153). These experiments ruled out the possibilities that HSV DNA replication factors might erroneously recognize an SV40 origin and that the sequence alteration in the origin domain of the integrated SV40 DNA in the hamster cell line Elona (Matz, 1987) was solely responsible for the amplification by HSV infection.

On the other hand, in this experimental system, extensive amplification occurred whenever a complete copy of SV40, either wild-type or variant, was inserted into the transfected plasmid. The absence of one of the two enhancer elements in pFR125 or a base substitution in pFR125 and pFR165 (arrow in Fig. 2a) had basically no influence on the results (Fig. 2c). Fig. 2(a) illustrates the inserts of the plasmids used and summarizes the results obtained.
(a) SV40

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Amplified

pFR187

pFR183

pFR160

pFR176

pFR153

pFR125

pFR165

pFR113

(b) 1 2 3 4 5 6

- + - + - + - + - + - + - +

(c) 1 2 3 4

- + - + - + - + - + - + - +

3.15 kb

1.0 kb
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Fig. 3. High Mr DNA sequences after amplification in HSV-infected BHK cells. Plasmids pSPT18 (vector; lanes 1 and 2), pFR106 (vector with HSV origin; lanes 3 and 4), and pFR165 (vector plus SV40; lanes 5 and 6) were transfected into BHK cells with a glycerol boost; after 20 h, cells were mock-infected (-) or infected with HSV (+). Twenty-nine h later, DNA was isolated, cleaved with BglII (lanes 1, 3, 5) or Asp718 (lanes 2, 4, 6), run on an agarose gel (0.8%), and blotted onto nitrocellulose. The filter was hybridized with a 32P-labelled pSPT18 DNA probe and autoradiographed. Restriction enzyme digests of HSV-1 DNA served as size markers in gel electrophoresis.

Additional Southern analyses were carried out with total DNA from HSV-infected and mock-infected cells cleaved with Asp718 or BglII. Asp718 separates the vector plasmid pSPT18 from the SV40 or HSV insert and BglII does not cleave within any of the plasmid constructions used. As an example, it can be seen in Fig. 3 that the radioactive pSPT18 probe detects a fragment comigrating with linearized pSPT18 in those lanes which contain Asp718-restricted DNA of HSV-1 DNA served as size markers in gel electrophoresis.

Fig. 2. Structure and properties of plasmids containing SV40 DNA from different sources. (a) SV40 sequences inserted in pSPT18 (vector sequences symbolized as dots). Arrows indicate a C to T substitution at position 5209. Boxes represent the 72 bp enhancer elements. pFR125 contains one repeat unit of amplified SV40 DNA from HSV-infected E10na cells, pFR160 and pFR187 are derived from pFR125. pFR165 contains the genome of the human SV40 isolate E10 (Brandner et al., 1977), pFR176 is a deletion subclone. pFR113 contains one full copy of a wild-type SV40 genome, pFR153 and pFR183 are derivatives of pFR113. DNA sequence analyses were carried out as described by Chen & Seeberg (1985). (b) Analysis of DNAs from plasmid transfection and virus superinfection experiments. BHK cells were transfected with the respective plasmid (12 h, no glycerol boost), infected with HSV (+) or mock-infected (-). Total cellular DNA was isolated (36 h p.i.); one-tenth of the DNA yield was treated with Asp718 and DpnI. Cleavage products were separated by electrophoresis on an 0.7% agarose gel, blotted onto a nitrocellulose filter and probed with 32P-labelled pSPT18 DNA. The filter was exposed to X-ray film (Kodak) using an intensifying screen (Kodak). Linearized pSPT18 DNA and the '1 kb ladder' (Bethesda Research Laboratories) were used as size markers in gel electrophoresis. Lane 1, pSPT18; lane 2, pFR187; lane 3, pFR183; lane 4, pFR160; lane 5, pFR176; lane 6, pFR153. (c) Comparison of amplification efficiency of three different SV40/pSPT18 recombinant plasmids (pFR125, 165, 113, lanes 1 to 3 respectively) with a plasmid harbouring HSV-1 or8 (pFR106, lane 4). Experimental details as in (b).
BHkB cells that had been transfected with pFR106 (HSV origin) or with pFR165 (SV40) and subsequently infected with HSV. The BglII cleavage products migrate very slowly in agarose gel indicating high $M_r$ forms of the amplified DNAs.

