Journal of Plant Protection Research ISSN 1427-4337

ORIGINAL ARTICLE

Topical treatment of LdMNPV-infected gypsy moth caterpillars with 18 nucleotides long antisense fragment from LdMNPV IAP3 gene triggers higher levels of apoptosis in infected cells and mortality of the pest

Volodymyr V. Oberemok[1], Kateryna V. Laikova[2], Alexey S. Zaitsev[1], Palmah M. Nyadar[1]*, Yuri I. Gninenko[3], Vladimir A. Gushchin[4,5], Valentin V. Makarov[6], Alexey A. Agranovsky[4,7]

[1] Department of Biochemistry, Taurida Academy of V.I. Vernadsky Crimean Federal University, 295007 Simferopol, Crimea
[2] Department of Biochemistry, Medical Academy of V.I. Vernadsky Crimean Federal University, 295051 Simferopol, Crimea
[3] All-Russian Research Institute for Silviculture and Mechanization of Forestry, 141200 Pushkino, Russia
[4] Department of Virology, Biological Faculty, Lomonosov Moscow State University, 119991 Moscow, Russia
[5] Genetics Department of Russian Centre of Forest Health, 141207 Pushkino, Russia
[6] Belozersky Institute of Physico-Chemical Biology, Lomonosov Moscow State University, 119991 Moscow, Russia
[7] Center of Bioengineering, Russian Academy of Sciences, 117312 Moscow, Russia

Vol. 57, No. 1: 18–24, 2017

DOI: 10.1515/jppr-2017-0003

Received: September 28, 2016
Accepted: January 30, 2017

*Corresponding address:
biopalmgene@gmail.com
dna.insecticides@yahoo.com

Abstract

The high efficiency of baculovirus infection is partially explained by the ability of the virus to suppress host defense machinery connected with the apoptosis pathway. Members of the baculovirus gene family, inhibitors of apoptosis (vIAPs), have been shown to inhibit apoptosis in baculovirus-infected cells. Here we showed that treatment of the LdMNPV-infected 1st instar gypsy moth (*Lymantria dispar*) caterpillars with sense (oligoBIR) and antisense (oligoRING) DNA oligonucleotides from the LdMNPV IAP3 gene induced elevated mortality of the insects. Apoptotic DNA ladder assay showed that the leading role in this phenomenon is played by the antisense oligoRING fragment of the vIAP3 gene. These results imply that the application of both antisense DNA oligonucleotides from vIAP genes and baculovirus preparations (one following the other) may be a potential method for plant protection against insect pests.

Key words: baculoviral infection, DNA insecticides, DNA oligonucleotides, forest and crop protection, gypsy moth, IAP genes

Introduction

Baculoviral infection is highly efficient in susceptible insect hosts. For *Autographa californica* multicapsid nuclear polyhedrosis virus it was found that 12 hours post infection, the viral transcripts comprised 38% of the total cellular mRNA (Clem and Passarelli 2013). In the course of infection, the death of host insects is delayed until baculovirus accomplishes its relatively slow reproduction. Baculoviruses are represented by two phenotypes, namely the budded virus and the occlusion-derived virus. The budded virus transmits viral infection from cell to cell whereas occlusion bodies (OBs) that contain occlusion derived virus (ODV) are responsible for insect to insect transmission and are produced in infected cell nuclei (Jehle *et al.* 2006) until they are completely filled up with the particles. As a result, one larva may contain about 10^{10} polyhedra comprising more than 30% of an insect's dry mass (Miller *et al.* 1983). To achieve such efficient infection rates, baculovirus induces pro-apoptotic DNA Damage Response (DDR) (Mitchell and Friesen

2012). Induction of the DDR by the viral replication increases virus yields up to 100,000-fold (Mitchell and Friesen 2012) and causes the so-called conflict of genomes (Weitzman *et al*. 2010). The DDR induces depletion of host inhibitor-of-apoptosis genes (hIAP) and thus promotes cell death (Vandergaast *et al*. 2011). To overcome the consequences of the DDR activation, baculoviruses employ special anti-apoptotic proteins (vIAPs) (Srinivasula and Ashwell 2008; Ikeda *et al*. 2011). These proteins either interfere directly with the cellular apoptotic proteins, or alter the activity of cellular genes, leading to an anti-apoptotic state (Agol 1997). Baculoviruses have two classes of anti-apoptotic genes, the gene for p35 caspase inhibitor and the IAP genes (IAP1-IAP5) (Bertin *et al*. 1996; Manji *et al*. 1997; Ikeda *et al*. 2013; Clem 2015). Most of the baculoviral IAPs with anti-apoptotic functions belong to the IAP3 group, with certain exceptions (Ikeda *et al*. 2011).

