Intracellular Niches of Microbes

A Pathogens Guide Through the Host Cell

Edited by
Ulrich E. Schaible and Albert Haas

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The Editors

Prof. Ulrich E. Schaible
Research Center Borstel
Zentrum für Medizin und Biowissenschaften (FZB)
Department of Molecular Infection Research
Parkallee 1 – 40
23845 Borstel
Germany

Prof. Albert Haas
University of Bonn
Cell Biology Institute
Ulrich-Haberland-Straße 61 a
53121 Bonn
Germany

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Foreword

Over the last thirty years, the combined use of bacterial genetics, molecular and cell biology, and more recently genomics, have illuminated our understanding of the virulence of the major human and veterinary pathogens. Among those, intracellular bacterial pathogens have played a dominant role in this endeavor because they recapitulate most of the existing steps of microbe-host interaction. The progress made has been impressive, not only conceptually, but also technically. Imaging, for instance, has often prepared and preceded the discoveries in this area where *seeing is believing*, it has also tremendously benefited from these models in return. Adherence of pathogens to cells, diversity of mechanisms of entry, a variety of mechanisms for intracellular survival and growth, from vacuolar rupture followed by escape into the cytoplasm, to remodeling of vacuoles to avoid phagolysomal fusion – a combinatorial synthesis of these steps of interaction has led to an amazing breadth of diversified strategies representing the complex solutions "developed" by nature for pathogens to achieve survival as species, or pathovars among these species, in the ferocious struggle for life. These microorganisms are exposed to harsh environmental conditions such as fighting against protozoan predators (i.e. amoebas) for the so called environmental pathogens like *Legionella*, or to the immune system that has appeared early in the world of multicellular organisms. *Nothing makes sense in biology if not seen under the angle of evolution* said Theodosius Dobzansky. The world of intracellular microbes is a perfect illustration of this statement. With the possible exception of environmental pathogens, intracellular pathogens bear in their genomes the traces of their construction under selective pressure of their host. It results in a complex mixture of gene acquisition and gene deletion that strongly differentiate them from their closest commensal cousins. A permanent flux of genes, largely perpetrated by bacteriophages and plasmids permanently occurs, particularly in the gut lumen of animals, thereby permanently offering microbes options to improve their fit with the host. It is difficult to decipher the timing of the genetic events that have led to such complex combinations. However, one would like to believe that some key steps have occurred at some stage, like the acquisition of a large pathogenicity island encoding invasive capacities, thereby propelling the microorganisms in a new hostile environment to which it was not prepared. Only the acquisition and/or loss of genes

allowing the pathogen to cope with these new conditions was able to secure its survival. *It is not the strongest of the species that survives. . .nor the most intelligent that survives. It is the one that is the most adaptable to change* said Darwin in *On the origin of species by way of natural selection*. It is interesting to consider that our "contemporary" pathogens still have a large capacity to evolve, but that only the stochastic acquisition of new genes by horizontal transfer can achieve the quantum leap changes that move the evolutionary process. It is also interesting to observe that several genomes of intracellular pathogens, particularly those that have become obligate intracellular parasites (i.e. *Rickettsia spp.*, *Chlamydia spp.*, *Mycobacterium leprae*) show highly degraded genomes, with massive amount of gene loss. Whether this is a true reflection of the need for compensatory gene deletion to the acquisition of new intracellular pathogenic properties, or simply the loss in absence of selective pressure of genes (i.e. metabolic pathways) that are no longer useful to the pathogen since it benefits from the cell nutrients, is still an open and debated question. In any event, the progression towards complete intracellular parasitism that is often accompanied by restriction in species specificity is a dominant feature of intracellular microorganisms that certainly needs further investigation.

Last but not least, it seems that the ultimate option for these microorganisms is to become true symbionts. Indeed, some molecular systems such as secretory apparatus that are essential to deliver virulence effectors are conserved in true symbionts, probably with the aim to deliver symbiotic effectors. The ultimate example is the absolute harnessing by eukaryotic cells of *Rickettsia spp*. to generate mitochondria as O_2 appeared on earth. No need to reach this extreme, however, *Wolbachia spp*. do parasitize insects in a very "stealthy" manner, and only recently was it observed that this symbiosis, beside its well known effect on fecundity, was stimulating a background level of innate immunity allowing these insects to resist viral infection.

The world of intracellular pathogens, and symbionts that should now be part of this global concept, appears as a gold mine of strategies whose purpose often still needs to be understood.

In an amazing series of contributions by renowned world experts, this volume provides the first integrated, coherent and exhaustive review of the biology of intracellular pathogens and symbionts. It offers a fascinating vision of the evolutionary logics that have led microorganisms to venture into cells and to adapt to the environment of this niche in a way that led to genomic modifications which made them for ever different and highly adapted to this particular life style. For some of them it evolved to the ultimate point of becoming obligate intracellular pathogens, or even symbionts.

Ulrich Schaible and Albert Haas should be congratulated for their exceptional vision of the field, contagious enthusiasm that helped assemble this key stone volume, and, last but not least, exceptional service to our community.

Philippe J. Sansonetti
Professeur au Collège de France
Professeur à l'Institut Pasteur

Contents

List of Contributors

Lee-Ann H. Allen
Departments of Microbiology and Internal Medicine
The Inflammation Program and the VA Medical Center
Iowa City, IA 52242
USA

Damien Balestrino
Institut Pasteur
Unité des Interactions Bactéries Cellules
75015 Paris
France
and
INSERM
U604
75015 Paris
France
and
INRA
USC2020
75015 Paris
France

Kristine von Bargen
Cell Biology Institute
University of Bonn
Ulrich-Haberland-Str. 61a
53121 Bonn
Germany

Martin Baumgartner
Division of Molecular Pathobiology
DCR-VPH
Vetsuisse Faculty Bern
University of Bern
Länggassstr. 122
3012 Bern
Switzerland

Ulrike Becken
Cell Biology Institute
University of Bonn
Ulrich-Haberland-Str. 61a
53121 Bonn
Germany

William J. Broughton
LBMPS
Sciences III
Université de Genève
30 Quai Ernest-Ansermet
1211 Geneva 4
Switzerland

Philip D. Butcher
Medical Microbiology
Centre for Infection
Division of Cellular & Molecular Medicine
St. George's University of London
Cranmer Terrace, Tooting
London SW17 0RE
UK

Gabriela Cosío
Program in Cell Biology
Hospital for Sick Children
555 University Avenue
Toronto, Ontario M5G 1X8
Canada

Pascale Cossart
Institut Pasteur
Unité des Interactions Bactéries Cellules
75015 Paris
France
and
INSERM
U604
75015 Paris
France
and
INRA
USC2020
75015 Paris
France

Deborah Dean
Center for Immunobiology and Vaccine Development
Children's Hospital Oakland Research Institute
Oakland, CA
USA
and
UCB and UCSF Joint Graduate Group in Bioengineering
University of California at Berkeley
Berkeley, CA
USA
and
University of California at San Francisco
San Francisco, CA
USA

and
Department of Medicine
University of California at San Francisco
San Francisco, CA
USA

William J. Deakin
LBMPS
Sciences III
Université de Genève
30 Quai Ernest Ansermet
1211 Geneva 4
Switzerland

Albert Descoteaux
INRS-Institut Armand-Frappier
Université du Québec
Laval, Quebec H7V 1B7
Canada

Régis Dieckmann
Département de Biochimie
Faculté des Sciences
Université de Genève
Sciences II
30 quai Ernest Ansermet
1211 Geneva 4
Switzerland

Dirk Dobbelaere
Division of Molecular Pathobiology
DCR-VPH
Vetsuisse Faculty Bern
University of Bern
Länggassstr. 122
3012 Bern
Switzerland

Heike Feldhaar
Lehrstuhl für Verhaltensbiologie
Universität Osnabrück
Barbarastr. 11
49076 Osnabrück
Germany

Stacey D. Gilk
Coxiella Pathogenesis Section
Laboratory of Intracellular Parasites
Rocky Mountain Laboratories
National Institute of Allergy and
Infectious Diseases
National Institutes of Health
Hamilton, MT 59840
USA

Jean-Pierre Gorvel
Centre d'Immunologie de
Marseille-Luminy
case 906
13288 Marseille Cedex 9
France

David B. Guiliano
Infectious and Tropical Diseases
Division of Infection and Immunity
Windeyer Building
46 Cleveland Street
London W1T 4JF
UK

