Investigation of promoter function in human and animal cells infected with human recombinant adenoviruses expressing rotavirus antigen VP7sc

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Human adenovirus (Ad) vectors are being used increasingly for a variety of applications in vaccination and gene therapy. The ability of vectors to enter cells and the efficiency of promoters expressing the therapeutic gene or vaccine antigen are critical to the outcome of such experiments. To identify promoters which might be suitable for use under a variety of conditions we have investigated the expression of a rotavirus antigen, VP7sc, employing several commonly used promoters carried in E1-substituted Ad vectors both in cell types which support virus replication and in cells which do not. Although not all gene constructions were identical, wide variations in promoter function were evident even in human 293 cells which support virus replication. The simian virus type 40 (SV40) early and β-actin promoters expressed poorly; the SV40 late promoter was somewhat better. The human IE94 cytomegalovirus (CMV) promoter and a modified Ad major late promoter were best, functioning equally well but with different kinetics. In other human cell lines the CMV promoter was more versatile, generally providing sustained expression at a significant level, in one case for at least 6 days. In addition, as mouse, rabbit and pig models of rotavirus infection are under investigation and VP7sc is a vaccine antigen, we also investigated the ability of the recombinant adenoviruses to infect cells from these and other sources. VP7sc expression was detected in several heterologous cell types, illustrating the ubiquity of the human Ad receptor and the versatility of human Ad as vectors when suitable promoters are used.

Introduction

For many years human adenoviruses (Ad) have been given orally as vaccines to combat infections at mucosal surfaces (Couch et al., 1963; Top et al., 1971). In part, because of their defined epidemiological record and an increasing understanding of their molecular properties, some Ad are being developed as vectors for recombinant vaccines (reviewed in Berkner, 1988; Graham & Prevec, 1992; Horwitz, 1990). Recently, there has also been increasing interest in using replication deficient Ad vectors to deliver a variety of potentially therapeutic genes (reviewed in Anderson, 1992; Mulligan, 1993).

We are investigating whether Ad expressing rotavirus antigens can induce protection against a rotavirus challenge. Rotaviruses which infect the gut are the principal cause of acute, severe diarrhoea in young animals and humans (reviewed in Kapikian & Chanock, 1990) but no effective vaccine against them presently exists (reviewed in Conner et al., 1994). Since Ad can be given orally, they may be suitable vectors for delivery of protective rotavirus antigens. We have previously used human Ad5 as a vector to express the rotavirus VP7sc gene (Andrew et al., 1990) inserted into the non-essential E3 region of the Ad genome (Graham & Prevec, 1992). This virus, which is replication-competent in permissive cells, induced a protective immune response in female mice which was passively transferred to the suckling offspring, affording them protection against challenge with the homologous rotavirus (Both et al., 1993). As mice are regarded as a semi-permissive host for human Ad (Mittal et al., 1993) this was an encouraging result. However, the Ad E3 region codes for a suite of proteins which interact with the immune system in various ways (Gooding, 1992; Wold & Gooding, 1991). Deletion of the E3 19 kDa glycoprotein gene in Ad5 induced severe immune pathology in the permissive host, the cotton rat (Ginsberg et al., 1989), raising concern about the safety of such vectors. In fact, if the data obtained in the cotton rat were to extrapolate to infection in humans, replication-competent, E3-substituted vectors with intact E1 regions may not be suitable for use.

An alternative approach is to develop recombinants in which foreign gene expression cassettes are inserted in
the E1 region of the Ad genome. As E1 codes for proteins which transactivate other viral and cellular promoters (Liu & Green, 1994), viruses which lack this region are replication-deficient in all but human 293 cells which provide the missing functions in trans (Graham et al., 1977). This strategy therefore offers advantages from a safety viewpoint but in terms of efficacy, the choice of promoter for driving foreign gene expression may be critical. In this work we have constructed several Ad in which the E1 region was substituted with gene cassettes in which VP7sc was expressed from different promoters. Our aim was to compare promoter efficiency and to investigate the kinetics of foreign gene expression in different cell types in order to identify promoters which might be suitable for use under a variety of conditions. Our results are also relevant to the development of vectors for vaccination and gene therapy.

Methods

Cells and viruses. Human 293 (embryonic kidney) cells (Graham et al., 1977) were maintained in MEM F-11 supplemented with 10% fetal calf serum (FCS). All recombinant Ad were propagated and titred in human 293 cells. Human fibroblast MRC-5 cells and mouse 3T3 cells were grown in Eagle’s MEM (alpha modification) supplemented with 10% FCS. HeLa cells were grown in Dulbecco’s MEM supplemented with 10% FCS. Ovine lung fibroblasts (CSL503 line), pig fibroblasts (PF10) and pig kidney cells (ATCC CCL33, PK15) were maintained in Eagle’s MEM plus 10, 10 and 5% FCS, respectively. Rabbit kidney cells (ATCC CCL37, RK-13) were maintained in MEM F-11 with 10% FCS.

Gene cassette constructions. Plasmids containing promoter/gene cassettes were constructed as follows. For construction of an E3-substituted vector a full-length SA11 VP7sc gene, as distinct from the gene with a short truncation (Andrew et al., 1990; Both et al., 1993), was cloned as an XhoI fragment into the SalI site of plasmid pAB26 (Hitt et al., 1995) (Fig. 1a). The complete VP7sc protein is efficiently transported to the cell surface (L. J. Lockett & G. W. Both, unpublished results). The protein has N- and C-terminal signal peptide and transmembrane anchor sequences derived from influenza haemagglutinin (Andrew et al., 1990). The gene was not flanked by exogenous promoter or polyadenylation sequences.

The BA7 and CMV7 cassettes (Fig. 1b) were constructed in plasmids derived from pCA2 and pCA3, respectively (Hitt et al., 1995). These plasmids contain sequences from the left-hand end (0-15 m.u.) of the Ad5 genome in which the E1 region is substituted between bp 342 and 3540 by the expression cassettes consisting of the human β-actin promoter (Gunning et al., 1987) or the human cytomegalovirus (CMV) immediate early promoter linked to the VP7sc gene and simian virus type 40 (SV40) polyadenylation sequences. These cassettes constructed later in the viruses AdCMV7L and AdBA7L are oriented in the right-to-left direction with respect to the Ad genome (Fig. 1b). An additional CMV7 cassette integrated into the genome of AdCMV7R in the right-to-left orientation was derived by modification of pCEP4 (Invitrogen). An XhoI–SalI fragment of 1.59 kb was prepared from pJC9VP7sc (Andrew et al., 1990). This contained the VP7sc gene and the SV40 polyadenylation sequences. The fragment was subcloned into the XhoI site of pCEP4 and the CMV promoter, gene and poly(A) sequences were amplified by PCR using primers 5’TAGTCGACCATATTCTCAT for the CMV region and 5’TAGAGGACTATAATCGC for the SV40 poly(A) region. The primers also carried ClaI sites to allow the fragment to be subcloned into the XbaI site of pXCX2 (Spessor et al., 1989).

Cassettes in which VP7sc was expressed via the Ad major late promoter (MLP7 cassettes, Fig. 1b) were constructed from pPyMLPX1 (obtained from J. Hassell, McGill University Montreal, Quebec), a derivative of pPyMT-1 (Davidson & Hassell, 1987). This plasmid carries the polyomavirus enhancer, Ad2 MLP and tripartite leader sequences (TLS) juxtaposed to 5’ and 3’ splice signals derived from the polyomavirus middle T antigen gene. A BamHI site and SV40 poly(A) signal follow. The VP7sc gene (XhoI fragment) from pJCVP7sc was cloned into the BamHI site using XhoI-BglII adaptors and the plasmid was propagated in a dcm- host E. coli GM48. The entire expression cassette was then excised as a ClaI fragment and inserted into pXCCL1 cut with ClaI. Plasmid pXCCL1 was derived from pXCX2 by the addition of a polylinker sequence (Hitt et al., 1995).

