Advances in Experimental Medicine and Biology 738

Carlos López-Larrea *Editor*

Self and Nonself

LANDES BIOSCIENCE

Springer

Self and Nonself

ADVANCES IN EXPERIMENTAL MEDICINE AND BIOLOGY

Recent Volumes in this Series

Volume 730
THE EFFECTS OF NOISE ON AQUATIC LIFE
Anthony Hawkins and Arthur Popper

Volume 731
SODIUM CALCIUM EXCHANGE
Lucio Annunziato and Simina Calin

Volume 732
REPRODUCTIVE HEALTH AND CANCER IN ADOLESCENTS AND YOUNG ADULTS
Gwendolyn P. Quinn and Susan T. Vadaparampil

Volume 733
NANO BIOTECHNOLOGY FOR BIOMEDICAL AND DIAGNOSTICS RESEARCH
Eran Zahavy, Arie Ordenlich, Shmuel Yitzhaki and Avigdor Shafferman

Volume 734
SYSTEMS BIOLOGY OF TUMOR DORMANCY
Heiko Enderling, Nava Almog and Lynn Hlatky

Volume 735
CURRENT TOPICS IN COMPLEMENT III
John D. Lambris

Volume 736
ADVANCES IN SYSTEMS BIOLOGY
Igor I. Goryanin and Andrew B. Goryachev

Volume 737
OXYGEN TRANSPORT TO TISSUE XXXIII
Martin Wolf, Hans Ulrich Bucher, Markus Rudin, Sabine Van Huffel, Ursula Wolf, Duane F. Bruley, David K. Harrison

Volume 738
SELF AND NONSELF
Carlos López-Larrea

Self and Nonself

Edited by

Carlos López-Larrea, PhD
Department of Immunology, Hospital Universitario Central de Asturias, Oviedo, Spain

Springer Science+Business Media, LLC

Landes Bioscience

Springer Science+Business Media, LLC
Landes Bioscience

Printed in the USA.

Springer Science+Business Media, LLC, 233 Spring Street, New York, New York 10013, USA
http://www.springer.com

Please address all inquiries to the publishers:
Landes Bioscience, 1806 Rio Grande, Austin, Texas 78701, USA
Phone: 512/ 637 6050; FAX: 512/ 637 6079
http://www.landesbioscience.com

The chapters in this book are available in the Madame Curie Bioscience Database.
http://www.landesbioscience.com/curie

Self and Nonself, edited by Carlos López-Larrea. Landes Bioscience / Springer Science+Business Media, LLC dual imprint / Springer series: Advances in Experimental Medicine and Biology.

ISBN: 978-1-4614-1679-1

Library of Congress Cataloging-in-Publication Data

Self and nonself / edited by Carlos López-Larrea.
p. ; cm. -- (Advances in experimental medicine and biology ; v. 738)
Includes bibliographical references and index.
ISBN 978-1-4614-1679-1
I. López-Larrea, Carlos, 1953- II. Series: Advances in experimental medicine and biology ; v. 738. 0065-2598
[DNLM: 1. Self Tolerance--immunology. 2. Adaptive Immunity. 3. Immunity, Innate. W1 AD559 v.738 2011 / QW 504]

616.07'9--dc23

2011035321

PREFACE

"It is an extraordinary fact that with many species, flowers fertilised with their own pollen are either absolutely or in some degree sterile; if fertilised with pollen from another flower on the same plant, they are sometimes, though rarely, a little more fertile; if fertilised with pollen from another individual or variety of the same species, they are fully fertile"

—Cross and Self-Fertilisation (Darwin, 1878)

In 1960 Sir Frank Macfarlane Burnet received the Noble Prize in Physiology and Medicine. He titled his Nobel Lecture "Immunological Recognition of Self" emphasizing the central argument of immunological tolerance in "How does the vertebrate organism recognize **self** from **nonself** in this the immunological sense—and how did the capacity evolve."

The concept of self is linked to the concept of biological self identity. All organisms, from bacteria to higher animals, possess recognition systems to defend themselves from nonself. Even in the context of the limited number of metazoan phyla that have been studied in detail, we can now describe many of the alternative mechanism of immune recognition that have emerged at varying points in phylogeny. Two different arms—the innate and adaptive immune system—have emerged at different moments in evolution, and they are conceptually different. The ultimate goals of immune biology include reconstructing the molecular networks underlying immune processes. This volume covers different aspects of the emergence of immune systems in the evolution of life.

The first part of the book focuses on the origin of the immune response during the development of multicellularity (Chapters 1-4). Bacteria have developed defense systems against viruses and conversely, viruses have devised escape mechanisms that allow infection. Most of the archaea and numerous bacteria possess an elaborate system of adaptive immunity known as the **CRISPR-Cas,** that confers resistance to mobile genetic elements. This continuous phage-host interaction is a strong selective pressure that triggers a rapid co-evolution of both entities. Nevertheless, the evolution of metazoans from their unicellular ancestors emerged as a novel self-identity that required mechanisms for cell adhesion and cell-to-cell communication. One of the most important cell adhesion

mechanisms for metazoan development is based on carbohydrate to carbohydrate self-assessment. The large variability of carbohydrates as the most exposed and dominant components of plasma membranes are involved in many cellular interactions essential for self-nonself recognition.

Since *cnidarians* are amongst the morphologically simplest metazoans, they are also the most suitable for studying the evolutionary origins of self-nonself recognition. A surprising characteristic is that they possess an exquisitely sophisticated histocompatibility system. When two allogeneic incompatible colonies come into direct contact, they develop inflammatory-like rejection lesions, called points of rejection (**POR**). The colonial ascidian *Botryllus schlosseri* manifests a unique allorecognition system that is controlled by a single histocompatibility **Fu/HC** locus, with a large number of expressed alleles, that also affects self-fertilization by sperm-egg incompatibility.

The second part of this volume covers immunity aspects of innate sensors (Chapters 5-11). Innate immunity is the dominant immune system found in plants, fungi, insects, and primitive multicellular organisms. In 1989, Janeway proposed that innate immune systems discriminate self and nonself through pathogen-associated molecular patterns (**PAMPs**). Some years later, Matzinger expanded Janeway's theory proposing the "danger signal theory", which states that the decision to respond or not to respond to a particular antigen depends on whether the antigen is "harmful or not" to our body.

