

# LYME DISEASE AND RELAPSING FEVER SPIROCHETES

**Genomics,  
Molecular Biology,  
Host Interactions  
and Disease Pathogenesis**

**Editors:**

**Justin D. Radolf**

Departments of Medicine, Pediatrics,  
Molecular Biology and Biophysics,  
Genetics and Genome Sciences,  
and Immunology  
UConn Health  
263 Farmington Avenue  
Farmington, CT 06030-3715  
USA

**D. Scott Samuels**

Division of Biological Sciences  
University of Montana  
32 Campus Dr  
Missoula MT 59812-4824  
USA



**Copyright © 2021**

Caister Academic Press, UK  
[www.caister.com](http://www.caister.com)

All rights reserved. No part of this publication may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, electronic, mechanical, photocopying, recording or otherwise, without the prior permission of the publisher. No claim to original government works.

ISBN: 978-1-913652-61-6 (paperback)

ISBN: 978-1-913652-62-3 (ebook)

DOI: <https://doi.org/10.21775/9781913652616>

**Cover image**

Spinning disk laser confocal micrograph showing endothelial activation in the murine cremaster microvasculature in *Cd1d<sup>-/-</sup>* mice 24 hours after intravenous injection of GFP-expressing *B. burgdorferi* (green). Adherent and crawling neutrophils, physically distanced from spirochetes, were stained with phycoerythrin-conjugated anti-Ly6G (red) and venules were stained with Alexa Fluor 647-conjugated anti-PECAM1 (blue). See Chapter 17. Image from Björn Petri, Xi Tan and George Chaconas. Imaging was supported by the Live Cell Imaging Laboratory, Snyder Institute for Chronic Diseases, The University of Calgary.

## Dedication

This book is dedicated to Jorge Benach, Alan Barbour, and the late Willy Burgdorferi for their seminal contributions – beginning with the discovery that started it all – and leading to lifetimes of accomplishments in *Borrelia* research and training successive generations of spirochetologists. We also gratefully acknowledge our colleagues and trainees for their passion and commitment.

## Current books of interest

- Veterinary Vaccines: Current Innovations and Future Trends ..... 2020
- Climate Change and Microbial Ecology..... 2020
- Alphaherpesviruses ..... 2020
- Legionellosis Diagnosis and Control in the Genomic Era ..... 2020
- Bacterial Viruses: Exploitation for Biocontrol and Therapeutics ..... 2020
- Microbial Biofilms: Current Research and Practical Implications ..... 2020
- Astrobiology: Current, Evolving and Emerging Perspectives ..... 2020
- *Chlamydia* Biology: From Genome to Disease ..... 2020
- Bats and Viruses: Current Research and Future Trends ..... 2020
- SUMOylation and Ubiquitination: Current and Emerging Concepts ..... 2019
- Avian Virology: Current Research and Future Trends ..... 2019
- Microbial Exopolysaccharides: Current Research and Developments ..... 2019
- Polymerase Chain Reaction: Theory and Technology..... 2019
- Pathogenic Streptococci: From Genomics to Systems Biology and Control ..... 2019
- Insect Molecular Virology: Advances and Emerging Trends ..... 2019
- Methyloproths and Methyloproth Communities ..... 2019
- Prions: Current Progress in Advanced Research (Second Edition) ..... 2019
- Microbiota: Current Research and Emerging Trends ..... 2019
- Microbial Ecology ..... 2019
- Porcine Viruses: From Pathogenesis to Strategies for Control ..... 2019
- *Lactobacillus* Genomics and Metabolic Engineering ..... 2019
- Cyanobacteria: Signaling and Regulation Systems ..... 2018
- Viruses of Microorganisms ..... 2018
- Protozoan Parasitism: From Omics to Prevention and Control ..... 2018
- Genes, Genetics and Transgenics for Virus Resistance in Plants ..... 2018
- Plant-Microbe Interactions in the Rhizosphere ..... 2018
- DNA Tumour Viruses: Virology, Pathogenesis and Vaccines ..... 2018
- Pathogenic *Escherichia coli*: Evolution, Omics, Detection and Control I..... 2018
- Postgraduate Handbook ..... 2018
- Enteroviruses: Omics, Molecular Biology, and Control ..... 2018
- Molecular Biology of Kinetoplastid Parasites ..... 2018
- Bacterial Evasion of the Host Immune System ..... 2017
- Illustrated Dictionary of Parasitology in the Post-Genomic Era ..... 2017
- Next-generation Sequencing and Bioinformatics for Plant Science ..... 2017
- Brewing Microbiology: Current Research, Omics and Microbial Ecology ..... 2017
- Metagenomics: Current Advances and Emerging Concepts ..... 2017
- The CRISPR/Cas System: Emerging Technology and Application ..... 2017
- *Bacillus*: Cellular and Molecular Biology (Third edition) ..... 2017
- Cyanobacteria: Omics and Manipulation ..... 2017

