Avian sarcoma virus RNA synthesis, RNA splicing and virus production in human foreskin fibroblasts: effect of co-infection with human cytomegalovirus

John B. Knight, Mark F. Stinski and C. Martin Stoltzfus*

Department of Microbiology, University of Iowa, Iowa City, Iowa 52242, U.S.A.

The level of RNA transcripts in human foreskin fibroblast (HFF) cells initiated from the avian sarcoma virus (ASV) long terminal repeat (LTR) promoter was stimulated more than 10-fold when the cells were also infected with human cytomegalovirus (HCMV). HCMV was able to stimulate transcription from the ASV LTR promoter even when all the LTR sequence upstream of the TATA box was deleted, suggesting that only the basal LTR promoter is required for the effect. There were no significant changes in the ASV RNA splicing pattern in stimulated and unstimulated HFF cells. The mRNAs showing an increase during HCMV stimulation included aberrantly spliced ASV RNA species as well as unspliced gag-pol, single-spliced env and single-spliced src mRNAs. This pattern was quite different from ASV splicing in chicken embryo fibroblasts (CEF) but typical of that seen in other mammalian cells. A dramatic increase in infectious ASV production from the normally non-permissive HFF was correlated with the increase in amount of ASV RNA in response to HCMV. Thus, there is not an absolute block to ASV production in human cells. However, infectious ASV production was inefficient in HCMV-stimulated HFF compared to that in CEF cells.

Introduction

Mammalian cells are normally non-permissive for avian sarcoma virus (ASV) infections (Temin, 1974; Varmus & Swanstrom, 1982). Inefficient transcription from the ASV long terminal repeat (LTR) promoter is considered to be one of the possible reasons for the non-permissiveness. The levels of ASV RNA in transformed rodent cells are 10- to 1000-fold lower than in transformed avian fibroblast cells (Bishop et al., 1976). Furthermore, little or no normal env mRNA is present because of aberrant ASV RNA splicing in the transformed (Bishop et al., 1976; Quintrell et al., 1980; Svoboda et al., 1983) and transfected (Berberich et al., 1990) rodent cells. These differences may explain, in part, the failure of ASV to complete productive infection normally in mammalian cells. To investigate ASV transcription and RNA processing further in non-permissive mammalian cells, we have carried out an analysis of RNA initiated from the ASV LTR promoter in human foreskin fibroblast (HFF) cells. We found that there was a more than 10-fold increase in the steady-state levels of ASV RNA in HFF cells when these cells were also infected with the herpesvirus human cytomegalovirus (HCMV). This allowed us to test whether the increased level of ASV transcription would result in changes in RNA splicing and in infectious virus production. No significant changes occurred in the pattern of ASV RNA splicing, which was aberrant but typical of ASV splicing in mammalian cells. We found, however, that concomitant with the increase in ASV RNA levels there was a dramatic increase in infectious ASV production in the normally non-permissive human cells. The HCMV-infected HFF cells were still relatively inefficient for infectious virus production compared to the permissive chicken embryo fibroblast (CEF) cells and this suggests that additional barriers inhibiting virus production in mammalian cells remain in place.

Methods

Plasmids and antibodies. pJTM14 is an infectious proviral clone of the Prague A strain of Rous sarcoma virus (RSV) contained in pUC18 (Miller & Stoltzfus, 1992). pJTM45 was constructed from pJTM14 by deletion of the env gene from nucleotide (nt) 5258 to nt 6983 (nucleotide numbering according to the Prague C sequence of Schwartz et al., 1983). p14R10e was constructed from parent pJTM14 by substitution of the env region from KpnI 4995 to Sall 6059 with the corresponding region from a Rous-associated virus (RAV-1) proviral clone (pRAV-10R) (Sealy et al., 1983). Ribonuclease mapping templates pMAP10 and p5'XH1 have been described previously (Berberich & Stoltzfus, 1991; Stoltzfus & Fogarty, 1989). The reporter plasmid pJCSVPA was constructed by inserting the chloramphenicol acetyltransferase (CAT) gene (cat) into an RSV (Pr-A) src cDNA clone downstream from the splice junction at nt 398, replacing src from nt 7127 to nt 8671. This
construct was then modified by deletion of all ASV sequence 3' to CAT, replacing the 3' LTR with the simian virus 40 (SV40) early poly(A) signal and small-t intron. RAV-1 and RAV-2 antisera (obtained from Dr Harriet Robinson, University of Massachusetts School of Medicine, Worcester, Mass., U.S.A.) were used at a concentration sufficient to neutralize more than 99.9% of ASV [present at a concentration of 5 x 10^4 focus-forming units (f.f.u.) per ml] of the same envelope subgroup.

