Expression of target genes by coinfection with replication-deficient viral vectors

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An in vivo transcription system was developed by coinfection of cells with replication-deficient viral vectors. Recombinant baculovirus (AcT7HCVLuc) and fowlpox virus (FPVT7HCVLuc) carrying a cDNA of the hepatitis C virus (HCV) minigene encoding the HCV 5' untranslated region (UTR), a luciferase gene and the 3' UTR, including the 98 nt extra sequence, under the control of the T7 promoter were constructed. The HCV minigene was synthesized in various cells by coinfection with one of these two viruses and recombinant baculovirus (AcCAT7) or adenovirus (AdexCAT7) expressing T7 RNA polymerase under the control of a mammalian promoter. Only a low level of luciferase expression was obtained in cells coinfected with AcT7HCVLuc and either AcCAT7 or AdexCAT7. In contrast, high-level luciferase expression was detected when the same cells were coinfected with FPVT7HCVLuc and either AcCAT7 or AdexCAT7. We further constructed a recombinant fowlpox virus with its HCV minigene extended to contain the whole HCV core protein region. Significantly high levels of expression of HCV core protein were detected in MT-2, COS7 and Vero cells by coinfection with the recombinant fowlpox virus and AdexCAT7. A coinfection system consisting of recombinant fowlpox virus and AdexCAT7 was established for high level of expression of a target gene in various cells.

Introduction

Eukaryotic expression systems utilizing virus vectors are preferred for functional expression of eukaryotic genes because accurate protein folding, glycosylation, phosphorylation and subunit assembly can occur in these systems. The regulated expression of foreign genes in mammalian cells under the control of bacteriophage T7 RNA polymerase has become an important and useful technique in molecular biology. There are various eukaryotic transient-expression systems based on recombinant viruses synthesizing T7 RNA polymerase in mammalian cells available today. For instance, vaccinia virus (VacT7) (Fuerst et al., 1986), modified vaccinia virus Ankara (MVAT7) (Wyatt et al., 1986), fowlpox virus (FPVT7) (Britton et al., 1996), baculovirus (AcCAT7) (Yap et al., 1997) and adenovirus (AdexCAT7) (Y. Aoki, H. Aizaki, T. Shimoike, H. Tani, K. Ishii, I. Saito, Y. Matsuura & T. Miyamura, unpublished results). It is well-known that the VacT7 system has been widely used for the recovery of infectious viruses from cDNA clones (Pattnaik et al., 1992; Schnell et al., 1994; Garcin et al., 1995; Lawson et al., 1995; Whelan et al., 1995; Yu et al., 1995). The mechanisms of processing of hepatitis C virus (HCV) polyprotein have also been studied using the VacT7 system (Grakoui et al., 1993; Selby et al., 1993). A derivative of vaccinia virus, MVAT7, was used for reconstitution of respiratory syncytial virus (Collins et al., 1995) and for rescuing measles virus (Schneider et al., 1997). In a previous communication, we reported the recovery of high titres of infectious poliovirus using the AcCAT7 expression system (Yap et al., 1997).

In the transient-expression system, plasmids containing the target genes flanked by the T7 promoter and termination sequences are introduced into the infected cells by transfection procedures. Although a high level of expression was obtained, the transfection procedure is one of the limiting factors for greater expression of target genes. In the VacT7 system, it has been shown that the efficiency of the system was enhanced when vaccinia virus was used to deliver the target gene (Fuerst et al., 1987). Since recombinant viruses are stable and can grow to high titres, coinfection with recombinant viruses is believed to produce a higher level of expression than that produced by transfection with plasmids. The VacT7 system offers an efficient and useful mammalian transient-expression system.
However, an extensive CPE caused by vaccinia virus sometimes interferes with analysis of the expressed products.

Since the discovery of HCV as a viral agent responsible for posttransfusion non-A, non-B hepatitis, characterization of the HCV genome structure and expression has progressed rapidly (Choo et al., 1989; Houghton, 1996; Rice, 1996; Shimotohno et al., 1995). However, the detailed mechanism of virus replication is still unknown.