Phylogenetic analysis of baculoviral IAP genes indicated their host origin. The capture of these genes from insect genome likely occurred at least twice in the course of evolution (Hughes 2002; Clem 2015). Despite the evolutionary relatedness of the vIAP proteins and hIAP proteins, there is an important structural distinction between them. In contrast to the hIAPs, which possess a specific N-terminal domain and are negatively regulated by signal-induced N-terminal degrons upon virus infection, the vIAPs do not have an equivalent N-terminal domain (Cerio *et al*. 2010; Vandergaast *at al*. 2015). This makes vIAPs more stable and active as apoptosis inhibitors (Cerio *et al*. 2010) controlling host cell fate. The conservative part of IAPs contains BIR (baculovirus IAP repeat) domain and RING (really interesting new gene) domain. BIRs interact with processed N-terminus of apoptosis inducer Hid (head involution defective) protein. In turn, RING domain functions as a module that confers ubiquitin protein ligase (E3) activity and, in conjunction with an ubiquitin activity enzyme (E1) and an ubiquitin conjugating enzyme (E2), catalyze the transfer of ubiquitin to target apoptosis proteins (Srinivasula and Ashwell 2008; Clem 2015).

Recently we tested IAP-specific DNA oligonucleotides (Oligo-IAPs) as potential insecticides. We used two Oligo-IAP sequences which were based on the fragments of BIR (sense chain) and RING (antisense chain) domains from *Lymantria dispar* multicapsid nuclear polyhedrosis virus (LdMNPV) IAP3 gene (Oberemok 2008a; Oberemok 2011; Simchuk *et al*. 2012; Oberemok and Skorokhod 2014; Oberemok and Nyadar 2015). The insecticidal effect of Oligo-IAP fragments was detected on LdMNPV-free caterpillars (Oberemok and Skorokhod 2014; Oberemok *et al*. 2015a, b; Oberemok *et al*. 2016). In our opinion, in the case of non-infected gypsy moth caterpillars, a host anti-apoptosis mRNA serves as target mRNA for complementary interaction with viral Oligo-IAP fragments initiating post-transcriptional silencing of the target host anti-apoptosis gene which subsequently leads to apoptosis and death of the insect. Our recent findings prove this hypothesis (Oberemok and Skorokhod 2014; Oberemok and Nyadar 2015; Oberemok *et al*. 2016). Interestingly, Yamada *et al*. (2012) found that the LdMNPV IAP3 gene induced apoptosis in Ld652Y cells in a transient expression assay but the authors say that it remains inconclusive as to whether virus Ld-IAP2 and Ld-IAP3 function as pro-apoptotic proteins in LdMNPV-infected Ld652Y cells or not. It would be interesting to study the fragments of other single-stranded fragments of LdMNPV IAP genes as DNA insecticides, such as Lp-Apsup (*L. dispar* apoptosis suppressor) (Yamada *et al*. 2011).

In our previous experiments not all LdMNPV-free gypsy moth caterpillars tested were sensitive to treatment with Oligo-IAP fragments (sense oligoBIR and antisense oligoRING) suggesting that individual insect populations may either possess different susceptibility to the specific oligonucleotides, or that an additional specific factor (or factors) might be necessary for activation of the cell apoptosis–anti-apoptosis system. In the absence of stress factors (e.g. viral infection) concentrations of the target anti-apoptosis mRNA would be low, and a pronounced insecticidal effect might not be generated by the antisense oligoRING fragment. For example, recently we showed that expression of host IAP-1 gene was significantly (3.45±1.16) higher in LdMNPV-infected control in comparison with non-infected control ($p < 0.05$) on the 14th day after the infection (to be published), thus, the cell apoptosis–anti-apoptosis system in LdMNPV-infected insects was more activated.

In this paper our main hypothesis is that in the case of LdMNPV-infected caterpillars, the mRNA of the LdMNPV IAP3 gene might be a complementary sequence interacting with oligoRING and initiating post-transcriptional silencing of the target baculoviral anti-apoptosis gene. Inactivation of expression of LdMNPV IAP3 gene might help infected host cells undergo apoptosis and the affected insect might die. Thus, in the case of LdMNPV-infected gypsy moth cells there always will be the target viral IAP3 mRNA to initiate post-transcriptional silencing in a manner similar to the mode of action of antisense oligonucleotides (Dias and Stein 2002). Relatively long double-stranded RNA fragments (Gu and Knipple 2013), and the DNA insecticides might work more often on LdMNPV-infected gypsy moth. In this work, to test the effect of Oligo-IAP fragments on LdMNPV-infected insects, the gypsy moth caterpillars were initially infected with LdMNPV, and then treated with DNA oligonucleotides.