**Cell culture and DNA transfections.** Secondary CEF cells isolated from chicken helper factor-negative, group-specific antigen-negative embryonated eggs (obtained from Spafas) were cultured in M-199 (Gibco) supplemented with 10% tryptose phosphate broth and 5% calf serum. Secondary HFF cells isolated from human foreskins were maintained in Dulbecco's MEM (Gibco) supplemented with heat-inactivated 10% newborn calf serum. All transfections were performed using the calcium phosphate procedure of Wigler et al. (1978). For RNA isolation and virus production, 25 μg of plasmid DNA was transfected; for CAT assays, 1 μg of DNA was transfected.

**RNA isolation and analysis.** Whole-cell RNA was isolated 48 h after transfection by the guanidinium hydrochloride method (Strohman et al., 1984). Lysate dilutions were performed such that all values shown were obtained in the linear range of the assay (less than 45% conversion to the acetylated form). Values were corrected relative to those obtained for pJSCSVpA (see Fig. 1) and are the result of at least two independent transfections for each construct. Measurements were made using a Radiomatic RTLC automated scanner.

**Infectivity assays.** Assays for production of infectious ASV particles in HFF cells were carried out as follows. Medium from HFF cultures (10 ml) was collected 48 h after transfection, filtered through a 0.45 pm filter and 1 ml aliquots were used to infect CEF seeded at 1 x 10^6 cells/60 mm plate. Infected cells were either overlaid with 0.8% agar 18 h after infection (direct assay) essentially according to the procedure of Vogt (1969) or transferred 3 days after infection to 100 mm plates. Foci were counted at 7 days post-infection (p.i.).

**Results**

**Stimulation of ASV LTR expression in HFF cells by HCMV**

Fig. 1(a) shows a schematic depiction of the CAT reporter plasmid pJSCSVpA in which cat was cloned downstream of the RSV (Pr-A) LTR. To determine the

![Image](https://via.placeholder.com/150)

Fig. 1. Stimulation of ASV transcription and effect of LTR deletions on the level of trans-activation by HCMV. HFF were transfected with 1 μg of pJSCSVpA or LTR mutant constructs 24 h prior to infection with HCMV or mock infection. CAT assays were performed at 24 h p.i. (a) CAT reporter plasmid pJSCSVpA. The RSV (Pr-A) fragment from HindIII (−251) to NcoI (7127) containing the 5' LTR was obtained from a spliced v-src cDNA construct (Chang & Stoltzfus, 1985). The cat reporter gene and the SV40 early polyadenylation signal were inserted downstream of nt 7127. (b) LTR deletion mutants. Deletions were made on parent construct pJSCSVpA using PCR. The sequence of each mutant clone in the LTR region was confirmed by dideoxynucleotide sequencing (Sanger et al., 1977)). Numbers assigned to clones indicate the number of bases deleted from a 17 bp upstream of the U3 3' boundary. The U3 boundary is at nt −234; numbering according to Schwartz et al. (1983). Construct dTATA is deleted in U3 from −234 to the 3' end of the TATA region, removing the TATA box. (c) Relative CAT activity is shown for each mutant in the presence (hatched) and absence (black) of HCMV stimulation.
Fig. 2. Effect of LTR deletions on mRNA produced in the presence and absence of HCMV stimulation. HFF were transfected with 25 μg of pJSCSVpA (wild-type) or mutant constructs 24 h prior to infection with HCMV. Total RNA was isolated at 24 h p.i. and analysed by ribonuclease protection assay. Protected fragments were electrophoresed on 5% polyacrylamide-urea gels and autoradiographed. (a) Lanes shown on the left and right sides of the map are derived from two independent experiments with each including pJSCSVpA (lanes 1 and 4) as a positive control. Hybridization of pJSCSVpA or mutant constructs with probe p5’XH1 results in protection of a 398 nt fragment corresponding to spliced mRNA initiating transcription at the +1 site. Protected fragments appear as doublets as a result of ribonuclease digestion conditions required to eliminate background produced with probe p5’XH1. Lanes 2, d136; lanes 3, d222; lanes 5, d67; lanes 6, dTATA; P, probe. (b) Probe p5’XH1 spans the LTR of RSV (Pr-A) from the beginning of U3 (−234) to XhoI 630. The fragment in JSCSVpA protected by the probe is shown at the bottom of the figure. (c) Quantification of mRNA steady-state levels. Radioactivity (c.p.m.) was quantified directly from the gels shown effect of HCMV infection on ASV expression, cells were transfected with pJSCSVpA followed 24 h later by HCMV infection at an m.o.i. of approximately 1. The level of cat expression at 48 h was used as an assay for expression from the LTR promoter. At this time, HCMV immediate early (IE), early and late proteins are expressed, but significant virus production has not yet occurred (Stinski, 1978). Fig. 1(c) shows that in HCMV-infected cells transfected with pJSCSVpA there was an approximately 20-fold increase in CAT levels relative to uninfected cells.

Because of the dramatic increase in CAT levels obtained in HCMV-infected cells, we determined whether the ASV LTR contained specific cis-acting signals responsive to HCMV stimulation. Mutants with nested deletions of the LTR were used to transfect HFF with and without subsequent HCMV infection. The deleted clones, shown in Fig. 1(b), removed regions of U3 that have been shown to enhance transcription (Cullen et al., 1985; Gilmarten & Parsons, 1983; Gowda et al., 1988; Laimins et al., 1984; Luciw et al., 1983; Norton & Coffin, 1987). Mutant d67 was deleted from nt −234 to nt −191, removing a portion of the ASV enhancer shown by DNA footprinting to interact with avian nuclear proteins referred to as EII, C/EBP or FIII (Ryden & Beemon, 1989; Sealy & Chalkley, 1987). Mutant d136 was deleted from nt −234 to nt −122, removing, in addition to the EII binding site, an inverted CCAAT motif which has been shown by DNA footprinting to interact with avian nuclear proteins EFI or FII (Goodwin, 1988; Sealy & Chalkley, 1987). Mutant d222 was deleted from the beginning of U3 to the 5’ end of the TATA motif which, in addition to deleting the elements described above, removed a core c-fos serum response element (CARG motif) (Zachow & Conklin, 1992). This region has been shown by DNA footprinting to interact with avian nuclear protein EEFIII (Boulden & Sealy, 1990). Mutant dTATA was deleted in the TATA motif as well as in all elements described above. Fig. 1(c) shows relative CAT activity as a measure of ASV LTR promoter expression for each of the deletion mutants in transfected HFF cells in the presence or absence of HCMV. As expected, deletion of regions containing sequences known to enhance transcription resulted in decreased LTR promoter activity in the HFF cells. However, in all of the mutants tested, even though the level of expression was greatly reduced as the enhancers

autoradiographed in (a) using an AMBIS image analysis system. Relative levels of mRNA species initiating transcription at the +1 site are shown for each deletion construct in the presence (hatched) or absence (black) of HCMV infection. Deletion mutants are shown in Fig. 1(b).
were deleted, a stimulation of cat expression still occurred upon HCMV infection. It is evident from these data that CAT expression was stimulated upon HCMV infection in the negative control construct, dTATA. The stimulation in CAT activity was observed even though this construct expressed no transcripts initiating at the bona fide ASV start site (see Fig. 2), and we attribute this increase in CAT expression to stimulation of non-specific initiation occurring within upstream plasmid DNA sequences.

