Insertion sites for recombinant vaccinia virus construction: effects on expression of a foreign protein

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The expression of antigens or other molecules from recombinant vaccinia viruses requires the insertion of coding sequence at specific sites in the viral genome. Here we investigate the influence of two different sites on the level of protein expressed during a viral infection. The level of immune response in mice to vaccinia virus-expressed murine interleukin 2 (IL-2) or IL-4 varied depending on whether the coding sequence was inserted into the vaccinia virus thymidine kinase (tk) gene or into the HindIII F fragment of the viral genome where herpes simplex virus (HSV) tk was used as a selectable marker. In each case the intensity of the response was greater when the relevant gene was expressed from the HindIII F insertion site. In order to quantify these differences a series of recombinant viruses expressing luciferase was constructed. Luciferase activity from coding sequence inserted into the HindIII F fragment was significantly higher than that from the tk gene insertion, provided HSV tk<sup>+</sup> constructs were compared. Insertion of a marker gene (HSV tk) into the HindIII F site with disruption of the F7L open reading frame led to a reduced level of luciferase expressed from the tk insert, despite more than 45 kb of intervening sequence. In mice, luciferase expression was higher from the HindIII F inserted gene than from the tk insert in both lungs and ovaries.

Introduction

Vaccinia virus, the prototypic orthopox virus, has been used extensively for more than a decade as a vector for the expression of foreign genes (reviewed in: Moss & Flexner, 1987; Paoletti, 1990; Smith & Mackett, 1992). Genes from a wide variety of sources have been inserted into non-essential regions of the viral genome with subsequent expression regulated by a vaccinia virus promoter located 5’ to the inserted sequence. A number of techniques have been used to distinguish recombinant vaccinia viruses from the background of parental virus used in the recombination reaction. The most widely used technique has been the insertional inactivation of the vaccinia virus thymidine kinase (tk) gene and identification of recombinants in the presence of BUdR (Mackett et al., 1984; Boyle et al., 1985). In order to coexpress two genes from the same recombinant, we have employed a strategy for the insertion of a second gene into the HindIII F region of a tk<sup>-</sup> vaccinia recombinant, using herpes simplex virus (HSV) tk as a selectable marker (Coupar et al., 1988). Insertion of DNA into the HindIII F region results in the disruption of open reading frame (ORF) F7L, which has potential to code for a protein of 80–92 amino acids (depending on the vaccinia virus strain) with no described function (Roseman & Slabaugh, 1990; Goebel et al., 1990). Deletion of the thymidine kinase function has been shown to reduce the virulence of the vaccinia virus infection in mice (Buller et al., 1985), while the restoration of some tk activity in double recombinants expressing the HSV tk has an intermediate effect on virus virulence (Andrew et al., 1989).

In experiments reported previously (Andrew & Coupar, 1992) we have expressed murine interleukin 2 (IL-2) and IL-4 from either the tk insertion site or the HindIII F insertion site. These two cytokines had quite different effects in mice. IL-2 had an attenuating effect in that it allowed immuno-compromised (nude or sublethally irradiated) mice to survive an otherwise lethal dose of vaccinia virus (Ramshaw et al., 1987; Flexner et al., 1987). In contrast, IL-4 expressed from a recombinant vaccinia virus resulted in shorter mean times to death in immunocompromised mice and, at high enough doses, was able to kill normal female mice (Andrew & Coupar, 1992).
Coexpression of IL-2 with IL-4 improved the survival rates of the mice. However, IL-2 expressed from the HindIII F insertion site was more effective in overcoming the effects of IL-4 than when the same gene, regulated by the same promoter, was expressed from the tk insertion site with IL-4 in the HindIII F region (Andrew & Coupar, 1992; unpublished observations). These effects were observed in vivo despite no detectable difference in the level of either cytokine expressed from each of the constructs in infected cell cultures.

In order to investigate potential insertion site effects more thoroughly and with a more readily quantifiable product, we have constructed a number of vaccinia virus recombinants where the firefly luciferase gene has been inserted into the tk gene or into the HindIII F region of the viral genome. In each case regulation of luciferase expression was under the control of the vaccinia 7.5 kDa promoter. Here we report the comparative levels of luciferase activity from different recombinant virus infections in cell culture and in a range of mouse tissues after intravenous inoculation.

