

## Peptidomic and proteomic approaches to investigate the defense reactions in *Drosophila melanogaster*.

Philippe BULET, Laurence SABATIER, Maurice CHARLET, Francine LEVY, David RABEL and Jules A. HOFFMANN

IBMC, UPR 9022 CNRS, 15, Rue René Descartes, 67084, Strasbourg cedex France  
P.Bulet@ibmc.u-strasbg.fr, L.Sabatier@ibmc.u-strasbg.fr.

Insects have developed an efficient host defense against microorganisms, which involves humoral and cellular mechanisms [Hoffmann & Reichhart, 1997]. Interestingly, recent data have highlighted similarities between pathogen recognition, signaling pathways, and effector mechanisms of innate immunity in *Drosophila melanogaster* and mammals [Hoffmann *et al.*, 1999]. Through its particularly convenient genetics, *Drosophila* appeared as a favorable model system for the analysis of the first line defense against microorganisms [for review see Meister *et al.*, 2000].

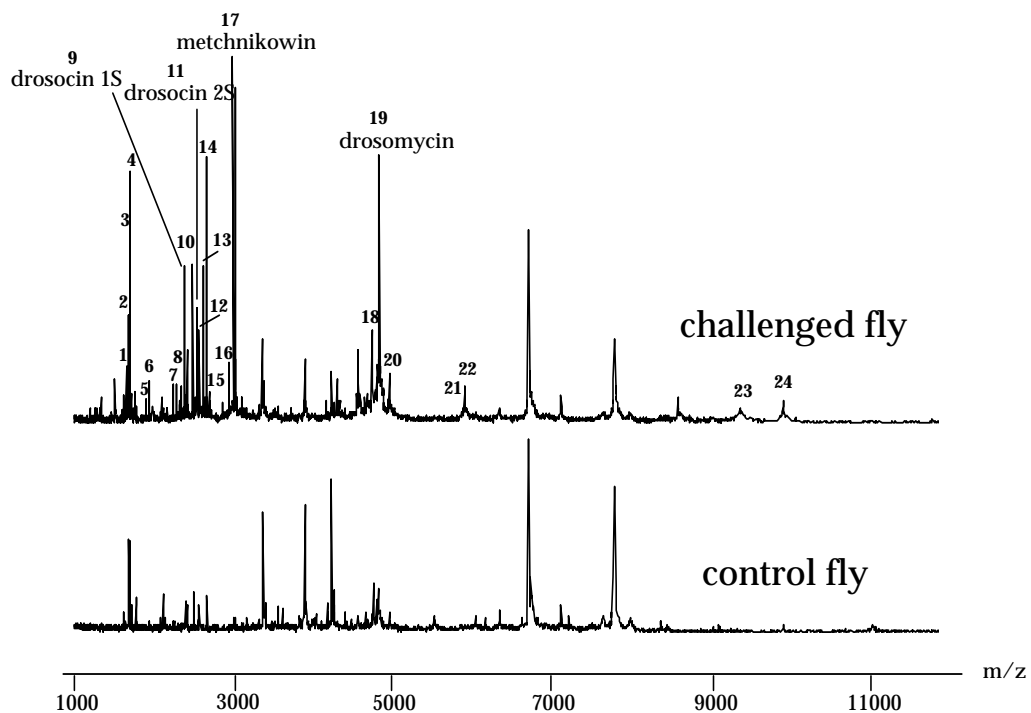


Figure 1: MALDI-TOF mass spectra of hemolymph from one control and one bacterial-challenged *Drosophila*

Taking advantages of the induction of the immune response in insects, a differential display analysis of the blood content (hemolymph) of immune-challenged versus unchallenged *Drosophila* was performed. Two strategies were developed: (1) a peptidomic analysis through MALDI-TOF MS and  $\mu$ -HPLC for molecules below 15 kDa, and (2) a proteomic analysis by 2D-gel electrophoresis, in gel digestion and data bank analysis for higher molecular weight compounds.

MALDI-TOF MS analysis led to the detection of a large number of molecules induced in the blood of a challenged fly compared to a control one (figure 1). Some of these *Drosophila* Immune-induced Molecules (DIMs) are corresponding to already identified defense compounds, antimicrobial peptides (drosocins, metchnikowin, drosomycin) [Uttenweiler-Joseph *et al.*, 1998].