Recognition is mainly based on a series of germ-line encoded pattern recognition receptors (**PRRs**) that have been selected during evolution to recognize nonself molecules present in microorganisms. Moreover, different types of intracellular sensors (Toll receptors) that recognize various forms of nucleic acids have been described in virus response. Similarly, plants utilize receptor-like proteins (kinases) as pattern recognition receptors which can detect conserved PAMPs. Charles Darwin made extensive observations of the pollination biology of a wide variety of plants. He carefully documented the consequences of self-pollination and described species that were self-sterile but that could easily be crossed with other plants of the same species. In fact, plants have evolved many complex mechanisms to prevent self-fertilization, and it is thought that this may partially explain the great success of the angiosperms. Self-incompatibility (**SI**) involves unique systems of cell-to-cell communication, cell-recognition and cell-to-cell rejection. Genetic studies show that a single polymorphic S-locus, encoding at least two components from both the pollen and pistil sides, controls the discrimination of self and nonself pollen.

Cell death is vital to the life of multicellular organisms, and it plays a role in the maintenance of population homeostasis of unicellular organisms. Apoptosis is the best known of these programs, and it has been suggested that it originated as part of a host defense mechanism. During apoptosis, cells maintain the integrity of their plasma membrane. In contrast, cell death by 'necrosis', which occurs in situations of uncontrolled tissue damage, is a 'passive' form of cell death which triggers inflammation. The component released during tissue injury, called damage-associated molecular patterns (**DAMPs**), also triggers innate immune response.

Recent evidence indicates that the cell homeostasis program is also triggered by inner sensors that intersect with the innate immune response. Here we also described how mechanisms such as the endoplasmic reticulum (**ER**) **stress** and **"autophagy**" are critical to restrict viral replication. Some viruses can also exploit these mechanisms. In

fact, regulating some aspects of these pathways, it is possible to favour viral replication and also inhibit the apoptotic machinery of infected cells.

The third part of this volume is dedicated to the emergence of adaptive systems in metazoans (Chapters 12-17). During vertebrate evolution, transposable elements have repeatedly contributed with regulatory and coding sequences to the host, leading to the emergence of new lineage-specific genes. Human endogenous retroviruses (as **HERVs**), represent vestiges of ancient infections that resulted in stable integration of the viral genome. These have occurred during the first evolutionary stages of jawed vertebrates due of the acquisition of different gene-related systems (**Igs**, **MHC**, **TCR**), and the recombinatorial mechanisms of generation of antigenic diversity (RAG genes) and lymphoid organs. Recent work has shown that jawless vertebrates have lymphocytes that express somatically diversified antigen receptors that contain leucine-rich-repeats, termed variable lymphocyte receptors (**VLRs**), and that the type of VLR expressed is specific to the lymphocyte lineage. However, during the millions of years of co-evolution with their respective hosts, viruses have extensively captured cellular genes. Cytomegaloviruses (**CMVs**) constitute an outstanding example of the many and varied encoded proteins directed to modulate both innate and adaptive immune responses

The last part of the volume describes the emergence of the **M**ajor **H**istocompatibility **C**omplex (**MHC**). The MHC is a multigene family that has arisen through recurrent expansion and contraction of genes, and a continuum of the evolutionary process is observed in the teleost fishes. This system contains genes encoding proteins involved with antigen presentation, playing an important role in the adaptive immune system. The study of how the MHC appeared in vertebrates during evolution and how it is organized in different species can help us clarify what features are essential for self-nonself recognition. On the other hand, the recent sequencing and assembly of the genomes of different organisms have shown that almost all vertebrates studied have one or more clusters of genes encoding odorant receptors (**OR**) in close physical linkage to MHC. Social signalling associated to MHC has been identified in over 20 species of vertebrates and is likely the basis for a vertebrate-wide chemosensory communication system.

This book presents an integrated view of self and nonself recognition systems in the context of evolution. I hope it will contribute to the conceptual discussion of the emergence of immune systems in nature. I am extremely grateful to all authors for their excellent contributions.

Carlos López-Larrea
Department of Immunology
Hospital Universitario Central de Asturias
Oviedo, Spain

ABOUT THE EDITOR...

CARLOS LÓPEZ-LARREA is Professor of Immunology (Oviedo, Spain) and currently Head of the Department of Immunology at the Hospital Universitario Central de Asturias (Oviedo, Spain). He is a world expert on spondyloarthropathies (SpA), in particular MHC and genetic factors that influence the development of the disease. The main research interests of his group also currently include the study of epigenetic mechanisms involved in autoimmune diseases and the role of innate immunity in organ transplantation tolerance. He is a member of several international scientific organizations and board member of several scientific journals. He has published more than 150 international papers and book chapters related to immunology and spondylaorthropathies.

PARTICIPANTS

Ana Angulo
Department of Cell Biology
Immunology and Neurosciences
Medical School
University of Barcelona
and
Institut d'Investigacions Biomèdiques
August Pi i Sunyer (IDIBAPS)
Barcelona
Spain

Vincenzo Calvanese
Department of Immunology and Oncology
National Center for Biotechnology
CNB-CSIC
Madrid
Spain

Nadia Danilova
Department of Molecular Cell
and Developmental Biology
University of California Los Angeles
Los Angeles, California
USA

Ian A. Dubery
Department of Biochemistry
University of Johannesburg
Auckland Park
South Africa

Pablo Engel
Department of Cell Biology
Immunology and Neurosciences
Medical School
University of Barcelona
and
Institut d'Investigacions Biomèdiques
August Pi i Sunyer (IDIBAPS)
Barcelona
Spain

Mario F. Fraga
Department of Immunology and Oncology
National Center for Biotechnology
CNB-CSIC
Madrid
Spain

Segundo González
Department of Functional Biology
University of Oviedo
IUOPA
Oviedo
Spain

Ju-Chi Huang
Department of Biochemistry
University of Johannesburg
Auckland Park
South Africa

Nao Jounai
Department of Molecular Biodefense Research
Yokohama City University Graduate School of Medicine
Yokohama
Japan

Philip J. Kear
Division of Biochemistry
University of Missouri
Columbia, Missouri
USA
and
Germplasm Enhancement and Crop Improvement Division
International Potato Center
Lima
Peru