**Methods**

- **Viruses and cells.** An L929 cell-adapted vaccinia virus WR strain has been described previously (Boyle et al., 1985). Human tk− 143B cells (Rhim et al., 1975) were used for recombinant virus construction and for virus titration, and stocks of vaccinia viruses were grown in CV1 cells.

- **Plasmids and construction of recombinant vaccinia viruses.** Firefly luciferase coding sequence was subcloned from pFC05 (Cameron & Jennings, 1989) into pBCB06 (Boyle et al., 1985) and pBCB07 (Andrew et al., 1987) for insertion into the vaccinia virus tk, and into pTK7.5A and pTK7.5B (Coupar et al., 1988) for HindIII F insertions. In each case the luciferase sequence was 3′ to the vaccinia virus 7.5 kDa promoter. Thymidine kinase negative vaccinia virus recombinants (VV-104 and VV-105) were constructed using VV-WR-L929 as described previously (Boyle et al., 1985) with insertion of the promoter-gene cassette between residues 92 and 93 of the 177 amino acid tk sequence and in both orientations relative to the tk sequence. Recombinants with the luciferase gene inserted into the HindIII F region (VV-128 to VV-131) were constructed using VV-PR8-HA6 (VV-128 and VV-129) or VV-PR8-NP6 (VV-130 and VV-131) (Andrew et al., 1986) as parental virus. Control recombinants (VV-163 and VV-164) resulted from the insertion of the HSV tk sequence alone into the HindIII F region of VV-104 or VV-105 using pFB-TK (Coupar et al., 1988). The F7L ORF was disrupted by insertion of a 1.8 kb fragment containing the HSV tk coding sequence, followed by the promoter-gene cassette in either orientation relative to F7L and HSV tk, after the 6th residue of the F7L putative product.

Recombinant viruses expressing human cytokines IL-2 and IL-4 and a control virus VV-HA-TK have been described previously (Ramshaw et al., 1987; Andrew & Coupar, 1992).

- **Mice.** CBA/H mice were inoculated intravenously (i.v.) with 10⁷ p.f.u. of virus and after 3 days organs were removed for luciferase assay and for vaccinia virus titrations.

- **Luciferase assays.** Infection of 143B cells with vaccinia virus recombinants was carried out in 96-well plates at a range of m.o.i.’s and for various times up to 9 h. Samples from quadruplicate wells were assayed for luciferase activity using a Promega luciferase assay kit. Assays were carried out using the luciferase assay reagent at a dilution of 1:20 and 1 min reaction time in a Berthold luminometer. A standard curve was prepared using dilutions of luciferase (Sigma) before each batch of samples was tested. Luciferase assays on mouse tissue samples were carried out using a 1:10 dilution of assay reagent for ovary samples and undiluted reagent for all other tissues. Duplicate assays were performed on each tissue sample. Comparisons of levels of luciferase activity were confided to data generated on the same day with the same batch of reagents and conditions. Luciferase activity is expressed in relative light units (RLU).

**Results**

**Expression of murine IL-2 and IL-4 and effects on morbidity and mortality in mice**

Using survival rates for nude mice inoculated with vaccinia virus or the rates of clearance of virus from CBA/H mice as parameters for virulence attenuation, vaccinia virus recombinants expressing murine IL-2 or IL-4 from either of two insertion sites were compared with each other and with control viruses. All recombinant viruses used had insertions at both sites in the viral genome to provide a comparable tk phenotype (HSV tk−). Vaccinia virus recombinants expressing murine IL-2 from coding sequence inserted either into the tk gene or into the HindIII F insertion site were more attenuated in nude and CBA/H mice when compared with control viruses or with viruses expressing IL-4 (Table 1). When the titre of virus in the ovaries of CBA/H mice was examined, it was found that titres were reduced in those mice which had received viruses expressing IL-2 (VV-IL2-TK and VV-HA-IL2) compared with those inoculated with control virus (VV-HA-TK) or viruses expressing IL-4 (VV-IL4-TK and VV-HA-IL4) (Table 1).

When IL-2 and IL-4 were coexpressed from the same virus with IL-2 coding sequence in the HindIII F site, 100% survival rates were observed in nude mice (Table 1). On the other hand, when the insertion sites for IL-2 and IL-4 sequences were reversed and IL-4 was expressed from the HindIII F site no mice survived. These results suggest that the dominant effect is provided by the expression product from the HindIII F insertion site. In the CBA/H mice there was no significant difference between the VV-IL2-IL4 and VV-IL4-IL2 groups and it appeared that the level of IL-2 expressed from both constructs was sufficient to allow the mice to survive.

