Egyptian IN VITRO and IN VIVO Baculovirus Complex Models for Recombination Studies

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Abstract - Three major lepidopteran pests in Egypt, namely Phthorimaea operculella, Spodoptera littoralis and Sesamia cretica, are infected by a specific granulovirus (GV) and also nucleopolyhedroviruses (NPVs) with different degrees in their respective pathogenicity and persistency. This local situation and the existence of several isolates of these different viruses offer exceptional potentialities for the development of genetic studies, because of the availability of susceptible cell lines established from embryonic cells of the three species.

Using experimental recombinations between GVs and also between GVs and NPVs will permit us to study the phenomena which regulate the viral specificity, to study at the genetic level the differential biological properties of the baculoviruses, and finally to increase their potentialities.

Key words - Potato tuber moth, Phthorimaea operculella, cotton leafworm, Spodoptera littoralis, maize stem borer, Sesamia cretica, granulovirus, nucleopolyhedrovirus, recombination, in vitro, cell line.

INTRODUCTION

Only baculoviruses can be used as biological control agents against insects until now. A special attention is given to these viruses in Egypt, because they are promising to control these strategic pests: the potato tuber moth Phthorimaea operculella (Zeller), a major pest in storage and fields, the polyphagous cotton leafworm Spodoptera littoralis (Boisdouval) and the maize stem borer Sesamia cretica (Lederer).

IN VIVO PERMISSIVENESS

Each of these three lepidoptera are infected by a specific granulovirus (GV) and also nucleopolyhedroviruses (NPVs) displaying different degrees in their respective pathogenicity and persistency. Many studies were developed on two potential NPVs. Several isolates of the Spodoptera littoralis-nucleopolyhedrovirus (SpliNPV) were detected in Egypt - They are only highly pathogenic and persistent for S. littoralis larvae (KHAMISS 1997). The best known nucleopolyhedrovirus isolated from Autographa californica (Speyer) (AcMNpV) is not originated from Egypt. However, a specific isolate is present on the cotton pink bollworm Pectinophora gossypiella (Saunders) (MONSARRAT et al. 1995). This virus
already used both as a biological control agent and for biotechnological studies, can infect locally \textit{S. cretica}. It acts at a high rate and when high doses were used. It can also cause post-natal mortalities on \textit{Ph. operculella} (TAHA et al. 1995).

The three granuloviruses are highly specific and no cross-infection could be detected \textit{in vivo}, even when high doses were used. On \textit{S. cretica}, one isolate (SecrGV) with a high pathogenicity could be identified in Egypt (FÉDÈRE et al. 1993). Two isolates of \textit{Spodoptera littoralis}-granulovirus (SpliGV) with late and slow pathogenicity, were recently found on \textit{S. littoralis} in Egypt. As no Egyptian isolate could be detected locally on \textit{Ph. operculella}, one of the numerous isolates described all over the world is actually used in biological control programmes. This is the highly pathogenic Tunisian isolate (PhopGV), a gift from the International Potato Center (ICP, Perou).

\begin{figure}
\centering
\includegraphics[width=0.8\textwidth]{fig1.png}
\caption{In vivo baculovirus replication models.}
\end{figure}

\textbf{IN VITRO PERMISSIVENESS}

\textit{In vitro} cultures have been used for a long time to replicate the nucleopolyhedroviruses but it is not the case for the granuloviruses. Only recently, did we obtain in our laboratory the \textit{in vitro} replication of several GVs once, under special conditions, the cell lines were established (LÉRY et al. 1997c). The \textit{in vitro} replication of PhopGV in an homologous cell line Po95, deriving from embryonic cells, induces a spectacular cytopathic effect and lysis (LÉRY et al. 1997b) allowing us to clone for the first time the Tunisian isolate and to determine its great heterogeneity (LÉRY et al. 1998).

\begin{table}
\centering
\caption{In vitro baculovirus replication models.}
\begin{tabular}{|l|c|c|c|c|c|c|}
\hline
\textbf{insect} & \textbf{cell line} & \textbf{PhopGV} & \textbf{SpliGV} & \textbf{SecrGV} & \textbf{SpliNPV} & \textbf{AcMNPV} \\
\hline
\textit{P. operculella} & Po93 & - & - & - & - & *** \\
& Po95 & * & ** & - & - & ** \\
\textit{S. littoralis} & S152 & - & - & ± & *** & - \\
& S196 & ** & ** & - & - & - \\
\textit{S. frugiperda} & Sf9 & - & - & ± & - & *** \\
\hline
\end{tabular}
\end{table}
Baculovirus recombinations

All the baculoviruses isolated on the three-pest complex could be multiplied on different lepidopteran cell lines established in our laboratory from the three hosts under our conditions of infection and transfection (LÉRY et al. 1997b) (Table I). Among all the cell lines established from embryos of *Ph. operculella*, only one (Po95) could replicate the homologous granulovirus but also the SpliGV. All these cell lines replicate AcMNPV (LÉRY et al. 1997a). The newly established cell line of *S. littoralis* deriving from ovarioles (SI96) replicates the two GVs, but also the SpliNPV. Finally, the cell line recently established from embryos of *S. cretica* (Sc98) replicates its homologous GV, but also AcMNPV. The permissiveness against the other viruses is under process for this cell line.

Considering these results, it is worthwhile to notice that granuloviruses are not as specific *in vitro* as they are *in vivo*, and that on the contrary, the SpliNPV is very specific both *in vitro* and *in vivo* in our models. This is not the normal rule and opinion for these two groups of viruses.

RECOMBINATIONS BETWEEN GVS AND GV-NPV

The phenomenons which regulate this specificity could be studied at the molecular level, using recombinations between different GVs, and also between GVs and NPVs. Such experiments are in process in our laboratory (Table II). The choice of the models used were directly suggested by the possible increase in the pathogenicity and host spectrum of the different recombinant viruses we could obtain. A recombinant AcMNPV which could infect *Ph. operculella* larvae, inducing drastic mortality, would be of great importance as an alternative virus to control the potato tuber moth. The construction of a PhopGV recombinant which could be replicated on *S. littoralis* larvae could be of great interest for its mass production. The obtainment of a recombinant SpliGV with an increase in its pathogenicity and speed of action on young instar larvae could be of value to control *S. littoralis*.

<table>
<thead>
<tr>
<th>Virus</th>
<th>Material</th>
<th>Cell line</th>
<th>1st filter</th>
<th>2nd filter</th>
<th>Amplification</th>
</tr>
</thead>
<tbody>
<tr>
<td>AcMNPV</td>
<td>Virus or DNA</td>
<td>Po95</td>
<td>S196</td>
<td>Sf9</td>
<td><em>Ph. operculella</em> or (S. cretica)</td>
</tr>
<tr>
<td>PhopGV</td>
<td>Virus or DNA</td>
<td>Po95</td>
<td>S196</td>
<td>Po95</td>
<td><em>Ph. operculella</em> or <em>S. littoralis</em></td>
</tr>
<tr>
<td>SpliGV</td>
<td>Total DNA</td>
<td>Po95</td>
<td>S196</td>
<td>S152</td>
<td><em>S. littoralis</em></td>
</tr>
<tr>
<td>PhopGV</td>
<td>Fragmented DNA</td>
<td>S196</td>
<td>Po95</td>
<td>S152</td>
<td><em>S. littoralis</em></td>
</tr>
<tr>
<td>SpliGV</td>
<td>Total DNA</td>
<td>S196</td>
<td>Po95</td>
<td></td>
<td><em>S. littoralis</em></td>
</tr>
<tr>
<td>SpliNPV</td>
<td>Virus or DNA</td>
<td>SI96</td>
<td>Po95</td>
<td></td>
<td><em>S. littoralis</em></td>
</tr>
</tbody>
</table>

After a systematic cloning of the recombinants obtained and a comparison of their DNA profiles, the genes responsible for the increase in potentialities could be determined, cloned and sequenced. The pool of genes constituted will permit to elaborate a new strategy to study at the genetic level the differential properties of these baculoviruses in relation with the ecological conditions. Deeper understanding of the phenomenons of specificity could be reached (CROIZIER et al. 1994).
X. Léry et al.

In a near future, baculoviruses might increase their potentialities, based on random recombinations between different baculoviruses or specific baculovirus gene insertions. They could be able to control the three main lepidopteran pests in Egypt.

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