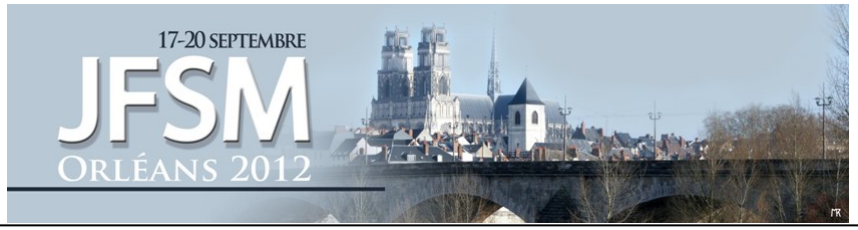


CONTRIBUTIONS OF MASS SPECTROMETRY IN THE UNDERSTANDING OF THE *DROSOPHILA* IMMUNE RESPONSE

Philippe Bulet, PhD

Present affiliation: AGIM Laboratory (AGe – Imaging - Modeling, FRE 3405, UJF-CNRS)

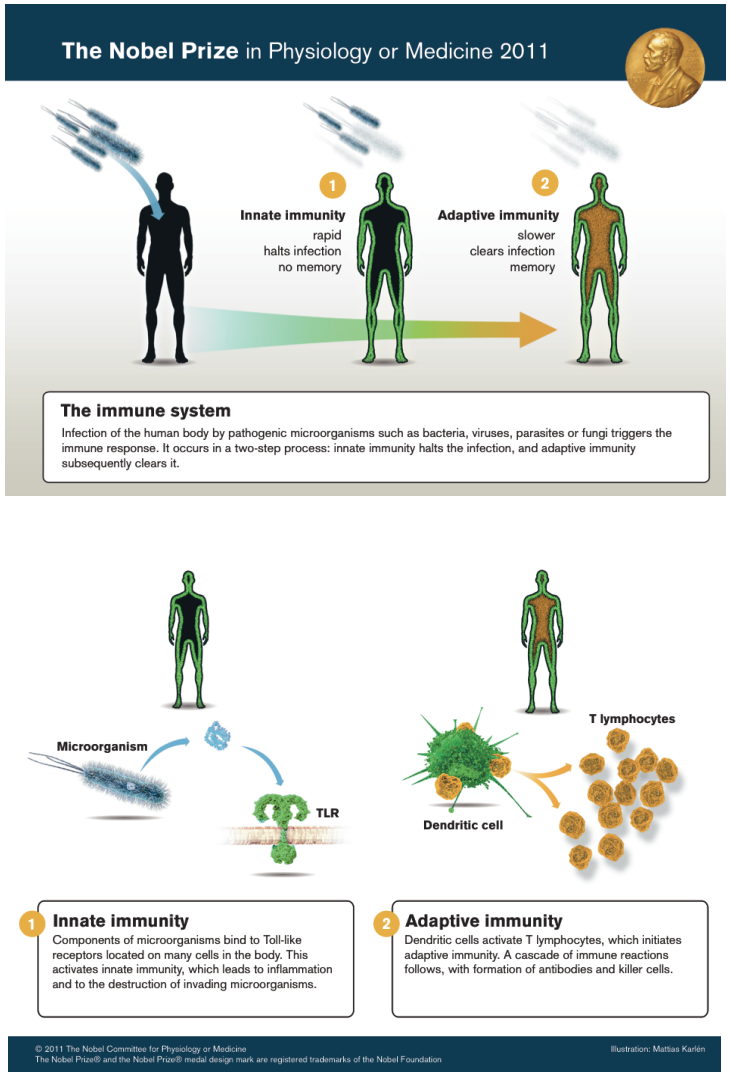
Contact addresses

Bâtiment Jean Roget, Faculté de Médecine, F-38700 La Tronche, France
Platform Biopark Archamps, Bat. Le Forum, F-74160 Archamps, France
Mail, philippe.bulet@agim.eu
Phone +33450432521, Mobile +33612777819

Content

- Introduction, Nobel Prize 2011
- The Toll Pathway
- Brief history on the defense peptides
- A question of scale down, MS as pivotal approach
- Two different strategies for peptidomics: functional assays *vs* differential profiling by MALDI-ms
  - *Drosophila* antimicrobial effectors (AMPs)
  - *Drosophila* immune-induced molecules (drosomycin, DIMs)
- Proteomic studies as a complement to peptidomics
- Conclusions
- Acknowledgement

The 2011 Nobel Prize in Physiology or Medicine

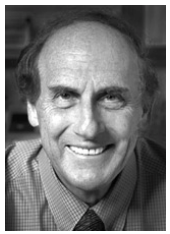


Bruce Beutler at the Scripps Research Institute in California
(1998, TLR mice model)

Jules Hoffmann at the French National Center for Scientific Research
(1996, Toll *drosophila* model)

HELPED TO ELUCIDATE INNATE IMMUNITY

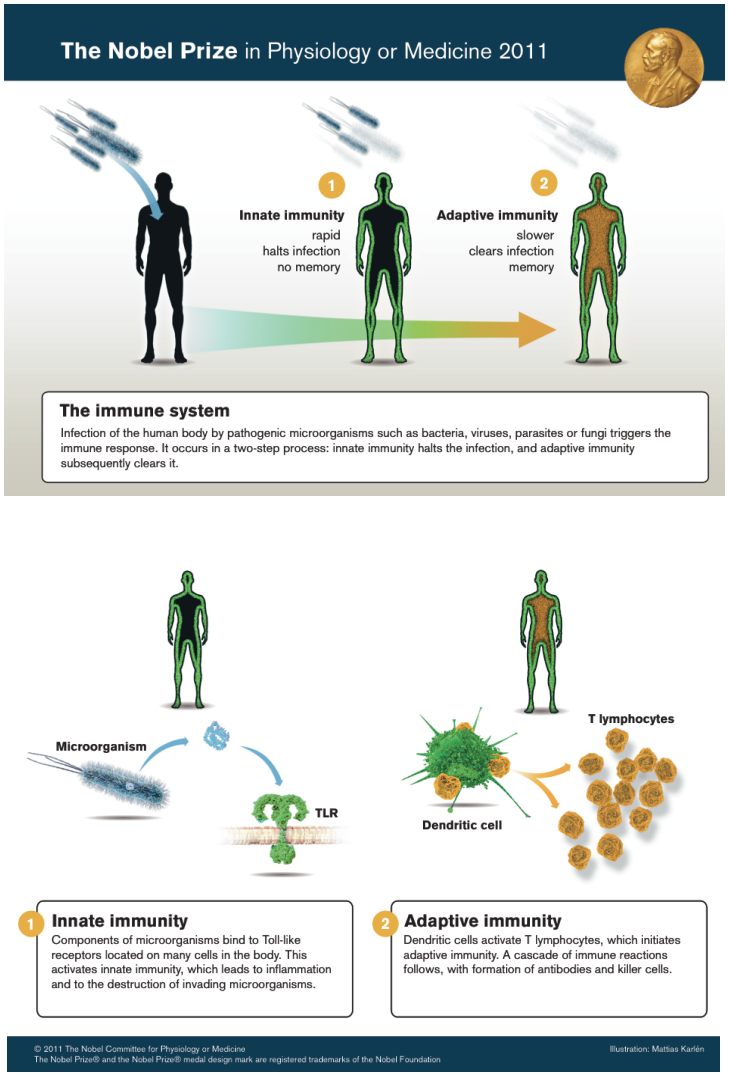
Discovery of the Toll pathway



Ralph Steinman at Rockefeller University in New York City
(1973, Dendritic cells, human model)

DISCOVERY OF DENDRITIC CELLS AS ACTIVATOR OF T CELLS

The 2011 Nobel Prize in Physiology or Medicine

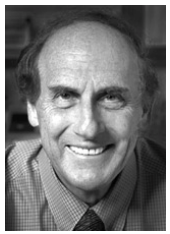


Bruce Beutler at the Scripps Research Institute in California
(1998, TLR mice model)

Jules Hoffmann at the French National Center for Scientific Research
(1996, Toll *drosophila* model)

HELPED TO ELUCIDATE INNATE IMMUNITY

Discovery of the Toll pathway

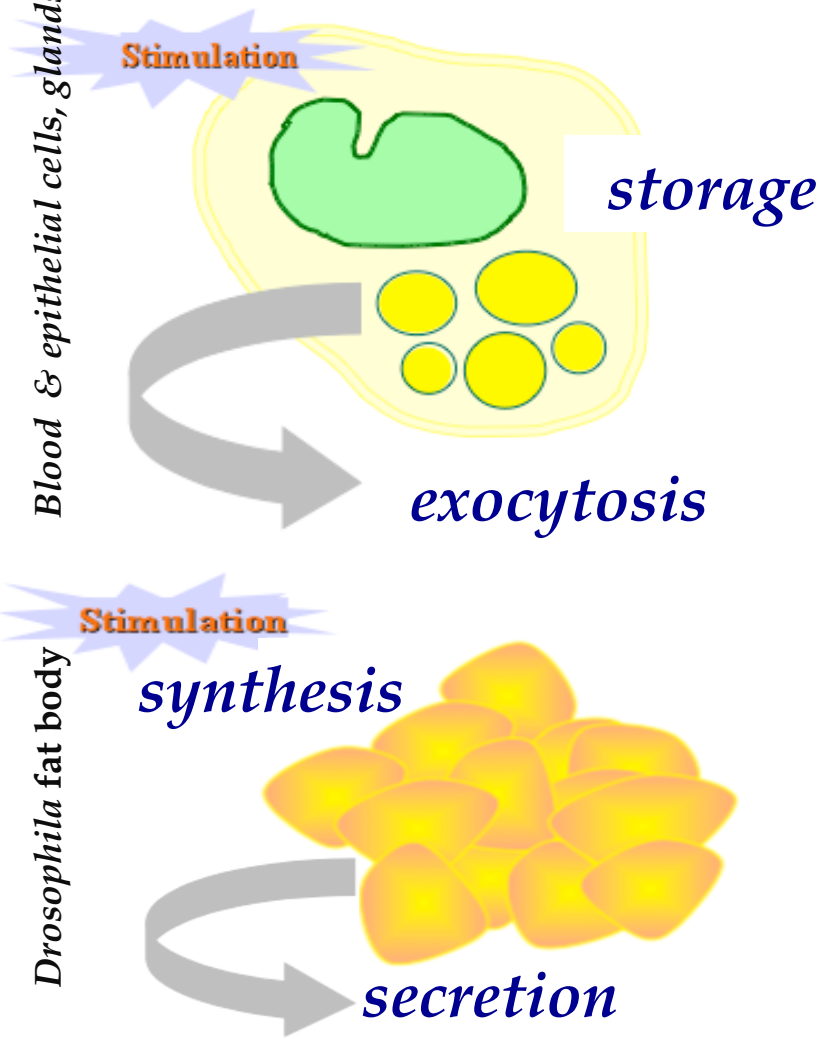


Ralph Steinman at Rockefeller University in New York City
(1973, Dendritic cells, human model)

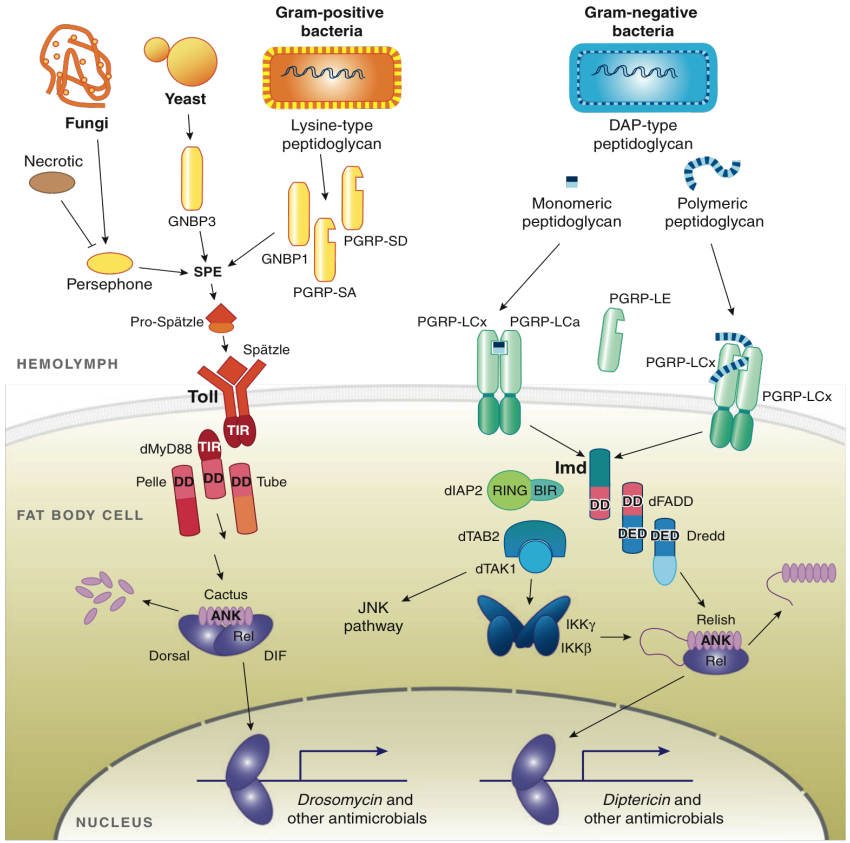
DISCOVERY OF DENDRITIC CELLS AS ACTIVATOR OF T CELLS

The *Drosophila* immune response

Constitutive or inducible by infectious and / or inflammatory stimuli



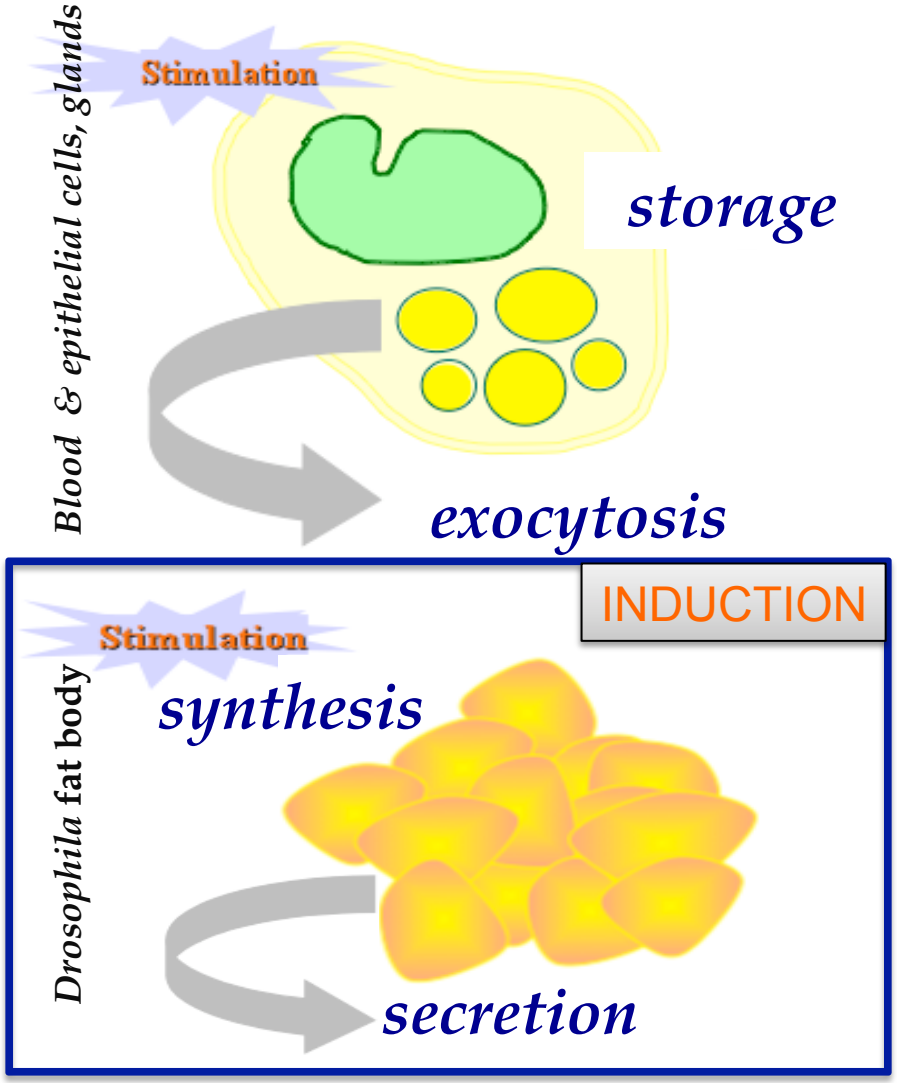
Two signaling pathways



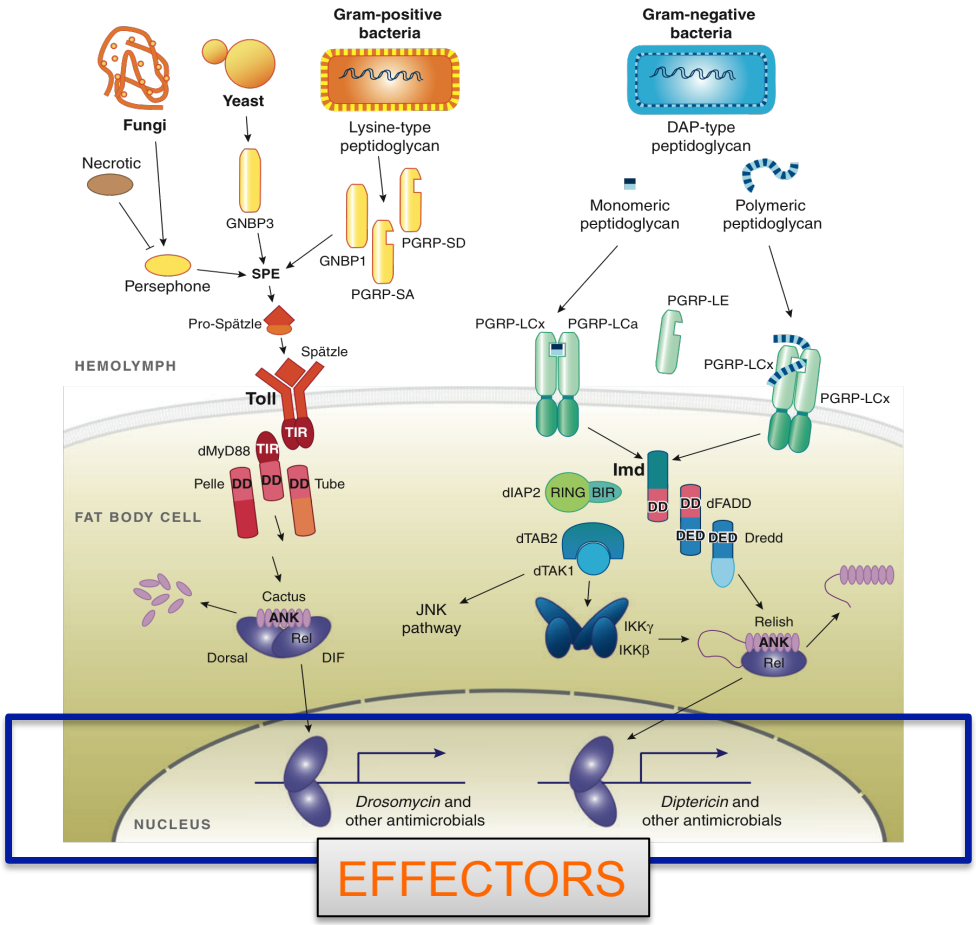
Lemaitre & Hoffmann, 2007 Ann Rev Immunol

The *Drosophila* immune response

Constitutive or inducible by infectious and / or inflammatory stimuli



Two signaling pathways



Lemaitre & Hoffmann, 2007 Ann Rev Immunol

Brief history on the defense peptides

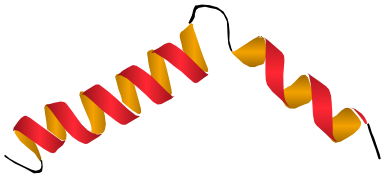
Founders of this discipline

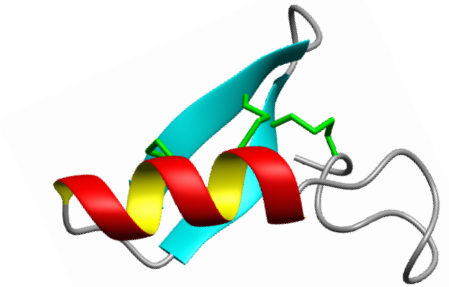
Hans Boman
(Stockholm, 1924 - 2008)

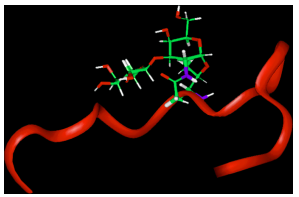
Shunji Natori
National Institute of Agrobiological Sciences, Tsukuba

Jules Hoffmann

Insect model	Size	Effectors, AMPs*
Hyalophora cecropia (lepidoptera)	100 – 110 mm	Cecropin (1981)
fleshfly	12 -15 mm	Insect defensins (1989)
blowfly		
drosophila	3 - 3.2 mm	Drosocin (1993)







** AMPs stands for AntiMicrobial Peptides*

Brief history on the defense peptides

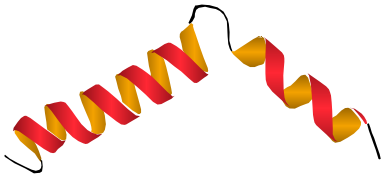
Founders of this discipline

Hans Boman
(Stockholm, 1924 - 2008)

Insect model **Size** **Effectors, AMPs***

Hyalophora cecropia (lepidoptera)

100 – 110 mm

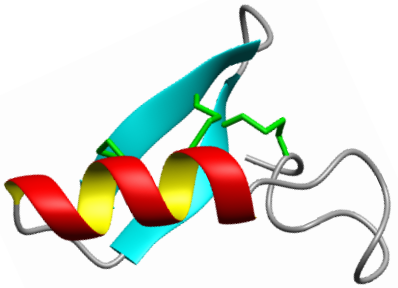


Cecropin (1981)

Shunji Natori
National Institute of Agrobiological Sciences, Tsukuba

fleshfly

12 -15 mm



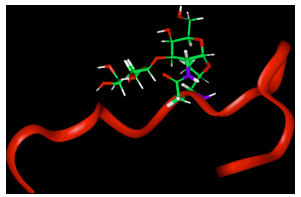
Jules Hoffmann

blowfly

drosophila

3 - 3.2 mm

Insect defensins (1989)



Drosocin (1993)

** AMPs stands for AntiMicrobial Peptides*

Optimization of the bioassays and the analytical strategies

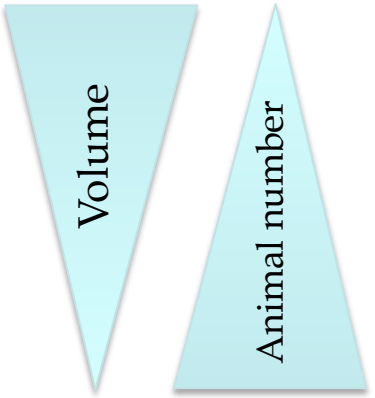
Liquid assays & MS as the key of the lock

blowfly 

12 -15 mm → Approx. 15 µL (larvae)

drosophila 

3 - 3.2 mm → 0. 1 µL (adult)