Attempts to quantify the levels of IL-2 and IL-4 expressed in infected cell culture supernatants from these recombinants showed no significant differences between any of the constructs for the expression of either cytokine (data not shown). Levels of virus replication as measured by one-step growth curves were not significantly different (data not shown).

**Construction and characterization of vaccinia virus recombinants expressing luciferase**

Vaccinia virus recombinants with the coding sequence for firefly luciferase inserted downstream from the vaccinia virus...
Table 1. Pathogenesis in mice of vaccinia virus recombinants expressing murine IL-2 or IL-4

Statistical significance of results is indicated as follows: a, $P < 0.001$ vs VV-HA-TK; b, $P < 0.001$ vs VV-IL4-TK; c, $P < 0.001$ vs VV-IL2-TK.

<table>
<thead>
<tr>
<th>Virus</th>
<th>Insertion site</th>
<th>Nude mice*</th>
<th>CBA/H mice†</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>No. of %</td>
<td>No. of %</td>
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<tr>
<td></td>
<td></td>
<td>mice</td>
<td>% Survival</td>
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<tr>
<td></td>
<td>tk</td>
<td>MTD ± SE†</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HindIII F</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VV-HA-TK</td>
<td>HA HSV tk</td>
<td>89</td>
<td>25.8</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VV-IL2-TK</td>
<td>IL-2 HSV tk</td>
<td>48</td>
<td>56.3</td>
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<td>HSV tk + IL-2</td>
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<tr>
<td>VV-IL4-TK</td>
<td>IL-4 HSV tk</td>
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</tr>
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<td></td>
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<td></td>
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<td>VV-HA-IL4</td>
<td>HSV tk + IL-4</td>
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<td>11.7</td>
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<tr>
<td>VV-IL2-IL4</td>
<td>IL-2 HSV tk + IL-4</td>
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<td>0</td>
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<td>VV-IL4-IL2</td>
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<td>100</td>
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</tbody>
</table>

* Nude mice were inoculated intravenously with $10^7$ p.f.u. of virus.
† CBA/H mice were inoculated intraperitoneally with $10^7$ p.f.u. of virus.
‡ Mean time to death ± standard error.
§ Number of mice used for assay of virus in ovaries.
∥ Virus titre in ovaries removed 5 days after virus inoculation ± standard deviation.

Fig. 1. Schematic representation of the viral genome for recombinant vaccinia viruses expressing luciferase. Arrows above the line indicate the relative positions and direction of transcription for the genes interrupted by insertions. Solid arrows represent promoter (7.5 kDa)–luciferase gene cassettes; open arrows represent the HSV tk gene sequence, which is transcribed from the F7L promoter; hatched arrows represent influenza virus haemagglutinin or nucleoprotein coding sequences. The number of separate recombinants constructed is shown in parentheses after each virus number.

7.5 kDa promoter, in either of two sites in the viral genome, are shown schematically in Fig. 1. Insertion into the tk gene provided recombinants with the luciferase coding sequence in either orientation relative to the tk gene (VV-104 and VV-105). In each case, four separate recombination reactions were carried out and recombinant viruses were plaque purified from each (A–D in each case). Recombinant viruses expressing luciferase from insertions in the HindIII F fragment were constructed using two tk− viruses expressing influenza virus HA (VV-128 and VV-129) or NP (VV-130 and VV-131) antigens. Once again both orientations of the insertion were used and recombinants were constructed in duplicate (A and B). Control viruses (VV-163A–D and VV-164A–D) with the HSV tk sequence inserted into the HindIII F region of VV-104A–D and VV-105A–D were constructed to provide the same tk phenotype as VV-128 to VV-131. The genome arrangement and homogeneity of each of the recombinants were examined by Southern blotting and probing with a number of radioactively labelled fragments of DNA, including the luciferase coding region and the wild-type vaccinia virus.
fragments covering each of the two insertion sites. All constructs were shown to have the expected patterns of hybridization and wild-type or parental vaccinia virus was not detected (data not shown).

In order to test for the effects of tk phenotype on virus replication in cell culture, one-step growth curves up to 24 h were carried out using VV-WR, VV-104A, VV-128A and VV-163A. No significant differences were found in the titre of virus recovered at any of the time-points tested (data not shown).