Gene cassettes SV40E7 and SV40L7 (Fig. 1b) in which VP7sc was linked to the SV40 virus early or late promoter, an SV40 intron and poly(A) sequence were derived as follows. The VP7sc XhoI fragment was cloned in the correct orientation into the SalI site of p3IΩ (Morgenstern & Land, 1990) between the SV40 early promoter and the SV40 t intervening sequence (IVS). The cassette was excised as a SpeI fragment and inserted into the XhoI site of pXCX2. The SV40L7 cassette was derived as an XbaI fragment by PCR amplification of the KpnI–SalI portion of plasmid pJC119 (Sprague et al., 1983), which carried the VP7sc gene in the XhoI site (Andrew et al., 1990), and transferred into pXCX2.

Construction of recombinant Ad. Recombinant virus AdE3-7R, in which a full-length VP7sc gene was incorporated into the E3 region in the left-to-right orientation with respect to the Ad5 genome, was constructed by recombination between plasmids pAB26, containing the VP7sc gene, and pF1G173 (Fig. 1a), using calcium phosphate transfection (Graham & van der Eb, 1973) as previously described (Both et al., 1993; Graham & Prevec, 1991). pF1G173 contains most of the Ad5 genome but has a KanB gene substitution in E3 and a lethal deletion nearby so that it is not infectious. The recombinant with the opposite orientation was not made because it was previously shown that this results in little or no expression (Johnson et al., 1988; Schneider et al., 1989; Both et al., 1993).

Viruses which carried foreign gene expression cassettes in the E1 region were constructed by recombination between plasmid pJM17 and the various plasmids which contained the gene expression cassettes (Fig. 1b). Plasmid pJM17 contains the Ad5d1309 genome with an insert which makes it too large to package (Graham & Prevec, 1991; McGreyer et al., 1988). For the major late promoter (MLP) and CMV promoters viruses containing gene cassettes inserted in the leftward (AdMLP7L and AdCMV7L) and rightward (AdMLP7R and AdCMV7R) orientations were obtained. For the viruses in which the VP7sc is driven by the β-actin promoter, SV40 early and late promoters, the gene cassettes were inserted in either the leftward (AdBA7L) or rightward (AdSV7R and AdSV7R) orientation, respectively (Fig. 1b). DNA from recombinant viruses was isolated and characterized by restriction enzyme digestion. Viruses displaying the correct structure were plaque purified before preparation and titration of stocks (Graham & Prevec, 1991).

Virus infections, radiolabelling and recovery of proteins. Cells were grown in monolayers in 60 mm dishes and infected when 60–80% confluent at a m.o.i. of 20–30 p.f.u./cell. After 30–60 min of adsorption, cells were fed with the appropriate medium. At the desired time p.i. (specified in the text) cells were incubated in DMEM lacking methionine for 1 h and then labelled in the same medium for 1–2 h in the presence of Trans35S-label (ICN; 50 μCi per dish). Cells were washed with PBS and harvested in radioimmunoprecipitation assay (RIPA) buffer. Radiolabelled VP7sc was recovered by immunoprecipitation using a polyclonal antiserum to SA11 rotavirus and
Fig. 1. Summary of plasmids, gene cassettes and recombinant Ad produced in this work. (a) E3 recombinants were constructed by recombination in vivo between pAB26, which carried the VP7sc gene (Andrew et al., 1990) in the Sall site, and pFG173, a non-infectious plasmid containing a KanR gene and a lethal deletion (indicated by the cross-hatching). (b) Expression cassettes in which the VP7sc gene was linked to various promoters are indicated. The promoters used were the IE94 from CMV, Ad2 MLP and TLS flanked by the enhancer and splice signals from polyomavirus middle T antigen regions, the human β-actin and SV40 early and SV40 late promoters. All cassettes terminate with the SV40 polyadenylation signal. Cassettes were inserted into plasmids pXCX2 or pXCJL-1 in one or both orientations and viruses were constructed by recombination in vivo with pJM17 (McGrory et al., 1988). E1-substituted Ad viruses in which the cassette was inserted in the leftward (L) or rightward (R) orientation are indicated.
proteins were analysed by gel electrophoresis as previously described (Both et al., 1993).