Materials and Methods

Three individual egg masses of gypsy moths were collected from a coniferous forest in the Vologda region (northwestern Russia), followed by randomization and rearing of insects under standard conditions. Two groups of 1st instar caterpillars were used in the experiments, the control non-infected group and the group pre-infected with LdMNPV. Insects in both groups were treated with sterile water, oligo(A)$_{18}$ (5'-dAAA AAA AAA AAA AAA AAA-3'), or Oligo-IAP fragments. An average of 20 caterpillars were subjected to a treatment (with water, oligo(A)$_{18}$, or Oligo-IAPs) in each group. Each experiment was independently repeated for every group in triplicate. Prior to the experiments, the gypsy moth caterpillars (grown on standard wheat germ-based diet at 25°C) were checked for LdMNPV infection using PCR with two oligonucleotide primers specific to the viral capsid gene p39: 5'-dACG TTC TCG TTG AAC GTG CTG-3' (forward primer), 5'-dCTG GTG AAC CAC AAA ACC CTG-3' (reverse primer) (Oberemok 2011; Oberemok *et al*. 2016). All tested caterpillars were free of the virus (Fig. S1, see lanes 1–10). HPLC-grade Oligo-IAP fragments were synthesized by Metabion International AG (Germany): 5'-dGCC GGC GGA ACT GGC CCA-3' (LdMNPV strain 3054, BIR domain, sense chain; http://www.ictvonline.org) and 5'-dCGA CGT GGT GGC ACG GCG-3' (LdMNPV strain 3054, RING domain, antisense chain; http://www.ictvonline.org). A sterile water solution of an equimolar mixture of Oligo-IAP fragments (100 pmol · µl^{-1}), oligo(A), or sterile water was applied as a small drop (0.3 µl) to the middle of a caterpillar's body (approximately 3rd–4th setae of the abdomen) with a pipette and was absorbed by the caterpillar's body for 15–20 min at room temperature. Caterpillars from the control groups were treated either with distilled water or oligo(A): 5'-dAAA AAA AAA AAA AAA AAA-3' synthesized by Metabion International AG (Germany). The 11-day period for rearing caterpillars after treatment with Oligo-DNAs was chosen as representative to show the dynamics of their action.

To infect gypsy moth caterpillars with LdMNPV, the baculovirus preparation "VIRIN-NSh" (Kyrgyzstan) was used. After 1-day starvation, the insects were fed a diet containing 30 000 polyhedra · mg^{-1} for 2 days. The caterpillars were then transferred to a non-infected diet. Phase contrast microscopy and Goryayev's chamber (hematocytometer) were used to count viral polyhedrons in the preparation. By weighing the remains of the infected diet, we calculated the virus dose of ca. 400–500 polyhedrons taken in by each caterpillar. The caterpillars were treated with water, oligo(A), or Oligo-IAP fragments 48 hours after infection with LdMNPV.

Quick Apoptotic DNA Ladder Detection Kit (Life Technologies, USA) was used according to the manufacturer's instructions to determine the level of apoptotic DNA fragmentation in LdMNPV-infected insects.

We used non-parametric Pearson's chi-squared test (χ^2) with Yates's correction to evaluate the significance of difference in mortality between caterpillars of experimental groups (STATISTICA 7 software).

Results and Discussion

The Oligo-IAP fragments did not have a statistically significant effect ($p > 0.05$) on the viability of LdMNPV-free caterpillars after 11 days of observation in comparison with water- and oligo(A)-treated controls, with recorded death of approximately 3–15% of the caterpillars in each group (Table 1).

On the 5th day after treatment with Oligo-IAP fragments, a significant increase in mortality of LdMNPV-infected caterpillars in comparison with water-treated controls was observed (Fig. 1 and Table 1). Although some elevated mortality of oligo(A)-treated caterpillars compared to water-treated control was recorded, the effect was statistically insignificant (Fig. 1 and Table 1). An average of 29.8%, 17.0%, and 3.0% of

Table 1. Statistical analysis of the insecticidal effect of Oligo-IAP and oligo(A)$_{18}$ on LdMNPV-infected and non-infected gypsy moth caterpillars. Pearson's chi-squared test (χ^2) with Yates's correction was used to evaluate the significance in each treatment. χ^2 values were calculated for experimental groups vs. water control group on the 5th day after the treatment; the experiment was performed in triplicate; *is given when $p < 0.01$

Experimental groups		χ^2 values	Number of caterpillars in each experimental group
Non-infected	Oligo-IAPs vs. water control	0.23	144
	oligo(A)$_{18}$ vs. water control	0.06	142
Infected	Oligo-IAPs vs. water control	12.01*	127
	oligo(A)$_{18}$ vs. water control	3.71	121