In this study, we constructed replication-deficient viral vectors, recombinant baculovirus and fowlpox virus, carrying the cDNA of the HCV minigenes with a reporter luciferase gene flanked by the T7 promoter and terminator regulatory elements. The HCV minigenes were synthesized in various cells by coinfecion with either the recombinant baculovirus or fowlpox virus and AcCAT7 or AdexCAT7. A high level of luciferase expression was detected in all the cell lines examined including the MT-2 cell line, which was reported by Kato et al. (1995) to be susceptible to HCV infection by coinfection with the recombinant fowlpox virus and AdexCAT7. In contrast, low luciferase activity was detected in cells coinfected with the recombinant baculovirus and AcCAT7 or AdexCAT7. The establishment of a coinfecion system consisting of replication-deficient viral vectors in this study may facilitate molecular analysis of the HCV replicative cycle.

Methods

**Bacteria, plasmids and cloning procedures.** *Escherichia coli* strain DH5α was used for selection and propagation of recombinant plasmids. pFTK5.8 vector was constructed by cloning a 5.5-kbp EcoRI fragment containing the nonessential thymidine kinase (TK) gene of fowlpox virus strain Beaudette into pUC19 (Y. Ueda, unpublished data). The unique site of NcoI in the middle of the TK gene of the vector was removed by digestion with NcoI, blunt-ended and ligated to the BglII linker to create a BglII site. The fragment containing the vaccinia virus p11 promoter and lacZ gene was excised from pSC10 (Chakrabarti et al., 1985) and inserted into the pFTK5.8 vector yielding pFTKlacZ. A T7HCVLuc expression cassette containing DNA encoding the HCV 5′ untranslated region (UTR) (nt 1–341 of the HCV genome), a luciferase gene, the 3′ UTR (nt 9354–9523 of HCV) including the 98 bp of 3′ extra sequences (Kolyhalov et al., 1996; Tanaka et al., 1996), and hepatitis D virus (HDV) ribozyme flanked by the T7 promoter at the 5′ end and the T7 terminator at the 3′ end was obtained by digestion with HindIII and Spel from pT7HCVLuc (Y. Aoki, H. Aizaki, T. Shimoike, H. Tani, K. Ishii, I. Saito, Y. Matsuura & T. Miyamura, unpublished results). Recombinant baculovirus (AcAlacZ) and adenovirus (AdexAlacZ) expressing the lacZ gene were constructed as described by Shoji et al. (1997) and Fujita et al. (1995), respectively. Various mammalian cell lines were used in this study, including human cell lines (HepG2, FLC-4, HeLa and MRC-5 cells), monkey cell lines (COS7, CV-1 and Vero), and a human lymphocyte cell line (MT-2). Dulbecco’s modified Eagle’s medium (GIBCO) containing 2 mmol L-glutamine, penicillin (50 IU/ml), streptomycin (50 µg/ml) and 10% foetal bovine serum was used for cultivation of all these cells except for MT-2 cells which were grown in RPMI-1640 medium. HepG2 and MRC-5 were purchased from Dainippon Pharmaceutical.

**β-Galactosidase activity in mammalian cells.** Various cell lines were infected with AcCAlacZ, AdexCAlacZ or FPVlacZ at an m.o.i. of 40 and cell extracts were harvested 48 h after infection. β-Galactosidase activity was measured with a β-galactosidase assay kit (Stratagene) with o-nitrophenyl β-D-galactopyranoside as a substrate (Nielsen et al., 1983) as described previously (Shoji et al., 1997). Protein concentration was determined with the BCA protein assay reagent (Pierce Chemical). The final β-galactosidase activity was expressed as units (nmol o-nitrophenyl β-D-galactopyranoside cleaved/min) per mg protein. For the qualitative analysis of β-galactosidase activity, at 48 h postinfection, cells were washed twice with PBS, fixed with 0.25% glutaraldehyde and subsequently stained with a solution containing 1 mg/ml X-Gal, 5 mmol K3Fe(CN)6, 5 mmol K4Fe(CN)6 and 2 mmol MgCl2 in PBS (Scholer et al., 1989).
In vivo expression of target genes by viral vectors

Fig. 1. Construction of recombinant fowlpox viruses FPVT7HCVLuc and FPVT7HCV09Luc and recombinant baculovirus AcT7HCVLuc. Transfer vector pFTKT7HCVLuc carrying the DNA encoding HCV 5' UTR, a luciferase gene, 3' UTR including the 3' extra sequence, ribozyme of HDV and T7 terminator under the control of the T7 promoter was constructed by insertion of the T7HCVLuc expression cassette cleaved from pT7HCVLuc and cloned into the transfer vector of fowlpox virus, pFTK5.8. pAcT7HCVLuc was constructed by insertion of the T7HCVLuc expression cassette into the polyhedrin promoter-deleted transfer vector of baculovirus pAcYM1. pFTKT7HCV09Luc containing the region of HCV core protein in addition to the T7HCVLuc expression cassette was obtained by insertion of the T7HCV09Luc fragment derived from pT7HCV09Luc. Recombinant fowlpox viruses and baculovirus were generated by homologous recombination as described in Methods.

Fig. 2. Schematic representation of in vivo expression of the HCV minigene used in this study. (1) Expression of the HCV minigene by co-infection with AcCAT7/AdexCAT7 and AcT7HCVLuc. (2) Expression by transfection of cells preinfected with AcCAT7/AdexCAT7 with pT7HCVLuc. (3) Expression by co-infection with AcCAT7/AdexCAT7 and FPVT7HCVLuc.
Expression of the reporter gene of the HCV minigene by a coinfection or transfection expression system. As shown in Fig. 2, monolayer cells were prepared in a 24-well plate and coinfected with different combinations of recombinant viruses at an m.o.i. of 50. Recombinant viruses were coinfected either simultaneously or at various time intervals after the initial infection. For transfection with a plasmid, cells were transfected with pT7HCVLuc 24 h later as described previously (Yap et al., 1997). After 48 h incubation, luciferase activity was determined using the Pica Gene Luciferase Assay kit (Toyo) as described by Yap et al. (1997). Relative light units (RLU) were measured with a luminometer (Berthold).

Expression of HCV core protein by a coinfection or transfection expression system in mammalian cells. MT-2, Vero and COS7 cells were simultaneously coinfected with AdexCAT7 and FPVT7HV09Luc at an m.o.i. of 50. To compare expression levels, cells were transfected with pT7HV09Luc 24 h after infection with AdexCAT7. At 48 h postinfection, cells were harvested and expression of HCV core protein was determined by Western blotting as described previously (Yap et al., 1997). Mouse monoclonal antibody raised against the HCV core protein was used as the first antibody. HCV core protein was detected with the ECL assay kit (Amersham). The amount of core protein expressed in cells was determined by fluorescent ELISA as described previously (Ruggieri et al., 1997).

Results
Expression of HCV minigene by coinfection with recombinant baculoviruses

Our previous studies (Yap et al., 1997) demonstrated that transient expression of luciferase was obtained by transfection with a plasmid containing the luciferase gene under the control of the T7 promoter in cells preinfected with baculovirus expressing T7 RNA polymerase, AcCAT7. In this study, to facilitate the analysis of HCV replication, we constructed a recombinant baculovirus (AcT7HCVLuc) containing the HCV minigene with an internal ribosome entry site in the region of the 5’ UTR, a luciferase gene, and a 3’ UTR under the control of the T7 promoter. Various mammalian cells were coinfected with AcCAT7 and AcT7HCVLuc either simultaneously or at various time intervals after the initial infection. The conditions of the baculovirus coinfection system required for optimal expression were examined. Single infection with AcT7HCVLuc was used as a control for background activity. Cells superinfected with AcCAT7 6 h after AcT7HCVLuc infection gave rise to the highest expression of luciferase. In general, equal m.o.i. (m.o.i. of 50) of these two viruses resulted in the highest level of luciferase expression (data not shown).

As shown in Fig. 3, a decrease in luciferase expression was detected in HepG2, HeLa, COS7 and Vero cells by coinfection with the baculoviruses compared with single infection with AcT7HCVLuc as background. However, in FLC-4, MRC-5 and CV-1 cells coinfected with the recombinant baculoviruses, expression of luciferase was 2–10 times higher than in cells singly infected with AcT7HCVLuc. Only traces of luciferase were expressed in MT-2 cells, which may be due to their low susceptibility to baculovirus infection (Shoji et al., 1997). The same result was obtained when the replication-deficient adenovirus expressing T7 RNA polymerase, AdexCAT7, was used instead of AcCAT7 in the coinfection system and coinfection with AdexCAT7 and an adenovirus carrying the HCV minigene under the control of the T7 promoter (data not shown).

Comparison of coinfection and transfection systems

To compare the efficiency of expression of the reporter gene by the coinfection and transfection systems, various mammalian cells preinfected with AcCAT7 or AdexCAT7 at an m.o.i. of 100 were transfected with pT7HCVLuc and the luciferase activity was determined 24 h after transfection. As shown in Fig. 4, transfection with pT7HCVLuc resulted in high
levels of luciferase activity in cells preinfected with either AcCAT7 or AdexCAT7, while no expression of luciferase was detected in MT-2 cells preinfected with AcCAT7 and transfected with pT7HCVLuc.

Susceptibility of mammalian cell lines to fowlpox virus infection

As shown above, no significantly higher expression of luciferase was detected in cells coinfect ed with recombinant baculovirus or adenovirus carrying the reporter gene and AcCAT7 or AdexCAT7 compared with cells transfected with the reporter plasmid. The most likely explanation for this could be translocation of the HCV minigene inserted on the viral DNA into the nucleus and inaccessibility of the T7 promoter in the viral sequence to T7 RNA polymerase. Therefore, to circumvent the transfer of viral DNA into the nucleus, a fowlpox virus (FPVT7HCVLuc) harbouring the HCV minigene under the control of the T7 promoter was constructed as an alternative replication-deficient vector to deliver the gene into the cytoplasm of the cells. Firstly, susceptibility of cells to fowlpox virus was determined by infecting them with recombinant fowlpox virus FPVlacZ possessing a lacZ gene under the control of the vaccinia virus P11 promoter. Expression of β-galactosidase in cells infected with FPVlacZ, AdexCALacZ and AcCALacZ was quantified by the β-galactosidase enzyme assay (Fig. 5).

A significant increase in β-galactosidase activity was detected in all the cell lines infected with FPVlacZ at an m.o.i. of 40 in comparison with the mock-infected control cells. Most dramatically, monkey cell lines, particularly Vero and COS7 cells, infected with the FPVlacZ expressed 1500-fold higher levels of β-galactosidase than the mock-infected control, whereas expression by MRC-5, HepG2, HeLa and CV-1 cells was 100–300 times that of the control. In contrast, FLC-4 and MT-2 cells expressed low levels of β-galactosidase in comparison with the other cells. All the cell lines exposed to AdexCALacZ expressed high levels of β-galactosidase, approximately 150- to 2500-fold that of the control. Similarly, infection with AcCALacZ also resulted in high yields of β-galactosidase in all the cell lines except MT-2 cells. These data demonstrate that mammalian cell lines are susceptible to fowlpox virus infection. In addition, no CPE was observed in cells inoculated with fowlpox virus, adenovirus or baculovirus at an m.o.i. of 40.

Examination of cells histochemically stained with X-Gal revealed that about 70% of MRC-5, COS7 and Vero cells...
infected with FPVlacZ were stained, whereas only 5–30% of MT-2, FLC-4, HeLa, CV-1 and HepG2 cells were stained. Exposure to AdexCAlacZ resulted in at least 80% of the cells of all cell lines being stained with X-Gal. Similarly, 50–80% of AcCALacZ-infected cells except MT-2 and CV-1 expressed β-galactosidase. Stained cells were not observed in uninfected cultures (data not shown).

Expression of the HCV minigene by co-infection with FPVT7HCVLuc and either AcCAT7 or AdexCAT7

Somogyi et al. (1993) showed that replication of fowlpox virus DNA does not occur in mammalian cells. Since most of the cell lines tested in this study were susceptible to fowlpox virus infection, cells were coinfected with FPVT7HCVLuc and AcCAT7 or AdexCAT7 under different conditions. The results presented here are those obtained under optimal conditions, i.e. for cells infected with the combination of AcCAT7 and FPVT7HCVLuc, AcCAT7 was added 6 h after FPVT7HCVLuc infection, whereas for the combination of AdexCAT7 and FPVT7HCVLuc, cells were infected simultaneously with both recombinant viruses at an m.o.i. of 50.

Coinfection of cells with AcCAT7 and FPVT7HCVLuc yielded a marked increase in expression of luciferase in HepG2, FLC-4, MRC-5, CV-1 and Vero cells, with approximately 10- to 1000-fold higher activity than in cells singly infected with FPVT7HCVLuc as control (Fig. 6). In contrast, no expression of luciferase was detected in MT-2 cells. This could be due to the low susceptibility of MT-2 cells to baculovirus and fowlpox virus infection. No substantial increase in luciferase activity was observed in HeLa and COS7 cells.