To confirm that the stimulation of ASV by HCMV was reflected at the RNA level, mRNA produced in HFF cells from the transfected LTR deletion mutants was analysed by ribonuclease protection mapping shown in Fig. 2(a). A 398 nt fragment from the +1 site to the splice junction resulted from hybridization protection of the probe which is given in Fig. 2(b). The protected fragment appears as a doublet as a result of ribonuclease digestion conditions required to eliminate background produced with probe p5′XH1. The faint bands shown migrating above 398 nt in Fig. 2(a) result from protection of the probe by transcripts initiating upstream of the LTR using a cryptic promoter(s) in the plasmid DNA. The single band in lane dTATA (+ HCMV) corresponds in length to such a product, which migrates slower than the 398 nt band, and correctly initiated mRNA was not detected in this construct. We attribute the small amount of CAT activity seen with this construct (see Fig. 1c) to translation of cat-containing mRNA species whose transcription is initiated from these cryptic promoters. It is of interest but of unknown significance, as yet, that the level of these transcripts was also increased in HCMV-infected cells. Protected fragments resulting from hybridization of the probe to correctly initiated ASV transcripts were quantified directly from the gels, and the results are shown in Fig. 2(c). The relative mRNA levels shown approximate to the relative CAT levels shown in Fig. 1(c). We conclude that the overall expression in HFF from the ASV LTR promoter was greatly improved by the presence of known enhancers in U3 but that

spliced. (c) Quantification of mRNA species was performed by scanning densitometry. RNA quantities are expressed as a percentage of the total ASV mRNA present in each lane. Densitometric values obtained from the autoradiograph shown in (a) were corrected for the number of U residues in each band to normalize mRNA molar ratios. Total ASV mRNA was calculated by combining values obtained for total unspliced, env single-spliced, env double-spliced and src single-spliced mRNA. Splicing of the cryptic splice donor site to the src splice acceptor results in hybridization-protection of a fragment comigrating with single spliced src on the gel (at 276 nt). Subtraction of the value obtained for this cryptic spliced env band from that obtained for spliced src provides an accurate measure of single-spliced (normal) src mRNA. This corrected value was used in all subsequent calculations.
significant (greater than 15-fold) stimulation of the LTR by HCMV still occurred when only the TATA box was present and not the U3 LTR enhancers.

Comparison of ASV mRNA levels and splicing patterns in stimulated and unstimulated HFF cells

Previous experiments have indicated that, because of aberrant viral RNA splicing, single-spliced ASV env mRNA levels are extremely low in a number of ASV-transformed rodent cells (Bishop et al., 1976; Svoboda et al., 1983) and in transfected mouse NIH-3T3 cells (Berberich et al., 1990). To determine env mRNA levels directly and to assay for the changes in the pattern of viral RNA splicing, we carried out an analysis of ASV RNA in uninfected and in HCMV-infected HFF cells that were transfected with ASV proviral DNA (pJTM14). Fig. 3 shows an autoradiogram of labelled fragments resulting from RNase protection of a probe which allowed simultaneous quantitative assays of all spliced and unspliced ASV mRNA species produced in HFF cells. As expected from the results shown in Fig. 1 and 2, an increase of more than 10-fold in ASV mRNA steady-state levels occurred in HCMV-infected HFF cells. The ASV RNA splicing pattern obtained in these cells was qualitatively and quantitatively different from that seen in CEF but similar to ASV RNA splicing in mouse NIH-3T3 cells (Berberich et al., 1990). The proportion of viral RNA molecules that were spliced was significantly increased in HFF compared to CEF (80 to 90% versus 30%). In addition, most of the viral RNA was spliced at the src 3′ splice site. A majority of the viral RNA spliced from the splice donor site to the splice site in HFF cells was also aberrantly spliced from a cryptic 5′ splice donor in the env coding sequence to the downstream src splice acceptor site, to form a double-spliced env mRNA species. In contrast to mouse NIH-3T3 cells, however, where little or no normal single-spliced env mRNA was detected (Berberich et al., 1990), we found a small amount of env mRNA to be present in HFF cells (5% of the viral RNA compared to approximately 14% in CEF cells). There were no significant changes in the ASV splicing pattern in the presence or absence of HCMV infection (Fig. 3). These data indicated that the effect of HCMV in the HFF cells was to increase the level of all ASV mRNAs, including the env mRNA.

Stimulation of infectious ASV production in HFF cells by HCMV

It has previously been proposed that the normally low level of transcription of ASV RNA in mammalian cells may be one of the barriers to infectious virus production

Table 1. Production of infectious ASV in HFF and dependence on the ASV env gene

<table>
<thead>
<tr>
<th>Plasmid*</th>
<th>HCMV infection†</th>
<th></th>
<th>Agar overlay</th>
<th>No overlay</th>
</tr>
</thead>
<tbody>
<tr>
<td>pJTM14 (ASV)</td>
<td>+ HCMV</td>
<td>15, 9</td>
<td>582, 466</td>
<td></td>
</tr>
<tr>
<td>- HCMV</td>
<td>0, 1</td>
<td>0, 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pJTM45 (env-)$</td>
<td>+ HCMV</td>
<td>0, 0</td>
<td>0, 0</td>
<td></td>
</tr>
<tr>
<td>- HCMV</td>
<td>0, 0</td>
<td>0, 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pUC18 (control)</td>
<td>+ HCMV</td>
<td>0, 0</td>
<td>0, 0</td>
<td></td>
</tr>
</tbody>
</table>

* HFF were transfected with either wild-type (JTM14) or env- (JTM45) proviral clones of ASV. pUC18 served as a negative control.
† Twenty-four h after transfection, HFF were infected with HCMV for 24 h prior to harvest of media for subsequent focus assay.
‡ One ml of filtered medium (0.45 μm filters) collected at 24 h p.i. was assayed for focus-forming activity on CEF. Assays shown in the first column were carried out in the presence of an agar overlay and represent a quantitative measurement of the number of infectious particles produced in HFF per ml of medium. In the assay shown in the second column, 60 mm plates of CEF were infected, the cells were split to 100 mm plates at 3 days p.i., and the foci were counted at 7 days. In this assay some virus spread occurs, thus increasing the sensitivity.

(Varmus & Swanstrom, 1982). Because the amount of ASV LTR-initiated RNA was amplified in HCMV-infected HFF cells to levels comparable to those seen in CEF without detectable changes in splicing, we tested whether this would result in the production of infectious virus from these mammalian cells. The data in Table 1 show that virus production in HFF cells was greatly enhanced when transfection was followed 24 h later by infection with HCMV. From data not shown, we determined that the infectious agent was heat-labile, as expected for a virus particle. The small number of foci observed in the absence of HCMV infection was not reproducible in all experiments. These foci were not tested for the production of virus particles but are assumed to reflect an extremely low level of virus production in HFF in the absence of HCMV.

Because previous experiments have indicated that ASV can be rescued by pseudotyping with the env glycoprotein of endogenous xenotropic and amphitropic mammalian retroviruses capable of infecting CEF (Levy, 1977; Weiss & Wong, 1977), it was important to show that the infectious agent produced by the stimulated human cells indeed contained authentic ASV env glycoproteins. We first assayed media collected after HFF cell transfection with an RSV proviral clone (pJTM45) in which a large region of the env gene was deleted. The results of this experiment, given in Table 1, demonstrated that an intact ASV env gene was required for formation of infectious particles in HFF cells. Second, we showed that virus produced in HFF cells was neutralized by incubation with specific anti-ASV serum prior to focus assay on CEF (Table 2). In this experiment, a Pr-
Table 2. Infectious ASV produced by HFF cells is neutralized by type-specific antiserum and excluded by superinfection

<table>
<thead>
<tr>
<th>Plasmid*</th>
<th>HCMV infection†</th>
<th>Number of foci on CEF‡</th>
<th>Number of foci on RAV-1-infected CEF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+ HCMV</td>
<td>+ anti-RAV-1</td>
<td>+ anti-RAV-2</td>
</tr>
<tr>
<td>p14R10e</td>
<td>+ HCMV</td>
<td>74, 35</td>
<td>0, 0</td>
</tr>
<tr>
<td>p14R10e</td>
<td>- HCMV</td>
<td>0, 1</td>
<td>0, 0</td>
</tr>
<tr>
<td>Prague A virus stock (10⁻⁴)</td>
<td>441, 478</td>
<td>0, 0</td>
<td>473, 502</td>
</tr>
<tr>
<td>Prague B virus stock</td>
<td>TNTC§</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

