Research Group: Molecular Genetics of Insects and Biotechnology

Research Staff

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Research Interests

1. Regulatory mechanisms controlling insect physiological functions.

(i) Oogenesis in lepidopteran insects: a model differentiation program induced by ecdysteroid hormones.
(ii) Mechanisms of immunosuppression in lepidopteran insects following parasitization by hymenopteran endoparasitoids: the role of interactions between proteins of hymenopteran endosymbiotic viruses and proteins of the hemocytes of the lepidopteran hosts.

(iii) Mechanisms controlling olfactory function in the malaria mosquito vector *Anopheles gambiae*.

(iv) Analysis of small RNA (miRNA, siRNA) pathways in lepidopteran insects.

(v) Analysis of the antiviral immune response against RNA virus infection in lepidopteran insects: small RNAs and “cytokines”.


(i) Viruses that express proteins toxic for the insect hosts.

(ii) Genetically modified baculoviruses as vectors for insect genetic transformation.

(iii) Genetically modified baculoviruses as vectors for gene therapy and cellular immunization applications.

(iv) Genetically modified RNA viruses for delivery of RNAi triggers.

3. Functional genomics.
(i) Systems for production of proteins of economic importance in lepidopteran insect and mammalian cell lines.

(ii) High throughput screening systems for detection of bioactive substances (activators and inhibitors of pharmacological targets) in chemical libraries and collections of natural products (plants and microorganisms).

4. Insect pest management.

(i) Cell-based assays for molting-accelerating compounds (ecdysone agonists): development, high-throughput screening and validation in larvicidal assays.

(ii) Functional expression and characterization of detoxification enzymes of insecticides.

(iii) Screening assays for identification of synergists/stabilizers of insecticides in natural plant extracts.


(v) Development of RNAi as tool for assessment of mechanism of insecticide resistance in insect larvae.

**Participation in research projects**


9) Key mechanisms of systemic RNA interference (RNAi) in insects. 2009-2012. FWO –
Vlaanderen F 6/12 (Belgium). Coordinator: G. Smagghe.


**Lab equipment**

**Insect cell culture**: incubators, BIOWAVE bioreactor, laminar flow, inverted microscope, inverted fluorescence microscope, microcentrifuges with cooling, osmometer.

**Insect culture** incubator and maintenance room (for silkmoth).

**Protein production**: affinity chromatography, antibody purification, HPLC.

**Biochemistry and molecular biology**: DNA, RNA and protein electrophoresis, microcentrifuges, electroporation apparatuses, sonicator, microphotospectrometer (Nanodrop).
Screening systems for detection of bioactive molecules: fluorescence/absorbance plate reader (Galaxy), fluorescence/luminescence/absorption plate reader (Tecan).

Collaborations

Dr. J. Vontas, FORTH - Institute of Molecular Biology & Biotechnology, Heraklion, Crete.

Dr. A. Kourti, Department of Biotechnology, Agricultural University of Athens, Athens, Greece.

Dr. R. Matsas, Laboratory of Cellular and Molecular Neurobiology, Hellenic Pasteur Institute, Athens, Greece.

Dr. K. Kalantidis, FORTH - Institute of Molecular Biology & Biotechnology, Heraklion, Crete.

Dr. M. Konstantopoulo, Chemical Ecology and Natural Products, NCSR Demokritos, Athens, Greece.

Dr. G. Smagghe, Faculty of Bioscience Engineering, Ghent University, Belgium.

Dr. J. Vanden Broeck, Animal Physiology and Neurobiology, University of Leuven, Belgium.

Dr. Y. Nakagawa, Graduate School of Agriculture, Kyoto University, Japan.

Dr. D. Zitnan, Institute of Zoology, Slovak Academy of Sciences, Bratislava, Slovakia.
Recent Progress:

Regulatory mechanisms controlling insect physiological functions

*The RNAi response in the silkmoth, Bombyx mori*

RNA interference (RNAi) has recently been developed as a potent reverse genetics technique to analyse gene function with possible application in insect pest control (3,13).

In the silkmoth, *B. mori* (Lepidoptera), no potent RNAi response is induced following injection or feeding of dsRNA (1). This observation prompted us to evaluate factors that could contribute to the (lack of) RNAi efficiency in the silkmoth, such as:

*Expression pattern of basic intracellular RNAi factors*

Expression studies suggested that the absence of R2D2 expression, an essential co-factor of Dicer-2 and Ago-2, may play a role in the refractoriness of the systemic RNAi response in *Bom byx* (2). However, functional studies indicate that the intracellular RNAi machinery can work efficiently in the absence of R2D2 in silmoth-derived Bm5 cells (5).
Expression of dsRNA-degrading enzymes

It was demonstrated that a non-specific DNA/RNA nuclease ("dsRNase") has a broad expression in many different tissues and is capable both to degrade dsRNA intracellularly and to interfere with dsRNA-mediated gene silencing (4).