Interestingly, when AdexCAT7 was substituted for AcCAT7 in the coinfection system, a dramatic increase in luciferase activity was detected in all the cell lines including MT-2 cells. This is in agreement with the results shown in Fig. 5 in which all the cell lines exhibited high susceptibility to adenovirus infection. In comparison with single infection with FPVT7HCVLuc, 100- to 5000-fold higher expression of luciferase was detected in cells coinfected with AdexCAT7. The time-course of luciferase synthesis in Vero cells by co-infection with AdexCAT7 and FPVT7HCVLuc shows that luciferase activity was detected 12 h after infection and peaked at 48 h, but decreased gradually thereafter (Fig. 7). Overall, coinfection of cells with AdexCAT7 and FPVT7HCVLuc

**Fig. 8.** Comparison of expression of reporter genes by co-infection with AdexCAT7 and FPVT7HCV09Luc and transfection with the plasmid. Cells were coinfected with AdexCAT7 and FPVT7HCV09Luc at an m.o.i. of 50 and harvested at 48 h postinfection. For transfection, cells were infected with AdexCAT7 at an m.o.i. of 100 then transfected with pT7HCV09Luc.

(a) The luciferase activity was measured as described in Methods. (b) The production of HCV core protein was quantified by fluorescent ELISA. Results represent the means of three independent experiments. SD are represented by vertical lines. Expression of HCV core protein was also examined by immunoblotting using anti-HCV core monoclonal antibody. Lane 1, cells coinfected with AdexCAT7 and FPVT7HCV09Luc; lane 2, cells infected with AdexCAT7 at an m.o.i. of 100 and subsequently transfected with pT7HCV09Luc; lane P, Vero cells infected with recombinant baculovirus AcCA39 (Shoji et al., 1997) expressing HCV core protein at an m.o.i. of 30 were used as a positive control; lane M, mock-infected Vero cells used as a negative control.
yielded higher expression of luciferase than coinfection with AcCAT7 and FPVT7HCVLuc.

When compared with transfection with the reporter plasmid, coinfection with AdexCAT7 and FPVT7HCVLuc induced higher expression of luciferase in all the cells except FLC-4 (Fig. 4). Vero, CV-1, COS7 and MRC-5 cells exhibited 200- to 5000-fold higher luciferase activity than those cells transfected with plasmid, whereas no substantial increase in luciferase activity could be detected in coinfected FLC-4 cells compared with the plasmid-transfected cells. This could be due to the low susceptibility of FLC-4 cells to fowlpox virus infection (Fig. 5). Similarly, superinfection of cells with AcCAT7 and FPVT7HCVLuc also caused higher expression of luciferase than transfection with the plasmid except in FLC-4 cells which showed no significant increase in expression.

Expression of HCV core protein by coinfection of cells with AdexCAT7 and FPVT7HCV09Luc

To determine the efficiency of the AdexCAT7 and recombinant fowlpox virus coinfection system for synthesizing HCV proteins, a recombinant fowlpox virus (FPVT7HCV09Luc) containing the entire core region of HCV, a luciferase gene and the 3' UTR under the control of the T7 promoter was constructed. MT-2, COS7 and Vero cells were coinfected with AdexCAT7 and FPVT7HCV09Luc at an m.o.i. of 50 and the cells were examined for expression of luciferase and HCV core protein. As demonstrated in Fig. 8(a), higher expression of luciferase was detected in the infected cells than in the plasmid-transfected cells. Similarly, the amounts of HCV core protein in cells coinfeected with the viruses (Fig. 8(b)) were consistently higher than those in cells transfected with the plasmid. Western blot analysis also revealed a high level of expression of properly processed 22 kDa HCV core protein in cells coinfeected with the viruses.

Discussion

In our previous communication, an efficient heterologous gene expression system in mammalian cells using an appropriately engineered recombinant baculovirus that synthesizes bacteriophage T7 RNA polymerase was described (Yap et al., 1997). A high level of luciferase expression was obtained in cells infected with recombinant baculovirus expressing T7 RNA polymerase after transfection with a plasmid containing a luciferase gene under the control of the T7 promoter. In this study, as depicted in Fig. 2, we used the recombinant baculovirus and fowlpox virus to introduce the HCV minigene, under the control of the T7 promoter, into cells pre- or coinfeected with AcCAT7 or AdexCAT7 to establish a coinfection system for greater gene expression and molecular biological studies of HCV.