Results

Expression of VP7sc from different promoters in human 293 cells

Viral promoters derived from SV40, human CMV and Ad as well as the cellular β-actin promoter have been widely used to drive expression of genes in various cell types. These promoters were assessed for their ability to drive VP7sc expression in recombinant Ad because of their availability and potential versatility. The level of expression of VP7sc from these promoters was first compared in human 293 cells which support replication of all the viruses under study, i.e. in these cells expression was not limited by the failure of the genome to replicate. Cells were infected with recombinant viruses using an m.o.i. of 30 p.f.u./cell. At 12 h p.i. cells were labelled for 2 h; the expressed protein was then recovered by immunoprecipitation and analysed by SDS gel electrophoresis. Under these conditions it was apparent that multiple forms of VP7sc (45 kDa and 41 kDa) were produced, as expected (Andrew et al., 1990; Both et al., 1993), and that the promoters varied greatly in activity. Expression from the β-actin promoter in AdBA7L was lowest (Fig. 2, lane 4); expression from the CMV7 cassette in AdCMV7L was significantly better, although most of the product was of the lower molecular mass form which is confined to the endoplasmic reticulum (Andrew et al., 1990) (Fig. 2, lane 5). In other experiments when AdCMV7L and AdCMV7R were compared, both forms of the protein were produced and expression from the rightward facing cassette was consistently better than that from the one facing leftward (data not shown). As the promoter fragments differed only in minor detail outside the promoter region, this suggests that a rightward orientation is preferable. The MLP7 cassette produced the highest level of expression. Again, there was a noticeable difference between cassettes in the leftward (AdMLP7L, lane 2) and rightward (AdMLP7R, lane 1) orientations. Expression by AdMLP7R was equal to that obtained with the AdE3-7R recombinant (Fig. 2, lane 3) in which the VP7sc gene was inserted in the E3 region without heterologous flanking sequences (Fig. 1a). It has not been determined whether in this virus expression is driven by the proximal E3 promoter or the distal, upstream MLP but this result is discussed further below.

In similar experiments the kinetics of VP7sc expression from different promoters was also examined in human 293 cells. Cells were infected with AdSVE7R, AdSVL7R, AdBA7L, AdCMV7R and AdE3-7R (Fig. 1) at an m.o.i. of 20 p.f.u./cell, radiolabelled and harvested at various times up to 20 h p.i., by which time the cells were beginning to show CPE as a result of the productive infection. Expression from the SV40 early promoter was barely detectable (data not shown) but significant expression was detected for AdSVL7R at 14 and 20 h p.i. (Fig. 3, lanes 3 and 4). Expression from the β-actin promoter in AdBA7L was again low and just detectable at 8, 14 and 20 h p.i. (Fig. 3, lanes 6, 7 and 8). The identity of the low molecular mass protein which appears in the 20 h samples (Fig. 3, lanes 4, 8, 12, 16, 19 and 20) is unknown but it also appears in cells infected with recombinant Ad which do not carry the VP7sc gene (data not shown) and is presumed, therefore, to be a cross-reactive Ad protein produced at late times in a permissive infection.

Infection with recombinants AdCMV7R, AdMLP7R and AdE3-7R produced expression profiles which reflected the different promoters used in each virus. For AdE3-7R expression was high at 5 h, peaked at 8 h and decreased thereafter (Fig. 3, lanes 9–12). With AdCMV7R, expression increased steadily from 5 to 14 h and declined slightly at 20 h (Fig. 3, lanes 13–16). In contrast, expression from the MLP in AdMLP7R was not detectable (or barely visible) at 8 h, maximal at 14 h and slightly lower at 20 h (Fig. 3, lanes 17–20). At both 14 and 20 h the expression level from this modified MLP exceeded that observed with any other promoter.

Expression of VP7sc from different promoters in other human cell types

As human 293 cells support replication of viruses which are defective in the E1 region, it was of interest to compare expression levels in other non-permissive cell
Gene expression from recombinant adenoviruses

Fig. 3. Kinetics of expression of VP7sc from various promoters in human 293 cells infected with recombinant Ad as indicated. Cells were radiolabelled and harvested at the times indicated. All lanes are taken from autoradiographs of the same length exposure derived from experiments carried out with identical infection and labelling protocols; the band intensities reflect promoter strengths.