DsRNA as (non-specific) “pathogen-activated molecular pattern” (PAMP)

It was observed that injection of dsRNA into the hemolymph induces the expression of genes of the RNAi machinery (Dicer-2, Ago-2) and dsRNase in the midgut, while the expression of the innate immune Toll9-1 receptor was inhibited (6). Ectopic expression of Toll9-1 receptor in Bm5 cells was observed to modulate the response against the PAMPs dsRNA and lipopolysaccharide (LPS) with respect to the expression of the RNAi machinery and innate immunity genes (12).

Persistent RNA virus infection

It is hypothesized that persistent virus infection can severely affect the function of the RNAi machinery according to several different molecular mechanisms (8). Since the Daizo strain of Bombyx was found to be persistently infected with cytoplasmic polyhedrosis virus (CPV), characterized with a segmented dsRNA genome (Cypovirus, Reoviridae), it was decided to investigate whether the persistent infection could affect the immune response (including RNAi) against pathogenic infection of the same virus. Analysis by next-generation sequencing reveals a unique response to dsRNA virus infection in the silkmoth, with no overlap with the classical innate immune pathways triggered by bacteria or fungi (15). More specifically, transcriptome analysis reveals a complex response to pathogenic BmCPV infection that involves differential expression of genes belonging to categories such as physical
barrier, immune response, proteolytic/metabolic enzymes, heat-shock proteins, hormonal signaling and uncharacterized proteins (15). Analysis of virus-derived small RNAs indicates a clear activation of the RNAi response against BmCPV infection, both in persistently and pathogenically infected larvae (15). The induction of the RNAi response, as indicated by the amounts of observed viral small RNAs, could be correlated with the severity of the viral infection (persistent versus pathogenic). Interestingly, earlier persistent infection did not seem to influence significantly the subsequent response to pathogenic infection (comparison with data from literature).
Amplification of cDNAs of core RNAi factors from silkworm tissues and the silkworm-derived Bm5 cell line

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<tr>
<th>Larval tissues</th>
<th>Pupal tissues</th>
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<tbody>
<tr>
<td>BmActin</td>
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<td>BmDrosha</td>
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<td>BmPasha</td>
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<td>BmDicer-1</td>
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<td>BmLOQS</td>
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<tr>
<td>BmAgo-1</td>
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<tr>
<td>BmDicer-2</td>
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<td>BmR2D2</td>
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<td>BmAub (SWI)</td>
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<td>BmTrax-B</td>
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<td>Bm5</td>
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miRNA pathway

siRNA pathway

piRNA pathway

auxiliary factors

Persistence of dsRNA incubation in silkworm midgut juice and hemolymph

Functional analysis of the RNAi response in Bm5 cells:
RNAi inhibition & stimulation

Transcriptional control of RNAi activity
To explore the interaction of bracovirus (CcBV) with its host, we have expressed CcBV proteins and tested their effect on the transcriptional and differentiating potential of the silkworm fat body. The results showed that some CcBV proteins can abrogate viral gene expression in transduced mammalian cells. This suggests that CcBV proteins may have potential for use in gene therapy applications.

Additionally, we have evaluated the potential of baculoviruses as gene transduction vectors for both mammalian and insect cells. Baculoviruses are large DNA viruses that are capable of infecting and transducing insect cells. They have several properties that make them very suitable for development as gene transduction vectors, such as large genome size, high efficiency of transduction, and the ability to produce high titers of budded virus in serum-free media.

In conclusion, the use of CcBV and Baculoviruses as gene transduction vectors offers a promising approach for research in insects. Further investigation into the mechanisms of action of CcBV proteins and the optimization of Baculovirus-based gene delivery systems are necessary to fully realize their potential for gene therapy and pest control applications.
Screening of Libraries of Synthetic Compounds and Plant Extracts for 20E Agonists and Antagonists

- **20E-responsive (GFP reporter) Bm5 cells**
  - Fluorescence induction: 20E AGONIST
  - No fluorescence induction: NO ACTIVITY or 20E ANTAGONIST

- Plant extracts (serial dilutions from 10 mg/ml to 1 ng/ml)
  - Treatment with RH-5994
  - Fluorescence induction: NO ACTIVITY