Kouji Kobiyama
Department of Molecular Biodefense Research
Yokohama City University Graduate School of Medicine
Yokohama
Japan

Jason L. Kubinak
Department of Biology
University of Utah
Salt Lake City, Utah
USA

Ester Lara
Cancer Epigenetics Laboratory
Instituto Universitario de Oncología del Principado de Asturias (IUOPA)
HUCA
Universidad de Oviedo
Oviedo
Spain

Carlos López-Larrea
Department of Immunology
Hospital Universitario Central de Asturias
Oviedo
Spain

Jesús Martínez-Borra
Department of Immunology
Hospital Universitario Central de Asturias
Oviedo
Spain

Bruce McClure
Division of Biochemistry
University of Missouri
Columbia, Missouri
USA

Gradimir N. Misevic
Gimmune GmbH
Zug
Switzerland

Nikola Misevic
Institute of Brain Research
University of Bremen
Bremen
Germany

Yasunobu Miyake
Division of Molecular Immunology
Medical Institute of Bioregulation
Kyushu University
Fukuoka
Japan

Cristina Muñoz-Pinedo
Bellvitge Biomedical Research Institute (IDIBELL)
L'Hospitalet
Barcelona
Spain

Aurora M. Nedelcu
University of New Brunswick
Biology Department
Fredericton, New Brunswick
Canada

Adam C. Nelson
Department of Biology
University of Utah
Salt Lake City, Utah
USA

Vipul M. Parmar
School of Biological and Biomedical Sciences
Durham University
Durham
UK

Octavian Popescu
Molecular Biology Center and Institute for Interdisciplinary Experimental Research
Babes-Bolyai University
Cluj-Napoca
and
Institute of Biology
Romanian Academy
Bucharest
Rumania

Wayne K. Potts
Department of Biology
University of Utah
Salt Lake City, Utah
USA

Baruch Rinkevich
Israel Oceanographic and Limnological Research
National Institute of Oceanography
Tel-Shikmona
Haifa
Israel

James S. Ruff
Department of Biology
University of Utah
Salt Lake City, Utah
USA

Natasha M. Sanabria
Department of Biochemistry
University of Johannesburg
Auckland Park
South Africa

Martin Schröder
School of Biological and Biomedical Sciences
Durham University
Durham
UK

Fumihiko Takeshita
Department of Molecular Biodefense Research
Yokohama City University Graduate School of Medicine
Yokohama
Japan

Luis Villarreal
Center for Virus Research
University of California
Irvine, California
USA

Sho Yamasaki
Division of Molecular Immunology
Medical Institute of Bioregulation
Kyushu University
Fukuoka
Japan

CONTENTS

ACKNOWLEDGMENTS

This work was supported in part by the Spanish grant FIS PI080566 from Instituto "Carlos III" (European FEDER founds), CAJASTUR and ASHISTO.

CHAPTER 1

THE ORIGIN OF THE BACTERIAL IMMUNE RESPONSE

Jesús Martínez-Borra,[1] Segundo González[2] and Carlos López-Larrea*[,1,3]

[1]Immunology Department. Hospital Universitario Central de Asturias, Oviedo, Spain; [2]Department of Functional Biology. University of Oviedo, IUOPA, Oviedo, Spain; [3]Fundación Renal "Iñigo Álvarez de Toledo", Hospital Universitario Central de Asturias, Madrid, Spain

**Corresponding Author: Carlos López-Larrea—Email: inmuno@hca.es*

Abstract: Bacteriophages are probably the oldest viruses, having appeared early during bacterial evolution. Therefore, bacteria and bacteriophages have a long history of co-evolution in which bacteria have developed multiple resistance mechanisms against bacteriophages. These mechanisms, that are very diverse and are in constant evolution, allow the survival of the bacteria. Bacteriophages have adapted to bacterial defense systems, devised strategies to evade these anti-phage mechanisms and restored their infective capacity. In this chapter, we review the bacterial strategies that hinder the phage infection as well as the counter-defense mechanisms developed by the bacteriophages as an evolutionary response to the antiviral systems.

INTRODUCTION

The immune system has developed during evolution to defend our organism against nonself entities such as microorganisms, some inert injurious materials and tumour cells. This system in jawed vertebrates (like mammals) is very complex and contains an innate and an adaptive immune response. Such a complex organization of immune system probably provided survival advantages: These animals are also complex anatomically, generally need a long time to reach their reproductive maturity, possess a higher mobility and have a diversified diet. These characteristics generate a higher exposure to pathogens. Other types of animals also possess an immune system, although more primitive. Thus, all metazoans, including plants and the simplest multicellular organisms like the Porifera, need to distinguish self from nonself to maintain their integrity. The distinction between self

Self and Nonself, edited by Carlos López-Larrea.

Table 1. Summary of some of the phage defense mechanisms described in this chapter and their respective counter defense systems developed by phages

Bacteriophage Resistance Mechanisms	Counter Defense Mechanisms	Examples
Restriction and modification systems		
Type I	Reduction of recognition sites	Point mutations
	Modified bases	Hydroxymetiluracil
	Occlusion restriction sites	DarA and DarB proteins
	Depletion of cofactors	S-adenosy metionine hydrolase
	Inhibition R-M enzymes	OCR proteins
Type II	Reduction of recognition sites	
	Modified bases	
Type III	Reduction of recognition sites	
	Occlusion restriction sites	DarA and DarB proteins
	Inhibition R-M enzymes	OCR proteins
Type IV	Inhibition of R-M enzymes	IPI proteins for GmrS-GmrD system
CRISPR	Inhibition of recognition	Mutated proto-spacer sequences
Abortive infection systems		
Lit proteins	Reversion of Lit action	Reprocessing host $tRNA^{lys}$
Rex system	rII exclusion	rII gene

and nonself prevent them from being deceived by pathogens, which, without the existence of the immune system, eventually would invade the body and destroy the individual. In the simplest metazoans, this destruction is avoided by distinguishing their cells from the cells coming from other colonies and maintaining their integrity, as happens in sponges.