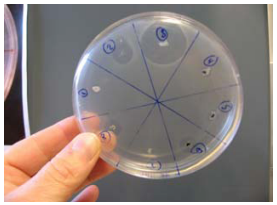


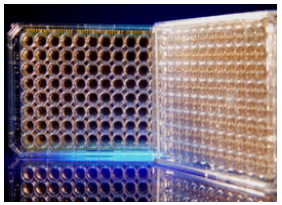
Functional assays

Solid assays

to

Liquid assays



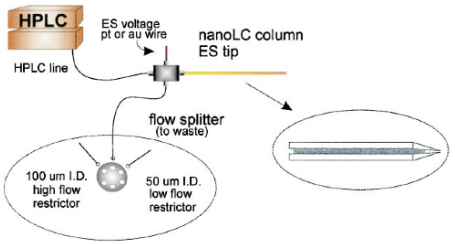


Analytical tools

Edman sequencing
Analytical - HPLC

to

MALDI* & ESI-MS
Nano to µscale HPLC



*1985 by Hillenkamp and Karas, 1987 K Tanaka

Two different strategies in parallel

functional assays & cDNA sequencing

versus

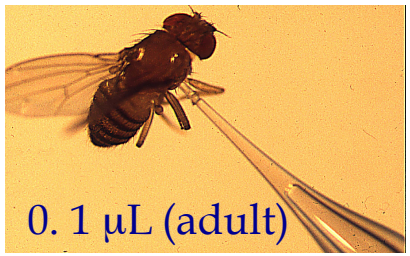
differential profiling by HPLC and MALDI-ms

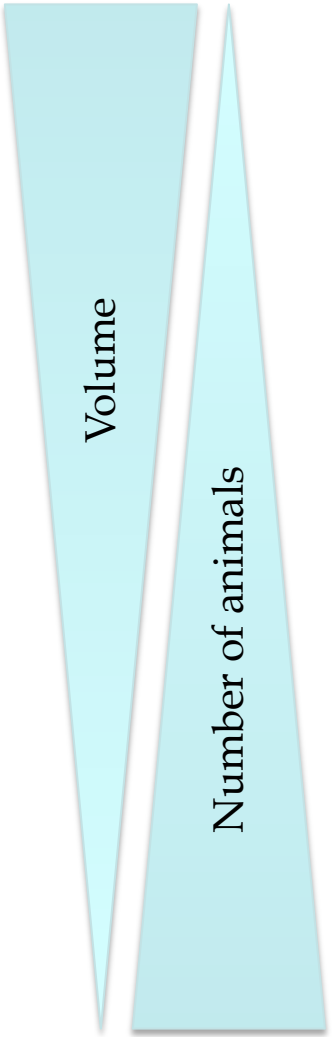
***Drosophila* antimicrobial effectors (AMPs), 1991**

***Drosophila* Immune-induced Molecules (DIMs)**

Discovery of the *drosophila* immune peptides

A question of scale down

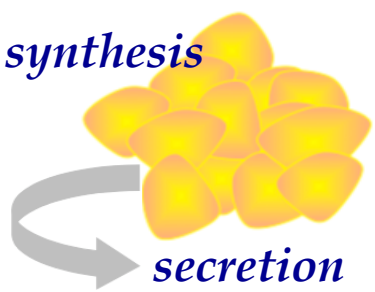
Insect model	Size	Volume of hemolymph (insect blood)
Hyalophora cecropia (lepidoptera) 	100 – 110 mm	Approx. 140 µL (caterpillar)
fleshfly 	12 -15 mm	Approx. 15 µL (larvae)
blowfly 	12 -15 mm	
drosophila 	3 - 3.2 mm	 0. 1 µL (adult)

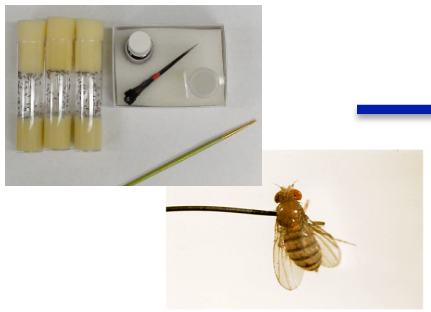


Drosophila antimicrobial effectors (AMPs) functional assays and cDNA sequencing

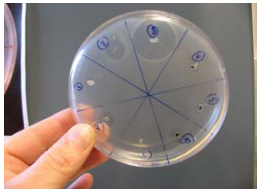
Drosophila adults

INDUCTION → **ISOLATION & CARACTERIZATION** → **ASSAYS**





From 10'000 – 30'000 individuals



Edman, MS

ESI-MS , GC-MS

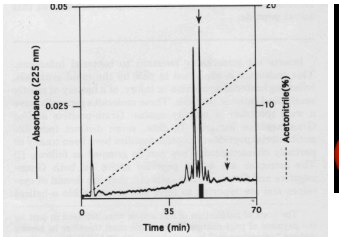
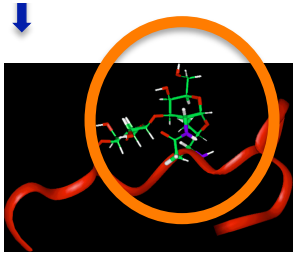


FIG. 1. **Final purification of the antibacterial peptide.** The peptide was submitted to reversed-phase HPLC on an Aquapore OD 300 C_{18} column (250 × 4.6 mm, Brownlee) eluted with a linear gradient of 8–20% acetonitrile in acidified water (*dotted line*) over 90 min at a flow rate of 2 ml/min. The absorbance was monitored at 225 nm (*full line*). *Arrow*, elution time of the native peptide; *dotted arrow*, elution time of a synthetic peptide corresponding to the amino acid sequence of the antibacterial peptide. The antibacterial activity of the different fractions was monitored by liquid growth inhibition assay (*column*).



drosocin (1993)

THE JOURNAL OF BIOLOGICAL CHEMISTRY
© 1993 by The American Society for Biochemistry and Molecular Biology, Inc.
Vol. 268, No. 20, Issue of July 15, pp. 14893–14897, 1993
Printed in U.S.A.

A Novel Inducible Antibacterial Peptide of *Drosophila* Carries an O-Glycosylated Substitution*

(Received for publication, February 24, 1993)

Philippe Bulet‡, Jean-Luc Dimarcq‡, Charles Hetru, Marie Lagueux, Maurice Charlet, Gilbert Hegy§¶, Alain Van Dorsselaer, and Jules A. Hoffmann‖

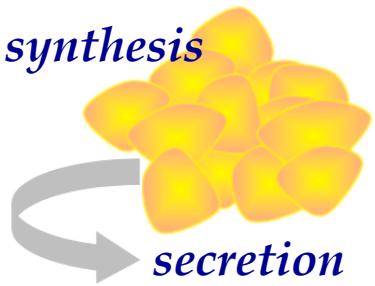
Drosophila antimicrobial effectors (AMPs) functional assays and cDNA sequencing

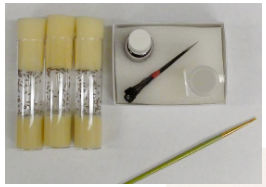
Drosophila adults

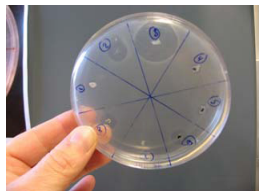
INDUCTION

ISOLATION & CARACTERIZATION

ASSAYS





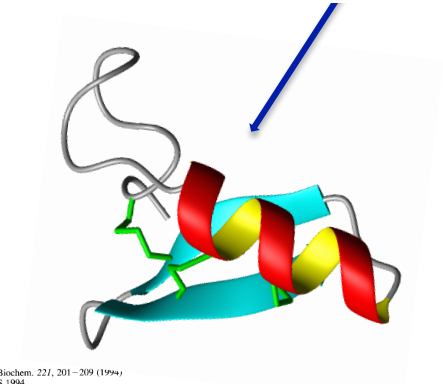


From 10'000 – 30'000 individuals

Edman, MS

ESI-MS , GC-MS

Edman, MS
cDNA sequencing



Eur. J. Biochem. 221, 201–209 (1994)
© FEBS 1994

defensins (1994)

Characterization and transcriptional profiles of a *Drosophila* gene encoding an insect defensin
A study in insect immunity

Jean-Luc DIMARCQ, Danièle HOFFMANN, Marie MEISTER, Philippe BULET, René LANOT, Jean-Marc REICHHART and Jules A. HOFFMANN

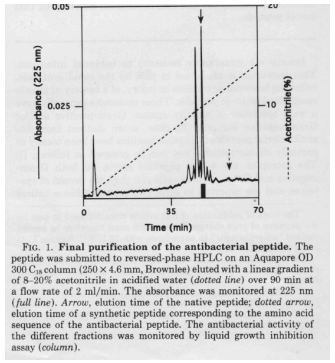
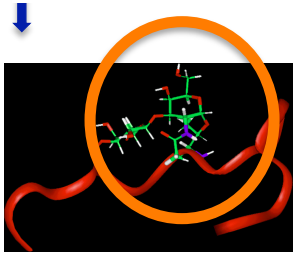


FIG. 1. **Final purification of the antibacterial peptide.** The peptide was submitted to reversed-phase HPLC on an Aquapore OD 300 C_{18} column (250 × 4.6 mm, Brownlee) eluted with a linear gradient of 8–20% acetonitrile in acidified water (*dotted line*) over 90 min at a flow rate of 2 ml/min. The absorbance was monitored at 225 nm (*full line*). *Arrow*, elution time of the native peptide; *dotted arrow*, elution time of a synthetic peptide corresponding to the amino acid sequence of the antibacterial peptide. The antibacterial activity of the different fractions was monitored by liquid growth inhibition assay (*column*).



drosocin (1993)

THE JOURNAL OF BIOLOGICAL CHEMISTRY
© 1993 by The American Society for Biochemistry and Molecular Biology, Inc.
Vol. 268, No. 20, Issue of July 15, pp. 14893–14897, 1993
Printed in U.S.A.

A Novel Inducible Antibacterial Peptide of *Drosophila* Carries an O-Glycosylated Substitution*

(Received for publication, February 24, 1993)

Philippe Bulet‡, Jean-Luc Dimarcq‡, Charles Hetru, Marie Lagueux, Maurice Charlet, Gilbert Hegy§¶, Alain Van Dorsselaer, and Jules A. Hoffmann‖

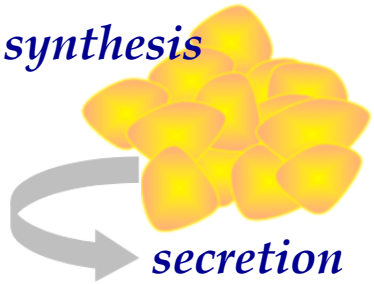
Drosophila antimicrobial effectors (AMPs) functional assays and cDNA sequencing

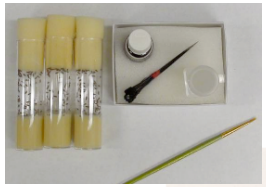
INDUCTION

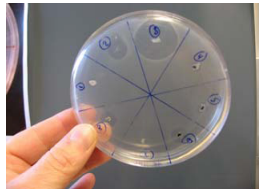
ISOLATION & CARACTERIZATION

ASSAYS

Drosophila adults







From 10'000 – 30'000 individuals

Edman, MS

ESI-MS , GC-MS

Edman, MS
cDNA sequencing

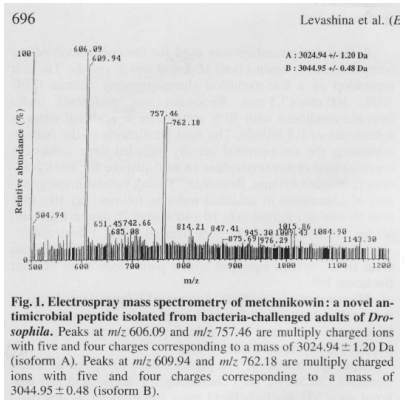


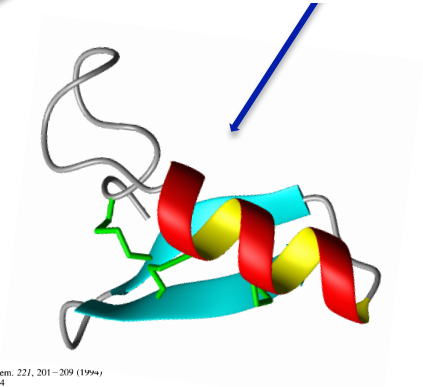
Fig. 1. Electrospray mass spectrometry of metchnikowin : a novel antimicrobial peptide isolated from bacteria-challenged adults of *Drosophila*. Peaks at *m/z* 606.09 and *m/z* 757.46 are multiply charged ions with five and four charges corresponding to a mass of 3024.94 ± 1.20 Da (isoform A). Peaks at *m/z* 609.94 and *m/z* 762.18 are multiply charged ions with five and four charges corresponding to a mass of 3044.95 ± 0.48 (isoform B).

Eur. J. Biochem. *233*, 694–700 (1995)
© FEBS 1995

Metchnikowins (1995)

Metchnikowin, a novel immune-inducible proline-rich peptide from *Drosophila* with antibacterial and antifungal properties

Elena A. LEVASHINA[1], Serge OHRESSER[2], Philippe BULET[2], Jean-Marc REICHHART[2], Charles HETRU[2] and Jules A. HOFFMANN[2]



Eur. J. Biochem. *221*, 201–209 (1994)
© FEBS 1994

defensins (1994)

Characterization and transcriptional profiles of a *Drosophila* gene encoding an insect defensin
A study in insect immunity

Jean-Luc DIMARCQ, Danièle HOFFMANN, Marie MEISTER, Philippe BULET, René LANOT, Jean-Marc REICHHART and Jules A. HOFFMANN

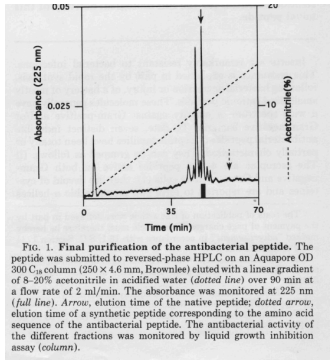
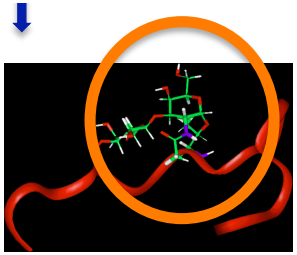


FIG. 1. **Final purification of the antibacterial peptide.** The peptide was submitted to reversed-phase HPLC on an Aquapore OD 300 C_{18} column (250 × 4.6 mm, Brownlee) eluted with a linear gradient of 8–20% acetonitrile in acidified water (*dotted line*) over 90 min at a flow rate of 2 ml/min. The absorbance was monitored at 225 nm (*full line*). *Arrow*, elution time of the native peptide; *dotted arrow*, elution time of a synthetic peptide corresponding to the amino acid sequence of the antibacterial peptide. The antibacterial activity of the different fractions was monitored by liquid growth inhibition assay (*column*).



drosocin (1993)

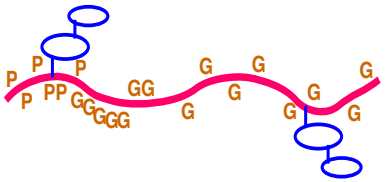
THE JOURNAL OF BIOLOGICAL CHEMISTRY
© 1993 by The American Society for Biochemistry and Molecular Biology, Inc.
Vol. 268, No. 20, Issue of July 15, pp. 14893–14897, 1993
Printed in U.S.A.

A Novel Inducible Antibacterial Peptide of *Drosophila* Carries an O-Glycosylated Substitution*

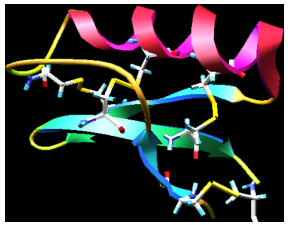
(Received for publication, February 24, 1993)

Philippe Bulet‡, Jean-Luc Dimarcq‡, Charles Hetru, Marie Lagueux, Maurice Charlet, Gilbert Hegy§¶, Alain Van Dorsselaer, and Jules A. Hoffmann‖

Drosophila AMPs



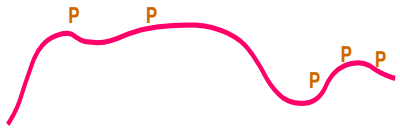
Diptericin
antibacterial



Drosomycin
antifungal

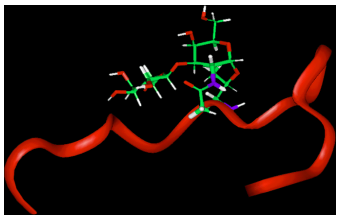
Attacine (cDNA)
antibacterial

Fat body



Metchnikowins
antifungal

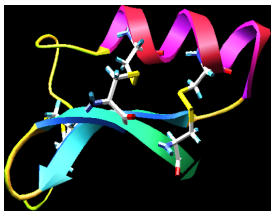
MPAC antibacterial



Drosocins
antibacterial

Cecropins
antimicrobial

Defensin
antimicrobial



Two different strategies in parallel

functional assays & cDNA sequencing

versus

differential profiling by HPLC and MALDI-ms

***Drosophila* antimicrobial effectors (AMPs)**

***Drosophila* Immune-induced Molecules**

(drosomycin, 1992 & DIMs 1994)

Differential analysis by HPLC: Drosomycin & Toll pathway

INDUCTION

DIFFERENTIAL ANALYSIS BY RP-HPLC
100'000 INFECTED DROSOPHILA ADULTS
20'000 NON INFECTED INDIVIDUALS

THE JOURNAL OF BIOLOGICAL CHEMISTRY
© 1994 by The American Society for Biochemistry and Molecular Biology, Inc.
Vol. 269, No. 52, Issue of December 30, pp. 33159–33163, 1994
Printed in U.S.A.

Insect Immunity

SEPTIC INJURY OF DROSOPHILA INDUCES THE SYNTHESIS OF A POTENT ANTIFUNGAL PEPTIDE WITH SEQUENCE HOMOLOGY TO PLANT ANTIFUNGAL PEPTIDES*

(Received for publication, September 2, 1994)

Pascale Fehlbaum‡, Philippe Bulet‡, Lydia Michaut‡, Marie Lagueux‡, Willem F. Broekaert§, Charles Hetru‡, and Jules A. Hoffmann‡¶

Drosomycin (1994)

Fungal growth inhibition

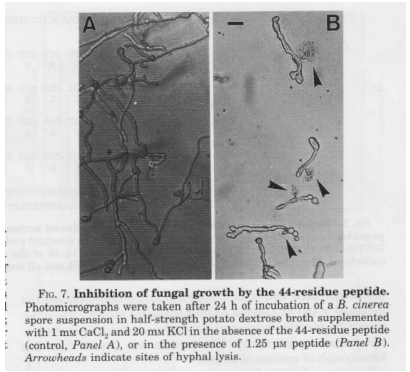


FIG. 7. **Inhibition of fungal growth by the 44-residue peptide.** Photomicrographs were taken after 24 h of incubation of a *B. cinerea* spore suspension in half-strength potato dextrose broth supplemented with 1 mM $CaCl_2$ and 20 mM KCl in the absence of the 44-residue peptide (control, *Panel A*), or in the presence of 1.25 μM peptide (*Panel B*). *Arrowheads* indicate sites of hyphal lysis.

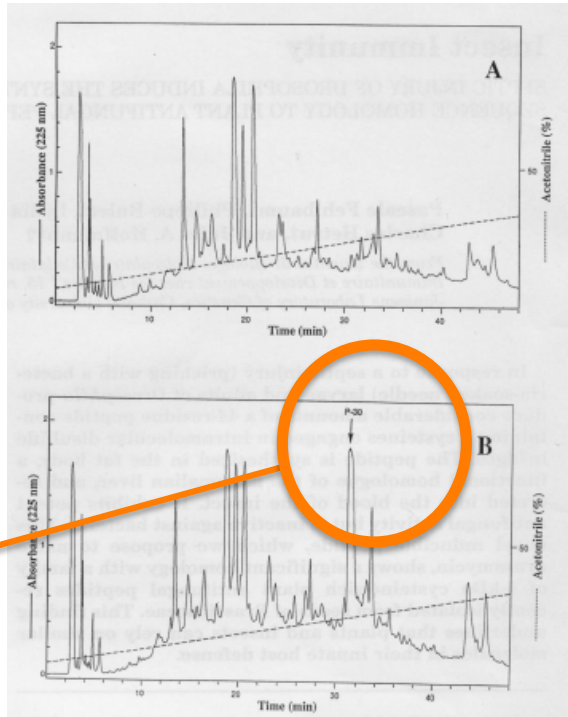


FIG. 1. **Reversed-phase HPLC separation of extracts of naive (*A*) and challenged adult males of *Drosophila* (*B*).** The material contained in fraction F-30% (see "Results") was loaded on an Aquapore $OD300C_{18}$ column and eluted with a linear gradient (*dotted line*) of acetonitrile in 25 mM ammonium acetate buffer pH 5.2. Absorbance was monitored at 225 nm (*solid line*). Note the appearance of an absorption peak P-30 in the extract of challenged insects.

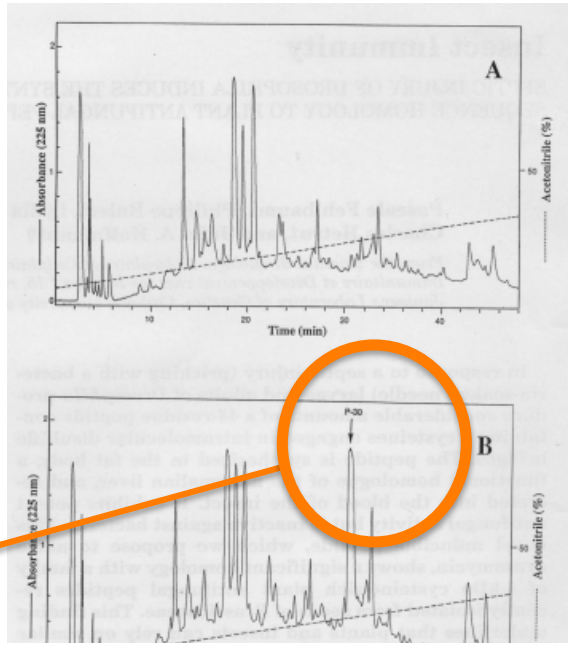
DCLSGRYKGP CAVWDNETCR RVCKEEGRSS GHCSPSLKCW CEGC

FIG. 2. **Amino acid sequence of the bacteria-induced 44-residue peptide.**

Differential analysis by HPLC: Drosomycin & Toll pathway

INDUCTION

DIFFERENTIAL ANALYSIS BY RP-HPLC
100′000 INFECTED DROSOPHILA ADULTS
20′000 NON INFECTED INDIVIDUALS



THE JOURNAL OF BIOLOGICAL CHEMISTRY
© 1994 by The American Society for Biochemistry and Molecular Biology, Inc.
Vol. 269, No. 52, Issue of December 30, pp. 33159–33163, 1994
Printed in U.S.A.