Gareth Griffiths
Electronmicroscopical Unit
for Biological Sciences
University of Oslo
P.O. Box 1062, Blindern
0316 Oslo
Norway

Sergio Grinstein
Program in Cell Biology
Hospital for Sick Children
555 University Avenue
Toronto, Ontario M5G 1X8
Canada

Roy Gross
Lehrstuhl für Mikrobiologie
Biozentrum
Universität Würzburg
Am Hubland
97074 Würzburg
Germany

Maximiliano G. Gutierrez
Helmholtz-Zentrum für
Infektionsforschung GmbH
Inhoffenstr. 7
38124 Braunschweig
Germany

Albert Haas
Cell Biology Institute
University of Bonn
Ulrich-Haberland-Str. 61a
53121 Bonn
Germany

Ted Hackstadt
Host–Parasite Interactions Section
Laboratory of Intracellular Parasites
Rocky Mountain Laboratories
National Institute of Allergy and
Infectious Diseases
National Institutes of Health
Hamilton, MT 59840
USA

Robert A. Heinzen
Coxiella Pathogenesis Section
Laboratory of Intracellular Parasites
Rocky Mountain Laboratories
National Institute of Allergy and
Infectious Diseases
National Institutes of Health
Hamilton, MT 59840
USA

Monica Hagedorn
Département de Biochimie
Faculté des Sciences
Université de Genève
Sciences II
30 quai Ernest Ansermet
1211 Geneva 4
Switzerland

Alyssa Ingmundson
Section of Microbial Pathogenesis
Yale University School of Medicine
Boyer Center for Molecular Medicine
295 Congress Avenue
New Haven, CT 06536
USA

Monika Kalde
Centre d'Immunologie de
Marseille-Luminy
case 906
13288 Marseille Cedex 9
France

Kumiko Kambara
LBMPS
Sciences III
Université de Genève
30 Quai Ernest Ansermet
1211 Geneva 4
Switzerland

Daniel S. Korbel
Centre for Digestive Diseases
The Blizard Institute
Barts and The London School of
Medicine and Dentistry
Queen Mary, University of London
4 Network Street
London E1 2AT
UK

and
London School of Hygiene and
Tropical Medicine
Department of Infectious and
Tropical Diseases
Immunology Unit
Keppel Street
London WC1 7HT
UK

Goran Kovacevic
Faculty of Science
University of Zagreb
Rooseveltov trg 6
10000 Zagreb
Croatia

Wolfgang Löffelhardt
Max F. Perutz Laboratories
University of Vienna
Department of Biochemistry
Dr. Bohrgasse 9
1030 Vienna
Austria

Klaus Lingelbach
FB Biology
Department of Parasitology
Philipps-University Marburg
Karl-von-Frisch-Str. 8
35043 Marburg
Germany

Julia Mallégol
INRS-Institut Armand-Frappier
Université du Québec
Laval, Quebec H7V 1B7
Canada

Christine Matte
INRS-Institut Armand-Frappier
Université du Québec
Laval, Quebec H7V 1B7
Canada

Edgardo Moreno
Programa de Investigación
en Enfermedades Tropicales
Escuela de Medicina Veterinaria
304–3000 Heredia
Costa Rica

Simon L. Newman
Division of Infectious Diseases
University of Cincinatti Col. Med.
P.O. Box 670560
Cincinatti, OH 45267-0560
USA

Yelena Oksov
Laboratory of Electron Microscopy
Lindsey F. Kimball Research Institute
New York Blood Center
New York, NY 10021
USA

Jude M. Przyborski
FB Biology
Department of Parasitology
Philipps-University Marburg
Karl-von-Frisch-Str. 8
35043 Marburg
Germany

Elena Rydkina
Departments of Microbiology/
Immunology and Medicine
University of Rochester Medical School
of Medicine and Dentistry
601 Elmwood Avenue
Rochester, NY 14642
USA

Yasuko Rikihisa
Department of Veterinary Biosciences
College of Veterinary Medicine
The Ohio State University
1925 Coffey Road
Columbus, OH 43210
USA

Craig R. Roy
Section of Microbial Pathogenesis
Yale University School of Medicine
Boyer Center for Molecular Medicine
295 Congress Avenue
New Haven, CT 06536
USA

