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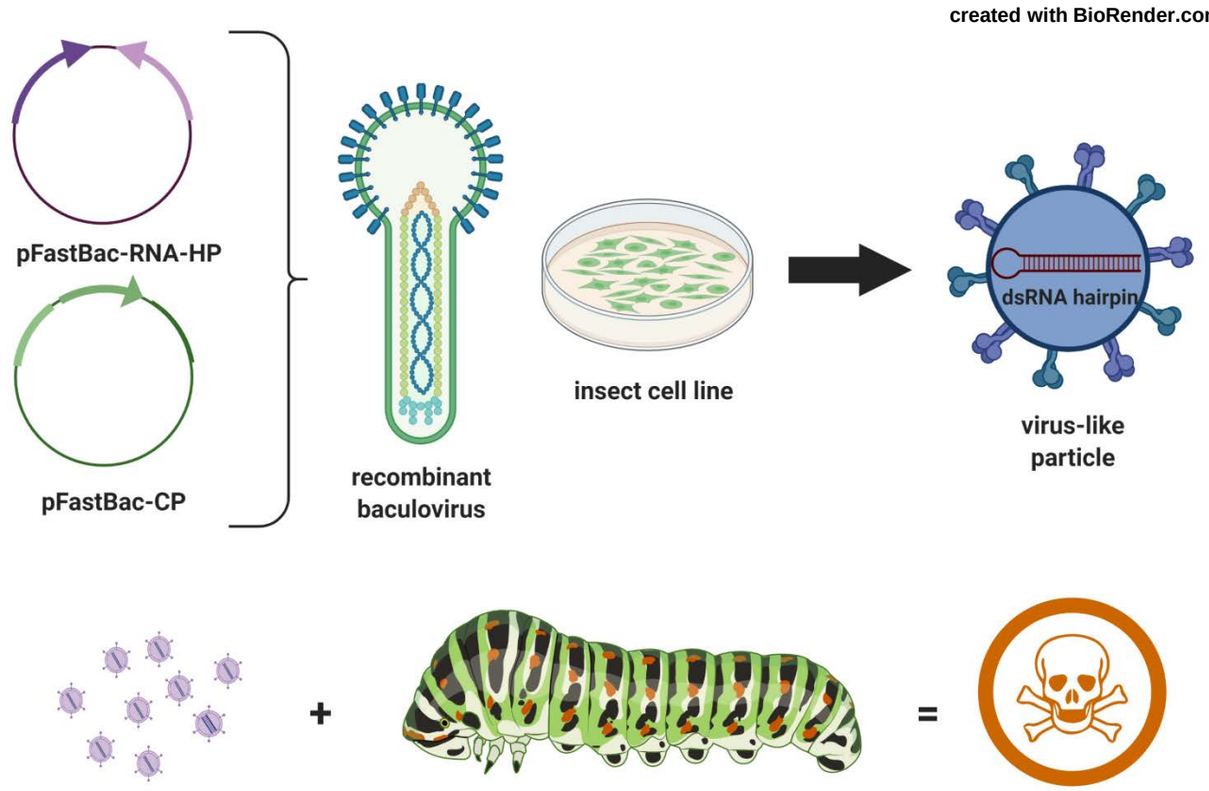
Hellenic Foundation for Research & Innovation

Description of the funded research project

1st Call for H.F.R.I. Research Projects to Support Faculty Members & Researchers and Procure High-Value Research Equipment

Title of the research project:
Viral-like Particles for Increased Delivery of RNAi in Insects



Principal Investigator:
Luc Swevers

Reader-friendly title:
Biotechnological platform for production of biomolecule complexes for safe pest control

Scientific Area:
Agricultural Sciences – Food Science & Technology

Institution and Country:
Research Center Greece

Host Institution:
NCSR “Demokritos”, Aghia Paraskevi (Athens)

Collaborating Institution(s):
South China Agricultural University, Guangzhou (China)

Budget: 152.000 €

Duration: 2 years

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Research Project Synopsis

RNA interference (RNAi) is a promising new approach for pest control that targets only the intended pests and is considered very safe with respect to the environment and human health. RNAi is based on the property of dsRNA to cause gene silencing of homologous RNA targets (RNA-based gene silencing) and specific dsRNAs can cause toxic effects when their sequences match those of essential cellular genes in insect pests.

While the capacity of specific dsRNA molecules to kill insect pests has been demonstrated in a few instances, a major obstacle remains the delivery of dsRNA to the insect cells in sufficient concentration to be able to cause silencing of essential genes.

