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BARD

FINAL REPORT

PROJECT NO. IS-2999-98

**Isolation of Baculoviruses with Expanded Spectrum of
Action Against Lepidopteran Pests**

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2002

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Date of Submission of the report:

BARD Project Number: IS-2999-98

Project Title: Isolation of baculoviruses with expanded spectrum of action against Lepidopteran pests

Investigators

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Institutions

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Keywords†not appearing in the title and in order of importance. Avoid abbreviations.

Abbreviations commonly used in the report, in alphabetical order

Budget: IS: \$ 138,000

US: \$ 127,000

Total: \$ 265,000

Signature
Principal Investigator

Signature
Authorizing Official, Principal Institution

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Publication Summary (numbers)

	Joint IS/US authorship	US Authors only	Israeli Authors only	Total
Refereed (published, in press, accepted)			1	1
Submitted, in review, in preparation	1	1	1	3
Invited review papers				
Book chapters				
Books				
Master theses		1		1
Ph.D. theses			1	1
Abstracts	1	6	3	10
Not refereed (proceedings, reports, etc.)				

Postdoctoral Training: †List the names and social security/identity numbers of all postdocs who received more than 50% of their funding by the grant.

Cooperation Summary (numbers)

	From US to Israel	From Israel to US	Together, elsewhere	Total
Short Visits & Meetings		1	5	6
Longer Visits (Sabbaticals)				

Description of Cooperation

Both partners utilized recombinant NPVs interchanged between them to study their respective non-permissive insect hosts-virus systems. (The American partner provided various recombinant viruses to the Israeli partner and the Israeli partner provided the *p49* gene and recombinant vAcMNPVs bearing this gene to the American partner). Besides exchanging materials both parties maintained continuous exchange of information using e-mail, correspondence and by meeting each other.

Patent Summary (numbers)

	Israeli inventor (s) only	US inventor (s) only	Joint IS/US inventors	Total
Submitted				
Issued (allowed)				
Licensed				

ABSTRACT

Our long-term goal is to learn to control (expand and restrict) the host range of baculoviruses. In this project our aim was to expand the host range of the prototype baculovirus *Autographa californica* nuclear polyhedrosis virus (AcMNPV) towards American and Israeli pests. To achieve this objective we studied AcMNPV infection in the non-permissive hosts *L. dispar* and *S. littoralis* (Ld652Y and SL2 cells, respectively) as a model system and the major barriers to viral replication. We isolated recombinant baculoviruses with expanded infectivity towards *L. dispar* and *S. littoralis* and tested their infectivity towards other Lepidopteran pests.

The restricted host range displayed by baculoviruses constitutes an obstacle to their further implementation in the control of diverse Lepidopteran pests, increasing the development costs. Our work points out that cellular defenses are major role blocks to AcMNPV replication in non- and semi-permissive hosts. Therefore a major determinant of baculovirus host range is the ability of the virus to effectively counter cellular defenses of host cells. This is exemplified by our findings showing that expressing the viral gene *Ldhfrf-1* overcomes global translation arrest in AcMNPV-infected Ld652Y cells. Our data suggests that Ld652Y cells have two anti-viral defense pathways, because they are subject to global translation arrest when infected with AcMNPV carrying a baculovirus apoptotic suppressor (e.g., wild type AcMNPV carrying *p35*, or recombinant AcMNPV carrying *Opiap*, *Cpiap*, or *p49* genes) but apoptose when infected with AcMNPV-lacking a functional apoptotic suppressor. We have yet to elucidate how *hrf-1* precludes the translation arrest mechanism(s) in AcMNPV-infected Ld652Y cells. Ribosomal profiles of AcMNPV infected Ld652Y cells suggested that translation initiation is a major control point, but we were unable to rule-out a contribution from a block in translation elongation. Phosphorylation of eIF-2 α did not appear to play a role in AcMNPV-induced translation arrest. Mutagenesis studies of *hrf-1* suggest that a highly acidic domain plays a role in precluding translation arrest. Our findings indicate that translation arrest may be linked to apoptosis either through common sensors of virus infection or as a consequence of late events in the virus life-cycle that occur only if apoptosis is suppressed.

AcMNPV replicates poorly in SL2 cells and induces apoptosis. Our studies in AcMNPV-infected SL2 cells led us to conclude that the steady-state levels of IE1 (product of the *ie1* gene, major AcMNPV-transactivator and multifunctional protein) relative to those of the immediate early viral protein IE0, play a critical role in regulating the viral infection. By increasing the IE1/IE0 ratio we achieved AcMNPV replication in *S. littoralis* and we were able to isolate recombinant AcMNPVs that replicated efficiently in *S. littoralis* cells and larvae. Our data that indicated that AcMNPV- infection may be regulated by an interaction between IE1 and IE0 (of previously unknown function). Indeed, we showed that IE1 associates with IE0 by using protein "pull down" and immunoprecipitation approaches. High steady state levels of "functional" IE1 resulted in increased expression of the apoptosis suppressor *p35* facilitating AcMNPV-replication in SL2 cells. Finally, we determined that IE0 accelerates the viral infection in AcMNPV-permissive cells.

Our results show that expressing viral genes that are able to overcome the insect-pest defense system enable to expand baculovirus host range. Scientifically, this project highlights the need to further study the anti-viral defenses of invertebrates not only to maximize the possibilities for manipulating baculovirus genomes, but to better understand the evolutionary underpinnings of the immune systems of vertebrates towards virus infection.

ACHIEVEMENTS

Significance

The high specificity of baculoviruses towards insects, encouraged the use of these friendly and environmentally safe agents in insect control programs and their registration as biological pesticides. However, the restricted host range displayed by baculoviruses (generally limited to single, or closely related, insect species) constitutes an obstacle to their further implementation in the control of diverse Lepidopteran pests and increases development costs. Our work points out that cellular defenses are major role blocks to AcMNPV replication in non- and semi-permissive hosts. Therefore a major determinant of baculovirus host range is the ability of the virus to effectively counter cellular defenses of host cells. We studied the blocks to AcMNPV replication in the non-permissive hosts *L. dispar* and *S. littoralis* (Ld652Y and SL2 cells, respectively). By combining our research efforts we were able to establish that:

1. When infected with AcMNPV Ld652Y cells have two anti-viral defense pathways, global translation and apoptosis. The former occurs only in the presence of an apoptosis suppressor gene (either from AcMNPV or from *S. littoralis* NPV, *Orgyia pseudotsugata* NPV, or *Cydia pomonella* GV). Apoptosis occurs in the absence of an apoptosis- suppressor gene. This indicates that the presence of an apoptosis suppressor gene contributes to the "signaling" of translational arrest.
2. Inhibition of apoptosis is not enough to achieve AcMNPV replication in SL2 cells and high "functional" steady state levels of IE1 (immediate-early 1), the major transactivator viral protein, are required to complete the replication cycle. Regulation of the above "functional" IE1 levels is mediated by IE0 (immediate early-0) viral protein of previously unknown function. This occurs probably by protein-protein interaction.
3. Infection of SL2 cells with AcMNPV expressing *hrf-1* does not relieve the block to AcMNPV replication. This suggests that the cellular response to the viral infection is cell-type specific (at least in part).
4. Recombinant baculoviruses that respond to the above cellular defense mechanisms are able to replicate efficiently in *L. dispar* and *S. littoralis*. Our preliminary data

suggests that some of the above findings can be extended towards other Lepidopteran pests (*H. zea* and *H. armigera*).

These results contribute to understanding the underlying mechanisms involved in viral infections enabling their future manipulations (i.e. augmenting or diminishing virulence) according to human needs.

Agricultural impact and estimated economic impact

The progress made in our study paves the way to obtain new potent means, i.e. AcMNPV with extended host range, to control Lepidopteran pests (compatible with integrated pest management programs), diminishing baculovirus pesticide-production costs. Lepidopteran insects (e.g. species of *Spodoptera* and *Helicoverpa*) constitute major world pests due to their polyphagous nature. The world pesticide market is mature with a growth of 1-2% per year from an estimated base of \$ 31 billion. From this market biologicals contribute about \$ 250 million with 60 % being *Bacillus thuringiensis*-based products. However, a variety of drivers are leading to a massive rate of increase of 25 % per year in biologicals. In many cases crops need to be supplemented by other pest control strategies and microbials could be used rather than broader spectrum synthetic compounds. Baculoviruses fit perfectly with these economic trends. Moreover, we envision that the distinct mode of action of baculoviruses will allow their utilization in situations where transgenic crops failed, partially or completely, to contain the pest and where transgenic crops are not available. Thus contributing to the reduction of environmental and human health risks associated with the extensive spread of chemical pesticides of broad spectrum of action.

Details of Cooperation

Both partners utilized recombinant NPVs interchanged between them to study their respective non-permissive insect hosts-virus systems. (The American partner provided various recombinant viruses to the Israeli partner and the Israeli partner provided the *p49* gene and recombinant vAcMNPVs bearing this gene to the American partner). Besides exchanging materials both parties maintained continuous exchange of information using e-mail, correspondence and by meeting in the U.S.A. in Colorado, Madison and Massachusetts during The American Society for Virology meetings (1999, 2000 and 2001) and in Mexico and The

Netherlands during The Society for Invertebrate Pathology meetings (2000 and 2001). Nor Chejanovsky visited Suzanne Thiem's lab to discuss the results obtained and devise future directions of the cooperation including applications to granting institutions (2003).

These facts improved the abilities of the American and the Israeli teams to make progress to achieve their common goals in understanding the factors that influence the ability of AcMNPV to replicate in non- and semi-permissive insect-systems, to facilitate implementation of baculoviruses for pest control in agriculture.

LIST OF PUBLICATIONS

1. Lu L., Du Q. and N. Chejanovsky (2003). Reduced expression of the immediate early protein IE0 enables efficient replication of *Autographa californica* multiple nucleopolyhedrovirus in poorly permissive *Spodoptera littoralis* cells. J. Virology, Vol. 77 (in press).
2. Thiem S. M. and N. Chejanovsky (2002). The role of baculovirus apoptotic suppressors in AcMNPV-mediated translation arrest in Ld652Y cells. (submitted).
3. Lu, L. and N. Chejanovsky (2002). Impact of the immediate-early 0 protein on the replication of the *Autographa californica* nucleopolyhedrovirus (in preparation).
4. Ikeda, M., Reimbold E. A. and S.M. Thiem. Functional analysis of the host – range gene , hrf -1, from *Lymantria dispar* nucleopolyhedrovirus (in preparation).

APPENDIX

I- Our studies in AcMNPV-infected SL2 cells that led us to conclude that the steady-state levels of IE1 (product of the *ie1* gene, major AcMNPV-transactivator and multifunctional protein) relative to those of the immediate early viral protein IE0 play a critical role in regulating the viral infection are described in publication 1. The impact of IE0 on AcMNPV infection is a paper in preparation and a brief summary of the data is included below.

1. Our recent data suggest that IE0 may interact with IE1 during infection. To investigate this interaction we performed immunoprecipitation studies with a specific anti-IE0 antibody that does not recognize IE1. For that purpose we engineered a recombinant plasmid able to express the first 54 amino acid residues present in IE0 and absent in IE1 (since IE1 amino acid