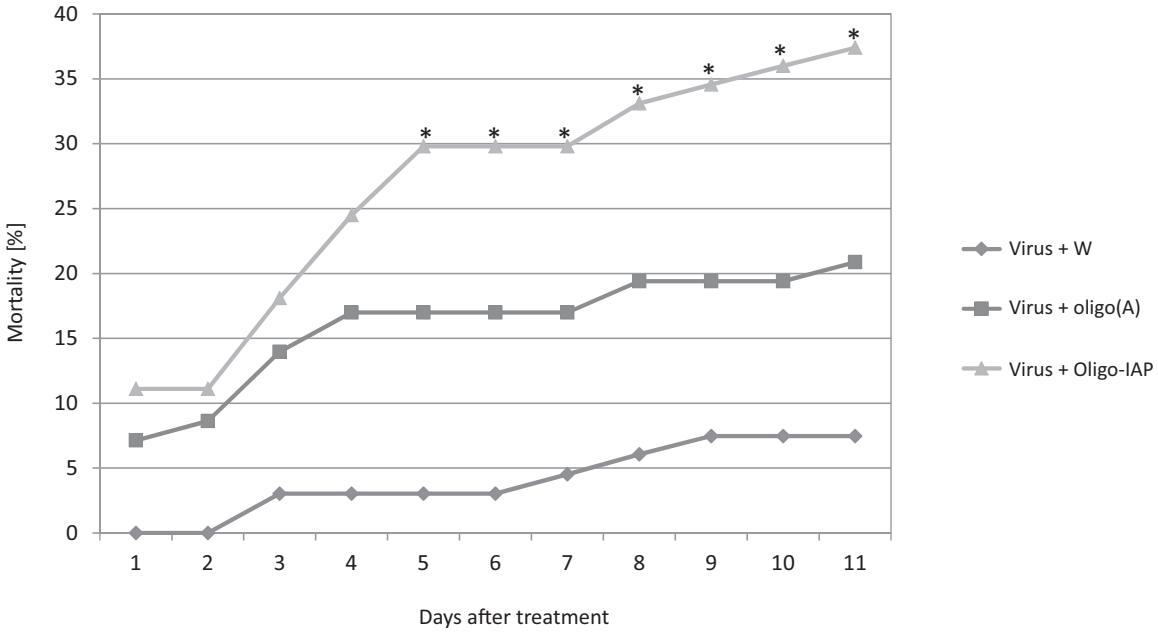

Fig. 1. Mortality in different groups of gypsy moth (*Lymantria dispar*) caterpillars pre-infected with LdMNPV and treated with distilled water (W), oligo(A), or DNA oligonucleotides from LdMNPV IAP-3 gene (Oligo-IAP); *is given when $p < 0.01$

caterpillars died in the groups treated, respectively, with Oligo-IAP fragments, oligo(A), and distilled water. Between the 5th and 11th days post-treatment, the percentage of dead insects reached 37.5%, 20.9%, and 7.5% in the groups treated with Oligo-IAP fragments, oligo(A), and distilled water, respectively. Again, only the treatment with Oligo-IAP fragments had a statistically significant effect on insect mortality ($\chi^2 = 13.1$; $p < 0.01$).

Although the mortality in the Oligo-IAP group of the infected caterpillars was significant, it did not reach very high levels. A plausible explanation is that not all the investigated caterpillars were successfully infected with the applied virus dose. Ebling *et al.* (2004) and Duan *et al.* (2011) reported that LD_{50} for the 2nd instar gypsy moth caterpillars is approximately 100–700 virus polyhedra per larva. Even though each caterpillar in our experiment acquired ca. 400–500 virus polyhedra, we detected LdMNPV by the PCR in only ca. 30% of the insects fed the infected diet (Fig. S2, see lanes 2 and 3). Obviously, the outcome of the application of DNA insecticides based on the LdMNPV IAP3 gene, particularly antisense oligoRING, depends on the dynamics of target mRNA synthesis and the breakdown of the target anti-apoptosis gene. In our opinion, in the case of LdMNPV-infected caterpillars, mRNA of LdMNPV IAP3 gene is a target complementary sequence that interacts with antisense oligoRING, initiating post-transcriptional silencing of a target anti-apoptosis gene in a manner similar to the mode of action of antisense oligonucleotides (Dias and Stein 2002) and relatively long dsRNAs (Gu and Knipple 2013) which subsequently leads to apoptosis of cells and death of the whole insect. Thus, we think that higher infection rates of caterpillars with LdMNPV will provide a substantially higher insecticidal effect of the preparation based on Oligo-IAP fragments.

To support the hypothesis that oligoRING played a major role in triggering apoptosis of LdMNPV-infected insect cells and the death of caterpillars from the Oligo-IAP group, we investigated levels of apoptotic DNA fragmentation in insects which died after 4–5 days (mortality peak) in all groups of the experiment. We did extra treatments of LdMNPV-infected caterpillars with oligoBIR an oligoRING separately. Extraction of DNA was made for each lane of the agarose gel from 3 caterpillars of every group of the experiment (Fig. 2).

