Virus Polypeptide Synthesis Induced by Herpes Simplex Virus in Non-permissive Rat XC Cells

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(Accepted 4 March 1983)

SUMMARY

The synthesis of virus polypeptides in rat XC cells infected with herpes simplex virus type 1 (HSV-1; 13VB4tsC75) was studied. At the permissive temperature the virus induced the synthesis, in a cascade fashion, of significant amounts of several early polypeptides (ICP 6, 8 and 39) and those late polypeptides that are relatively resistant to inhibition by phosphonoacetic acid in HEp2 cells (ICP 5, 11, 25, 29, 43 and 44). The infectious cycle appeared to become arrested in XC cells at about 7 to 9 h post-infection, because the relative concentrations of early and latest polypeptides labelled thereafter remained constant and the levels of several of the late virus polypeptides were severely reduced (ICP 2, 10, 24 and 26) or not synthesized at all (ICP 32, 34 and 37). When XC cells were infected at a very high m.o.i., only a small amount of virus DNA synthesis could be detected; the synthesis of cellular DNA was not impaired and the infected XC cells continued to replicate for several weeks at least. When XC cells were infected at the non-permissive temperature, only the immediate-early (IE) ICP 4 could be detected while IE ICP 0 and 22 were not observed. Infection of XC cells with HSV-1 (MP) also resulted in the production of early and late viral polypeptides. On the other hand, in XC cells infected with HSV-1 (F) and HSV-1 (HFEM), the synthesis of virus polypeptides could not be detected.

INTRODUCTION

The present study deals with the interaction of herpes simplex virus type 1 (HSV-1) with the Rous sarcoma virus-transformed rat cell line XC (Svoboda, 1960). It is accepted that XC cells infected with HSV-1 do not lyse and do not produce infectious progeny; however, the nature of the replicative block and the step at which it acts remain unknown. Furthermore, except for a general agreement that HSV-1 is able to attach to the XC cell plasma membrane, the literature concerning this system appears to be contradictory. Docherty et al. (1973) and Campbell et al. (1974) found no signs of penetration or of virus-induced macromolecules, whereas we found that penetration of HSV-1 into XC cells occurred at high rates although mainly, or even only, by viropexis instead of membrane fusion (Epstein et al., 1980). Becker et al. (1974) have claimed that virus particles were not able to penetrate XC cells unless they had been previously grown on permissive rat cells, in which case the block was entirely removed and XC cells produced infectious particles. On the other hand, at least two groups have found that penetration of HSV-1 into XC cells is not impaired but that the block occurs after this step, after the synthesis of virus-specific RNA (Padgett et al., 1978) and proteins (Garfinkle & McAuslan, 1973, 1974), but before the onset of virus DNA replication. Although Garfinkle & McAuslan (1973, 1974) clearly demonstrated that the virus thymidine kinase and other virus antigens were synthesized in HSV-1-infected XC cells, detailed results on the patterns of HSV-1-induced polypeptides in these cells have not been shown.

In this study we report that the pattern of virus expression in XC cells can vary according to the virus strain employed. Working with a virus able to synthesize significant levels of virus
proteins in these cells, we present data showing that early and late virus polypeptides were synthesized in a cascade fashion similar to that in permissive cells but that the virus cycle seemed to be prematurely arrested and that the synthesis of some late polypeptides was very depressed or completely absent.

METHODS

Cells. Serially propagated human epidermoid carcinoma no. 2 (HEp2) cells, obtained from Dr B. Roizman (Chicago, U.S.A.) were grown in Eagle's minimal essential medium (MEM) supplemented with 10% inactivated newborn calf serum. The XC cell line was obtained from the American Type Culture Collection, and grown in MEM supplemented with 10% tryptose phosphate broth (TPB) and 10% inactivated foetal calf serum (FCS). 

Viruses. HSV-1 (F) (Heine et al., 1974), HSV-1 (MP) (Hoggan & Roizman, 1959), HSV-1 (13VB4tsC75) (Honess et al., 1980) and HSV-1 (HFEM) (Honess et al., 1980) were employed in this study. HSV-1 (F) and HSV-1 (MP) were obtained from Dr B. Roizman; HSV-1 (13VB4tsC75) was obtained from Dr R. W. Honess, Mill Hill, London, U.K.; HSV-1 (HFEM) (Dr A. Buchan, Birmingham, U.K.) was obtained from Dr R. Manservigi, Ferrara, Italy. All the strains were propagated in our laboratory at 33 °C on HEp2 cells at very low multiplicity of infection (m.o.i.) and all of them gave comparable yields of infectious virus (10⁹ to 10¹⁰ p.f.u./ml). HSV-1 (13VB4tsC75) is thermosensitive (39 °C) for the IE polypeptide ICP 4 (Honess et al., 1980). In all experiments described in this work, HEp2 and XC cells were infected at multiplicities given in the text and incubated for 1 h. The inoculum was then removed and cells were rinsed and overlaid with Medium 199 + 1% FCS. 

Radioisotopes. L-[14C]Isoleucine, L-[14C]leucine and L-[14C]valine (350 mCi/mmol each) were purchased from New England Nuclear. [Me-3H]Thymidine (40 Ci/mmol) was purchased from Commissariat à l'Energie Atomique (Saclay, France).

Analysis of polypeptides in HSV-1- and mock-infected cells. Cells were rinsed twice with Medium 199 without isoleucine, leucine and valine, supplemented with 1% dialysed FCS, and overlaid with the same medium containing the radioactive precursors [14C]isoleucine, [14C]leucine and [14C]valine (5 μCi/ml) at the times indicated in the text. At the end of the labelling period the cells were rinsed, scraped off, resuspended in phosphate-buffered saline (PBS), disrupted by addition of SDS and 2-mercaptoethanol to 2% and 1% final concentrations respectively, disrupted by three cycles of ultrasonication (30 s each time) and then subjected to electophoresis in linear 9% or 9-5 to 15% gradient polyacrylamide slab gels (PAGE). In experiments in which the sensitivity of late polypeptide synthesis to phosphonoacetic acid (PAA) was determined, HEp2 and XC monolayers were pretreated with 300 μg/ml PAA for 3 h before infection and that level of PAA was maintained throughout the course of the infection. The polypeptides were designated according to Honess & Roizman (1973) as modified by Morse et al. (1978).

Synthesis of DNA in infected cells. Confluent monolayers of HSV-1- or mock-infected XC cells were labelled with [3H]thymidine (40 Ci/mmol) at the times indicated in the text. At the end of the labelling period the cells were scraped off, rinsed with TES buffer (0.01 M-Tris–HCl pH 7.4, 0.001 M-EDTA, 0.1 M-NaCl) and resuspended in the same buffer. Cells were then digested with heat-treated Pronase (200 μg/ml) for 30 min and suspended in 0.1% SDS–0.2% Sarkosyl. The DNA was banded in CsCl equilibrium density gradients in a Beckman 50Ti rotor at 36000 rev/min and 20 °C for 60 h. Fractions were collected from the bottom of the gradients and the radioactivity insoluble in 5% trichloroacetic acid determined. 

RESULTS

Virus polypeptide synthesis in XC cells infected with different strains of HSV-1

Replicate semi-confluent cultures of XC cells were infected at an m.o.i. of 50 with different strains of HSV-1 as listed in the legend to Fig. 1. Cells were incubated at 33 °C and labelled with 14C-amino acids between 5 and 7 h post-infection. The autoradiogram of the electrophoretically separated polypeptides is shown in Fig. 1: the relevant result of this experiment is that HSV-1 strains differed in their ability to induce the synthesis of virus polypeptides in XC cells. In the case of HSV-1 (MP) and HSV-1 (13VB4tsC75), early and late polypeptides were readily detected, whereas in XC cells infected with HSV-1 (F) and HSV-1 (HFEM), the synthesis of virus-induced polypeptides was not apparent.

Patterns of virus polypeptide synthesis in XC cells infected with HSV-1 (13VB4tsC75)

We decided to use HSV-1 (13VB4tsC75) for further study because of its convenience for the study of the immediate-early (IE) class of virus polypeptides, rather than HSV-1 (MP), despite a clearer pattern of polypeptide synthesis after infection of XC cells by the latter virus (Fig. 1). It
**HSV polypeptide synthesis in XC cells**

Fig. 1. Autoradiographic images of electrophoretically separated polypeptides labelled with $^{14}$C-labelled valine, leucine and isoleucine in cells infected at an m.o.i. of 50 with different strains of HSV-1 and maintained at 33 °C. Lanes 1 to 5, XC cells uninfected (U) or infected respectively with HSV-1 strains F, 13VB4tsC75 (C75), HFEM or MP and labelled between 5 and 7 h post-infection. Lanes 6 and 7, HEp2 cells infected with HSV-1 (13VB4tsC75) and labelled between 3 and 5 h post-infection (lane 6) or 7 and 9 h post-infection (lane 7). Samples were subjected to electrophoresis in 9.5 to 15% gradient polyacrylamide slab gels.

may be noted that both these viruses gave essentially identical results in XC cells at 33 °C (data not shown).

In an initial experiment, XC cells were infected with HSV-1 (13VB4tsC75) at 33 °C at increasing m.o.i. and labelled as above between 5 and 7 h post-infection. Fig. 2 shows that no virus-induced polypeptides could be detected in XC cells infected at an m.o.i. of 5. However, at an m.o.i. of 50, several virus polypeptides were produced (ICP 5, 6, 10, 11, 20, 24, 25, 29, 39, 40, 43 and 44). Increasing the m.o.i. to 500 resulted essentially in a quantitative increase in the synthesis of the polypeptides observed at an m.o.i. of 50; ICP 2 and 26 were also visible. Some of the virus polypeptides (ICP 20, 29, 40 and 43) migrate very near to host polypeptides in XC cells, a fact that hinders the quantitative analysis of these polypeptides (especially ICP 20) in some.
Fig. 2. Autoradiographic images of electrophoretically separated polypeptides labelled between 5 and 7 h post-infection with $^{14}$C-labelled valine, leucine and isoleucine in cells infected at different m.o.i. and maintained at 33 °C. Lanes 1 to 4, XC cells uninfected (U) or infected with HSV-1 (13VB4tsC75) at m.o.i.s of 5, 50 and 500. Lanes 5 and 6, HEp2 cells infected with 13VB4tsC75 at an m.o.i. of 50 and labelled between 3 and 5 h post-infection (lane 5) or 5 and 7 h post-infection (lane 6). Samples were subjected to electrophoresis in 9.5 to 15% gradient polyacrylamide slab gels.

In the next experiment, XC cells were infected with HSV-1 (13VB4tsC75) at 33 °C at an m.o.i.
HSV polypeptide synthesis in XC cells

Fig. 3. Autoradiographic images of electrophoretically separated polypeptides from cells infected at 33 °C with HSV-1 (13VB4tsC75) at an m.o.i. of 100 and labelled at different times post-infection with 14C-labelled valine, leucine and isoleucine. Lanes 1 to 6, HEp2 uninfected (U) or infected cells; lanes 7 to 13, XC uninfected (U) or infected cells. The labelling intervals were 2 to 4 h (lanes 3 and 9), 4 to 6 h (lanes 4 and 10), 7 to 9 h (lanes 5 and 11), 10 to 12 h (lanes 6 and 12) and 13 to 15 h post-infection (lane 13). Lanes 2 and 8, Hep2 and XC cells respectively, infected and maintained at 39 °C (39) and labelled between 5 and 7 h post-infection. Samples were subjected to electrophoresis in 9% linear polyacrylamide slab gels.

of 100 and labelled for 2-h intervals at different times after infection, as indicated in the legend to Fig. 3. HEp2 cells infected and labelled under similar conditions were used as controls for the kinetics of virus-induced polypeptide production. Also, a set of XC and HEp2 cells were infected and maintained at 39 °C and labelled between 5 and 7 h post-infection. The relevant features of this experiment were as follows.