The strategy that is employed in the project to stimulate the uptake of dsRNA by insects relies on their encapsulation in viral-like particles (VLPs). Advantage is taken of the co-evolution between viruses and hosts in which the uptake of the former by the latter has been optimized over extended periods of evolutionary time. By packaging dsRNA in VLPs it is believed that superior delivery vehicles can be generated that will cause robust gene silencing and associated toxicity.

In the project, the baculovirus expression vector system (BEVS) will be employed to express VLPs and dsRNA simultaneously and conditions will be evaluated for the efficient encapsulation of dsRNA in VLPs. After purification with ultracentrifugation, dsRNA-VLP complexes will be evaluated for cellular uptake and capacity to induce gene silencing in insect cell lines and after feeding to insect larvae. VLPs will be based on naturally occurring viruses with dsRNA genome that infect lepidopteran insects and the silkworm (*Bombyx mori*) will be used as the lepidopteran insect model.

Project originality

Two approaches are considered for the delivery of “toxic” dsRNAs to insect pests. “Host-induced gene silencing” (HIGS) relies on the production of RNA hairpins (with dsRNA structure) that target relevant insect pests in transgenic crops. This and other strategies that are based on “genetically modified organisms” (GMOs) however meet high social resistance and severe regulatory obstacles in Europe and are therefore not considered as viable. The second approach is based on sprays of formulations of (biotechnologically produced) dsRNAs directly on the plant surface (“spray-induced gene silencing”, SIGS).

The SIGS approach faces considerable challenges that relate to stability of dsRNA in the environment and insect tissues (mainly the gut) and the efficient uptake of dsRNA by the insect cells. To enhance dsRNA uptake, several formulations have been tested that include natural polymers such as chitosan and synthetic chemical nanoparticles consisting of polymers and lipid mixtures. Challenges in SIGS approaches that remain include specificity and efficiency of dsRNA delivery as well as safety with respect to accumulation in the environment.

DsRNA-VLPs conceptually represent an ideal non-GMO strategy for specific and efficient delivery of dsRNA to insect pests with minimal impact on the environment. Both dsRNA and VLPs represent naturally occurring molecules (nucleic acid and protein, respectively) that are rapidly degraded in nature. VLPs will encapsulate dsRNAs and provide protection against nucleases and other environmental insults. Because of co-evolution between viruses and hosts, engineered VLPs can be predicted to permeate insect targets efficiently in a species-specific manner. Finally, VLPs do not contain replicating genetic material – while they resemble viruses in other features, they represent an approach that is definitely non-GMO.

Expected results & Research Project Impact

The baculovirus expression vector system (BEVS) will be employed to express both viral capsid proteins and dsRNA molecules. The ultimate goal will be the production of VLPs that do not only encapsulate and protect dsRNA but also retain all other useful properties of viruses such as entry into the insect body and penetration of cellular membranes. To achieve these ambitious goals, several important challenges are apparent.

First, to acquire all necessary properties, VLPs of different capsid proteins need to be assembled. Particular capsid proteins will assemble spontaneously into the protective shell that forms the basic structure on which other capsid proteins with functions necessary for cell binding and membrane penetration are recruited. This will require "MultiBac" constructs in which several capsid proteins are expressed in the appropriate stoichiometry.

Second, the capacity of the BEVS for the manufacture of dsRNA at high levels remains unexplored. High production of dsRNA may require additional engineering of both virus and cell lines to neutralize nuclease activity and to protect dsRNA by specific binding proteins.

Third, a strategy for efficient recruitment of co-produced dsRNAs into assembling VLPs needs to be devised. In the project, binding proteins with high affinity for particular RNA secondary structures will be incorporated into capsid proteins as fusions. In parallel, RNA hairpins will contain specific features for high-affinity interaction.

The project presents the unique concept of dsRNA-VLPs to increase the efficiency of dsRNA delivery in target insect pests. While major challenges lie ahead, a new paradigm is explored that in the long run could shift strategies for insect pest control towards biotechnological solutions that are based on natural pathogen-host interactions and simultaneously avoid potential risks associated with replicating genetic material.

The importance of this funding

New strategies for insect pest control are urgently needed that are based on new mode of actions and that have minimal adverse effects. The technique of RNAi can be promoted as part of the solution but its efficiency needs to be improved to become economically viable.

DsRNA-VLPs represent an entirely new approach for efficient delivery of RNAi in insects. While VLPs have many applications in medicine and veterinary science, e.g. for drug delivery and vaccination, their employment in agriculture remains negligible. In this project, the challenge is undertaken to use VLPs for efficient and specific delivery of toxic dsRNA molecules to insect pests. For this purpose, the BEVS is harnessed for its well-documented capacity to produce macromolecular complexes at high yield. The project therefore pioneers a new biotechnological platform for delivery of macromolecules to insects that, if successful, may have many applications in insect population management and pest control.

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