Insect Immunity

SEPTIC INJURY OF DROSOPHILA INDUCES THE SYNTHESIS OF A POTENT ANTIFUNGAL PEPTIDE WITH SEQUENCE HOMOLOGY TO PLANT ANTIFUNGAL PEPTIDES*

(Received for publication, September 2, 1994)

Pascale Fehlbaum‡, Philippe Bulet‡, Lydia Michaut‡, Marie Lagueux‡, Willem F. Broekaert§, Charles Hetru‡, and Jules A. Hoffmann‡¶

Drosomycin (1994)

Fungal growth inhibition

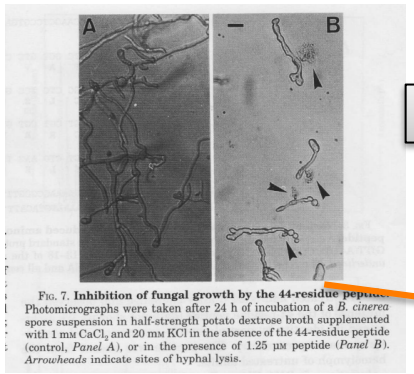
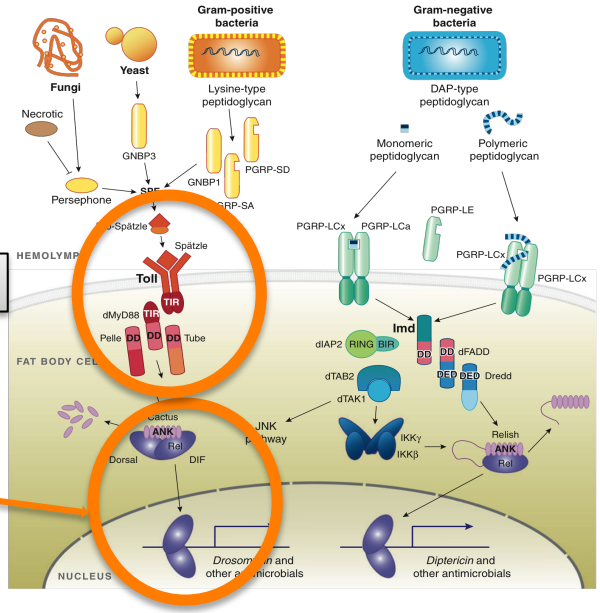


FIG. 7. **Inhibition of fungal growth by the 44-residue peptide.** Photomicrographs were taken after 24 h of incubation of a *B. cinerea* spore suspension in half-strength potato dextrose broth supplemented with 1 mM $CaCl_2$ and 20 mM KCl in the absence of the 44-residue peptide (control, *Panel A*), or in the presence of 1.25 μM peptide (*Panel B*). *Arrowheads* indicate sites of hyphal lysis.

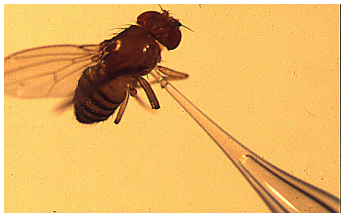
TOLL PATHWAY

EFFECTOR



Differential analysis by MALDI – MS body fluid from a single fly

septic injury
(M. luteus & E. coli)



Hemolymph collection
0.1 µL

SAMPLE PREPARATION

Dried droplet (purified molecules)
(matrix solution / sample)

Thick layer (mixture, body fluid)
(M1 NC/4HCCA– Sample – M2 4HCCA)

Thin layer (HPLC fractions)
(M1 4HCCA– Fraction)

Proc. Natl. Acad. Sci. USA
Vol. 95, pp. 11342–11347, September 1998
Immunology

Differential display of peptides induced during the immune response of *Drosophila*: A matrix-assisted laser desorption ionization time-of-flight mass spectrometry study

SANDRINE UTTENWEILER-JOSEPH*, MARC MONIATTE†, MARIE LAGUEUX*, ALAIN VAN DORSSELAER†, JULES A. HOFFMANN*, AND PHILIPPE BULET*‡

CHAPTER 11

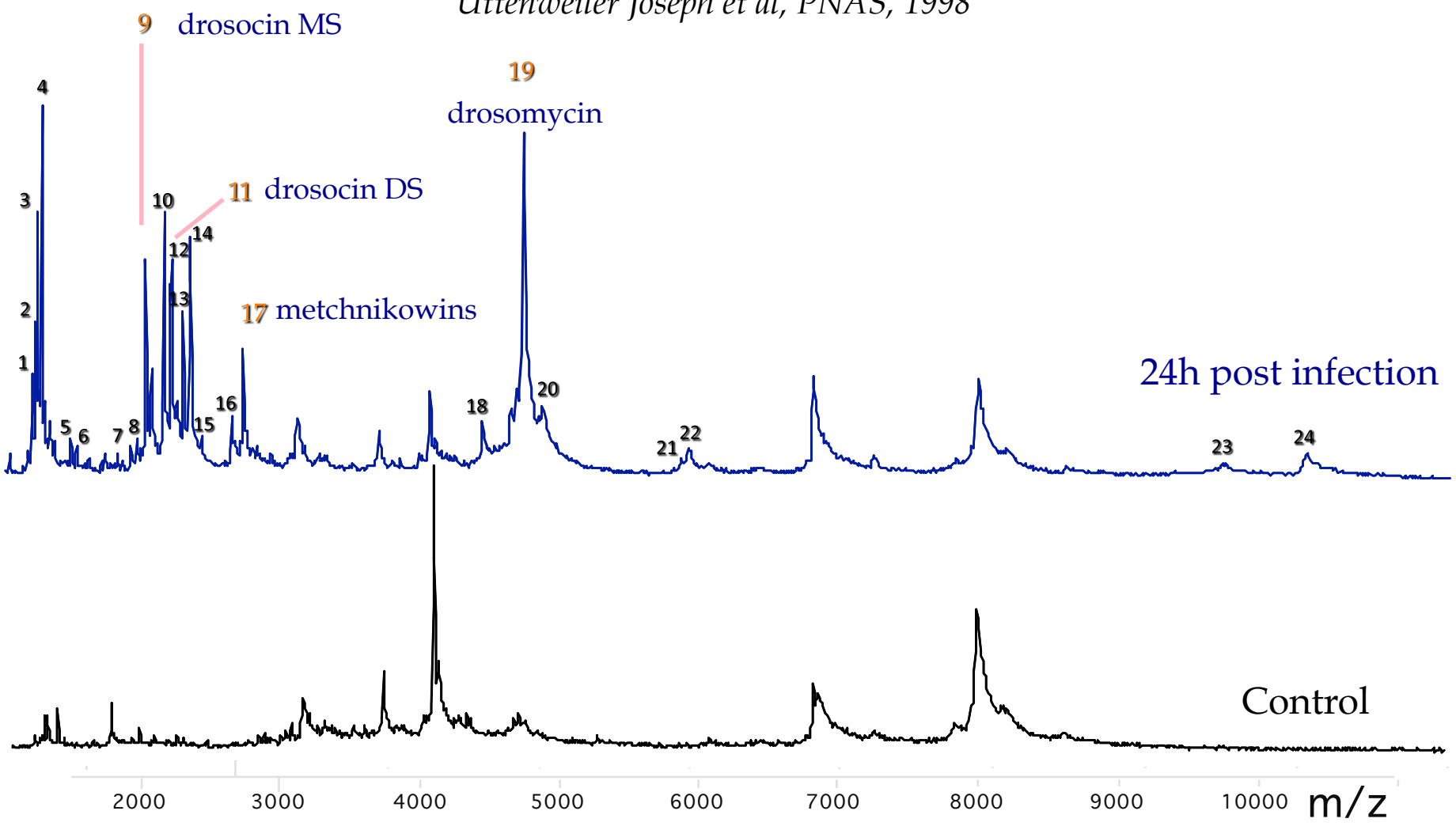
A MALDI-TOF Mass Spectrometry Approach to Investigate the Defense Reactions in *Drosophila melanogaster*, an Insect Model for the Study of Innate Immunity

PH. BULET[1] and S. UTTENWEILER-JOSEPH[2]

Differential analysis by MALDI – MS

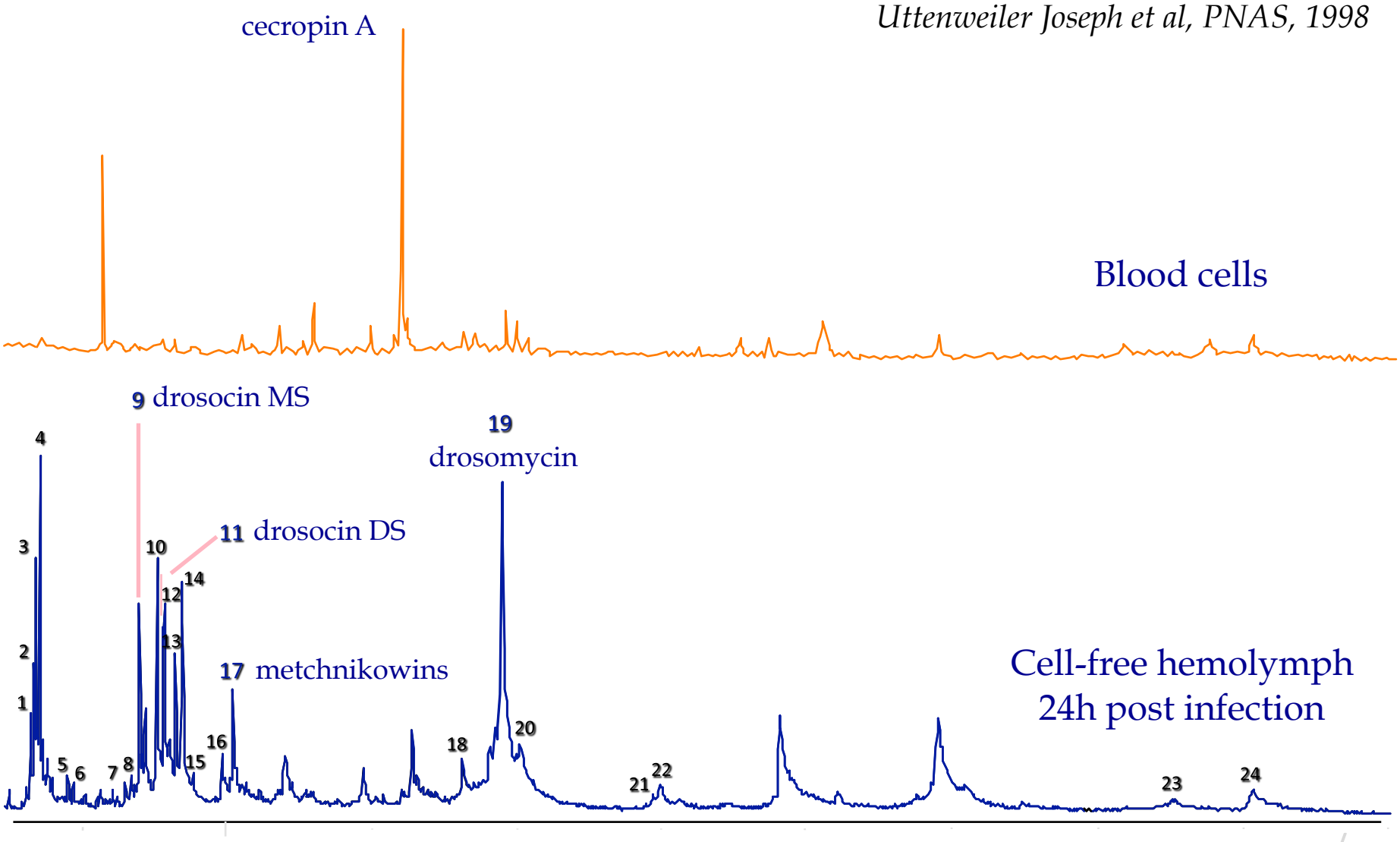
Molecular mass fingerprint (blood from one fly)