Due to viral infection in caterpillars in all experimental groups we detected apoptosis as an antiviral response (Clarke and Clem 2003). However the intensity of the apoptotic ladder (DNA fragments of multiples of 180–200 bp) varied. The strongest apoptotic response to applied DNA oligonucleotides was detected in Oligo-IAP and oligoRING groups. In caterpillars from the oligoRING group we detected deep degradation of the apoptotic DNA ladder by nucleases (Vanyushin 2001). Nucleases completely cleaved higher fractions (HF) of insect genomic DNA and formed one fragment around 100–180 bp long (Fig. 2), which indicates that oligoRING triggered the quickest apoptotic reactions in response to all investigated oligonucleotides and obviously played the pivotal role in causing apoptosis and significant mortality of caterpillars in the Oligo-IAP group. We decided to name this phenomenon as VOVA (Virus before Oligonucleotide – Vent to Apoptosis) effect.

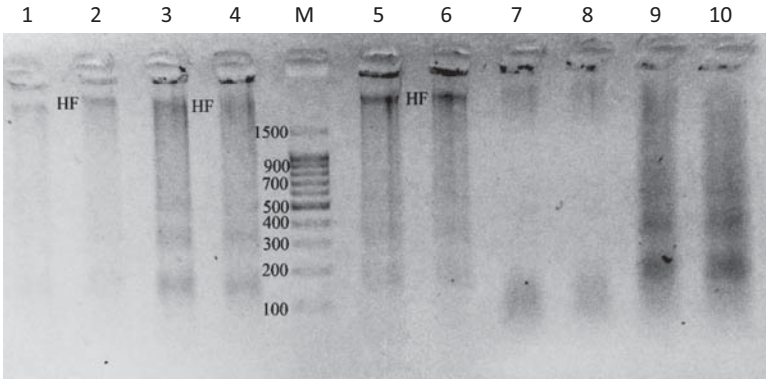

Fig. 2. Electrophoregram of apoptotic DNA ladder extracted from tissues of dead LdMNPV-infected caterpillars in different groups of experiment: 1, 2 – virus + water; 3, 4 – virus + oligo(A); M – DNA ladder (1 kb) + 1,500; 5, 6 – oligo-BIR; 7, 8 – oligoRING; 9, 10 – Oligo-IAP; HF – higher fraction of genomic DNA

Of note, in the oligoBIR group, apoptotic DNA ladders were of the same intensity as those of the water-controls whereas caterpillars from the oligo(A) group showed more pronounced apoptotic response which correlates with higher insect mortality in this group. To date, we do not know how exactly oligo(A) leads to pronounced formation of apoptotic DNA ladder in insect cells but obviously it cannot serve as a reliable control oligonucleotide in our further experiments.

In the same vein, recently we found that treatment of LdMNPV-infected gypsy moth larvae with oligoRING leads to significantly decreased expression of host IAP1 gene on the 14th day by more than 30 times (to be published). This indicates that the stronger apoptotic processes are triggered in LdMNPV-infected cells in response to the applied oligoRING fragment.

Comparing our approach of DNA insecticides, on both LdMNPV-infected and LdMNPV-free gypsy moth caterpillars (Oberemok and Skorokhod 2014; Oberemok *et al.* 2016) with the RNAi approach (Terenius *et al.* 2011; Gu and Knipple 2013) we see a number of advantages. First, the advantage of using short (approximately18 nucleotides long) insect-specific DNA insecticides compared to using relatively long double-stranded RNA fragments because dsRNA is cleaved in cells into numerous, unpredictable and short (21–23 nucleotides) siRNAs that have abundant direct sequence matches throughout the genomes of most non-target organisms (Lundgren and Duan 2013). It is difficult to solve this problem to guarantee specificity of RNAi preparations for crop protection. Second, topical application of the DNA insecticides is a convenient way of insect pest control. It might be impossible to use DNA insecticides against cryptic feeding insects and adult beetles because elytra could provide some protection from a contact insecticide. Nevertheless, DNA insecticides look very promising for insect pest control of lepidopteran pests at the caterpillar stage, especially during early larval instars when the insects' exoskeleton is thin. Third, gene silencing by feeding or injection of double-stranded RNA requires high concentrations for success. This issue could be resolved with the topical application which we have described here of DNA insecticides based on short antisense DNA fragments acting in substantially lower concentrations. In most studies "standard" amounts of double-stranded RNA, injected to achieve RNAi in lepidopterans, varies between 1 and 100 μg (Terenius *et al.* 2011). For comparison, in experiments with DNA insecticides we use topically 3–30 pmol of viral 18 nucleotides long DNA fragments per *L. dispar* caterpillar which corresponds to approximately 3–30 ng of DNA (per 0.7–12 mg of caterpillar biomass). Thus, ssDNA insecticide works in substantially lower concentrations and accordingly may be cheaper in comparison with RNA preparations for insect pest control. Fourth, the presence of the 2'-OH group makes the hydrolysis of RNA much more facile than hydrolysis of DNA (Thorp 2000). Thus, DNA insecticides will be more stable than RNA preparations in nature and will have an insecticidal effect before they are degraded.