* HFF were transfected with p14R10e or pUC18 (negative control). p14R10e was constructed from parent construct pJTM14 by substitution of the region from Kpnl (4995) to SalI (6059) (env determinants) with the corresponding region from an RAV-1 proviral clone (pRAV10R). † Twenty-four h after transfection, HFF were infected with HCMV for 24 h prior to harvest of media for subsequent focus assay. ‡ One ml of filtered medium was incubated at 37°C for 1 h in the presence of anti-RAV-1, anti-RAV-2 or control chicken antiserum. Treated medium was then used to infect CEF as described in Table 1. Foci were counted at 7 days using the assays with no agar overlay in Table 1 for p14R10e transfections. Virus stock was assayed using the agar overlay assay in Table 1. Two independent transfections were carried out for each condition and the results of each assay are given. Plasmid pUC18 served as a negative control. § TNTC, Too numerous to count; ND, not determined.

A–RAV-1 hybrid proviral clone (p14R10e) was constructed from parent plasmid pJTM14 by substitution of the type-specific sequences of the env glycoprotein gene (Bova et al., 1988) with the corresponding region from an RAV-1 proviral clone. The data given in Table 2 showed that ASV produced in HFF cells was specifically inhibited by anti-RAV-1 serum and was resistant to neutralization by either anti-RAV-2 serum or non-immune chicken serum. It was also resistant to inactivation by anti-HCMV serum (data not shown). Third, we showed that the ASV produced in HFF did not infect and transform CEF that were chronically infected with an avian leukosis virus (ALV) of the same envelope subgroup (Table 2). Prior infection of CEF with RAV-1, a subgroup A ALV, completely inhibited focus formation by ASV produced in transfections of HFF with p14R10e. This indicated that the ASV produced in HFF cells contained a subgroup A envelope. As shown in Table 2, a subgroup B ASV (Prague B) was capable of infecting and transforming both RAV-1-infected and uninfected CEF. From these data, we conclude that the focus-forming particles produced in HFF cells contained ASV env proteins and did not arise because of rescue by an endogenous mammalian virus. Thus, the stimulation of ASV RNA transcription in HFF cells was correlated with a large increase in the production of infectious ASV bearing ASV env proteins in the HCMV-infected cells. We propose that an increase in the steady-state levels of mRNAs from the ASV LTR promoter in HCMV-infected cells plays a major role in increased infectious ASV production from the normally non-permissive HFF cells.

**Discussion**

We showed that HCMV strongly stimulates the level of ASV RNA in HFF cells. Although steady-state levels of RNA initiated from the ASV LTR decreased, as expected, in response to deletions in U3, constructs with these deletions still were stimulated by HCMV even when all U3 sequences lying upstream of the TATA box were deleted. This suggests that only the basal ASV LTR promoter is necessary for enhancement by HCMV of the steady-state levels of ASV RNA and that this may reflect an effect of HCMV infection on the general transcriptional machinery of the HFF cells. Although an effect of HCMV infection on the stability of ASV RNA is a formal possibility, we believe that this is unlikely because of the magnitude of the effect and the fact that the ASV RNA is relatively stable in the absence of HCMV in both avian and mammalian cells (Berberich et al., 1990; Stoltzfus et al., 1983).

A number of other viral and cellular genes have been shown to be trans-activated by herpesviruses. For example, HCMV has been shown to upregulate the steady-state RNA levels of c-fos, c-myc and hsp70; these genes responded to stimulation by HCMV, as we showed above with the RSV LTR, when only the basal promoter sequence containing the TATA sequence but no upstream enhancers were present (Hagemeier et al., 1992). In some cases, such as with human immunodeficiency virus type 1, trans-activation has been shown to result from the action of the HCMV IE proteins (Barry et al., 1990; Biegalke & Geballe, 1991; Davis et al., 1987; Markovitz et al., 1989; Rando et al., 1990). Although we noticed a small stimulation of the ASV LTR (two- to threefold) when cotransfected with plasmids coding for HCMV IE proteins (J. Knight, unpublished data), IE1 and IE2 cannot account for the extent of the increase in ASV RNA mediated by HCMV infection. It is also of interest that production of ALV in CEF has been shown to increase when accompanied by infection with the avian herpesvirus Marek's disease virus (MDV) (Pulaski et al., 1992). This increase was correlated with a concomitant increase in the level of RNA initiated from the ALV LTR promoter, suggesting that MDV may also encode or induce factors that act to increase transcription from the ALV LTR (Tieber et al., 1990).