Uttenweiler Joseph et al, PNAS, 1998



Differential analysis by MALDI – MS
cell free hemolymph vs blood cells

Uttenweiler Joseph et al, PNAS, 1998



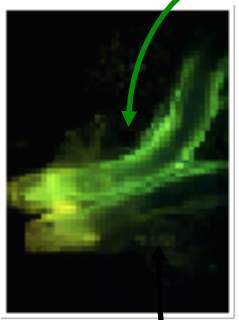
Detection by MALDI-TOF MS of drosomycin in *Drosophila* trachea

The EMBO Journal Vol.17 No.5 pp.1217–1227, 1998

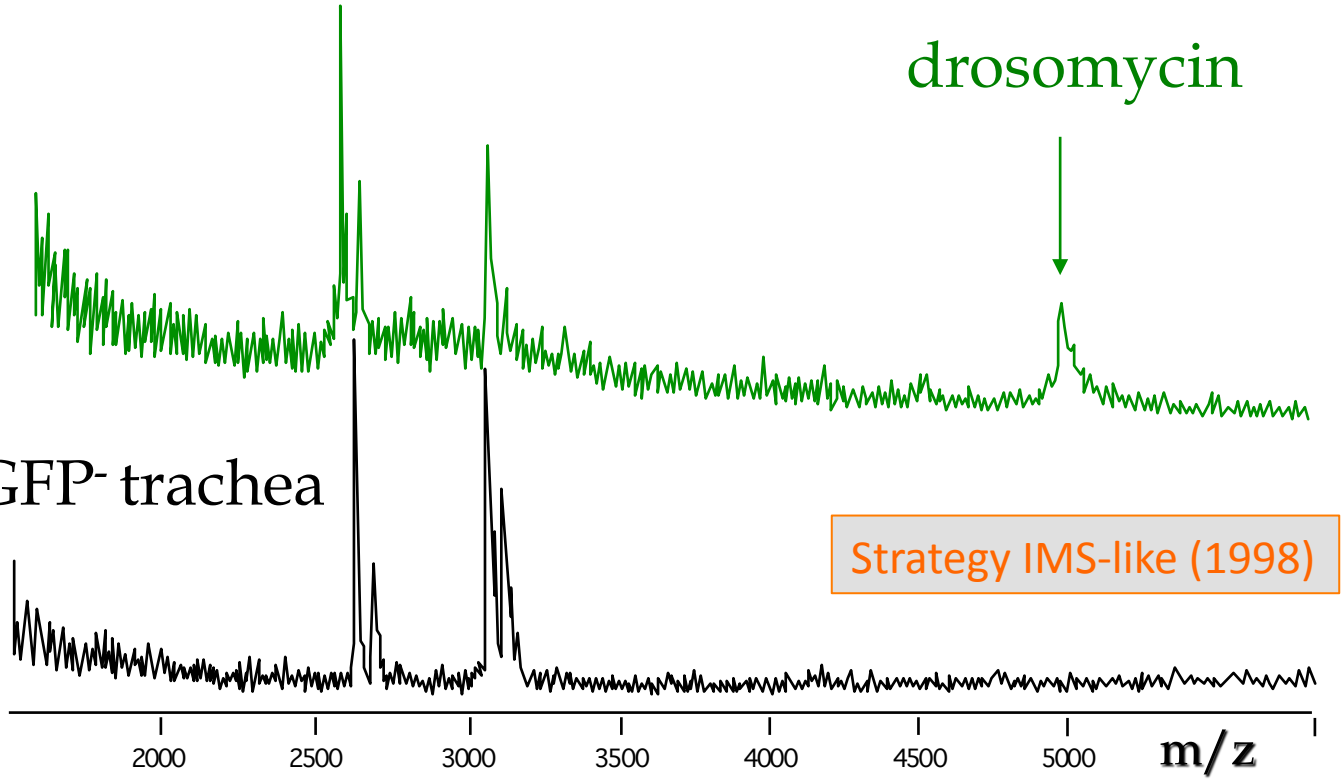
A drosomycin–GFP reporter transgene reveals a local immune response in *Drosophila* that is not dependent on the *Toll* pathway

D.Ferrandon[1], A.C.Jung, M.-C.Criqui[2], B.Lemaitre, S.Uttenweiler-Joseph, L.Michaut[3], J.-M.Reichhart and J.A.Hoffmann

Drosomycin-GFP$^+$ trachea

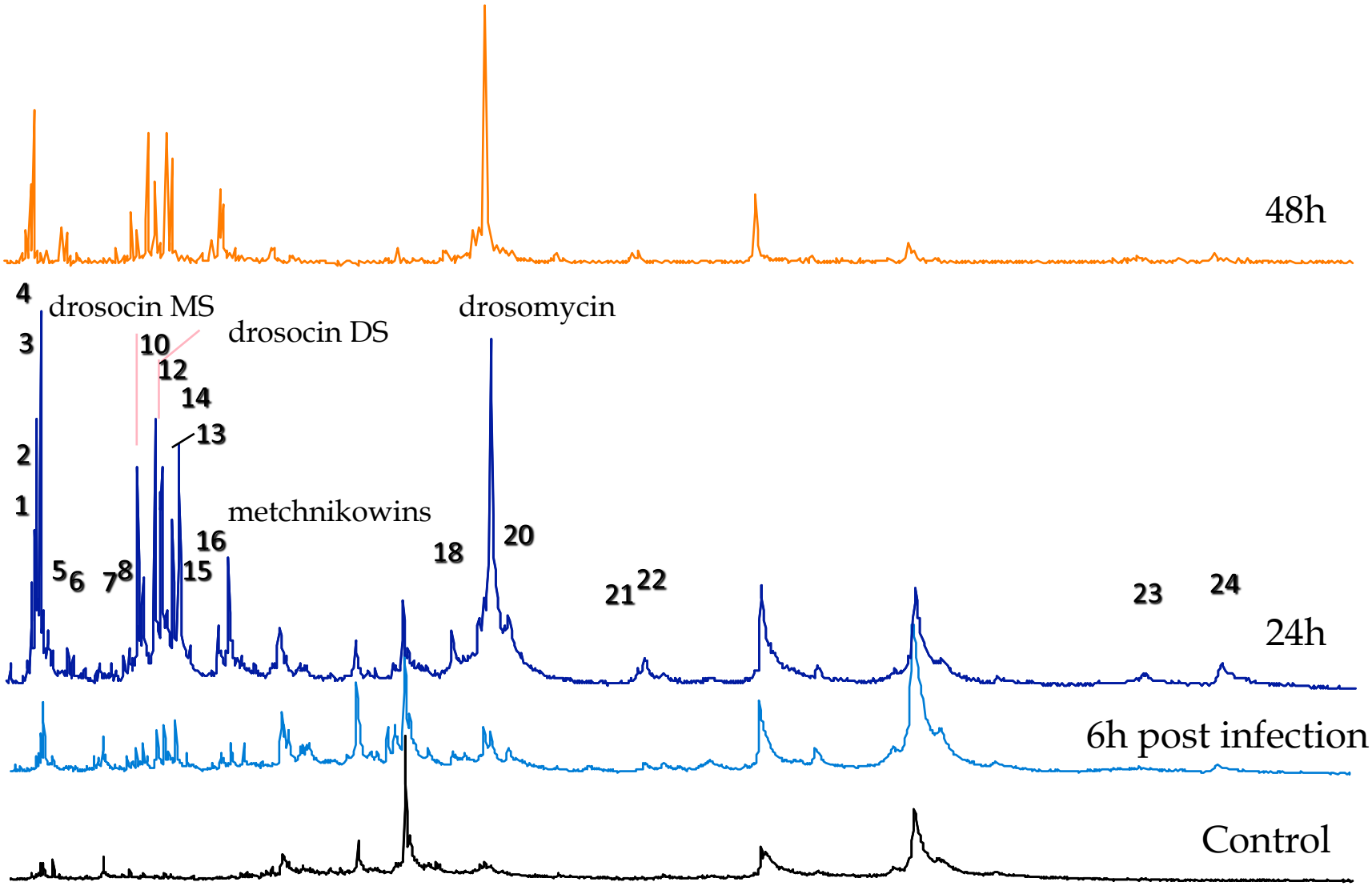


Drosomycin-GFP$^-$ trachea

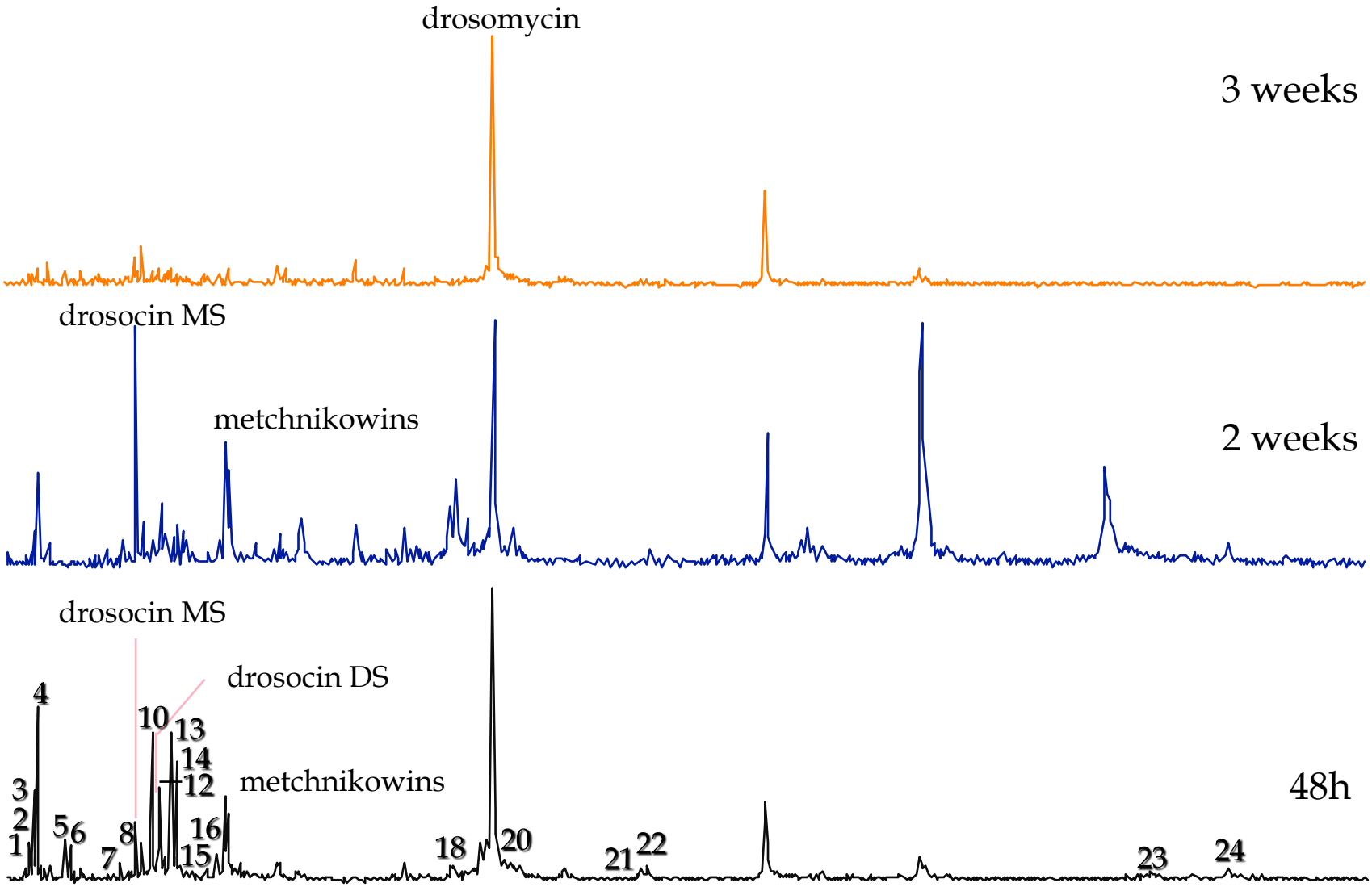


Strategy IMS-like (1998)