Baculoviruses are ubiquitous in the environment and are known to be an important regulator of insect populations. These characteristics make them promising candidates for the biological control of insect pests acting effectively but slowly. They also have minimal off-target impacts (Oberemok and Skorokhod 2014; Oberemok *et al.* 2015a; Oberemok *et al.* 2015b) in comparison with chemical insecticides (Lozowicka *et al.* 2015; Oberemok *et al.* 2015b). The data of this work indicate that the specific DNA oligonucleotides may interfere with expression of vIAP genes to induce stronger apoptosis in infected insects. This hypothesis is also supported by the finding that the oligoRING fragment applied here decreases the gypsy moth body mass and up-regulates the pro-apoptotic genes (Oberemok *et al.* 2016). Further studies of the exact mode of action of Oligo-IAP fragments may reveal their potential as DNA

insecticides, as well as unravel possible ways to modify the baculovirus genomes to enhance their effect against the insect pests. The results indicate the possibility of using both antisense Oligo-IAP fragments of the LdMNPV IAP3 gene and LdMNPV preparations (one following the other) to control gypsy moth and to encourage this principle in plant protection against insect pests. Since LdMNPV is known to be transmitted transovarially (Oberemok 2008b; Bakhvalov *et al.* 2012), this approach could be useful in places where LdMNPV was used the previous year. Thus, alternation of LdMNPV preparations (first year) and antisense fragments of vIAP genes (second year) could be applied.

We see one more promising applications of this approach in medicine for treatment of cancers caused by viruses where we could trigger apoptosis in cancer cells using antisense Oligo-IAP fragments of cancer viruses (for example, Epstein-Barr virus).

Acknowledgements

This research was supported by the Project of Development Program of V.I. Vernadsky Crimean Federal University network "Academic mobility of young scientists of Russia – AMMUR" GSU/2016/3.

References

Agol V.I. 1997. How viruses cause disease. Soros's Educational Magazine 9: 27.

Bakhvalov S.A., Martem'yanov V.V., Bakhvalova V.N., Morozova O.V. 2012. Detection of the nuclear polyhedrosis virus DNA in samples from eggs and caterpillars at different stages of the gypsy moth *Lymantria dispar* (L.) population dynamics. Voprosy Virusologii 57 (4): 35–37.

Bertin J., Mendrysa S.M., La Count D.J., Gaur S., Krebs J.F., Armstrong R.C., Tomaselli K.J., Friesen P.D. 1996. Apoptotic supression by baculovirus P35 involves cleavage by and inhibition of a virus induced CED – 3/ICE-like protease. Journal of Virology 70 (9): 6251–6259.

Cerio R.J., Vandergaast R., Friesen P.D. 2010. Host insect inhibitor-of-apoptosis SfIAP functionally replaces baculovirus IAP but is differentially regulated by its N-terminal leader. Journal of Virology 84 (21): 11448–11460.

Clarke T.E., Clem R.J. 2003. Insect defenses against virus infection: the role of apoptosis. International Reviews of Immunology 22 (5–6): 401–424.

Clem R.J., Passarelli L.A. 2013. Baculoviruses: Sophisticated Pathogens of Insects. PLoS Pathogens. Available on: http://dx.doi.org/10.1371/journal.ppat.1003729. [Accessed: November 14, 2013].

Clem R.J. 2015. Viral IAPs, then and now. Seminars in Cell & Developmental Biology 39: 72–79.

Duan L., Otvos I.S., Xu L.B., Conder N., Wang Y. 2011. Comparison of the activities of three LdMNPV isolates in the laboratory against the Chinese strain of Asian gypsy moth. Open Entomology Journal 5 (1): 24–30.

Dias N., Stein C.A. 2002. Antisense oligonucleotides: basic concepts and mechanism. Molecular Cancer Therapeutics 1 (5): 347–355.

Ebling P.M., Otvos I.S., Conder N. 2004. Comparative activity of three isolates of LdMNPV against two strains of *Lymantria dispar*. Canadian Entomologist 136 (5): 737–747.

Gu L., Knipple D.C. 2013. Recent advances in RNA interference research in insects: Implications for future insect pest management strategies. Crop Protection 45: 36–40.

Hughes A.L. 2002. Evolution of inhibitors of apoptosis in baculoviruses and their insect hosts infection. Infection, Genetics and Evolution 2 (1): 3–10.