(i) At 33 °C, a cascade sequence could be observed for the appearance of virus polypeptides in XC cells: the synthesis of early polypeptides (ICP 6, 8 and 39) was followed by the synthesis of
the first population of late polypeptides (ICP 5, 11, 25, 29, 43 and 44) (compare lanes 4 and 11). However, several differences between infected XC and HEp2 cells were evident: first, the production of virus polypeptides in XC cells appeared to lag about 2 h behind that in HEp2 cells; secondly, IE ICP 4, strongly present in HEp2 cells until at least 4 to 6 h post-infection, could not be detected in XC cells; thirdly, the virus cycle seemed to be arrested in XC cells at about 7 to 9 h after infection, as labelling between 10 and 12, or 13 and 15 h produced essentially the same results as labelling between 7 and 9 h post-infection; the relative concentrations of early and late polypeptides remained constant and several of the latest polypeptides detected in HEp2 cells (lane 6) (ICP 2, 20, 24, 32, 37 and 40) could not be observed in XC cells infected at an m.o.i. of 100. For some of them (ICP 2, 20, 24 and 40), their absence from Fig. 3 is presumably a matter of m.o.i. as they can be detected, in reduced amounts, when XC cells are infected at higher multiplicities (Fig. 2, lane 4; Fig. 4, lane 4).

(ii) To analyse further the production of the IE class of virus polypeptides in XC cells, we have taken advantage of the fact that HSV-1 (13VB4tsC75) normally overproduces this class of virus polypeptides at the non-permissive temperature (39 °C). Fig. 3 shows that whereas in HEp2 cells the accumulation of ICP 4, 6, 0, 22 and 27 at 39 °C could be readily detected (lane 2), in infected XC cells only ICP 4 and 6 seemed to be present and in very reduced amounts (lane 8). The presence of ICP 27 could not be evaluated from this experiment because of the presence of a co-migrating cellular protein. On the other hand, the synthesis of ICP 0 and 22 could not be detected in XC cells. A band migrating close to ICP 22 (labelled with a dot) can be seen to be more intense at 39 °C (lane 8) than at 33 °C, but this band seems to migrate slightly more slowly than ICP 22; it can also be detected in uninfected XC cells and it did not decrease with time after infection. This band might correspond to a heat-shock protein or even to a stress protein induced by the virus (Notarianni & Preston, 1982).

**Effect of PAA on the synthesis of early and late polypeptides in XC cells**

To analyse further the synthesis of early and late groups of virus polypeptides, XC cells were infected at an m.o.i. of 200 with HSV-1 (13VB4tsC75) and incubated at 33 °C in the presence or absence of PAA as indicated in the legend to Fig. 4. Infected cells were labelled between 10 and 12 h post-infection; the autoradiogram of the electrophoretically separated polypeptides revealed the following.

(i) The polypeptides which were the most sensitive to PAA inhibition in HEp2 cells (ICP 2, 10, 20, 32, 34, 37 and 40) were, with the exception of ICP 20, much reduced or absent in XC cells in the absence of PAA (lane 4). Conversely, several of the major late polypeptides observed in XC cells in lane 4 (ICP 5, 11, 25 and 29) were less sensitive to inhibition by PAA in HEp2 cells. These results suggest that the polypeptides that are synthesized in very low amounts, or that appear to be absent, in infected XC cells (in the absence of PAA) are those that are the most dependent on the synthesis of virus DNA.

(ii) However, adding PAA to XC cells (lane 3) had a significant effect on the production of virus polypeptides: several of the late polypeptides (including ICP 5, 11, 20, 25, 29 and 44) were inhibited to some degree whereas the early polypeptides ICP 6, 8 and 39 were slightly increased relative to controls without PAA, suggesting that the virus was blocked by PAA in an earlier phase of the cycle. As it is widely accepted that the major action of PAA is to block virus DNA synthesis by selective inhibition of the virus DNA polymerase, our results suggest that some virus DNA synthesis, presumably mediated by the virus polymerase, is taking place in XC cells.