Our results also show that, when the absence of receptors for ASV on HFF cells was bypassed by
transfection of ASV proviral DNA, significant levels of infectious virus were produced in the normally non-permissive cells also infected with HCMV and this correlated with stimulation of ASV RNA levels. In addition to this large increase in ASV RNA levels, it is possible that other physiological changes in the cells resulting from HCMV infection may also contribute to the observed enhancement of infectious ASV production. Most previous reports have indicated that ASV-transformed mammalian cells do not produce any infectious virus (Temin, 1974; Varmus & Swanstrom, 1982), although very small amounts of infectious ASV have previously been reported to be produced from certain ASV-transformed rodent cell lines (Geryk et al., 1984). Levy (1977) reported that a preparation of ASV which was env-pseudotyped with a xenotropic mouse leukaemia virus (MuLV) was able to infect and transform a variety of mammalian cells because of the change in the normal ASV host range. Similar results using other mammalian-tropic helper viruses were reported by Weiss & Wong (1977). It was noted in the experiments of Levy (1977) that small amounts of ASV were produced from these infected mammalian cell lines. However, production of virus was transient and dependent upon the presence of co-infecting MuLV to supply the env glycoprotein for ASV. In contrast, we have shown that ASV produced in the HFF cells contains authentic ASV env glycoproteins and therefore, ASV production in HFF cells does not result from rescue by env glycoproteins of an endogenous mammalian virus. Thus, we have shown that there is not an absolute block to infectious virus production in human cells.

The amounts of infectious virus, although greatly increased in the HCMV-infected HFF cells compared to the uninfected cells, still appear to be relatively low (10 to 15 f.f.u. per ml in the direct assay; see Table 1) compared to permissive CEF cells. We have shown that a CEF culture cotransfected under the same conditions with a replication-defective ASV genome and a non-packagable helper virus construct produces approximately 10^3 infectious particles per ml in a 48 h period (J. Knight, unpublished data). Therefore, as a first approximation, this would suggest that the level of infectious ASV produced in HCMV-infected HFF is approximately 1% of the levels normally present in transfected CEF. We have shown above that the levels of unspliced gag-pol genomic RNA and env mRNA are approximately 20% and 30%, respectively, of the levels normally present in transfected CEF. Thus, the apparent lower efficiency of virus production in HFF cells can be accounted for partially by the reduced amounts of these viral mRNAs and genomic RNA. The failure to carry out other steps adequately in the virus life cycle in HFF may further explain the observed inefficiency of virus production. One of these is the failure to process the gag polyprotein precursor Pr76^agg efficiently. Several reports have indicated that no mature cleavage products of Pr76^agg are detected in ASV-transformed mammalian cells or in the cell culture medium (Eisenman et al., 1975; Vogt et al., 1975). A more recent study however has indicated that wild-type Pr76^agg is cleaved and released from monkey COS cells, albeit inefficiently, when the gag gene was expressed at high levels from an SV40-based plasmid. The efficiency of gag protein cleavage was increased when a myristylation signal was present at the N terminus of the gag precursor (Wills et al., 1989). Since we have shown above that infectious virus is produced in HFF cells, our results also indicate that at least some gag protein cleavage must occur in human cells in the absence of a myristylation signal. We have not yet tested this, but would expect an increase of virus production in HFF cells in response to a gag myristylation signal. Since the levels of ASV RNA in the HCMV-stimulated cells were enhanced to levels comparable to those seen in CEF, it should also be possible to use this system to examine ASV protein processing and requirements for particle assembly of ASV in human cells.

This work was supported by Public Health Service grants CA28051 from the National Cancer Institute (C.M.S.) and AI13562 from the National Institute of Allergy and Infectious Diseases (M.F.S.). J.B.K. was supported in part by National Research Service Award GM07228.

References