MALDI-TOF MS time course analysis of appearance of the DIMs upon bacterial infection



MALDI-TOF MS time course analysis of disappearence of the DIMs



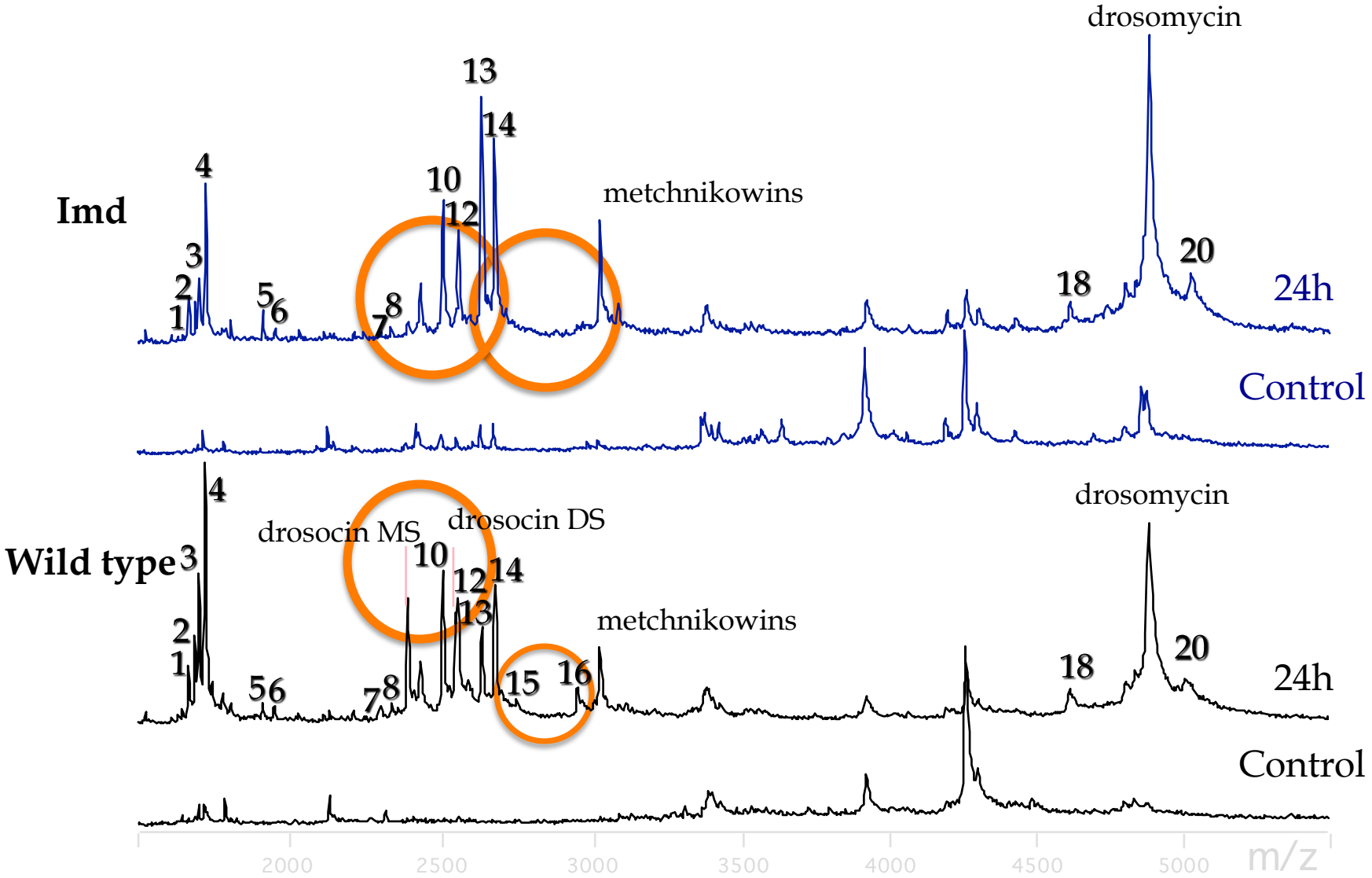
Induction of DIMs in mutants with an alterated immune response

Mutant strains:

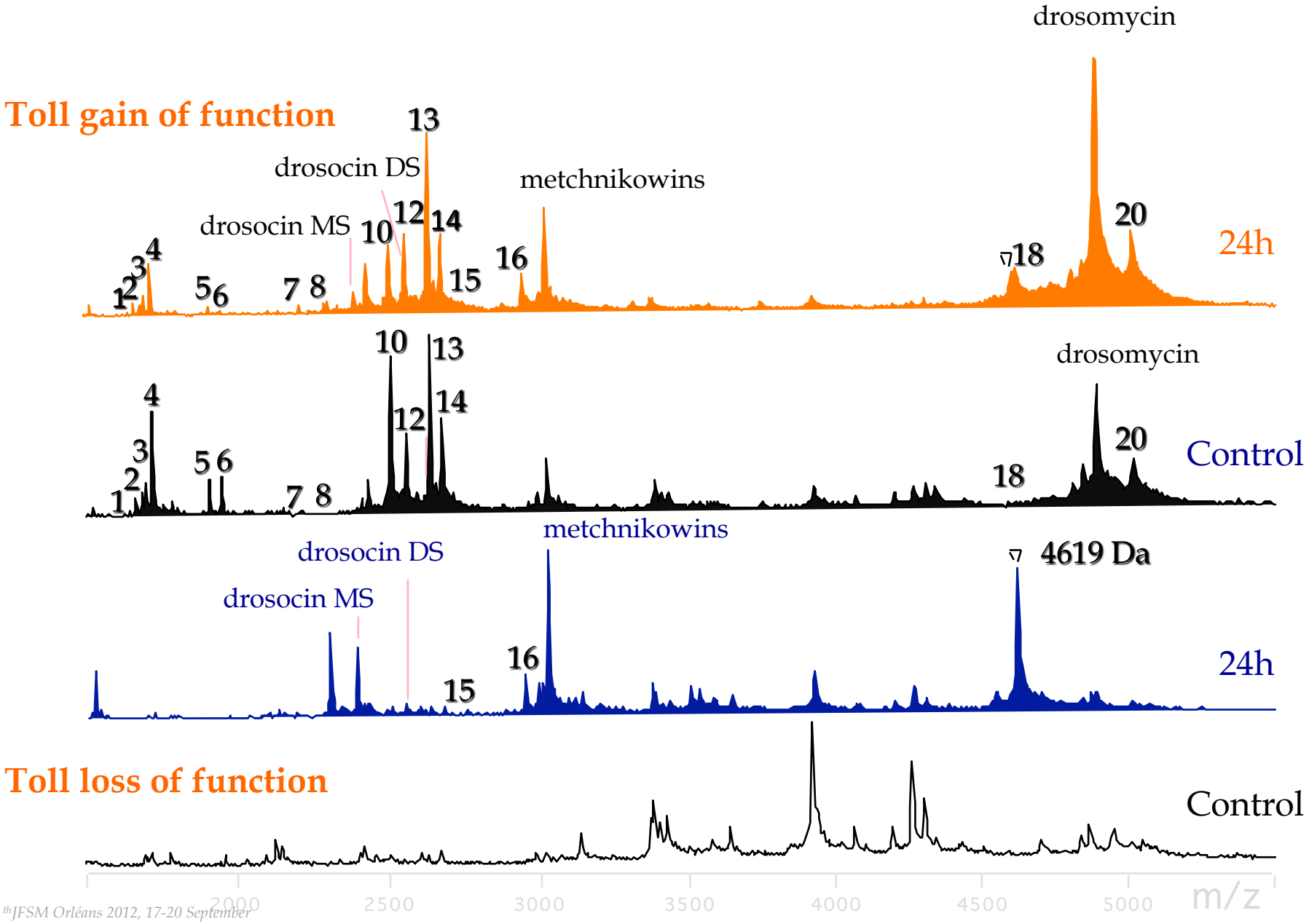
Imd, Toll-loss and Toll-gain of function

Imd mutants

Four DIMs are controlled by the Imd pathway: DIMs -9, -11, -15 and -16



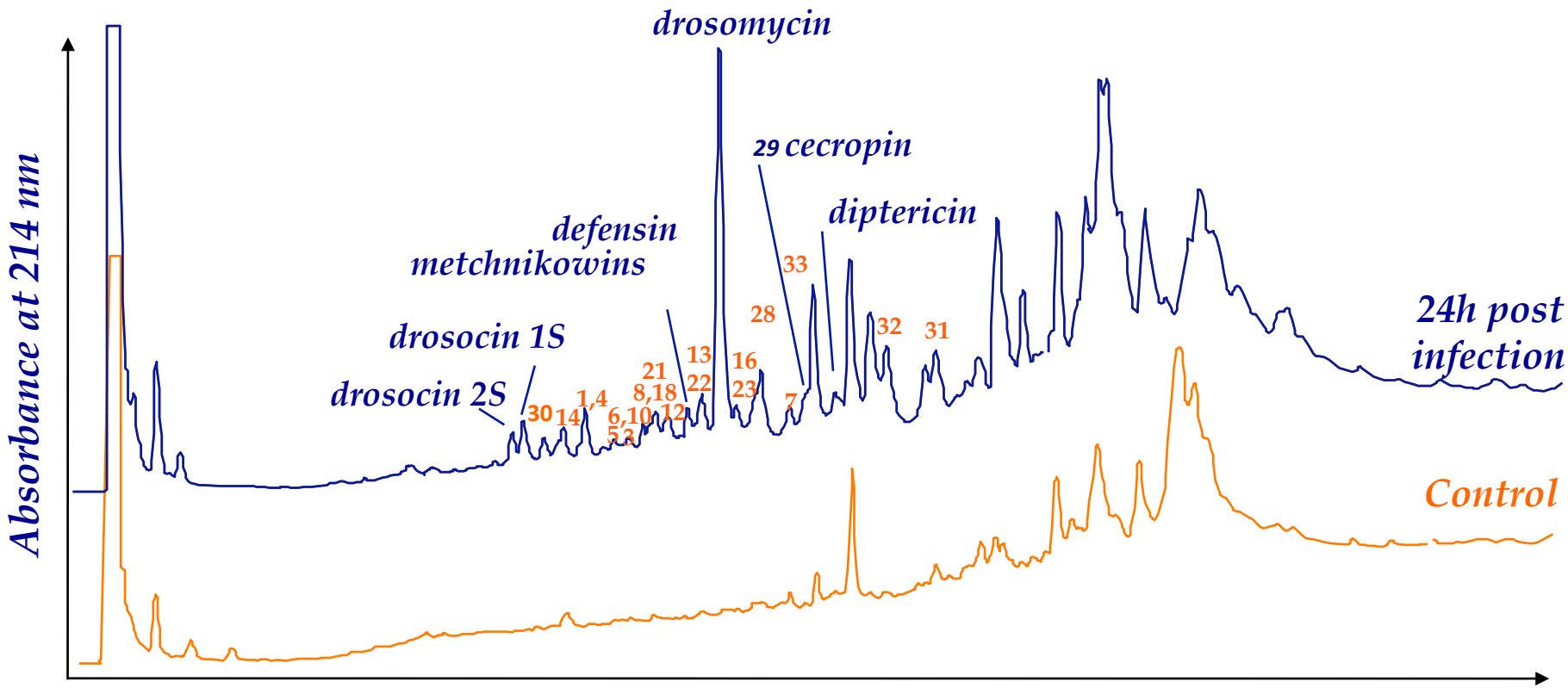
Toll mutants



Differential analysis by µ-HPLC & MALDI – mass fingerprint

HPLC off line or on line LC-ms (20 flies)