**Requirements for HSV-induced amplification of plasmids**

Once it had been established that transfected plasmid DNAs could be driven into replication after HSV infection by virtue of inserted SV40 DNA and that the SV40 origin domain alone was obviously not able to substitute for an HSV origin, it was asked whether there was any cryptic *cis* element somewhere in the SV40 genome with functional HSV origin properties (a rather unlikely possibility) or whether both the SV40 origin and T antigen were necessary. For example, pFR113 containing the complete wild-type SV40 genome loses its ability to be amplified simply by a deletion of 3 bp from the hairpin structure of the core origin (pFR197 in Fig. 4). Amplification, however, occurs with a construction from which the late region had been deleted (pFR190). The activity can be abolished either by deleting the 3' portion of the coding region for T antigen (pFR214) from pFR190 or by removing the regulatory region (pFR216). Small t antigen is very unlikely to be involved since a deletion within the coding sequence of the gene (pFR235 in Fig. 4a) does not abolish HSV-dependent amplification activity. These results indicate that amplification of plasmid DNA is due to the concerted action of the SV40 origin of replication, the SV40 T antigen, and HSV functions.

In this context, it was not surprising that amplification could be achieved by simultaneous transfection of the SV40 origin-defective plasmid (pFR197) together with a plasmid exclusively bearing the SV40 origin core sequences (Fig. 5). From the size of the detected restriction fragment (3.22 kb) it is clear that it is in fact the origin-bearing plasmid that undergoes replication. Amplification of pFR197 would have produced a 4.63 kb fragment (Fig. 5).

Furthermore, it was demonstrated that HSV-induced amplification of a plasmid containing both the intact SV40 origin and the early region (pFR190) was efficiently inhibited by PAA (Fig. 5b). This is in accordance with earlier results assigning the HSV-encoded DNA polymerase a major role in amplification of SV40 DNA in transformed hamster cells (Matz *et al*., 1984). The possibility that the inhibition might be due to an effect of the drug on cellular DNA polymerase is rather unlikely because the drug does not abolish the activity of a PAA-resistant HSV mutant, IP2. Further evidence for the importance of the viral DNA polymerase is provided by the fact that the temperature-sensitive mutant tsH, which has a defect in the structural gene for DNA polymerase, fails to amplify the SV40 test plasmid pFR190 as well as the HSV origv-containing plasmid pFR106 at the non-permissive temperature. An additional ts mutant, namely tsS, behaves differently: it is unable to replicate the HSV origin-bearing plasmid pFR106 at the non-permissive temperature as expected for a conditional-lethal DNA-negative mutant, yet it is able to replicate the SV40 test plasmid pFR190 at both the permissive and the non-permissive temperatures. The following conclusions can be drawn from these results. First, HSV-induced amplification of heterologous DNA can take place in the absence of HSV DNA replication. Secondly, HSV DNA polymerase itself is responsible for amplification of SV40 DNA rather than the products of late genes that are expressed after the onset of viral DNA replication from progeny DNA copies. Thirdly, the product of the gene affected by the mutation in tsS is not required for amplification of foreign DNA. This mutant has been characterized phenotypically by Marsden *et al*., (1976), and its ts lesion has been physically mapped (Stow *et al*., 1978; Matz *et al*., 1983) to a location on the genome where one of the seven essential genes for HSV DNA replication was recently identified, namely UL9 (McGeoch *et al*., 1988; Wu *et al*., 1988). Olivo *et al*., (1988) have presented evidence that the UL9 gene encodes an HSV-specific origin-binding protein that had previously been detected in nuclear extracts of HSV-infected Vero cells (Elias *et al*., 1986). A temperature-labile HSV origin-binding in tsS-infected cells would be the simplest explanation for the apparently paradoxical fact that the virus is able to promote replication of foreign DNA sequences while being incapable of replicating its own DNA under identical conditions.
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Fig. 4. SV40 elements required for HSV-dependent plasmid amplification. (a) Segments of the SV40 wild-type genome. Recombinant plasmids were constructed from pFR113 as described in Methods. K, KpnI; E, EcoRI; B, BamHI; P, PstI; X, BstXI; O, position 0/5243 (origin of DNA replication); N, NcoI. Boxes 't' and 'T' represent coding sequences for small t antigen (5163 to 4642) and large T antigen (5163 to 4918, and 4571 to 2694), respectively. The deletion in pFR235 has not been determined by nucleotide sequencing, but fine structure restriction analyses have made it sure that it lies within the limits of the large T intron. The deletion in pFR197 affects nucleotides number 5239 to 5241. Nucleotide numbers correspond to van Heuverswyn & Fiers (1979). Vector sequences are symbolized by dots. (b) Properties of the SV40 clones. BHK cells were transfected with plasmid DNAs (12 h, no glycerol boost) and superinfected with HSV-1 (+) or mock-infected (−). Total cell DNA was purified (36 h p.i.), treated with Asp718 and DpnI and analysed by Southern blot hybridization using radiolabelled pSPT18 as probe. Size markers on gel electrophoresis were Asp718-linearized pSPT18 (6.15 kb), Asp718-linearized pSPT18 (3.15 kb), and the '1 kb ladder' (Bethesda Research Laboratories). Lanes 1, pFR235; lanes 2, pFR197; lanes 3, pFR216; lanes 4, pFR214; lanes 5, pFR190.
Fig. 5. An SV40 origin-defective plasmid (pFR197; lanes 2) supports HSV-induced amplification of a plasmid bearing the SV40 core origin (pFR183; lanes 1). Lanes 3, pFR197 plus pFR183. Plasmid DNAs were introduced by transfection (12 h, with glycerol boost) into BHK cells, which were subsequently either mock-infected (−) or infected with HSV (+). Total cellular DNA was isolated (32.5 h p.i.), restricted with EcoRI, and subjected to Southern analysis after electrophoresis on 0.8% agarose gel, using radioactively labelled pSPT18 as probe. The band at 3.22 kb corresponds to the size of linearized pFR183; the arrow at 4.63 kb points to a position where the EcoRI cleavage product of pFR197 would be expected had it been amplified (see Fig. 4a for map position of EcoRI site in SV40 DNA).