Sanjeev K. Sahni
Departments of Microbiology/
Immunology and Medicine
University of Rochester Medical School
of Medicine and Dentistry
601 Elmwood, Avenue
Rochester NY 14642
USA

Philippe Sansonetti
Unité de Pathogénie Microbienne
Moléculaire
Institut Pasteur
Paris
France
and
Unité 786
Institut National de la Santé et
de la Recherche Médicale
Inserm U786
Paris
France

Ulrich E. Schaible
Research Center Borstel
Leibniz-Zentrum für Medizin und
Biowissenschaften
Department of Molecular Infection
Research
Parkallee 1-40
23845 Borstel
Germany

and
London School of Hygiene and
Tropical Medicine
Department of Infectious and
Tropical Diseases
Immunology Unit
Keppel Street
London WC1 7HT
UK

Bianca E. Schneider
Infectious and Tropical Diseases
Immunology Unit
London School of Hygiene and
Tropical Medicine
Keppel Street
London WC1 7HT
UK

Grant S. Schulert
Departments of Microbiology and
Internal Medicine
The Inflammation Program and the
VA Medical Center
Iowa City, IA 52242
USA

L. David Sibley
Department of Molecular Microbiology
Washington University School of
Medicine
St. Louis, MO 63110
USA

David J. Silverman
Department of Microbiology and
Immunology
University of Maryland School of
Medicine
Baltimore, MD
USA

Thierry Soldati
Département de Biochimie
Faculté des Sciences
Université de Genève
Sciences II
30 quai Ernest Ansermet
1211 Geneva 4
Switzerland

Naraporn Somboonna
Center for Immunobiology and
Vaccine Development
Children's Hospital Oakland Research
Institute
Oakland, CA
USA
and
UCB and UCSF Joint Graduate Group
in Bioengineering
University of California at Berkeley
Berkeley, CA
USA
and
University of California at San Francisco
San Francisco, CA
USA
and
National Center for Genetic
Engineering and Biotechnology
National Science and Technology
Development Agency
Pathumthani 12120
Thailand

Olivia Steele-Mortimer
Laboratory of Intracellular Parasites
National Institutes of Allergy and
Infectious Diseases
National Institutes of Health
Rocky Mountain Laboratories
Hamilton, MT 59840
USA

Jürgen M. Steiner
Max F. Perutz Laboratories
University of Vienna
Department of Biochemistry
Dr. Bohrgasse 9
1030 Vienna
Austria

Guy Tran Van Nhieu
Inserm U 971
Unité de Communications
Intercellulaires et Infections
Microbiennos
Collège de France
11, Place Marcelin Berthelot
75005 Paris Cedex
France

Michael Steinert
Institut für Mikrobiologie
Technische Universität Braunschweig
Spielmannstr. 7
38106 Braunschweig
Germany

Joanne M. Stevens
Division of Microbiology
Institute for Animal Health
Compton
Berkshire RG20 7NN
UK

Mark P. Stevens
Division of Microbiology
Institute for Animal Health
Compton
Berkshire RG20 7NN
UK

Martin C. Taylor
London School of Hygiene and
Tropical Medicine
Keppel Street
London WC1 7HT
UK

Daniel E. Voth
Coxiella Pathogenesis Section
Laboratory of Intracellular Parasites
Rocky Mountain Laboratories
National Institute of Allergy and
Infectious Diseases
National Institutes of Health
Hamilton, MT 59840
USA

Simon J. Waddell
Medical Microbiology
Centre for Infection
Division of Cellular & Molecular
Medicine
St. George's University of London
Cranmer Terrace, Tooting
London SW17 0RE
UK

Markus Winterberg
FB Biology
Department of Parasitology
Philipps-University Marburg
Karl-von-Frisch-Str. 8
35043 Marburg
Germany

Part I
General Aspects

1
Introduction: The Evolution of Intracellular Life Forms and their Niches

Ulrich E. Schaible and Albert Haas

> "As species are produced and exterminated by slowly acting and still existing causes, and not by miraculous acts of creation and by catastrophes; and as the most important of all causes of organic change is one which is almost independent of altered and perhaps suddenly altered physical conditions, namely, the mutual relation of organism to organism, – the improvement of one being entailing the improvement or extermination of others". Charles Darwin