Ikeda M., Yamada H., Ito H., Kobayashi M. 2011. Baculovirus IAP1 induces caspase-dependent apoptosis in insect cells. Journal of General Virology 92 (11): 2654–2663.

Ikeda M., Yamada H., Hamajima R., Kobayashi M. 2013. Baculovirus genes modulating intracellular innate antiviral immunity of lepidopteran insect cells. Virology 435 (1): 1–13.

Jehle J.A., Blissard G.W., Bonning B.C., Cory J.S., Herniou E.A., Rohrmann G.F., Theilmann D.A., Thiem S.M., Vlak J.M. 2006. On the classification and nomenclature of baculoviruses: a proposal for revision. Archives of Virology 151 (7): 1257–1266.

Lozowicka B., Hrynko I., Kaczyński P., Rutkowska E., Jankowska M., Mojsak P. 2015. Occurrence of pesticide residues in fruit from Podlasie (Poland) in 2012. Journal of Plant Protection Research 55 (2): 142–145.

Lundgren J.G., Duan J.J. 2013. RNAi-based insecticidal crops: potential effects on non-target species. BioScience 63 (8): 657–665.

Manji G.A., Hozar R.R., La Count D.J., Friesen P.D. 1997. Baculovirus inhibitor of apoptosis functions at or upstream of the apoptotic supressor P35 to prevent programmed cell death. Journal of Virology 71 (6): 4509–4516.

Miller L.K., Lingg A.J., Bulla L.A.J. 1983. Bacterial viral and fungal insecticides. Science 219 (4585): 715–721.

Mitchell J.K., Friesen P.D. 2012. Baculoviruses modulate a pro-apoptotic DNA damage response to promote virus multiplication. Journal of Virology 86 (24): 13542–13553.

Oberemok V.V. 2008a. Method of elimination of phyllophagous insects from order Lepidoptera. Patent 36445 Ukraine, the applicant and the owner of the patent Taurida National V.I Vernadsky University. № u 2008 0674.

Oberemok V.V. 2008b. [Proof of transovarial transmission of *Lymantria dispar* nucleopolyhedrovirus (Fam. Baculoviridae) with the RAPD-PCR method.] Zhurnal Obshcheo Biologii 69 (5): 397–400. (in Russian)

Oberemok V.V. 2011. DNA markers in the study of the relationship between nuclear polyhedrosis virus and its host *Lymantria dispar*. Summary of Ph.D. thesis. Taras Shevchenko National University of Kyiv, Kyiv, Ukraine, 22 pp. (in Ukrainian)

Oberemok V.V., Skorokhod O.A. 2014. Single-stranded DNA fragments of insect-specific nuclear polyhedrosis virus act as selective DNA insecticides for gypsy moth control. Pesticide Biochemistry and Physiology 113: 1–7.

Oberemok V.V., Laikova K.V., Gninenko Yu.I., Zaitsev A.S., Nyadar P.M., Adeyemi T.A. 2015a. A short history of insecticides. Journal of Plant Protection Research 55 (3): 221–226.

Oberemok V.V., Zaitsev A.S., Levchenko N.N., Nyadar P.M. 2015b. A brief review of most widely used modern insecticides and prospects for the creation of DNA insecticides. Entomological Review 95 (7): 824–831.

Oberemok V.V., Nyadar P.M. 2015. Investigation of mode of action of DNA insecticides on the basis of LdMNPV IAP3 gene. Turkish Journal of Biology 39: 258–264.

Oberemok V.V., Laikova K.V., Zaitsev A.S., Gushchin V.A., Skorokhod O.A. 2016. The RING for gypsy moth control: topical application of fragment of its nuclear polyhedrosis virus anti-apoptosis gene as insecticide. Pesticide Biochemistry and Physiology 131: 32–39.

Simchuk A.P., Oberemok V.V., Ivashov A.V. 2012. Genetics of interactions among moths, their host plants and enemies in crimean oak forests, and its perspective for their control. p. 187–205. In: "Moths: Types, Ecological Significance and Control" (L. Cauterruccio, ed.) Nova Science Publisher, N.Y., USA, 286 pp.

Srinivasula S.M., Ashwell J.D. 2008. IAPs: what's in a name? Molecular Cell 30 (2): 123–135.

Terenius O., Papanicolaou A., Garbutt J.S., Eleftherianos I., Huvenne H., Kanginakudru S., Albrechtsen M., An C., Aymeric J.L. 2011. RNA interference in Lepidoptera: an overview of successful and unsuccessful studies and implications for experimental design. Journal of Insect Physiology 57 (2): 231–245.

Thorp H.H. 2000. The importance of being r: greater oxidative stability of RNA compared with DNA. Chemical Biology 7 (2): 33–36.

