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 silkworm).

**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.
Dr. D. Martin, Institute of Evolutionary Biology, Barcelona, Spain.

Dr. J. Sun, College of Animal Science, South China Agricultural University, Guangzhou, People's Republic of China.

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 silkmoth-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>BmActin</td>
<td>BmActin</td>
</tr>
<tr>
<td>BmDrosha</td>
<td>BmDrosha</td>
</tr>
<tr>
<td>BmPasha</td>
<td>BmPasha</td>
</tr>
<tr>
<td>BmDicer-1</td>
<td>BmDicer-1</td>
</tr>
<tr>
<td>BmYOG5</td>
<td>BmYOG5</td>
</tr>
<tr>
<td>BmAgo-1</td>
<td>BmAgo-1</td>
</tr>
<tr>
<td>BmDicer-2</td>
<td>BmDicer-2</td>
</tr>
<tr>
<td>BmR2D2</td>
<td>BmR2D2</td>
</tr>
<tr>
<td>BmAgo-2</td>
<td>BmAgo-2</td>
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<tr>
<td>BmTranslin</td>
<td>BmTranslin</td>
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<td>BmTrax-B</td>
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### Persistence of dsRNA incubation in silkmoth midgut juice and hemolymph

- **MQ** in midgut juice and hemolymph after 30', 10', 20', 30'

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

- Bm5 cells expressing luciferase + dsRNA targeting luciferase → Luminescence
- dsRNA targeting expression vector for
- miRNA targeting silRNA auxiliary factors
- siRNA factors
- recovery of luminescence?
- stimulation of luminescence inhibition?

- "RNAi-of-the-RNAi" stimulation of RNAi

### Graphs

- Relative Luminescence (%) vs dsLuc (ng/ml)
  - 0, 3.125, 6.25, 12.5, 25, 50
Molting accelerating compounds (MACs) or ecdysone agonists act as insecticides by inducing a premature, lethal moult in the target insects. Because 20-hydroxy-ecdysone (20E), and are not cleared efficiently from the insect body, the insect MACs can induce the moult at much lower concentrations than the natural hormone.

Insect Pest Management Functional Characterization of a Juvenile Hormone Esterase Related Gene in the Moth Transformed lepidopteran cells expressing a protein of the pollutant bisphenol A against arthropods (9), coleopteran (4), dipteran (3) compounds in the screen was validated in toxicity tests on larvae of the cotton leafworm, *A cell-based screening system for MACs was developed based on the activation of an ecdysone agonist receptor. MACs can induce the moult at much lower concentrations than the natural hormone, 20-hydroxy-ecdysone (20E), and are not cleared efficiently from the insect body, the insect MACs can induce the moult at much lower concentrations than the natural hormone.

Recent advances in molecular biology and genetic manipulation of insect viruses include the development of hybrid PiggyBac-baculovirus vectors that can be used to engineer baculoviruses as gene transduction vectors for both mammalian and insect cells. Baculovirus vectors were engineered with mammalian expression cassettes that could drive GFP and therapeutic protein expression in cell lines and primary Schwann cells. The use of baculoviruses as gene transduction vectors for both mammalian and insect cells has been made possible by the development of high-titer production systems for these viruses and the availability of transgenic insect cell lines. This has allowed for the use of baculoviruses as tools for expression of secreted and membrane-anchored proteins and high throughput screening platforms for drug and insecticide discovery.

The role of juvenile hormone esterase (JHE) genes in the regulation of molting and metamorphosis in insects is well established. The juvenile hormone esterase-related gene (JHERG) in the moth, *Bombyx mori*, has been shown to have a functional and spatial difference between the various members of this family. The JHERG gene was recently characterized in detail, and its expression pattern was found to be consistent with its role in the regulation of molting and metamorphosis.

For the parasitization of lepidopteran insects by parasitoid hymenopterans, the immune system plays a crucial role in the survival of the parasitoid larvae. The expression of polydnavirus-encoded proteins in lepidopteran larvae by the endoparasitoid *Cotesia congregata* has been shown to interfere with the host's induced innate immune responses. Introducing transcriptional ankyrin-repeat proteins into the host cell has been shown to interfere with host cellular and humoral immune responses.

To study the mechanisms of immunosuppression in lepidopteran insects following parasitization by the braconid wasp *Drezen JM, Huguet E.*, the ankyrin-repeat protein CcBV Anks have been shown to exhibit differential inhibition on the functional and spatial differences between the various members of this family. The expression of an ankyrin-repeat protein has been shown to interfere with host cellular and humoral immune responses.

The role of the Toll 9-1 gene in the immune response of lepidopteran insects has been studied extensively. The expression of the Toll 9-1 gene in the midgut of *Bombyx mori* has been shown to be associated with antiviral immune response and prospects for insect pest control.

The transcriptome analysis of *Bombyx* larval midgut during persistent and pathogenic cytoplasmic polyhedrosis virus infection has been performed, and the expression of RNA interference-related genes has been shown to be associated with the antiviral immune response and prospects for insect pest control.
Insect Molecular Genetics and Biotechnology - Institute of Biosciences & Applications

Screening of Libraries of Synthetic Compounds and Plant Extracts for 20E Agonists and Antagonists using Transformed or Transfected Cell Lines.

Publications:


