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EGYPTIAN *IN VITRO* AND *IN VIVO* BACULOVIRUS COMPLEX MODELS FOR RECOMBINATION STUDIES

Xavier LÉRY, Ahmed TAHA, Omaima KHAMISS, Adly NOUR-El-DIN & Joseph GIANNOTTI

Laboratoire d'Entomovirologie, Faculté d'Agriculture-ORSTOM, BO 26, Giza Code 12211, Cairo, Egypt

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 phenomenons 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 three strategical pests: the potato tuber moth *Phthorimaea operculella* (Zeller), a major pest in storage and fields, the polyphagous cotton leafworm *Spodoptera littoralis* (Boisduval) 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 developped 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 *S. cretica*. It acts at a high rate and when high doses were used. It can also cause post-natal mortalities on *Ph. operculella* (TAHA *et al.* 1995).

The three granuloviruses are highly specific and no cross-infection could be detected *in vivo*, even when high doses were used. On *S. cretica*, one isolate (SecrGV) with a high pathogenicity could be identified in Egypt (FÉDIÈRE *et al.* 1993). Two isolates of *Spodoptera littoralis*-granulovirus (SpliGV) with late and slow pathogenicity, were recently found on *S. littoralis* in Egypt. As no Egyptian isolate could be detected locally on *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).

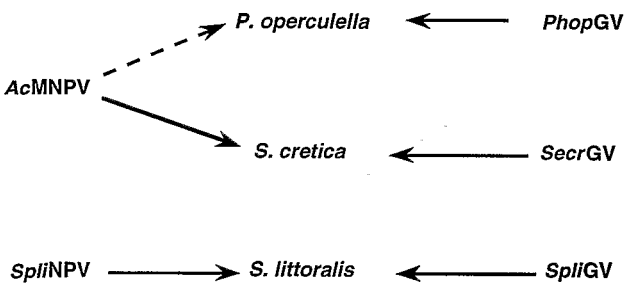

Fig. 1. – *In vivo* baculovirus replication models.

Such a local situation, regarding the differential pathogenicity, specificity and persistency of these baculoviruses (Fig. 1), and the existence of several isolates for these different viruses, offer exceptional potentialities for the development of genetic studies (KONDO & MAEDA 1991). For that purpose, the availability of susceptible cell lines was determinant.

IN VITRO PERMISSIVENESS

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 *in vitro* replication of several GVs once, under special conditions, the cell lines were established (LÉRY *et al.* 1997c). The *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).

Table I. – *In vitro* baculovirus replication models.

insect	cell line	PhopGV	SpliGV	SecrGV	SpliNPV	AcMNPV
P. operculella	Po93	–	–	–	–	***
	Po95	*	**	–	–	**
S. littoralis	Sl52	–	–	±	***	–
	Sl96	**	**	–	**	–
S. cretica	Sc98	?	?	**	?	***
S. frugiperda	Sf9	–	–	±	–	***

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 (Sl96) 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 worsthwhile 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 II. – *In vitro* recombinations between GVs and GV-NPV.

virus	material	cell line	1st filter	2nd filter	amplification
AcMNPV	virus or DNA				*Ph. opercullella*
		Po95	Sl96	Sf9	or
PhopGV	virus or DNA				*(S. cretica)*
SpliGV	total DNA				
		Po95	Sl96	*Ph. operculella*	*S. littoralis*
PhopGV	fragmented DNA				
SpliGV	fragmented DNA				
		Sl96	Po95	*S. littoralis*	*Ph. operculella*
PhopGV	total DNA				
SpliGV	virus or DNA				
		Sl96	Po95	Sl52	*S. littoralis*
SpliNPV	virus or DNA				

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).

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.

REFERENCES

CROIZIER G., CROIZIER L., ARGAUD O. & POUDEVIGNE D. 1994. – Extension of *Autographa californica* nuclear polyhedrosis virus host range by interspecific replacement of a short DNA sequence in the p143 helicase gene. – *Proceedings of the National Academy of Sciences*, USA 91: 48-52.

FÉDIÈRE G., TAHA A.A., ABOL-ELA S., LÉRY X., ZEDDAM J.L., VEYRUNES J.C. & GIANNOTTI J. 1993. – Mise en évidence d'un virus de granulose chez *Sesamia cretica* Led. (Lepidoptera: Noctuidae) principal ravageur du maïs d'Afrique du Nord-Est: caractérisation de l'ADN génomique et diagnostic viral. – *Comptes-Rendus Hebdomadaires des Séances de l'Académie des Sciences*, Paris 316: 1350-1354.

KHAMISS O. 1997. – Studies on the natural genomic interference of *Spodoptera littoralis* baculoviruses and their stability in the presence of insecticides. – Thesis, Faculty of Agriculture, University of Cairo, Egypt, 172 pp.

KONDO A., MAEDA S. 1991. – Host range expansion by recombination of the baculoviruses *Bombyx mori* nuclear polyhedrosis virus and *Autographa californica* nuclear polyhedrosis virus. – *Journal of Virology* 65: 3625-3632.

LÉRY X., El-TARRAS A., ABOL-ELA S. & GIANNOTTI J. 1997a. – Yield and activity of *Autographa californica* nuclear polyhedrosis virus and *Phthorimaea operculella* ganulosis virus in cloned and uncloned cell lines of *P. operculella*. – *Cytotechnology* 26: 103-110.

LÉRY X., GIANNOTTI J., TAHA A., RAVALEC M. & ABOL-ELA S. 1997b. – Multiplication of a granulosis virus isolated from the potato tuber moth in a new established cell line of *Phthorimaea operculella*. – *In Vitro Cell Development Biology* 33: 640-646.

LÉRY X., KHAMISS O., NOUR-El-DIN A., ABOL-ELA S. & GIANNOTTI, J. 1997c. – Multiplication of *Spodoptera littoralis* granulosis virus in a cell line established from *Phthorimaea operculella*. – *Acta Virologica* 41: 169-174

LÉRY X., ABOL-ELA S. & GIANNOTTI J. 1998. – Genetic heterogeneity of the *Phthorimaea operculella* granulovirus: restriction analysis of wild-type isolates and clones obtained *in vitro*. – *Acta Virologica* 42: 13-21.

MONSARRAT A., El-HUSSEINI M.G. & ABDEL-HAMID I. 1995. – Studies on new isolate of *Autographa californica* nuclear polyhedrosis virus on the pink bollworm *Pectinophora gossypiella* Saunders. – *Journal of the Union Arab Biology* 3: 387-399.

TAHA A., GIANNOTTI J., LÉRY X., RAVALEC M. & ABOL-ELA S. 1995. – Host range of the *Autographa californica* multicapsid nuclear polyhedrosis virus (AcMNPV) in relation to granulosis viruses susceptible lepidopteran species in Egypt. – *Egyptian Journal of Biological Pest Control* 5: 127-130.