(a) kDa

(b) kDa

Fig. 4. Expression of VP7sc from various promoters in (a) MRC-5 and (b) A549 cells infected with recombinant adenoviruses. Cells were radiolabelled and harvested at 22-24 h p.i. All lanes were from the same autoradiograph.

Different results were obtained when HeLa cells were infected at an m.o.i. of 20 p.f.u./cell, then radiolabelled and harvested at 5, 8, 14, 26 and 50 h p.i. In these cells, surprisingly, expression from AdMLP7R was barely detectable, a small amount being evident at 50 h p.i. (Fig. 5a, lanes 2–6) while expression from AdE3-7R was transient. Little was detected after 8 h p.i. (Fig. 5a, lanes 7–10) and the virus had a rapid CPE which was evident after 14 h p.i. Under these conditions two proteins of 25 kDa and 14 kDa were produced by 14 h p.i. (Fig. 5a, lanes 9 and 10). These were not degradation products of VP7sc as they were also immunoprecipitated from cells infected with Ad5 (data not shown). They are assumed to be cross-reactive viral proteins. In contrast to AdMLP7R and AdE3-7R, AdCMV7R expressed VP7sc consistently with a broad peak of activity between 14 and 50 h p.i. (Fig. 5b, lanes 2–6). These results are in contrast to the
observations made in A549 cells where the CMV promoter failed to function while expression from the MLP promoter was clearly detectable.

Expression of VP7sc from different promoters in non-human cell types

As AdCMV7R produced sustained expression of VP7sc in some human cell types, this virus was also tested for expression in murine, porcine and lapine cells since mouse, piglet and rabbit models of rotavirus infection have been established for vaccine studies (Schaller et al., 1992; Ward et al., 1990; Conner et al., 1993). When mouse 3T3 or pig fibroblast (PF10) cells were infected with AdE3-7R or AdCMV7R at an m.o.i. of 20 p.f.u./cell and harvested after radiolabelling at 26 h p.i., no expression of VP7sc was detected (data not shown). When mouse kidney cells (PK15) were infected with AdE3-7R, AdCMV7R or AdMLP7R and labelled and harvested at 8, 14, 26 and 50 h p.i., expression was detected in AdE3-7R-infected cells (Fig. 6a, lanes 2–5), proving that human Ad5 was capable of entering these porcine cells. However, expression from the E1-substituted AdCMV7R and AdMLP7R recombinants over the same time course was barely detectable by 50 h p.i. (Fig. 6a, lanes 6, 9 and 10–13, respectively). Thus, the human CMV and AdMLP promoters do not appear to function well in these porcine kidney cells, at least when inserted in E1 replication-deficient vectors.

When a similar experiment was carried out using rabbit kidney cells expression was observed with all three viruses. AdE3-7R produced a pronounced CPE by 28 h and expression decreased as a result (Fig. 6b, lanes 1–4). AdCMV7R produced sustained high level expression over 50 h (Fig. 6b, lanes 5–8), whereas for AdMLP7R expression was detectable at 14 h and highest after 50 h (Fig. 6b, lanes 9–12). To further investigate the infection capabilities of human Ad5 and promoter function ovine lung fibroblast cells (CSL503), available from parallel studies of an ovine adenovirus (Boyle et al., 1994), were also infected with AdCMV7R or AdMLP7R (m.o.i. 20 p.f.u./cell). Sustained VP7sc expression over 74 h was
Gene expression from recombinant adenoviruses

Fig. 7. Infection of ovine lung fibroblast (CSL503) cells with human recombinants (a) AdCMV7R or (b) AdMLP7R. Cells were radiolabelled and harvested at the times indicated. UC shows uninfected cell controls. Lanes 7 contain marker proteins.

Fig. 8. Sustained expression of VP7sc in HeLa cells infected with AdCMV7R. Cells were radiolabelled and harvested over a 6 day period. Left-hand lanes contain marker proteins.

detected with both viruses (Fig. 7a, b, lanes 3-6), indicating that the human virus infected these cells and that the CMV and MLP promoters were functional. However, the kinetics of expression from the MLP (Fig. 7b) were distinctly different from those seen in replication-permissive human 293 cells. Sustained expression at a level similar to that seen for AdCMV7R was observed, a pattern distinct from that seen in productively infected cells (Fig. 3, lanes 17-20).

Sustained expression by the CMV promoter

Given the consistent level of expression observed from the human CMV promoter in different cell types, we examined the longevity of expression from this promoter. HeLa cells were seeded in 60 mm dishes at 60% confluency and infected with AdCMV7R at an m.o.i. of 20 p.f.u./cell. One plate of cells was labelled and harvested after 24 h and additional plates were labelled and harvested daily over a 6 day period. By 3 days the cells had reached confluence and on the fourth day the medium was changed. Total VP7sc expressed in each plate of cells was recovered by immunoprecipitation and dissolved in the same volume. One fifth of each sample was examined by gel electrophoresis. Expression was clearly observed, being maximal at 24 h p.i. (Fig. 8, lane 2) but sustained over the 6 day time course of the experiment (Fig. 8, lanes 3-7). After 6 days the level of expression was still about 30% of that seen after 24 h.

Discussion

Ad vectors are being used increasingly for a variety of applications in vaccination and gene therapy. In both cases vector choice and promoter function will strongly influence the outcome of experimental approaches. For vaccination, the majority of work has been done using human Ad vectors which carry foreign gene cassettes in the E3 region of the genome as this is non-essential for replication in vitro (Lewis et al., 1973; reviewed in Berkner, 1988; Graham & Prevec, 1992). In these viruses it has not proved necessary to introduce a promoter to drive foreign gene expression as genes inserted in the left-to-right orientation are efficiently expressed, either from the upstream MLP or E3 promoters in the viral genome (Both et al., 1993; Morin et al., 1987; reviewed in Graham & Prevec, 1992). However, genes inserted at the right-hand end of the genome between the E4 promoter and the ITR require a promoter for expression (Chanda et al., 1990; Mason et al., 1990). In many cases the homologous Ad MLP/TLS was used to promote gene expression. This was also true for viruses in which the expression cassette was inserted into the E1 region of the genome in place of the E1A/E1B sequences (Levrero et al., 1991; and reviewed in Graham & Prevec, 1992). However, the immediate early (IE94) human CMV promoter has also been used to create E1-substituted viruses (Bacchetti & Graham, 1993; Jacobs et al., 1992; Morsy et al., 1993; York et al., 1994). Such vectors are replication-deficient except in human 293 cells which provide the missing E1 functions in trans (Graham et al., 1977). Despite being replication-deficient, an E1-substituted Ad recombinant expressing a flavivirus non-structural protein induced a significant protective immune response in vaccinated mice (Jacobs et al., 1992). This work suggested that vaccination strategies using safer, replication-deficient viruses might be possible if
expression by the vector were optimized and/or prolonged. Our aim therefore, was to compare promoter efficiencies and to investigate the kinetics of foreign gene expression in different cell types to identify versatile promoters suitable for use principally in vaccine vectors.

Using a modified rotavirus antigen VP7sc as a reporter (Andrew et al., 1990) we have assessed the promoter activity of the human β-actin, SV40 early and late, CMV and a modified Ad MLP in the context of an Ad infection in cells which fully support Ad replication and in cells which do not. A replication-competent E3-substituted virus which expressed VP7sc (Both et al., 1993) was also included. We found clear differences in the levels and kinetics of expression of VP7sc depending on the vector construct and the cell line. In human 293 cells, which supported replication of all viruses tested, viruses in which β-actin or SV40 promoters were used expressed only low levels of VP7sc in the context of the gene constructions used. The AdMLP recombinant produced the highest level of expression but the E3 and CMV recombinants also expressed VP7sc efficiently. However, there was a noticeable difference in the kinetics of VP7sc expression. AdCMV7R and AdE3-7R recombinants expressed VP7sc at 5 h p.i., the earliest time monitored, and continued throughout the experiment. However, the AdMLP7R recombinant did not express at early times and only expressed strongly at 14 and 20 h p.i. In previous work (Both et al., 1993) VP7sc expression was observed at 7 h p.i. with Ad5/7.4, a virus very closely related to AdE3-7R, suggesting that expression in the AdE37R virus probably initiated from the E3 promoter, though whether the MLP was used later during the infection is not clear. This conclusion differs from an earlier study using a similar E3 recombinant in which it was concluded that the MLP drove expression of a firefly luciferase reporter gene inserted at this site. Expression in the latter case was largely AraC sensitive, implying that DNA replication was needed (Mittal et al., 1993). It is becoming apparent (S. K. Mittal and others, unpublished results) that the kinetics of expression from E3 inserts can depend critically on the reporter gene itself.