The immune system can be defined, in a broad way, as a system to distinguish self from nonself, whose function is to preserve the individual integrity both from an excessive competition for nutrients and from pathogenic assaults. The unicellular microorganisms, like bacteria, are able to perform this distinction. They can detect the presence of competitors that use the same nutrients and kill them by secreting anti-microbial substances. They are also able to detect and eliminate their own intracellular parasites.[1,2] This is, for example, the well-known function of the restriction enzymes, which evolved to destroy the invading bacteriophage genome while the bacterial DNA remained unharmed. In this chapter, we review the mechanisms used by bacteria to avoid a bacteriophage attack and destroy the phages once they enter inside the bacteria. The bacteriophages have an important role in bacterial evolution and have led to a great variety of defense mechanisms. Bacteriophages, for their part, have developed counter defense mechanisms to evade the bacterial antiviral mechanisms (Table 1, Fig. 1). We will also discuss how these defense mechanisms resemble those of more evolved forms of life.

BACTERIOPHAGE BIOLOGY

Most bacteria are infected by viruses called bacteriophages (also known as phages), which only have bacteria as a host (reviewed in ref. 3). Like other viruses, phages are

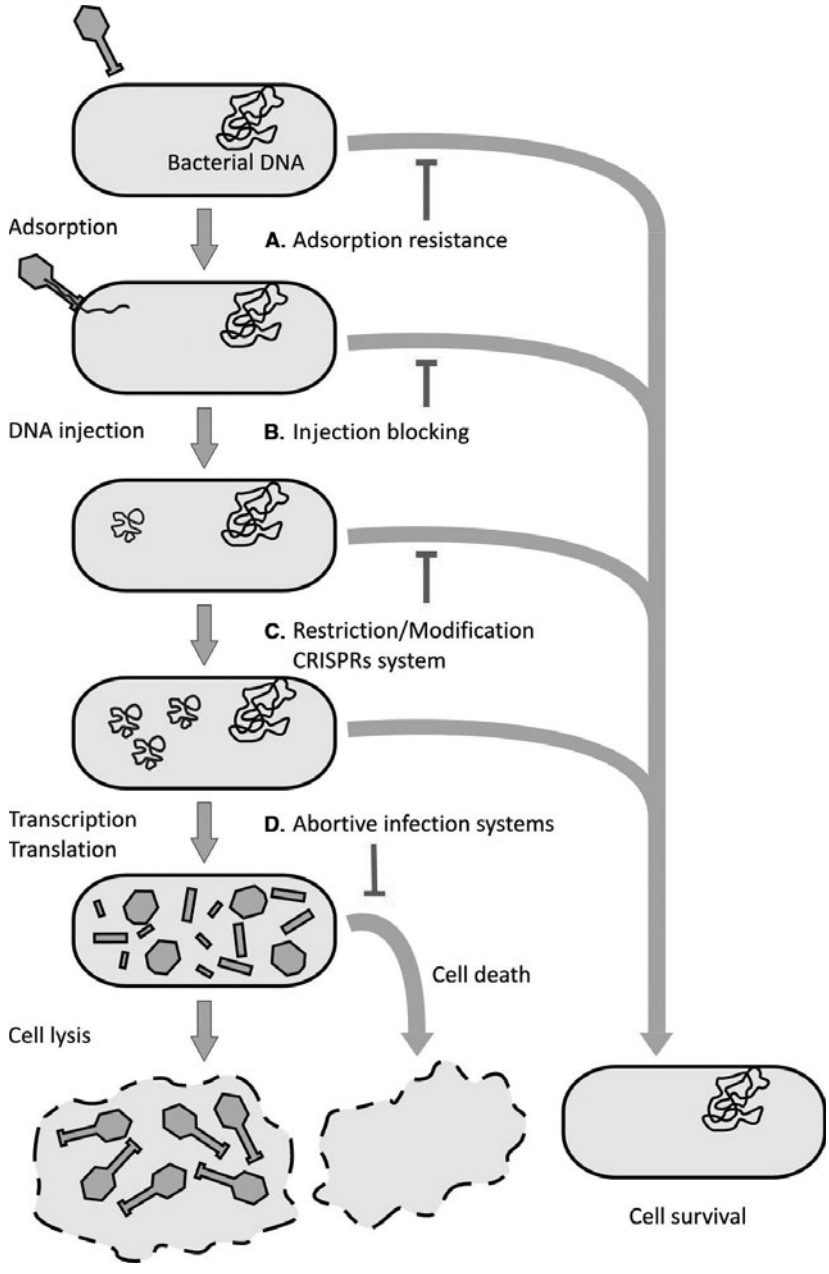

Figure 1. Phage infection stages and antiphage mechanism. Each stage of the phage cycle can be specifically inhibited by an antiphage mechanism. A) Absorption is blocked by several strategies that interfere with the recognition of a phage with its receptor. B) The DNA injection into the host also can by blocked. C) Restriction-modification system can detect and destroy foreign DNA while the host DNA remains undamaged. CRIPRS recognize phage DNA when the phage has infected previously the bacteria. D) Abortive infection systems affect the last stages of the phage cycle (replication, transcription or translation). These mechanisms lead to the death of the infected bacteria but protects the bacterial population.

parasites that only can live in their host cell. However, many phages can survive in the absence of the appropriate host for years and remain able to infect bacteria. Phages need an appropriate host that is generally a group of bacteria of one species, although some phages can infect several related species. Their host range is very broad and so, they can infect any bacteria group, including gram positive, gram negative and archaea, the latter being infected by specific viruses that are called archaephages. Phages are ubiquitously distributed; they can be found in all habitats where the bacteria or archaea can proliferate, including places with extremes temperature, pH, or salinity. Phages are probably the most abundant biological entities. They are perhaps the oldest viruses, having appeared before the split of the two bacterial kingdoms, bacteria and archaea. They are very diverse in structure, which indicates they have a polyphyletic origin.

The phage virion consists of a capsid make up of proteins or lipoproteins that enclose the genetic material (the nucleocapsid). Their structure can be tailed, polyhedral, filamentous, or pleomorphic. Most of the phages described (95% of all phages) belong to the Order *Caudovirales* or tailed phages. The *Caudovirales* include the families *Myoviridae*, *Siphoviridae* and *Podoviridae*. Phages of these families are formed by an icosahedral head and a tail. The tail of the phage varies in length and can be contractile or not, depending on the family. These phages contain linear double-stranded DNA (dsDNA). The polyhedral, filamentous and pleomorphic phages comprise another ten families of tailless phages, with very few species described to date. A few species have not been assigned to any group yet. The phage capsid has different shapes and that include families with linear or circular dsDNA, two families with circular single-stranded DNA (ssDNA) and two families with RNA as genetic material, one of them with linear ssRNA and other with segmented dsRNA.[4]

The long period of time in contact between bacteria and phages could explain the lysogeny, a complex phenomenon that probably has required a long co-evolution of phages and their hosts.[3] Phages can be grouped into virulent and temperate phenotypes on the basis of their infection cycle.[5] After the infection, virulent phages have a lytic cycle: They immediately start the production of new viral particles using the bacterial molecular machinery and liberate new phages by lysing the host cell. Temperate phages have two alternative cycles when they infect the bacteria. They can follow a lytic cycle as aforementioned or alternatively, they can enter a lysogenic cycle: The phage remains in a quiescent state, their genetic material (known as a prophage) integrates into the bacterial genome or remains as a plasmid and replicates at the same time as the host. The phage stays in this state until a specific factor (dependent on the host's metabolic state) triggers the phage to leave this state and to enter a lytic cycle. Lytic phages affect all aspects of host metabolism: They modulate transcription and translation of proteins and alter the membrane and genomic integrity. On the contrary, the lysogenic cycle is not toxic to the host until it switches to the lytic cycle. The lysogenic cycle can be beneficial for the bacteria by preventing infection from other phages and providing resistance to antibiotics. As a consequence of this long contact between phages and their host, the majority of gram-positive and gram-negative bacteria contain prophages. In fact, prophages can constitute 3-10% of the genome of bacteria[6] and are the main contributor to genomic diversity in some species. These prophages become a stable part of the bacteria genome and they can be functional or defective.

We will discuss the mechanisms developed by the bacteria to protect themselves from these viruses. For this, it is interesting to consider that the phage infection involves several steps and at each step, the bacteria have developed resistance mechanisms to try to avoid or stop the infection. The steps in a phage lytic cycle are adsorption, genome injection, genome replication, phage transcription, translation, assembly and lysis.

In some cases, phage infection is possible only in a certain phase of the host's growth cycle. For example, the infectious cycle of *Bordetella*, a bacterium that causes respiratory infections, has two phases: The Bvg+ phase which expresses the virulence and colonization factors that are necessary for respiratory tract colonization and the Bvg- phase which expresses genes for ex vivo growth and survival but not genes for colonization. The bacteriophage BPP-1 can infect bacteria in the Bvg+ phase because it specifically expresses the adhesion protein pertactin, which is not expressed in the Bvg- phase. However, the BPP-1 phage has developed a mechanism to infect at different stages in the infectious cycles of *Bordetella*. The tropism in this phage is determined by the gene *mtd* (major tropism determinant), but *mtd* suffers the action of a reverse transcriptase enzyme that acts as a diversity-generating retroelement, since its only purpose appears to be to generate changes in the sequence of that gene. In fact, new phages have been found in which changes in the *mtd* gene have allowed the infection of the Bvg- phase *Bordetella*.[7,8]

PHASES OF THE IMMUNE RESPONSE

Bacteria, as the rest of organisms, are susceptible to being attacked by pathogens. Comparing the defense systems developed by the different organisms shows similarities between them, which indicates the existence of basic mechanisms that have appeared independently by convergent evolution both in higher muticellular organisms and in unicellular organisms. Considering the different aspects of the immune system, the self-nonself distinction is employed to maintain the host's integrity by detecting the presence of competitors that use the same nutrients (a defense mechanism present in unicellular organisms) or by detecting pathogens that try to colonize the organism (a defense mechanism present in both unicellular and multicellular organisms). Bearing in mind the latter aspect, when a pathogenic agent tries to invade an organism, the defense mechanisms act in two phases: (1) The first phase consists of trying to prevent or limit the injury when the microorganisms are outside the host and (2) a second phase in which the organism tries to destroy the internalized pathogens. Regarding the first mechanism, the antimicrobial peptides (defensins) and proteins (hemolysin, lysozyme) are some of the earliest forms of defense. Thus, defensins or defensin-like antimicrobial peptides have been found in animals (primitive vertebrates, arthropods, fishes, frogs, mammals, etc) and in plants. These types of peptides are also produced by bacteria, which use the peptides to thwart competing microorganisms or to avoid a phage attack. This first phase also includes the anatomical and physiological barriers that hinder the pathogen from penetrating the host body (skin or other membranes, mucus, etc). These barriers are present both in unicellular and pluricellular organisms and are a way to prohibit pathogens from gaining access to the inner tissues (or the cytoplasm) at the beginning of the infection. In jawed vertebrates the immune response has three phases. The first phase includes the non-induced (passive) and nonspecific response prior to the pathogen's entry into the body. The second phase is the innate response that occurs when the pathogen has been able to invade the organism. This innate response has a medium grade of specificity since it recognizes some specific structures that are shared by a broad range of pathogens. The third phase consists of the adaptive immune response that develops highly specific responses toward a specific pathogen. One of the characteristics of the adaptive immune response is the memory. Surprisingly, some bacterial defense mechanisms against phages possess this property.

Thus, as we describe below, in the Pgl and CRISPRs systems, the phage that infects a bacteria is recognized in the subsequent attacks, which allows a better response that avoid a new infection. In the following sections, we describe the different defense strategies used by the bacteria to respond to the biological agents that threaten them.

BACTERIAL IMMUNE RESPONSE BEFORE PHAGE ENTRY

As we mentioned above, microorganisms use antimicrobial peptides against a competing microorganism, especially under conditions of nutrient depletion. These peptides also play a role in the defense against bacteriophages in some cases. For example, the micromicin J25, a peptide secreted by Enterobacteria under conditions of nutrient depletion, are directed against related bacterial strains. The mechanism of action of Micromicin J25 involves the inhibition of RNA polymerase and altering the electric potential of cell membranes. This peptide has an additional function in the defense against phages: It affects FhuA, an *E. coli* outer membrane protein. FhuA is an iron transporter that serves as a receptor for the unrelated coliphages T1, T5 and φ80. FhuA is required for injection of phage DNA into the target bacteria. Micromicin J25 blocks phage infection by inhibiting the binding of the phages to PhuA and preventing phage adhesion.[9,10]

The outer membrane protein OmpA serves as a receptor for several T-even-like phages in *E. coli*. Some *E. coli* strains inhibit the injection of these phages by producing a protein in the outer membrane called Trat, which interacts with OmpA. The interaction of Tract with OmpA decreases the binding of phage and its injection into the bacteria.[11]

Bacteriophage super infection refers to the same bacteria cell being infection by more than one bacteriophage in a sequential manner. We have mentioned previously that there is an abundance of prophage or prophage-remnant sequences in the bacterial genome. Some phages have mechanisms to prevent a superinfection. Sfi21, a *Streptococcus thermophilus* temperate phage, encodes the superinfection exclusion gene *orf203*. This protection is effective against many virulent phages, but it does not affect their own infection.[12] The mechanism of sie_{2009} exhibits the same characteristics as *orf203*, is expressed in the temperate *Lactococcus lactic* bacteriophage Tuc2009 and encodes bacteriophage resistance mechanism that blocks bacteriophages from injecting their genome and capsid.[13]

Biofilms are aggregates of microbial cells encased in an external matrix secreted by the own microbes.[14] The matrix is composed of an exopolysaccharide that is the main macromolecular component, although it can also contain proteins and other components. However, water is the main constituent of the matrix. Biofilms are formed in response to certain external insults as a protection mechanism and require the bacterial community to produce signals that co-ordinate the production of the biofilm's components. When the complex has been formed, the bacterial cells are metabolically inactive, since the extracellular matrix only allows a slow diffusion of nutrients. There are relatively few studies on the effect of biofilms on the capacity of phage infection.[15] Extracellular polymers may prevent access of the phage to the cell surface in some cases.[16,17] However, some factors allow the phage access to the bacterial membranes in the biofilm. Thus, biofilms' structures contain some 'channels' that can be used by the phages. Furthermore, many prophages contain genes encoding biofilm-degrading enzymes, such as polysaccharases or polysaccharide lyases. These enzymes allow the phage to spread through the extracellular matrix and promote viral access to the bacterial surface and the infection. Biofim formation as well as other activities like expression of virulence factors, sporulation, or antibiotic

formation is controlled by quorum sensing (QS). Quorum sensing is a process of intercellular communication that enables the bacteria to detect their population cell density and the population density prescribes a co-ordinated gene expression throughout the population. Indirect evidence links QS and the regulation of the lytic/lysogenic switch.[18] Many pathologic bacteria use QS to escape host defense mechanisms.[18] It would be interesting to elucidate whether QS have also any relationship with the bacterial antiphage response.

BACTERIAL IMMUNE RESPONSE AFTER PHAGE ENTRY

Restriction and Modification Enzymes

The restriction and modification (R-M) systems were discovered in the 1950s during investigations into the ability of certain bacteria to avoid the propagation of viruses that were able to infect other strains of bacteria. Thus, restriction enzymes were one of the first mechanisms involved in the microbial immune system to be discovered and appeared to be exclusive to unicellular organisms.[19] The protection against invading DNA is probably the main function of these systems although they could also participate in other processes such as DNA repair.[20] There is no clear evidence of these alternate functions. On the other hand, R-M systems are non-essentials for bacteria since strains deficient for this system are defective only in the susceptibility to phage infection. R-M systems consist of two activities performed generally by separate enzymes: Restriction endonuclease (REase) and methyltransferase (MTase). The nonself DNA is recognized by the endonucleases that interact with specific DNA sequences and trigger the cleavage of the nonself DNA. Their own bacterial DNA is protected by the MTases that methylate the adenine or cytosine within the specific sequence recognized by the restriction enzyme. As the methylation generally confers protection from cleavage, only the foreign DNA is recognized by the endonuclease. The four groups of R-M systems (Type I-Type IV) differ in enzyme activity, cofactor requirements, recognition sequences and cleavage sites.[21,22] Type I enzymes recognize unmethylated substrates, require ATP, S-adenosyl methionine (SAM) and Mg^{2+}. They cleave the DNA at variable locations away from the recognition site. Type II enzymes are the most numerous. They recognize specific DNA sequences and cleave it at a constant position, generally at the recognition site. They require Mg^{2+} as a cofactor. Type III enzymes need ATP, SAM and Mg^{2+}. They cleave at a specific distance away from the recognition site. Type IV enzymes require Mg^{2+} and recognize only modified DNA (methylated, hydroxymethylated and glucosyl-hydroxymethylated), cleaving both DNA strands twice and excising the recognition site.

The R-M systems are common in bacteria from all taxonomic groups, which indicates the importance of this defensive system. However, phages have developed anti-restriction strategies to avoid cleavage of their DNA.[1] The bacteriophage's most simple approach is to avoid the endonuclease recognition. Phages have evolved by modifying their sequence and accumulating point mutations, which reduce the number of recognition sites.[23] The genomes of some phages contain unusual bases like 5-hydroxymethyluracil instead of thymine or hydroxmethylcytosine (HMC) instead of cytosine and also glucosylated HMC. These bases, which are absent from the host genome, protect the phage genome against restriction enzymes since R-M systems are generally unable to recognize sequences containing this modified bases. However, bacteria have evolved to be able to recognize these modified phage DNA using the modification-dependent systems (MDS), which are also a nuclease-based

host defense mechanism directed towards modified bases. Although only a few DMS systems are described, the glucose-modified restriction (Gmr) S-GmrD system, a Type IV R-M systems, only recognize glucosylated hydroxymethylcytosine-modified DNA.[24,25] In this case, the host DNA is not recognized and there is no need for host DNA protection. The co-evolution of the attack and defense mechanisms has spurred the development of the internal protein I (IPI) which also inhibits the GmrS-GmrD system. When the bacteriophage T4 infects a *E. coli* strain encoding the *gmrs*/*gmrd* genes, its genome is degraded. However, some T4 bacteriophages possess the *ip1* gene in their genome which encodes the IPI protein. In this case the *Ip1*-containing phage is able to successfully infect the bacteria. The gmrS/gmrD genes encode two proteins, GmrS and GmrD that form a complex and degrade the glucosylated hydroxymethylcytosine-modified DNA. The IPI protein binds to the GmrS—GmrD complex, inhibits its activity and prevents the digestion of the T4 DNA.

Another phage resistance system is the phase-variable, phage-growth limitation (Pgl) system, which is an unusual phage resistance mechanism present in *Streptomyces coelicolor*.[26] This phase variation mechanism is a method used by bacteria for adapting promptly to new environments. The Pgl system consists of reversible variations of protein expressions. *S. coelicolor* A3(2) frequently varies from Pgl^+ to Pgl^- and vice versa. This variation mechanism allows changes in the phenotype much more quickly that those produced by mutational changes in the genome and is associated generally with bacterial immune evasion, especially against infection by phages. The phage φC31 can infect *S. coelicolor* (Pgl-) and produce viable progeny. In contrast, infection of a Pgl^+ strain produced phages that are severely attenuated in a subsequent infection. The mechanism of this resistance system is not completely understood, but has been proposed that Pgl^+ strains modify phage and this modification is recognized in a second infection by Pgl^+ hosts but not Pgl- hosts.[27]

Additional resistance mechanisms affect the enzymatic activity of the R-M systems. One of these mechanisms is the depletion of intracellular cofactors that are necessary for enzyme activity. For example, Type I and Type III R-M enzymes require SAM for their activity. Phage T3 encodes a SAM hydrolase which eliminates intracellular SAM pools, inhibits the enzyme activity and allows phage survival.[28] Another strategy is the production of proteins that interfere directly with the enzyme activity. For example, phage T3 and T4 encode the overcome classical restriction (Ocr) proteins, which bind specifically to Type I R-M enzymes and inhibit their activity. Because dimeric Ocr protein mimics approximately 20 bp of B-form DNA in the shape and charge distribution, Ocr acts as an anti-restriction protein by binding to Type I DNA restriction enzymes. The binding of Ocr to the Type I restriction enzymes prevents their binding to their DNA target and competitively inhibiting the action of the enzymes.[29] These resistance mechanisms are only effective when expressed soon after the entry of the phage bacteria into the host and thus, Ocr and SAM hydrolase are some of the first proteins to be expressed by phage T7 and T3, respectively.

CRISPRs

Many prokaryotes can acquire heritable immunity to phages by incorporating viral DNA into their own genome. This mechanism of anti-viral defense is known by the acronym CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats). The phage

or plasmid DNA sequences are integrated between repeated sequences in the CRISPR locus of the genome of prokaryote. This integrated DNA provides further interference for the exogenous genetic elements in a manner analogous to RNA interference (RNAi) in eukaryotic organisms. CRISPRs system may evolve rapidly, by acquiring new phage sequences to adapt to highly dynamic viral population.[30,31] Nevertheless, CRISPR system imposes a strong selective pressure on phages and has led to rapid mutation of viral genomes. CRISPR provides one explanation for the high evolutionary rates observed in phages. This primitive system of immune defense was discovered by comparative genome analysis in 1987 in the bacterium *E.coli by* Ishino and colleagues.[32] They found 14 repeats of 29 base pairs that were interspersed by 32-33 base pairs nonrepeating DNA sequences that were adjacent to the isozyme-converting alkaline phosphatase gene in *E.coli*. Computational analyses later revealed that the CRISPR system is present in the genomes of approximately 40% of bacteria and 90% of archaea.[33,34]

CRISPR systems are composed by multiple short DNA repeats that are separated by similarly sized non-repetitive DNA sequences termed "spacers". Each cluster is flanked by a varying number of genes called *CAS* (CRISPR-associated) genes.[32] Although many prokaryote genomes contain a single CRISPR locus, *Mathanocaldococcus jannaschii* has 18 loci, totalling more than 1% of the genome.[35] DNA repeats are composed of 24 to 47 base pairs.[36] Despite being divergent between species, the number of repeats per array varies from 2 to 249. Some groups of repeats contain a short palindrome (5-7 base pairs), hence the name palindrome in the CRISPR acronym. These palindromes likely contribute to RNA stem-loop secondary structure. Many repeats also have a conserved 3′terminus GAAA (C/G). Both structures are suggested to act as a binding site for cas proteins.[37] DNA repeats are interspaced by non repetitive spacers of DNA sequence of 26 to 72 base pairs.[35] The spacers are usually unique in a genome; a few exceptions, which are thought to have resulted from duplications, have been found to match sequences in phage genomes.[38] These spacers can be acquired from phages and subsequently help to protect the cell from infection. Removal or addition of particular spacers modified the phage-resistance phenotype of the cell.[30] CRISPR systems also comprise a leader A/T-rich, noncoding sequence, which is located immediately upstream of the first repeat and likely acts as the promoter for the transcription of the repeat-spacer array into a CRISPR transcript, the precrRNA.[39,40]

Cas genes are present in genomes of prokaryotes containing CRISPRs, but are absent from genomes that lack CRISPRs. More than forty different cas protein families have been described.[41] Particular combinations of *Cas* genes are found together, along with characteristic subclasses of CRISPR repeat sequences. These combinations appear to represent distinct CRISPR/Cas subtypes. Several different subtypes may occur in a single genome. Some Cas proteins are involved in the acquisition of novel spacers; others provide CRISPR-encoded phage resistance and interfere with invasive genetic elements. CRISPR-associated gene 1 (cas1) encodes the only universally conserved protein component of CRISPR systems.[42] Cas1 appears to be a double-stranded DNA endonuclease that produces double-stranded DNA fragments of approximately 80 base pairs in length. Its endonuclease activity suggests that it is part of the machinery for processing foreign nucleic acids. CRISPR1-associated *cas7* gene is involved in the integration of novel spacers after phage exposure.[30] Cas2 may act as a sequence-specific endoribonuclease that cleaves uracil-rich single-stranded RNAs (ssRNAs).[43]

The exact mechanism of the anti-phage or anti-plasmid activity of the CRISPR system is not fully characterized (Fig. 2). However, exogenous DNA is apparently processed

