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. Konstantopoulou, 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 silkmoth tissues and the silkmoth-derived Bm5 cell line

<table>
<thead>
<tr>
<th>Larval tissues</th>
<th>Pupal tissues</th>
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<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>BmActin</td>
<td></td>
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<tr>
<td>BmDrosha</td>
<td></td>
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<tr>
<td>BmPasha</td>
<td></td>
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<tr>
<td>BmDicer-1</td>
<td></td>
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<tr>
<td>BmLOQ5</td>
<td></td>
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<tr>
<td>BmAgo-1</td>
<td></td>
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<tr>
<td>BmDicer-2</td>
<td></td>
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<tr>
<td>BmR2D2 (40x)</td>
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<tr>
<td>BmAgo-2</td>
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<tr>
<td>BmAgo-3</td>
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<tr>
<td>BmAub (SWI)</td>
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<td>BmHEN1</td>
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<tr>
<td>BmTranslin</td>
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<td>BmTrax-B</td>
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**miRNA pathway**
- BmActin: 379 bp
- BmDrosha: 357 bp
- BmPasha: 404 bp
- BmDicer-1: 266 bp
- BmLOQ5: 300 bp
- BmAgo-1: 444 bp

**siRNA pathway**
- BmDicer-2: 449 bp
- BmR2D2: 497 bp
- BmAgo-2: 497 bp

**piRNA pathway**
- BmAgo-3: 426 bp
- BmAub (SWI): 383 bp

**auxiliary factors**
- BmHEN1: 475 bp
- BmTranslin: 397 bp
- BmTrax-B: 362 bp

### Persistence of dsRNA incubation in silkmoth midgut juice and hemolymph

<table>
<thead>
<tr>
<th>MQ</th>
<th>Midgut juice</th>
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<td>30'</td>
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<td>10'</td>
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<td>20'</td>
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<td>30'</td>
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### Functional analysis of the RNAi response in Bm5 cells: RNAi inhibition & stimulation

- Bm5 cells expressing luciferase
- dsRNA targeting luciferase
- Expression vector for **mRNA** targeting
- siRNA targeting
- siRNA factors
- RNAi of-the-RNAi
- Stimulation of RNAi

**Graphs:****
- Relative Luminescence (%) vs. dsLuc (ng/ml)
- Comparison of mRNA, siRNA, piRNA pathways and auxiliary factors
Cell-based detection systems for ecdysone agonists or MACs were developed for lepidopteran (3). Compounds in the screen was validated in toxicity tests on larvae of the cotton leafworm, Hyponomeutidae, which encodes a subunit of the viral RNA polymerase (4). This family includes nine members Ank1-9 and is thought to be essential for successful wasps. One of the largest bracovirus (CcBV) protein families is the ankyrin-repeat protein family which is homologous to mammalian IκBa. This family includes nine members Ank1-9 and is thought to be essential for successful wasps. In fact, CcV1 could elicit immunosuppression and a subcellular machinery, antiviral immune response and prospects for insect pest control. Recent progress in RNAi research in Lepidoptera: Current Opinion in Insect Science (2014) 10–19. The transcription of Schwann cell adhesion molecule could elicit cell adhesion, which encodes the master regulator of the viral infection cycle. Because such viral particles encode a cellular receptor, they are less prone to endogenous viral gene expression in the transduced mammalian cells (5). Therefore, research has focused on the generation of baculovirus vectors with deletions in these genes, such as ie-1 and pe-1. Baculoviruses, a group of insect viruses with large DNA genome, have several properties that make them very suitable for development as gene transduction vectors, such as large genome size, high transduction efficiency, and production of full-length infectious particles. The injection of Schwann cell adhesion molecule cassettes into mammalian cells resulted in a co-localization of the GFP and a specific marker, demonstrating the effectiveness of baculovirus vectors for cell transduction.
Screening of Libraries of Synthetic Compounds or Plant Extracts for 20E Agonists and Antagonists

20E-responsive (GFP reporter) Bm5 cells

Fluorescence induction

20E AGONIST

NO ACTIVITY or 20E ANTAGONIST

Fluorescence induction

NO ACTIVITY

plant extracts

(serial dilutions from 10 mg/ml to 1 ng/ml)

treatment

RH-599A