In fact, our initial purpose was to develop an in vivo transcription system with recombinant baculoviruses AcCAT7 and AcT7HCVLuc. However, it was noticed that even under optimal conditions, coinfection of cells with the baculoviruses and adenoviruses did not give rise to a substantial increase in luciferase expression in comparison with transfection with the plasmid (Fig. 3). This is in marked contrast with the results obtained by coinfection with vaccinia viruses in which expression of the reporter gene was 14-fold higher than that induced by transfection with the reporter plasmid (Fuerst et al., 1987). It was found that although coinfeected FLC-4 cells expressed a considerably higher level of luciferase than singly infected cells, an equivalent level of luciferase expression was obtained by transfection with the plasmid. Similar results were observed when AdexCAT7 was substituted for AcCAT7 in the coinfection system (data not shown).

The baculovirus genome seems to have a cryptic promoter which can be recognized by cellular RNA polymerases. In our previous study, we showed that infectious poliovirus was recovered after single infection with a baculovirus containing the entire CDNA of poliovirus (Yap et al., 1997). Similarly, in this study, single infection with AcT7HCVLuc in the absence of T7 RNA polymerase resulted in a high level of luciferase expression in HepG2, HeLa, COS7, Vero and FLC-4 cells. DNA of AeNPV enters the nucleus of susceptible mammalian cells immediately after the infection and remains in the cell nucleus for at least 24 h (Tijia et al., 1983). Therefore, the high expression of luciferase in cells singly infected with AcT7HCVLuc may be due to the entry of baculovirus DNA into the nucleus, followed by activation of its cryptic promoters by cellular RNA polymerases.

Although a high level of T7 RNA polymerase was synthesized, lower luciferase expression was observed in most of the cells, except FLC-4, MRC-5 and CV-1 cells, after coinfection with baculoviruses rather than after single infection (Fig. 3). This may be attributable to the transport of the baculovirus DNA possessing the reporter gene into the nucleus, where it becomes inaccessible to the T7 RNA polymerase needed for transcription. Furthermore, competition for cellular RNA polymerase in the nucleus may occur between DNAs of AcT7HCVLuc and AcCAT7 and lead to reduced expression of luciferase. The same phenomenon was observed in cells coinfeected with AdexCAT7 and adenovirus harbouring the HCV minigene (data not shown). This is consistent with the finding by Tomanin et al. (1997) in which very little expression of β-galactosidase was detected in A549 and MRC-5 cells after coinfection with adenovirus expressing T7 RNA polymerase and reporter adenovirus carrying a reporter gene under the control of the T7 promoter. Particles of adenovirus have been reported to be transported to the nucleus and the release of viral DNA appeared to occur in a nuclear pocket (Dales & Chardonnet, 1973; Luftig & Weihing, 1975). However, it is also possible that the decrease in expression of luciferase observed in this study was caused by saturation or modification of baculovirus receptors on the cell surface by the first infection. Baluda (1959) had indeed demonstrated a loss of
virus receptors resulting from a modification of virus receptor sites in homologous interference by ultraviolet-irradiated Newcastle disease virus (NDV).

Viral interference, which is also termed intrinsic interference, has been reported in relation to reduced multiplication of NDV observed when cells were coinfected with NDV and other viruses (Marcus & Carver, 1967; Seto & Carver, 1969). The induction of intrinsic interference between RNA viruses such as influenza A and B viruses (Mikheeva & Ghendon, 1982) or swine influenza virus with fowl plaque virus (Rott et al., 1972; Rott & Scholtissek, 1981) has also been reported. It has been suggested that viral interference occurs at the level of translation of the viral mRNA (Hattman & Hofschneider, 1967, 1968; Saxton & Stevens, 1972) and also at a stage of primary transcription as reported by Mikheeva & Ghendon (1982). In this study, the reduced expression of luciferase in cells coinfected with baculoviruses seemed to coincide with interference occurring between the two viruses. However, viral interference described above involved only viruses that can multiply in cells to produce infectious virion particles. Since the baculoviruses used in this study are unable to replicate in mammalian cells, other factors that may be responsible for the decreased expression of luciferase in the coinfected cells cannot be excluded.