Expression of luciferase activity in recombinant vaccinia virus-infected cells

In an initial series of experiments the levels of luciferase activity in lysates of 143B cells infected with vaccinia virus recombinants were measured at a range of m.o.i. values (0.5–10 p.f.u. per cell) and times post-infection (p.i.) up to 9 h. Further experiments were carried out using quadruplicate wells infected at 5 and 1 p.f.u. per cell for 7–8 h. Fig. 2(a) shows no significant difference in the level of activity produced by the four recombinants VV-104A–D, where the luciferase coding sequence is inserted into the tk gene of vaccinia virus. However, VV-105B consistently showed very low levels of luciferase activity when compared with the other three recombinants constructed in parallel (VV-105A, C and D). Southern blotting and probing of the DNA from VV-105B did not reveal any obvious reason for this low level of luciferase activity.

Fig. 2. Luciferase activity in cell lysates at 8 h p.i. with 5 p.f.u. per cell. (a) Recombinants expressing luciferase from the tk insertion site (tk−); (b) recombinants expressing luciferase from the HindIII F insertion site (HSV tk+). Means of assays on quadruplicate infections with standard errors are shown.

Fig. 3. Effect of HSV tk insertion into HindIII F on luciferase expression from the tk insertion site. Luciferase activity from cell lysates at 8 h p.i. and m.o.i. 1 p.f.u. per cell. Pairs of recombinants are shown with the tk− recombinant in open bars and the same virus modified by insertion of the HSV tk sequence into the HindIII F region in solid bars. Means of assays on quadruplicate infections with standard errors are shown.
expression and further investigation was not carried out. Data derived using VV-105B were excluded from the calculation of means for any particular group of recombinants. Orientation of the inserted DNA relative to tk did not appear to affect the level of expression of luciferase (VV-104 versus VV-105).

Levels of luciferase activity using recombinants with the coding sequence inserted in the HindIII F region are shown in 2(b). Pairs of recombinants constructed in parallel did not show significant differences in the level of luciferase expressed, although recombinants constructed using VV-PR8-HA did appear to express marginally higher levels than those based on VV-PR8-NP. In this case, orientation of the luciferase coding sequence may have some effect in that recombinants with luciferase in the same orientation as the inserted HSV tk marker and the vaccinia ORF F7L expressed slightly higher levels than those in the opposite orientation (VV-128 versus VV-129 and VV-130 versus VV-131). This trend was consistent over several experiments at a range of m.o.i.’s and time-points and was not related to the order of processing of the samples during the luciferase assay. Although levels of luciferase expression from the HindIII F insertion site were higher than those from the tk− recombinants the range in values for the HindIII F group was such that there was no significant difference between the two groups, which had different tk phenotypes.

In order to provide recombinants with the same tk phenotype and equivalent manipulations of the viral genome, the eight recombinants with luciferase insertions in the tk locus (VV-104A–D and VV-105A–D) were used for insertion of the selectable marker HSV tk into the HindIII F region. When the resultant recombinants, VV-163A–D and VV-164A–D, were compared with their parental viruses, surprisingly, the results showed a significant drop in the level of luciferase expressed in the double recombinants. Results from a representative experiment are shown in Fig. 3. Apart from VV-164B, which was constructed using VV-105B (already shown to express abnormally low levels of luciferase), each recombinant with an insertion into and interruption of F7L and expressing HSV tk expressed significantly lower levels of luciferase than its tk negative parent. The reduction in activity ranged from 43% for VV-104D/VV-163D to 76% for VV-105D/VV-164D.

Comparisons of the levels of luciferase expressed from each of the three groups of constructs in two separate experiments are shown in Fig. 4. In each case, higher levels of luciferase were detected when the coding sequence was inserted into the HindIII F region (VV-128 to VV-131) than when luciferase was expressed from the tk locus, either with (VV-163 and VV-164) or without (VV-104 and VV-105) an insertion in the HindIII F site. Although the difference between the tk− group and the HindIII F luciferase recombinants was not significant, comparison between the two groups with the same tk phenotype which differed only in the site of insertion of the luciferase gene showed a significant difference (P < 0.0005), with higher levels expressed from an insertion into the HindIII F region than from insertions into the tk gene (Fig. 4).

Fig. 5 shows time-courses for one construct from each group at either 5 or 1 p.f.u. per cell. Higher levels of expression from the HindIII F insertion site were detected as early as 3 h p.i. and maintained throughout the time-course of the experiment.

Luciferase expression in mouse tissues

Groups of female CBA/H mice were inoculated i.v. with 10^7 p.f.u. of recombinant or wild-type vaccinia virus. Three days p.i. the mice were sacrificed and organs removed for luciferase assay and virus titrations. Vaccinia virus has been shown to replicate to high levels in the ovaries of mice after i.v.
inoculation (Karupiah et al., 1990). Fig. 6(a) shows the levels of luciferase detected in ovaries of mice inoculated with VV-104A, VV-163A or VV-128A compared with wild-type virus (VV-WR). The relative levels of expression are comparable with those detected after infection of cells in culture, with higher levels of expression from the HindIII F inserted luciferase sequence. The data are presented after correction for the level of virus detected in the same tissue sample, which averaged $10^6$ p.f.u./mouse ovaries (Table 2). Since high levels of luciferase were detected in the ovaries, luciferase assays were carried out using a 1:10 dilution of the assay reagent and are therefore not directly comparable with data from other tissues.

Fig. 6(b) shows the results obtained for lungs, spleens and livers of the same animals. In these tissues the levels of both virus and luciferase were lower than in ovaries (Table 2) and luciferase assays were carried out using undiluted reagent. Spleens and livers showed low levels of luciferase activity irrespective of recombinant. However, in the lung samples there was a dramatic increase in the level of activity detected when luciferase was expressed from the HindIII F insertion site compared with either of the tk locus constructs, which were at background levels. Data from a separate experiment confirmed the high level of activity in lungs of mice inoculated with VV-128A (Fig. 6(c)).
expression observed by Davison & Moss (1989) when the promoter-gene insert was in the same, rather than the opposite, orientation compared with tk were not observed. Recombinant viruses with luciferase expression from the HindIII F insertion site did show higher levels of expression when the promoter-luciferase gene cassette was in the same orientation as F7L (Fig. 2b); however, the F7L promoter has been shown to be relatively weak (Coupar et al., 1987) and is located more than 2 kb upstream of the luciferase coding sequence.

When vaccinia virus recombinants which differed only in the insertion site for the luciferase coding sequence were compared in mice, once again there was a significantly higher level of expression from the HindIII F site in both ovaries and lungs (Fig. 6). In other organs tested the levels of luciferase detected were relatively low and no significant differences were seen between the constructs.

An unexpected result was the decreased level of luciferase expressed from the tk insertion site when a second insertion was made into the HindIII F site (Fig. 3). In eight pairs of recombinants where the only modification was the insertion of HSV tk into the HindIII F site, with consequent interruption of the F7L ORF, the level of luciferase from a coding sequence inserted into the vaccinia virus tk gene was reduced, despite the two sites being more than 45 kb apart (Fig. 3). This suggests an effect on expression from the tk locus either due to the expression of HSV tk or to the lack of expression of the F7L gene product. At present we have no data to support or exclude either hypothesis, nor has a function for the F7L product been demonstrated. No significant differences in the levels of virus replication in cell culture for any of the recombinants or wild-type virus were detected in one-step growth curves. In vivo levels of virus replication vary with tk phenotype, with the HSV tk+ constructs having a level of virulence intermediate between the wild-type vaccinia tk and the attenuated tk− viruses (Andrew et al., 1989), thus emphasizing the importance of comparisons between recombinants with the same tk phenotype.

A number of studies have included data for expression of multiple products from a single recombinant vaccinia virus

### Table 2. Virus titres in selected organs of CBA/H mice 3 days post-inoculation with recombinant viruses

<table>
<thead>
<tr>
<th>Virus</th>
<th>Insertion site (luc)</th>
<th>TK phenotype</th>
<th>Vaccinia virus log titre*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ovaries</td>
</tr>
<tr>
<td>VV-WR</td>
<td>Not applicable</td>
<td>tk+</td>
<td>7.13 ± 0.10</td>
</tr>
<tr>
<td>VV-104A</td>
<td>tk</td>
<td>tk−</td>
<td>6.21 ± 0.27</td>
</tr>
<tr>
<td>VV-163A</td>
<td>tk</td>
<td>HSV tk+</td>
<td>6.43 ± 0.15</td>
</tr>
<tr>
<td>VV-128A</td>
<td>HindIII F</td>
<td>HSV tk+</td>
<td>6.52 ± 0.14</td>
</tr>
</tbody>
</table>

* Geometric means per group of six mice with standard errors.