1.1
A Short History of Theories and Discoveries

The complex mutual relationship between intracellular microbes and their host cells is a challenging field of research and requires the perspective of evolution biology. The individual host–microbe interactions covered in this book all raise the following questions: how do microbes enter, survive and proliferate in, and how do they exit host cells? And how can intracellular niches be characterized and what are the benefits of intracellular life for the microbes and its consequences for the host cell? The question, however, is how and under what selective pressure did these interactions evolve? The year 2009 marks the 200th birthday of Charles Darwin (1809–1882; 12th February 1809), and, more importantly, the 150th anniversary of the publication of his most important book *The Origin of Species by Means of Natural Selection* (24th November 1859) [1]. In this eminent and highly disputed and provocatively revolutionary work, Darwin outlined the concept of evolution by natural selection in the struggle of life. The concept of interspecies competition as the driving force for the evolution of all bacterial, animal and plant species laid the basis for modern day biology.

Louis Pasteur (1822–1895) and others proved that microbial life did not arise spontaneously and miraculously, but rather due to the omnipresence of microorganisms, an important fact for food preservation and the consequential establishment of sterilization techniques. The seminal work of the nineteenth-century microbiologists

set the path to study the novel complexity of interspecies interactions in natural science and medical research. Although infectious diseases were an important determinant for human history, causing migration, settlement and conflict behavior, it was not until the nineteenth century that infectious agents were identified as causative agents for certain diseases rather than the diseases being of mysterious origins. The time between the end of the nineteenth and the beginning of the twentieth century was the high season of bacteriology, during which a huge number of microbial species were identified using newly developed culture techniques. Many of these microbes were associated with humans, animals or plants, and they were either pathogens, beneficial symbionts or commensals. A number of those microbes had chosen other unicellular or multicellular organisms as their ecological niches. Finally, infectious diseases were recognized as the driving force for the evolution of the innate and, in vertebrates, the acquired immune systems (Chapter 12).

Robert Koch (1843–1910) and his colleagues identified the first intracellular pathogenic bacterium, the tubercle bacillus (*Mycobacterium tuberculosis*). In the late nineteenth century tuberculosis was the prime cause of death in the metropolitan areas of Europe and North America, stirring up intensive medical and scientific interest. At around the same time, an important virulence trait of the tubercle bacillus, that is, living in macrophages, was described by Elie Metchnikoff (1845–1916), the founder of phagocyte biology. This is still a prime topic in tuberculosis research today (see Chapter 19). Metchnikoff was the first to observe the phagocytosis of bacteria by phagocytes in 1883 during his time at the Viennese Institute of Zoology and he also pointed out the importance of these cells in host response and inflammation [2, 3]. The term macrophage was attributed to him and made him the founder of innate immunity. In 1908, he received the Nobel Prize for his achievements. Metchnikoff was also the first to observe tubercle bacilli thriving intracellularly in macrophages (Figure 1.1) [4]. However, it was not until the last quarter of the twentieth century that scientists started to study the virulence factors of pathogens, and that intracellular pathogens (and symbionts) were highlighted for their unique capabilities to survive within and manipulate their host cells.

The identification of intracellular survival mechanisms was made possible by novel techniques in cell biology and the arrival of modern molecular genetics. J. A. Armstrong and Philip D'Arcy Hart [5, 6] were the first to show inhibition of phagolysosome fusion by the tubercle bacillus. Similar peculiarities of *Toxoplasma gondii*- and *Chlamydia psittaci*-containing vacuoles were published in 1979 and 1981, respectively [7, 8]. In the last decade of the twentieth century, many virulence traits of intracellular microbes were elucidated. Genome analyses and molecular techniques, paired with novel model systems such as yeast two-hybrid screening technology, uncovered pathogenicity islands and plasmids, virulence factors, as well as host cell target structures. It was discovered that throughout evolution there must have been a tremendous horizontal gene transfer between different microbes as well as between bacteria and eukaryotes (Chapter 2). Many of those pathogens and their virulence traits will be covered in this book. Some important intracellular microbes, such as *M. leprae*, *Chlamydia* and *Rickettsia*, are not yet accessible to