The primary structure of 19 over 20 new peptides was established by combining a biochemical approach ( $\mu$ -HPLC, N- and C-terminal sequencing, proteolytic treatment), nanoES-Ion Trap MS, cDNA cloning and data bank searches. Recent sequencing of the *Drosophila* genome confirmed and/or completed the data obtained. In fact, some DIMs can be classified into two main groups: (i) three small-size peptides containing a disulfide bridge (DIMs 1-3), (ii) six homologous small-size N-terminally blocked molecules deprived of cysteine-residues (DIMs 10,12, 13 and their related C-terminally truncated forms 5, 6 and 8). Height additional DIMs can not be linked to the previous families. In addition, two peptides related to already known antimicrobial peptides were characterized. Except these two last peptides, none of these molecules have clear-cut similarities to already reported peptides.

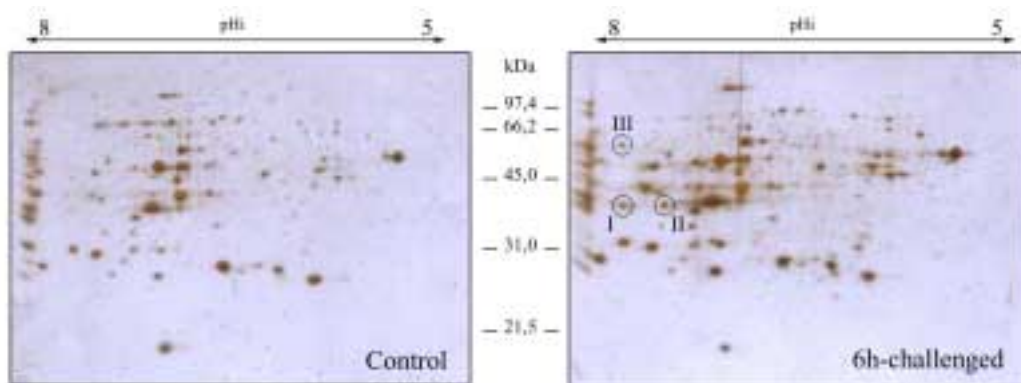


Figure 2: 2D gel electrophoresis of the hemolymph from 25 control and 25 bacterial-challenged *Drosophila*

Data bank analysis of the *Drosophila* genome revealed that most of the DIMs were found to be synthesized as single precursor molecules (preprosequence). However, DIMs 10, 12, 13 and 24 were found to derive from a large precursor by proteolytic processing at a conserved dibasic cleavage site.

The protein content of *Drosophila* hemolymph before and after an experimental infection was monitored by 2D-gel electrophoresis. From the hemolymph of 25 flies, more than 130 proteins were detected after silver staining and three were clearly up regulated after the immune-challenge (I, II, III, figure 2).

After excision, in gel digestion, extraction of the cleavage products, MALDI-TOF mass spectrometry analysis and data bank searching, only the protein III was identified. This protein corresponds to UTP-glucose-1-phosphate-uridylyl-transferase, an ubiquitous enzyme up regulated in various stress conditions in plants as well as in microorganisms. To improve sensitivity and resolution, this study has recently been extended to larger gels (hemolymph from 200 control or infected flies).

The precise function in the *Drosophila* defense reactions of all the molecules characterized by these differential analysis (peptidomic or proteomic) is presently under study using experiments such as *in situ* hybridization, over-expression, RNAi, mutagenesis and production of large quantities of peptides and proteins for *in vitro* and *in vivo* assays.

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Meister M, Hetru C, Hoffmann JA (2000) The Antimicrobial Host Defense of *Drosophila*. Current Topics in Microbiology and Immunology, 248, 17-36, Origin and Evolution of the Vertebrate Immune System, Edts L. Du Pasquier & G.W. Litman, Springer-Verlag, Berlin.

Uttenweiler-Joseph S, Moniatte M, Lagueux M, Van Dorsselaer A, Hoffmann JA and Bulet P (1998) Differential display of peptides induces during the immune response of *Drosophila*: a matrix-assisted laser desorption ionization time-of-flight mass spectrometry study. Proc. Natl. Acad. Sci. USA, 95, 11342-11347.