Vandergaast R., Schultz K.L., Cerio R.J., Friesen P.D. 2011. Active depletion of host cell inhibitor-of-apoptosis proteins triggers apoptosis upon baculovirus DNA replication. Journal of Virology 85 (16): 8348–8358.

Vandergaast R., Mitchell J.K., Byers N.M., Friesen P.D. 2015. Insect inhibitor of apoptosis iaps are negatively regulated by signal-induced N-terminal degrons absent within viral IAPs. Journal of Virology 89 (8): 4481–4493.

Vanyushin B.F. 2001. Apoptosis of plants. Uspehi Biologicheskoi Khimii 41: 3–38. (in Russian)

Weitzman M.D., Lilley C.E., Chaurushiya M.S. 2010. Genomes in conflict: maintaining genome integrity during virus infection. Annual Review Microbiology 64: 61–81.

Yamada H., Shibuya M., Kobayashi M., Ikeda M. 2012. Baculovirus *Lymantria dispar* multiple nucleopolyhedrovirus IAP2 and IAP3 do not suppress apoptosis, but trigger apoptosis of insect cells in a transient expression assay. Virus Genes 45 (2): 370–379.

Yamada H., Shibuya M., Kobayashi M., Ikeda M. 2011. Identification of a novel apoptosis suppressor gene from the baculovirus *Lymantria dispar* multi capsid nucleopolyhedrovirus. Journal of Virology 85 (10): 5237–5242.

Supplemental information

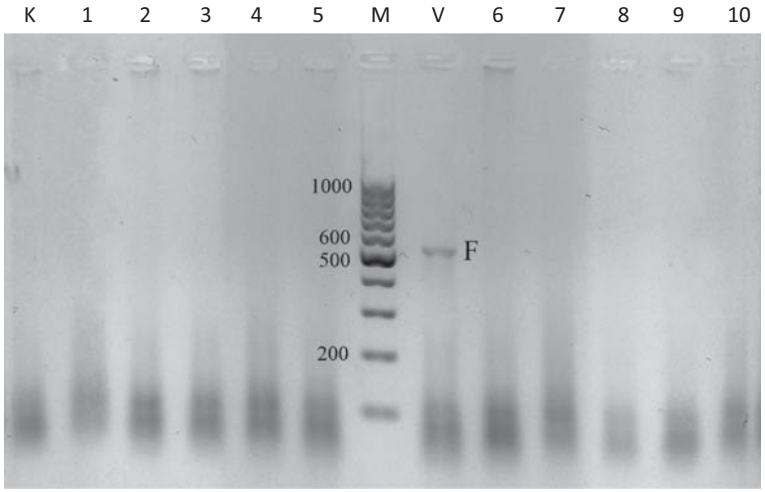

Fig. S1. Electrophoregram of DNA amplification products of gypsy moth: K – control; M – DNA ladder (1 kb); 1–10 – DNA spectra of different individuals + primers for detection of LdMNPV; V (positive control) – LdMNPV ("VIRIN-NSh", Kyrgyzstan) + primers for detection of LdMNPV; F – fragment of LdMNPV genome 524 bp long

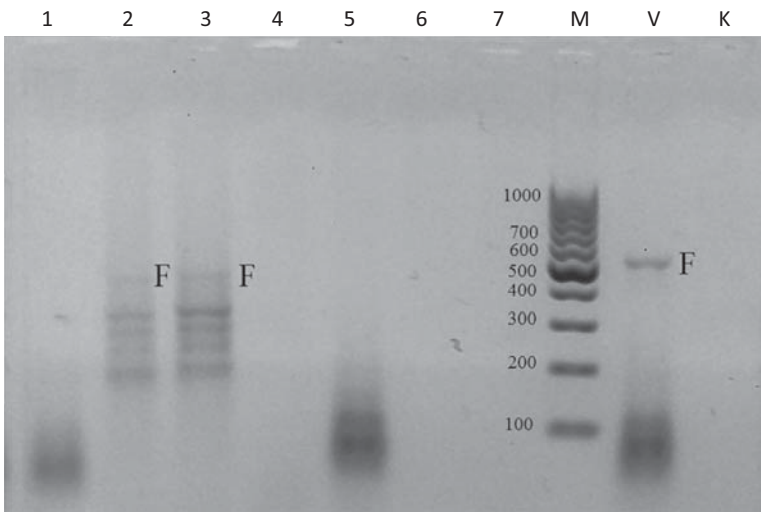

Fig. S2. Electrophoregram of DNA amplification products of LdMNPV-infected gypsy moth caterpillars: K – control; M – DNA ladder (1 kb); 1–7 – DNA spectra of infected with LdMNPV individuals from control group (5 days after the infection) + primers for detection of LdMNPV; V (positive control) – LdMNPV ("VIRIN-NSh", Kyrgyzstan) + primers for detection of LdMNPV; F – fragment of LdMNPV genome 524 bp long