Expression of Recombinant Vaccinia Virus-derived Alphavirus Proteins in Mosquito Cells

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SUMMARY

A recombinant vaccinia virus strain which contains and expresses a 26S cDNA insert encoding Sindbis virus structural proteins (VV:3S) was used to infect a continuous line of Aedes albopictus mosquito cells. There were not visible cytopathic effects due to the virus infection and the cells continued to grow normally. However, examination of the proteins present in the cytoplasm of the infected cells with Sindbis virus-specific antisera revealed that Sindbis virus proteins were being synthesized and processed. These results are discussed with respect to (i) vaccinia virus as a non-lethal expression vector to deliver and express eukaryotic genetic information in insect cell systems and (ii) using this system (VV:3S) to dissect various facets of togavirus–insect cell interactions.

Sindbis virus (SV), an alpha-togavirus, has the interesting ability to initiate productive infections in both mammalian and insect cells (Stollar, 1980). Although the replicative cycle of SV is similar within the two different hosts, there are some unique facets of the SV–insect cell system. First, although the pattern of glycosylated amino acid residues in the SV envelope proteins is similar to that observed in vertebrate cells, the extent of glycosylation is quite different (Hseih & Robbins, 1984), yet the proteins apparently carry out similar functions (Pollack & Atkinson, 1983). Second, SV infections may result in acute productive phase and/or a low-level persistent phase that lasts for many cell generations without killing the cells (Stollar, 1980). Third, both SV (Riedel & Brown, 1979) and Semliki Forest virus (Newton & Dalgarno, 1983) infections have been reported to induce the production of an antiviral factor that renders cells resistant to acute virus infections. This activity differs from interferon in that it is both cell- and virus-specific. It is presently unknown which of the SV-encoded gene products are responsible for the above phenomena. One possible approach towards answering this question would be to utilize a cloning and expression vector to study the expression of individual SV genes, or sets of genes, in insect cells to ascertain the role of each. Most of the traditional vector systems currently available, e.g. papovaviruses (Mulligan et al., 1979) or retroviruses (Wei et al., 1981), are designed for, and limited to, use in mammalian cell systems. One potential solution to this problem may be to try a different vector system, namely vaccinia virus.

Vaccinia virus (VV) has a number of unique biological attributes that contribute to its utility as a eukaryotic cloning and expression vector (Smith & Moss, 1984), not the least of which is a very broad host range which allows genes to be shuttled between different strains and species of cells. Furthermore, previous experiments have shown that even in cell lines that do not support a lytic VV infection, i.e. non-permissive hosts, VV is able to initiate early phases of its replication cycle and to direct the regulated expression of its early gene products (Hruby et al., 1980). The extent to which VV is able to cross species barriers has not yet been exhaustively tested but, to date, no apparent limitations have been identified. We have recently constructed a recombinant VV which contains and expresses an intact 26S cDNA insert that encodes the SV structural proteins.
Fig. 1. Expression of SV proteins. Monolayers of mosquito (AA) or monkey (BSC) cells were mock-infected (MI), or infected at a multiplicity of 10 p.f.u./cell with wild-type VV (WT), VV:3S (3S) or Sindbis virus (SV). The infected monolayers were labelled with 10 μCi/ml [35S]methionine from 0 to 24 h post-infection. Cell-free extracts were prepared and the radioactive proteins immunoprecipitated with either preimmune (P) or polyclonal anti-SV (S) serum. The immunoprecipitated proteins were then analysed by SDS-polyacrylamide gel (10%).