**DNA synthesis in infected XC cells**

To test the above-mentioned conclusions directly, XC cells were infected at an m.o.i. of 200 with HSV-1 (F), HSV-1 (MP) or HSV-1 (13VB4tsC75) and labelled from 3 to 24 h after infection with [3H]thymidine as described in Table 1. Virus and cellular DNA were separated in CsCl equilibrium density gradients as described in Methods and were identified according to their buoyant densities of 1.726 g/ml (virus) and 1.700 g/ml (cellular). As summarized in Table 1, a small amount of labelled virus DNA was detected in XC cells infected with HSV-1.
**HSV polypeptide synthesis in XC cells**

Fig. 4. Autoradiographic images of electrophoretically separated polypeptides labelled with $^{14}$C-labelled valine, leucine and isoleucine between 10 and 12 h post-infection in cells infected with HSV-1 (13VB4tsC75) at an m.o.i. of 200 and maintained at 33 °C. Lanes 1 and 2, HEp2 infected cells; lanes 3 and 4, XC infected cells. Lanes 1 and 3, infected cells were incubated in the presence of 300 μg/ml PAA which was added 3 h before infection; lanes 2 and 4, no PAA was added. Samples were subjected to electrophoresis in 9% linear polyacrylamide slab gels.

 HSV polypeptide synthesis in XC cells

(13VB4tsC75) or HSV-1 (MP). This virus DNA represented about 10% of the virus DNA labelled in HEp2 cells and corresponded to no more than 5% of total labelled DNA in XC cells. Synthesis of cellular DNA was not inhibited in XC cells, whereas in HEp2 cells up to 75% inhibition was observed after infection. No virus DNA synthesis could be detected in XC cells infected with HSV-1 (F) at an m.o.i. of 200. In a similar experiment carried out at an m.o.i. of 50, no labelled virus DNA could be observed in XC cells after infection by any of the above viruses (data not shown).

It may be noted that, in spite of some degree of cellular detachment, XC cells infected at high m.o.i. with HSV-1 (13VB4tsC75) or HSV-1 (MP) replicated for several weeks without producing virus particles.
Table 1. DNA synthesis in cells infected with different strains of HSV-1*

<table>
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<tr>
<th>Cell</th>
<th>Virus</th>
<th>Virus DNA (ct/min)</th>
<th>Cell DNA (ct/min)</th>
<th>% Virus DNA †</th>
<th>% Cell DNA inhibition ‡</th>
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<td>-</td>
<td>11500</td>
<td>-</td>
<td>-</td>
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<td>3400</td>
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<td>2800</td>
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<td>75</td>
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<tr>
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</table>

* HEp2 and XC cells were infected at an m.o.i. of 200 with HSV-1 strains F, 13VB4tsC75 or MP and maintained at 33 °C. [3H]Thymidine (5 μCi/ml) was added at 3 h post-infection. At 24 h post-infection, the cells were scraped from the flasks. The DNAs were extracted and banded in CsCl equilibrium density gradients as described in Methods.

† (Virus DNA/virus DNA + cell DNA) × 100.
‡ 100 - (cell DNA from infected cells/cell DNA from uninfected cells) × 100.

DISCUSSION

In this report we present patterns of virus polypeptides induced by HSV-1 in non-permissive XC cells. As shown in Fig. 1, when XC cells were infected with HSV-1 (MP) or (13VB4tsC75) (and also with strains 13VB4, mP or Justin; results not shown) the virus induced the synthesis of significant amounts of a wide range of virus polypeptides, whereas when XC cells were similarly infected with HSV-1 (F) or HSV-1 (HFEM), the synthesis of virus polypeptides could not be observed. The different groups who have previously worked on this system (see Introduction) carried out their experiments with different strains of HSV-1. Our observations suggest that this might be the reason for the contradictory results they have reported. Why different strains of HSV-1 behave differently in XC cells is not yet understood and is now under investigation. For the rest of the discussion we will consider only the pattern of expression of HSV-1 (13VB4tsC75) in XC cells.