Uttenweiler Joseph et al, PNAS, 1998



Induction upon infection of 75 *DIMs

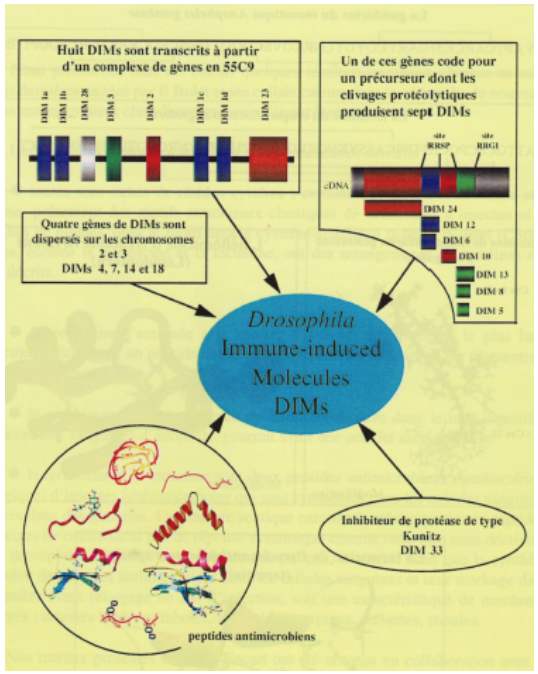
* *Drosophila Immune-induced Molecules*

Identification & characterization of 33 novel DIMs

Chromatography

Mass spectrometry

Characterization



Proteolyses

Carboxypeptidase

Data bank search

Results of the differential display by µ-HPLC and MALDI-TOF MS

After a 24h microbial-challenge at least 75 DIMs are detected

✓ By differential analysis by MALDI-Tof MS, 24 DIMs were detected (DIM-1 1,666 Da to DIM-24 10,031 Da). Five are antimicrobial compounds (metchinikowins, drosomycin, drosocin, diptericin, MPAC)

✓ Most of the DIMs are controlled by the Toll pathway, except DIM-9, -11, -15 (drosocins) and DIM-16 (MPAC) under the Imd pathway. One (metchnikowin) is regulated by both pathways

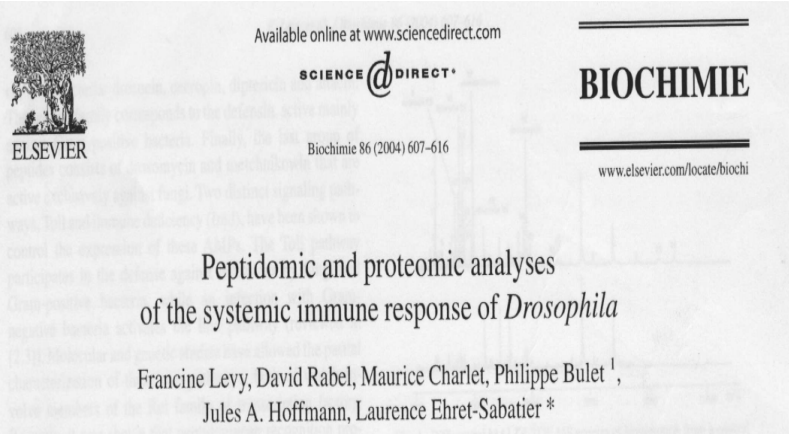
✓ By HPLC and MALDI-Tof MS, 70 are potentially novel molecules of the *Drosophila* immune response:
- 30 have a mass < 3 kDa
- 20 have a mass between 3-6 kDa and 25 between 6-15 kDa
- 33 were characterized, some are antimicrobial peptides with PTMs (glycoform of drosomycin and diptericin)

✓ Few molecules are repressed.

Proteomic studies (molecules > 15 kDa) to complement peptidomic investigations

Laurence Sabatier, Francine Levy

(early 2000)



Available online at www.sciencedirect.com

SCIENCE DIRECT

BIOCHIMIE

ELSEVIER

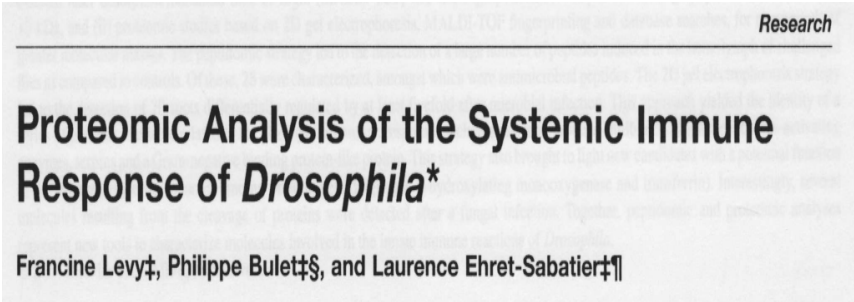
Biochimie 86 (2004) 607–616

www.elsevier.com/locate/biochi

Peptidomic and proteomic analyses of the systemic immune response of *Drosophila*

Francine Levy, David Rabel, Maurice Charlet, Philippe Bulet [1], Jules A. Hoffmann, Laurence Ehret-Sabatier *

Published in Molecular and Cellular Proteomics, 3.2, 2004



Research

Proteomic Analysis of the Systemic Immune Response of *Drosophila**

Francine Levy‡, Philippe Bulet‡§, and Laurence Ehret-Sabatier‡¶

Differential analyses through proteomics studies

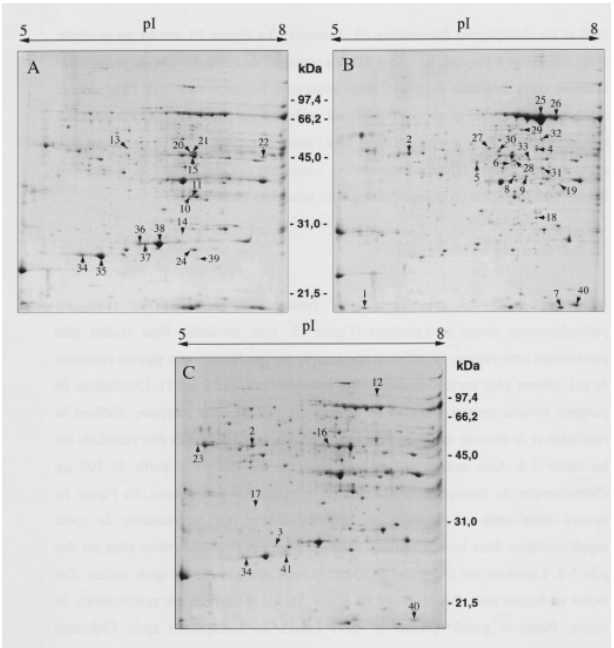
Published in Molecular and Cellular Proteomics, 3.2, 2004

Research

Proteomic Analysis of the Systemic Immune Response of *Drosophila**

Francine Levy‡, Philippe Bulet‡§, and Laurence Ehret-Sabatier‡¶

2D map of colloidal Coomassie-stained proteins from *Drosophila* adult blood (600 flies, 300 mg of proteins). Map A, control flies. Map B, flies infected with a fungus (72h). Map C, flies inoculated with G+ bacteria (6h). Over 160 spots were detected in Map A (versus 350 in silver stained gels).



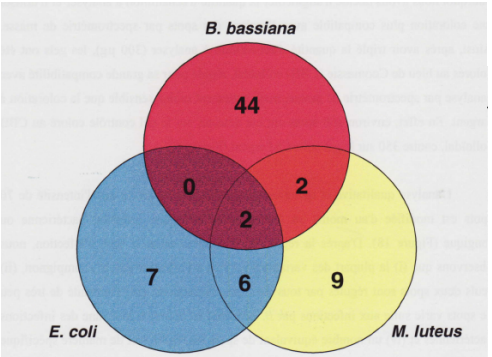


FIG. 4. **Schematic representation of the number of spots specifically regulated by each type of infection.** PDQuest analysis of colloidal Coomassie-stained spots regulated at least 5-fold (up- or down-regulated) on 2D gels after each infection (*B. bassiana*, *M. luteus*, or *E. coli*) and MS analysis allowed the determination of which proteins have an expression level altered by one, two, or three types of infection. Proteins that were identified are given in Table II.

70 over the 160 proteins were found to be up- or down-regulated (factor of five) after a bacterial or fungal challenge.

This includes proteins from many functional classes:

- proteases (ProPO-AEs, zymogens and CLIP-domain-containing proteases)
- serpins (Nec involved in the Toll pathway)
- recognition proteins (GNBP-like proteins) and signal transducing proteins (phosphatidylethanolamine binding proteins, PEBP)
- complement-like proteins (TEPs, thiesther-containing proteins)
- molecules involved in the iron metabolism were identified such as transferrin (up-regulated) and ferritins (down-regulated)
- olfactory proteins, two pherokines were found up-regulated upon a viral infection (Sabatier et al, EJB, 2003)
- amidation enzyme (Phm, peptidylglycine –hydroxylating monooxygenase).

Conclusions

MS was ubiquitous in our investigations on *drosophila* immune response

- Molecular mass determination, purity control, structure elucidation, identi
- Identification of PTMs (glycosylations, S-S bridging, etc.)
- Control of integrity between native peptides and recombinant or synthetic versions
- Discovery process of the immune effectors (75 DIMs including 7 families of AMPs), discrimination between DIMs regulated by the Toll or Imd pathways
- Peptidomics ands proteomics studies (body fluids and tissues)
- Kinetic of appearance / disappearance of the immune effectors
- Quantitation of the AMPs by a combination of MS, HPLC and CE.

Acknowledgments

UPR 9022, RIDI

Present Director JM Reichhart

Former Director JA Hoffmann

My colleagues

Danielle Hoffmann

Charles Hetru

Jean Luc Dimarcq

Marie Lagueux

Jean Lambert

Maurice Charlet

Laurence Sabatier

Nathalie Boulanger

Martine Schneider

and many others

UMR 7178, LSMBO

Present Director A Van Dorsselaer

The colleague

Jean Marc Strub

PhD students & Post Doc

RIDI

Serge Ohresser

Pascale Fehlbaum

Sandrine Uttenweiler-Joseph

Francine Levy

David Rabel

Jacopo Vizioli

LSMBO

Gilbert Hegy

Marc Moniatte

Nukhet Cavusoglu

Nathalie Carte

Thank you for your attention
and
for inviting me to the 29th JFMS