### Discussion

The level of expression of a particular protein from a coding sequence inserted into the genome of vaccinia virus has generally been assumed to be dependent on the promoter sequence used and the post-transcriptional modifications and translational requirements of that protein. Here we show that when the same promoter-gene cassette is inserted at two different sites within the viral genome different levels of product are produced. In mice, vaccinia virus-expressed IL-2 and IL-4 have been shown to have quite different effects. Whilst IL-2 attenuated the virus and allowed immunocompromised mice to recover from an otherwise lethal infection (Ramshaw et al., 1987; Flexner et al., 1987), expression of IL-4 shortened the mean time to death in immunocompromised animals (Andrew & Coupar, 1992). Here we show that expression of either cytokine under the control of the 7.5 kDa promoter, and the outcome of the immune response in mice, was influenced by the location of the inserted genes in the viral genome (Table 1). When both cytokines were expressed from the same virus, the effect attributed to the cytokine expressed from the HindIII F insertion site predominated over that of the cytokine expressed from the TK gene insertion site as judged by survival rates in nude mice (Andrew & Coupar, 1992; Table 1). In CBA/H mice, the level of IL-2 expressed from either site appeared to be sufficient for significant levels of virus clearance despite the coexpression of IL-4, perhaps reflecting the complexity of the balance of cytokines functioning in vivo.

Using luciferase as a quantifiable reporter gene we have demonstrated that in infected cells the level of expression from the HindIII F insertion site is higher than from the tk insertion site, provided that the tk phenotype is the same (Figs 4 and 5). In tk insertion site constructs the orientation of the inserted sequence relative to the tk gene and its promoter does not appear to have a significant influence on the level of luciferase expressed. Although in VV-105, where the possibility of readthrough transcription from the tk promoter exists, the levels are marginally higher (Fig. 2a) the increased levels of
(Perkus et al., 1985; Coupar et al., 1988; Morrison et al., 1990; Wild et al., 1992) but with no direct comparison of effects of insertion site on the expression of a particular product. However, in cases where the same gene has been expressed from different loci within the vaccinia virus genome (e.g. Kunke et al., 1993; Flexner et al., 1987) no significant effect of insertion site was demonstrated. Flexner et al. (1987) inserted the coding sequence for human IL-2 at three separate sites (HindIII C, tk and vaccinia virus HA) within the viral genome and found that the site of insertion was not critical to the attenuating effects observed in mice. Levels of expression of IL-2 produced by these recombinants were not compared. Kunke et al. (1993) constructed vaccinia virus recombinants coexpressing hepatitis B virus surface (HBsAg) and core (HBCag) antigens in two sites, interrupting the tk gene and the K1L host-range gene. Reversal of the locations of HBsAg and HBCag in a second construct did not significantly affect the level of expression of either antigen as determined by ELISA or immunoblot analysis (Kunke et al., 1993). On the other hand, Bembridge et al. (1998) did find differences in the levels of expression of respiratory syncytial virus (RSV) F protein and murine cytokines IL-2, IL-4 or IFN-γ which were coexpressed from the tk locus or the VP37 locus. Levels of expression assessed by ELISA were lower from the tk insertion site than from the VP37 site in each case (Bembridge et al., 1998). However, as different promoters were associated with each locus (expression from the tk site being regulated by the 7.5 kDa promoter and that from the VP37 by a synthetic early/late promoter), direct comparison of the insertion sites is difficult. More recently, Bennett et al. (1999) have shown differences in the levels of expression of the Clostridium perfringens α-toxin expressed from the tk or the B13R (serpin-2 gene) loci. In each case expression was regulated by the 7.5 kDa promoter and in the tk locus, where higher levels of expression were detected, the insert was in the same orientation as the tk gene, with the potential for enhanced transcription.

The data presented here suggest than in the construction of vaccinia virus recombinants, particularly for the coexpression of antigens or immunomodulators as vaccines, the selection of sites for insertion of the foreign genes may be critical to the outcome in vivo.

We thank Biba Horvatic, Rhonda Voysey and Vikki Holmes for expert technical assistance.

References


Received 27 July 1999; Accepted 3 November 1999