The patterns of IE polypeptides observed in HEp2 and in XC cells were very different. Not only was ICP 4 not detected in XC cells at 33 °C (despite its strong presence in HEp2 cells) but infection at the non-permissive temperature only allowed the accumulation of very reduced amounts of ICP 4 whereas ICP 0 and perhaps ICP 22 could not be detected at all in these cells. Also, no IE polypeptides were observed in XC cells after reversal of cycloheximide blocks at 33 °C (data not shown). The low level of synthesis or accumulation of IE polypeptides might indicate that some positive controls acting on IE genes or their products are not present in XC cells.

A group of virus polypeptides were consistently observed in significant amounts in XC cells. This group comprised the early polypeptides (ICP 6, 8 and 39) and the late polypeptides relatively resistant to PAA inhibition in HEp2 cells (ICP 5, 11, 25, 29, 43 and 44). Other late polypeptides, such as ICP 2, 10, 24 and 26, were sometimes detected in XC cells but generally in low amounts, even when the cells were infected at very high m.o.i., whereas other late polypeptides, notably ICP 32, 34 and 37, were not detected at all in XC cells. Most of these polypeptides were severely depressed or not expressed by PAA in HEp2 cells. These results, together with the kinetic analysis and the observation that only a low level of virus DNA is synthesized in XC cells, suggest that the virus cycle is blocked in XC cells at the stage of DNA synthesis. Our results further suggest that the presence of both the virus DNA polymerase and ICP 8 [a polypeptide described as playing a regulatory role on the synthesis of virus DNA (Conley et al., 1981)] is not sufficient to ensure the synthesis of normal amounts of virus DNA.

While the virus polypeptides synthesized in XC cells by HSV-1 (13VB4tsC75) cover a large fraction of the HSV-1 genome, several of the most obvious products (ICP 5, 8, 11, 39, 43 and 44) are encoded by the region 0.2 to 0.5 map units. On the other hand, some of the polypeptides severely depressed or not expressed (ICP 2, 10, 23 and 32) cluster in the region 0.5 to 0.7 map.
units (Morse et al., 1978). This region also codes for the structural gene of gC (Ruyechan et al., 1979) which, as already quoted, is absent from cells infected with HSV-1 (13VB4tsC75) (Honess et al., 1980) and appears to contain sequences controlling the inhibition of host macromolecular synthesis.

Fenwick et al. (1979) have found that the functions controlling the inhibition of synthesis of cell DNA and cell polypeptides map to the same region of the genome (0.52 to 0.59 map units) while Halliburton (1980) mapped the functions controlling host polypeptide synthesis to 0.69 to 0.75 map units. In XC cells infected with 13VB4tsC75, the inhibition of cellular polypeptides and cellular DNA appeared to be dissociated. The inhibition of host polypeptide synthesis, although less marked than in HEp2 cells, took place in XC cells and was dose-dependent, while the inhibition of host DNA synthesis was not impaired even at an m.o.i. of 200, suggesting that the two functions could be separately controlled.

In conclusion, in spite of the large fraction of virus polypeptides that was synthesized in XC cells infected with HSV-1 (13VB4tsC75), the cells avoided massive lysis and continued to replicate. Our observations raise several points of interest that are now being investigated. These include the difference of behaviour observed between some of the HSV-1 strains, the possibility of the establishment of a latent or chronic infection in XC cells, and the absence of synthesis of one or more of the IE virus polypeptides.

We are grateful to Y. Michal for excellent technical assistance and to T. Greenland for help in the preparation of the manuscript. This work was supported by Institut National de la Santé et de la Recherche Medicale (I.N.S.E.R.M.) contract P. R. C. (Cancérogénèse) from the Franco-American Research Program and contract no. 80 70448 CAN from Délégation Générale à la Recherche Scientifique et Technique (Cancérologie et Pharmacologie).

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*(Received 10 November 1982)*