In HeLa cells substantial expression from the AdE3-7R recombinant was detected only at 5 and 8 h p.i. although this may have been due to a more rapid onset of CPE, as suggested by the appearance of cross-reacting proteins (Fig. 5, lanes 9 and 10) which are produced late in infection. The results with HeLa cells also highlight the differences in promoter efficiency in cells which cannot support replication of defective Ad recombinants. In these cells the AdMLP7R recombinant failed to express whereas the AdCMV7R expressed reasonable levels of VP7sc over a 50 h period with no evidence of CPE. Clear differences in promoter function were also evident from the studies in A549 and MRC-5 cells, although in these cell types the kinetics of expression were not examined. In A549 cells the AdMLP recombinants expressed significant levels of VP7sc whereas the AdCMV7L virus did not. In contrast, in MRC-5 cells expression from both the AdCMV and AdMLP recombinants was comparable, but much inferior to the replication-competent AdE3 virus. In several cell types, the MLP7 gene cassette consistently expressed at a higher level in the rightward facing compared with the leftward orientation. These data illustrate that in the absence of viral replication gene cassette orientation and the choice of promoter is critical to the level of expression in different human cell types. The CMV promoter expressed VP7sc consistently when the viral vector was unable to produce a lytic infection and appears to be more versatile than the modified Ad2 MLP also used in this work. Thus, the cumulative amount of antigen expressed by a replication-deficient virus may equal or exceed that produced during a lytic infection. This conclusion is in accord with earlier work (Levrero et al., 1991) but our data emphasize the need for careful promoter selection, especially for applications in gene therapy where expression in a certain cell type may be required. These studies are also relevant to the problem of testing potential rotavirus vaccines based on expression of protective antigens by recombinant human Ad. Animal models of rotavirus infection are under investigation for mice, rabbits and gnotobiotic piglets (Conner et al., 1993; Schaller et al., 1992; Ward et al., 1990). It was therefore of interest that both pig and rabbit kidney epithelial cells could be infected by human Ad5. In contrast, we observed no expression in mouse 3T3 fibroblasts, although it is well established that mouse cells support a limited amount of Ad5 replication. Ad recombinants have previously been shown to induce seroconversion in mice (Both et al., 1993; Jacobs et al., 1992).

We also observed significant differences in the efficiency of expression by recombinant viruses in the animal cells used in this work. In particular, in rabbit kidney cells AdCMV7R exhibited a sustained high level of expression, producing more VP7sc in total than did AdE3-7R. In porcine kidney cells, however, only AdE3-7R produced significant levels of antigen; the E1-substituted viruses expressed poorly, indicating that the AdMLP and human CMV promoters did not function well.

It is well known that the level of rotavirus antibodies in serum correlates poorly with protection against rotavirus infection in the gut (reviewed in Offit, 1994). Therefore, it may be necessary to induce mucosal immunity to obtain protection. The choice of vector and
promoter might then be critical to the success of such vaccination experiments.

We thank Richard Bellamy for antisera to rotavirus SA11. This work was supported by grants from the WHO/UNDP Programme for Vaccine Development, the Natural Sciences and Engineering Research Council and the Medical Research Council (MRC) of Canada. F. L. G. is a Terry Fox Research Scientist of the National Cancer Institute of Canada and V. K. was the recipient of a Visiting Scientist award from the MRC.

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(Received 3 January 1995; Accepted 2 March 1995)