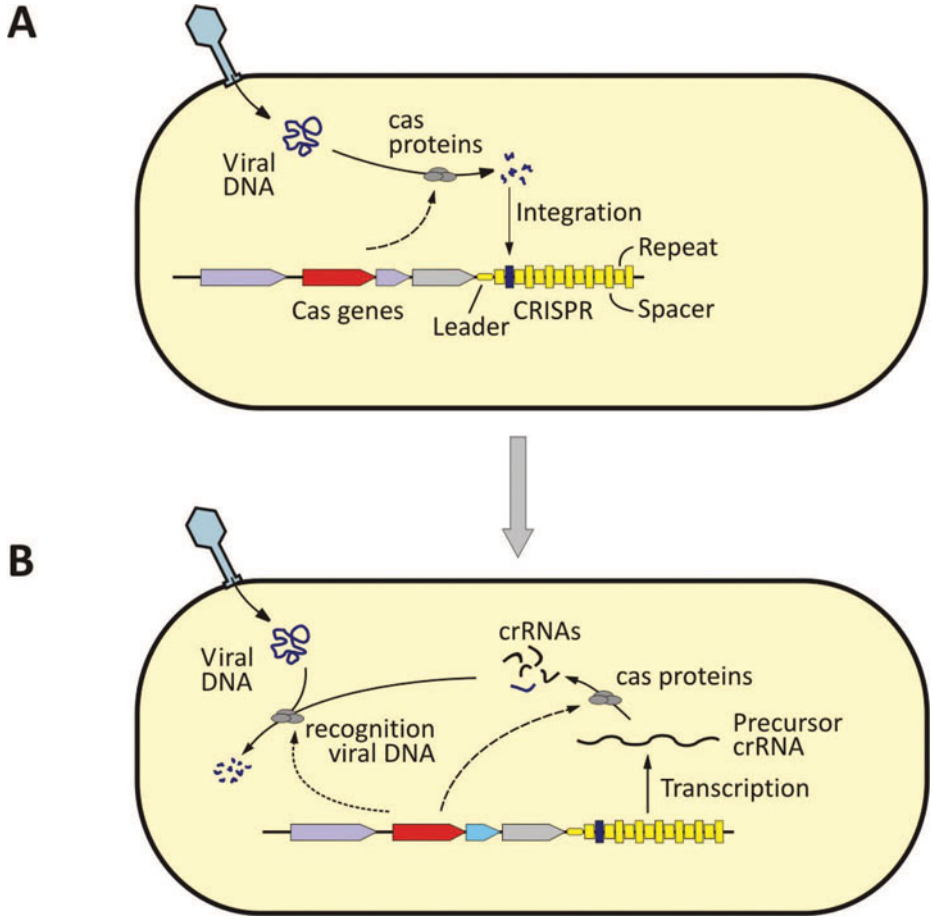

Figure 2. CRIPRS mode of action. A) Viral infection starts with the injection of phage DNA. Cas protein complex recognizes viral DNA and generates small DNA fragments using an unknown mechanism. Some of the small DNA fragments generated from the virus can be incorporated in the CRISPR locus as a new spacer, having then acquired the bacterial immunity against that virus. B) CRISPR repeat and spacer cluster is transcribed into a precrRNA that is processed by the cas protein complex into crRNAs, which are composed of a spacer and two half repeats. A new infecting phage is recognized when there is a crRNAs complementary to it. In this case, the cas protein complex, along with the respective crRNA recognize and destroy the invading DNA by an unknown mechanism.

by proteins encoded by some of the cas proteins into small elements (of about 30 base pairs in length), which are then inserted into the CRISPR locus near the leader sequence. The repeat-spacer array is constitutively transcribed into a full-length precrRNA and subsequently processed into specific small RNA molecules that correspond to a spacer flanked by two partial repeats.[40,44,45] The crRNAs seem to specifically guide the cas interference machinery toward foreign nucleic acid molecules that match its sequence, which leads ultimately to degradation of the invading element.[44] It has been proposed that they may act in a manner analogous to RNAi in eukaryotic organism. However, in spite of many similarities between CRISPR systems and eukaryotic system, key differences exist.

First, the enzymatic machinery differs between RNAi and CRISPR system.[46] Second, the crRNAs are larger than the short RNA duplexes generated by eukoryotic organisms (typically 21 to 28 nucleotides in length) because the CRISPR spacer (23 to 47 nucleotides) is flanked by partial repeats. Finally, RNAi involves RNA-dependent transcription, generation of double stranded RNA and use of the cleaved target RNA, in contrast to the CRISPR systems.

Although CRISPRs represents an effector element of a very primitive immune system, prokaryotes have the same dilemma as eukaryotic organisms. They also have to discriminate between self and nonself to avoid autoimmune disease. CRISPR systems have to target foreign extra-chromosomal material, but they have to avoid targeting their own spacer DNA. The mechanism is not fully understood, nevertheless, it has been proposed that in *Staphylococcus epidermidis,* target crRNA mismatches at specific positions outside of the spacer sequence leads to interference, but extensive pairing between crRNA and CRISPR DNA repeats prevents interference-targeting of their own prokaryote DNA and autoimmunity.[47]

ABORTIVE INFECTION (ABI) SYSTEMS

Abi sytems avoid phage infection in the remaining steps (replication, transcription and translation). They are peculiar in comparison with other resistance mechanisms as eventually they result in the death of the host bacteria. The Abi systems exhibit more specific nonself recognition than the aforementioned systems, such as the R-M systems. However, the immune response preserves the individual but the Abi systems destroys not only the phage but eventually also the host. Contrarily to other defense mechanisms, the Abi system protects the population, but not the individual. Many types of Abi systems have been described, especially in lactic bacteria, in which the phage-resistance mechanism has been studied at length due to the bacteria's economic importance. The mechanisms of these systems appear to be variable and details remain unclear. One example of this system in *E. coli* is the Rex system: Abi acts as a phage sensor and induces cell death by producing the loss of membrane potential. Another Abi system involves the PrrC protein, a ribonuclease specific for the host $tRNA^{lys}$: PrrC becomes active after T4 infection and produces cell death. The Lit protein is a metalloprotease that is also activated by T4. Lit specifically but relatively slowly cleaves the host's elongation factor (EF)-TU, inhibits protein synthesis and induces bacterial death (reviewed in ref. 48).

As with the rest of the resistance mechanisms, phages also have developed methods to circumvent the Abi systems. The *rII* gene allows phage T4 to survive the action of the Rex system.[49] The action of the Lit protein is reversed in some T4 phage by repairing the host $tRNA^{lys}$ with RNA ligase activity.[50]

CONCLUSION AND FUTURE PROSPECTS

The presence of an immune response is probably a characteristic of all living beings since all organisms can be attacked by pathogens. Regardless of the environment, bacteria are exposed to phages, which can infect them. Bacteriophages are the most abundant living entities and exceed the number of bacteria by about 10 times. For these reasons, they are very important in the regulation of the microbial balance and pose their most