Fig. 6. Influence of herpesviral DNA replication on SV40 plasmid amplification. (a) Sensitivity of DNA amplification to PAA. BHK cells were transfected (12 h, with glycerol boost) with pFR190 (see Fig. 4a) and subsequently infected with the drug-resistant HSV-1 mutant IP2 (lane 1) or with HSV-1 17syn+ (lane 2). Phosphonoacetic acid was added at a final concentration of 100 μg/ml to cell cultures when they were infected or mock-infected (lane 3), i.e. immediately after the adsorption period. DNAs were isolated (32.5 h p.i.), cleaved with Asp718 and analysed by Southern blotting with radioactive pSPT18 as probe. The bands at 6.15 kb correspond to the linearized full-length DNA of pFR190. +, PAA present; −, control, no PAA. (b) DNA-negative HSV-1 mutants. BHK cells were transfected with pFR190 (lanes 1, 2, 5, 6 and 9) or with pFR106 (lanes 3, 4, 7 and 8). Cells were incubated at 37 °C for 12 h (no glycerol boost), infected with tsS (lanes 1 to 4), tsH (lanes 5 to 8) or wild-type strain 17syn+ (lane 9), and kept at the permissive (33 °C; even-numbered lanes) or at the non-permissive (39 °C; odd-numbered lanes) temperature for 28 h. DNA was isolated, cleaved with Asp718 and DpnI, and subjected to Southern blot analysis as described above.
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DISCUSSION

DNA replication of HSV is thought to initiate at distinct loci within the genome and to proceed in a rolling-circle manner (Becker et al., 1978). Several strategies have led to the identification of the origins of replication. Evidence was derived by the analysis of repeat units present in defective interfering particles (Frenkel, 1981) defining two classes of defective DNA. It has been shown that subgenomic segments of the standard viral genome, after circularization in vitro and transfection, were amplified by superinfection with standard virus (Biswal et al., 1978). This procedure has subsequently been refined by using molecularly cloned restriction fragments (Stow, 1982). Meanwhile, several groups have succeeded in defining very precisely the sequence elements of HSV replication origins (Stow & McMonagle, 1983; Gray & Kaerner, 1984; Weller et al., 1985; Lockshon & Galloway, 1986) and cleavage/packaging signals (Spaete & Frenkel, 1982, 1985; Stow et al., 1983).