Table 1. One-step vaccinia virus growth in monkey and mosquito cells*

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Time (h)</th>
<th>Titre (p.f.u./dish)</th>
<th>Relative yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSC40</td>
<td>0</td>
<td>3.06 × 10⁶</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>4.14 × 10⁸</td>
<td>135.29</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>5.32 × 10⁸</td>
<td>173.86</td>
</tr>
<tr>
<td>A. albopictus</td>
<td>0</td>
<td>1.16 × 10⁶</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>4.82 × 10⁵</td>
<td>0.42</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>4.06 × 10⁵</td>
<td>0.35</td>
</tr>
</tbody>
</table>

* Sixty mm dishes of BSC40 cells (4.6 × 10⁶ cells/dish) and A. albopictus cells (7 × 10⁶) were infected at a multiplicity of 10 p.f.u./cell with wild-type VV. After adsorption the infected BSC40 cells were incubated at 37 °C and the infected A. albopictus cells were placed at 31 °C. Crude lysates were prepared from individual dishes at 0, 24 and 48 h post-infection and the titre of infectious virus was determined by plaque assay on monolayers of BSC40 cells.
polypeptides E1, E2 and C (Rice et al., 1985). It was therefore of interest to determine if this recombinant (VV:3S) would be able to infect insect cells and express the SV proteins, and if so whether the SV proteins would be expressed in a manner consistent with a bona fide wild-type SV infection.

A continuous line of Aedes albopictus mosquito cells, was obtained and adapted to growth in Eagle’s MEM and maintained at 28 °C. Monolayers of mosquito cells were infected at a multiplicity of 10 p.f.u./cell with wild-type VV (Hruby et al., 1979). After viral adsorption the infected monolayers were placed at 31 °C. This temperature was chosen as a compromise between the normal growth temperature of the cells and the optimal growth temperature of VV, 37 °C. The monolayers were observed for several days and there was no visible cytopathic effect nor did any viral plaques develop that could be seen by either neutral red or crystal violet staining (data not shown). Mosquito cells infected in this manner with VV were able to continue growing through numerous subcultivations with no apparent reduction in their growth rate. To
determine if any VV replication was occurring, single-step infections were set up in both mosquito cells and a permissive monkey kidney cell line, BSC40. The data in Table 1 demonstrate that whereas the BSC40 cells supported a vigorous VV infection (174 p.f.u./cell), there was no evidence of VV replication in the mosquito cells (0.4 p.f.u./cell) after 48 h of infection. By all the criteria listed above, mosquito cells seemed to be non-permissive for a productive VV infection. However, it was still possible that VV was capable of expressing some of its genetic information under these conditions.

Monolayers of mosquito cells were infected with wild-type VV, VV:3S or SV in the presence of $[^{35}S]$methionine. VV-infected cell extracts were then examined by immunoprecipitation with polyclonal antisera directed against SV structural proteins followed by polyacrylamide gel electrophoresis (Studier, 1973) and fluorography (Bonner & Laskey, 1974) to see whether any SV proteins were being expressed (Fig. 1). It was apparent that VV:3S directed the synthesis of immunoreactive polypeptides that co-migrated with authentic SV structural proteins. These proteins were not present in the control lanes (MI or WT) and were not precipitated by preimmune sera. The identity of the VV:3S-encoded proteins was confirmed by immunoprecipitation with monospecific antisera directed against each of the individual SV proteins (data not shown). It can be seen that the SV proteins derived from the VV:3S recombinant co-migrated with authentic SV proteins, whether they are synthesized in mosquito cells (Fig. 1) or mammalian cells (Rice et al., 1985). This implies that the differences in glycosylation patterns characteristic of SV proteins made in insect cells are conserved on the recombinant-derived proteins.

To examine the kinetics of VV:3S-derived SV protein expression in infected mosquito cells, a pulse labelling experiment was carried out (Fig. 2). A low level of SV protein expression was evident during the first 2 h of infection. The rate of synthesis increased markedly after that with a peak of SV protein expression apparent between 4 and 6 h post-infection. Synthesis of the SV proteins continued at later time points but at a reduced rate.

The results of these experiments are important for two reasons. First, they demonstrate that VV may be used as vector system with which to mobilize genetic information into insect cells and express it, without the lethal side-effects of a productive infection. Second, it will now be of interest to use the VV:3S recombinant to study SV protein glycosylation and transport, and to determine which of the SV proteins are involved with the induction of the antiviral factor and persistent infection.

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REFERENCES


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