Fowlpox virus is a prototypic virus of the genus Avipoxvirus and has been used as a live attenuated vaccine against the disease in poultry (Tripathy & Hanson, 1975). It has been reported that replication of this poxvirus is blocked in mammalian cells (Taylor & Pauletti, 1988; Somogyi et al., 1993). Recombinant fowlpox viruses have been used in expression of rabies virus glycoprotein (Taylor et al., 1988), tumour-associated antigen (Wang et al., 1995) and cytokines (Leong et al., 1994). In this study, we constructed a recombinant fowlpox virus containing the HCV minigene under the control of the T7 promoter.

It was noticeable that the level of β-galactosidase expression was cell type-dependent. Even though the same m.o.i. of fowlpox virus was used, different levels of β-galactosidase expression were obtained. Examination of the susceptibility of cells to fowlpox virus revealed that Vero and COS7 cells are much more susceptible to infection than other cell lines (Fig. 5). Interestingly, the human T-lymphocyte cell line MT-2, which is permissive for HCV replication (Kato et al., 1995; Mizutani et al., 1996; Sugiyama et al., 1997), was also shown to be susceptible to fowlpox virus infection. All the cell lines examined in this study appeared to be highly susceptible to adenovirus infection. In fact, adenovirus has been shown to have a broad host range in mammalian cells (Dulbecco & Ginsberg, 1988). Despite the inability of baculovirus to replicate in mammalian cells, mammalian cells other than MT-2 cells were highly susceptible to AcCAT7 infection, as reported previously (Boyce & Bucher, 1996; Shoji et al., 1997).

Coinfection with FPVT7HCVLuc and AdexCAT7 yielded high levels of luciferase expression regardless of cell type. This coinfecion resulted in approximately 200- to 5000-fold higher level of luciferase expression than in cells transfected with the plasmid. A similar finding was obtained in cells coinfected with AcCAT7 and FPVT7HCVLuc but the degree of increase in luciferase activity was lower than that observed in coinfecion with AdexCAT7. The most likely explanation for this dramatic increase with fowlpox virus is the uncoating of fowlpox virus DNA occurring in the cytoplasm which enables the T7 promoter on the viral genome to be assessed by T7 RNA polymerase. Interference also seemed to occur when cells were coinfected with FPVT7HCVLuc and AcCAT7 simultaneously, and resulted in lower expression of luciferase (data not shown) than observed under the optimal conditions in which cells were superinfected with AcCAT7 6 h after infection with FPVT7-HCVLuc.

In this study, we have demonstrated that the MT-2 cell line is less susceptible to fowlpox virus infection than to adenovirus infection (Fig. 5). It is noteworthy that, despite the low susceptibility of MT-2 cells to fowlpox virus infection, a high level of luciferase expression was detected in MT-2 cells after coinfection with FPVT7HCVLuc and AdexCAT7 (Fig. 6). In addition, about 12.5 ng HCV core protein per mg protein was obtained in coinfected MT-2 cells. In comparison with the vaccinia virus coinfection system, in which 1.5 µg HIV gp160 protein per 3 × 10⁸ cells was produced in CV-1 cells (Fuerst et al., 1987), low production of HCV core protein was observed in MT-2, COS7 and Vero cells after coinfection with the viruses. This may be due to the modified T7 promoter which contains only one G residue in the 3’ end of the promoter used in this study compared with three G residues in the authentic one. Furthermore, the inability of fowlpox virus to replicate in the mammalian cells may result in lower copy number of the HCV minigene for transcription by T7 RNA polymerase. A fowlpox virus expressing T7 RNA polymerase has been established by Britton et al. (1996) and it offers an alternative to another hybrid T7 system for transient expression of foreign genes. However, overall the susceptibility of mammalian cells, especially MT-2 cells, to fowlpox virus infection was lower than that to adenovirus infection (Fig. 5); therefore, the FPVT7 hybrid system was not used in the present study.

Fowlpox virus particles have the capacity to incorporate a large foreign gene and deliver it into the cytoplasm of the cells. Therefore, it is even possible to construct a recombinant fowlpox virus harbouring the entire HCV genome under the T7 promoter. In addition, fowlpox virus infection is abortive in mammalian cells with no production of infectious virus. By combining these useful characteristics of fowlpox virus as a vector and the high transcriptase activity of T7 RNA polymerase produced by adenovirus, a potential tool for in vivo transcription of HCV RNA in various cell lines can be established.

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