Circumstantial evidence exists for replicative activation by HSV gene functions of heterologous sequences, i.e. DNA species apparently lacking any herpesvirus-specific origin of replication. Chromosome analyses with HSV-infected cells revealed various areas of damage and reorganization compatible with amplification of genetic material of the host cell (Nachtigal et al., 1975; Pratt et al., 1984; Chenet-Monte et al., 1986/87). A typical example of aberrant DNA replication caused by HSV is the fact that SV40 DNA integrated into the genome of transformed cells can be extensively amplified (Schlehofer et al., 1983, 1986; Matz et al., 1985). This is not simply due to induction of papovavirus replication. To some extent, amplification occurs intrachromosomally according to an onion skin model, expanding to the cellular sequences flanking the SV40 integration site (Gerspach & Matz, 1988). The majority of amplified SV40 DNA sequences accumulate in concatemeric forms in the size range of HSV DNA indicating a rolling-circle mode of replication, typical of herpesvirus DNA replication (Matz, 1987). Since it has also been shown earlier (Matz et al., 1984) that SV40 DNA amplification in transformed cells was strictly linked with the HSV-coded DNA polymerase, an active role of the herpesvirus in this process is evident. Additional data indicated an interaction of HSV gene functions with the SV40 origin and T antigen in SV40-transformed cell lines (Matz et al., 1985), but it had not been proven until recently by Danovich & Frenkel (1988) and by the data presented here that SV40 DNA itself was the target for HSV-induced amplification. Also, it had to be ruled out that neither the transformed phenotype of the cells nor the integrated status of SV40 DNA were prerequisites for the HSV-mediated process.

The data presented here clearly demonstrate that SV40 sequences are in fact sufficient to enable a DNA molecule to be replicated in the presence of functions provided in trans by HSV. In simian cells that are permissive for SV40 growth, transfected SV40 DNA is replicated by host factors provided that the origin of replication is intact and that the T antigen is functional (Myers & Tjian, 1980). The same requirements are needed for HSV-induced amplification of SV40 DNA in hamster cells with the exception that the host factors are substituted at least partially by HSV functions.

It still remains to be investigated in which way these HSV functions interact with heterologous DNA sequences. One speculation is that HSV-encoded DNA replication factors (Challberg, 1986) are attracted by DNA–protein complexes composed of replication origins and origin-binding proteins, in our particular case SV40 large T antigen and the SV40 origin. The results obtained with the tsS mutant (Fig. 6b) strongly support this hypothesis: HSV origin-dependent plasmid DNA replication is inhibited under non-permissive conditions whereas amplification of SV40 DNA, a species not related to HSV, is permitted. Once initiated, replication of heterologous DNA proceeds in a manner typical of herpesviral DNA replication. This is an explanation for the occurrence of large SV40 concatemers in transformed hamster cells and the amazing speed at which they arise (Matz, 1987; Matz et al., 1984) as soon as HSV is involved.

An interesting question remains to be answered, as to whether specific host cell DNA sequences can also be mobilized and over-replicated under the control of herpesviral gene functions. In addition to gene amplification, various types of rearrangements of heterologous DNA are imaginable. One such example is represented by the observation that lysates of HSV-
infected cells contain an activity that causes pBR322 molecules to form large network structures (Leary & Francke, 1984). The data of Danovich & Frenkel (1988) also point in this direction. They showed clearly that prokaryotic plasmids devoid of any eukaryotic replication origin are rendered resistant to DpnI cleavage after transfection into mammalian cells and subsequent HSV infection. Similar observations were made in context with this present work; in DNA preparations from HSV-infected cells slightly more plasmid DNA was detectable than in the corresponding uninfected control cell DNA. It has to be ruled out that this is due to a stabilizing effect of the virus infection in preventing intracellular DNA degradation by conformational changes in the transfected plasmid. Experiences of others (Rao & Martin, 1988) indicate that replication in a eukaryotic cell is not the only way to make a dam-methylated bacterial plasmid resistant to DpnI. Thus, in this paper a given plasmid construction was considered as amplification-positive only when the autoradiographic signal obtained with HSV-infected cell DNA exceeded that with mock-infected cell DNA by a factor of greater than 10. If, eventually, it is shown unambiguously that HSV in fact induces low grade amplification of DNA sequences apparently in the absence of corresponding origin-binding proteins, this would be of considerable importance for future studies of interactions between virus and the host cell. But even if this were not the case, the simple fact of HSV having the potential to induce detectable changes in the structure of an unrelated DNA molecule is important enough to be investigated in more detail.

In conclusion, the main object of this paper is to draw attention to perhaps not unimportant phenomena in the scenario of herpesvirus action and to stimulate discussions on the biological relevance of herpesvirus-caused alterations of cellular DNA (Galloway & McDougall, 1983; Macnab, 1987; zur Hausen, 1983).

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