# Contents

<b>Chapter 1</b> .....	<b>1</b>
The World-Wide Saga of Lyme Borreliosis	
<i>Jorge L. Benach and Juan Carlos García-Moncó</i>	
<b>Chapter 2</b> .....	<b>17</b>
Multipartite Genome of Lyme Disease <i>Borrelia</i> : Structure, Variation and Prophages	
<i>Ira Schwartz, Gabriele Margos, Sherwood R. Casjens, Wei-Gang Qiu and Christian H. Eggers</i>	
<b>Chapter 3</b> .....	<b>63</b>
Genomics of Relapsing Fever Spirochetes	
<i>Luke C. Kingry</i>	
<b>Chapter 4</b> .....	<b>73</b>
Replication of the <i>Borrelia burgdorferi</i> Genome	
<i>Kerri Kobryn</i>	
<b>Chapter 5</b> .....	<b>87</b>
Gene Regulation and Transcriptomics	
<i>D. Scott Samuels, Meghan C. Lybecker, X. Frank Yang, Zhiming Ouyang, Travis J. Bourret, William K. Boyle, Brian Stevenson, Dan Drecktrah and Melissa J. Caimano</i>	
<b>Chapter 6</b> .....	<b>131</b>
Metabolism and Physiology of <i>Borrelia</i>	
<i>Frank C. Gherardini, Daniel P. Dulebohn, Travis J. Bourret and Crystal L. Richards</i>	
<b>Chapter 7</b> .....	<b>181</b>
Structure, Function, Biogenesis and Maintenance of the <i>Borrelia</i> Cell Envelope	
<i>Wolfram R. Zückert, Brandon L. Jutras, Alvaro M. Toledo and Sven Bergström</i>	
<b>Chapter 8</b> .....	<b>221</b>
Dancing with the Star: <i>Borrelia burgdorferi</i> , a Solo Dancer with All the Right Moves	
<i>Ching Woon Sze, Hui Xu, Md A. Motaleb, Charles W. Wolgemuth, Jun Liu, Nyles W. Charon and Chunhao Li</i>	
<b>Chapter 9</b> .....	<b>251</b>
Evolutionary Genetics of <i>Borrelia</i>	
<i>Zachary J. Oppler, Kayleigh R. O'Keeffe, Karen D. McCoy and Dustin Brisson</i>	
<b>Chapter10</b> .....	<b>267</b>
Perpetuation of Borreliæ	
<i>Sam R. Telford III and Heidi K. Goethert</i>	
<b>Chapter11</b> .....	<b>307</b>
Interactions Between Ticks and Lyme Disease Spirochetes	
<i>Utpal Pal, Chrysoula Kitsou, Dan Drecktrah, Özlem Büyüktanir Yaş and Erol Fikrig</i>	
<b>Chapter12</b> .....	<b>339</b>
Biology and Molecular Biology of <i>Ixodes scapularis</i>	
<i>Daniel E. Sonenshine and Ladislav Šimo</i>	

<b>Chapter13</b> .....	<b>369</b>
Lyme Disease Pathogenesis <i>Jenifer Coburn, Brandon Garcia, Linden T. Hu, Mollie W. Jewett, Peter Kraiczy, Steven J. Norris and Jon Skare</i>	
<b>Chapter14</b> .....	<b>415</b>
Pathogenesis of Relapsing Fever <i>Job Lopez, Joppe W. Hovius and Sven Bergström</i>	
<b>Chapter15</b> .....	<b>447</b>
Animal Models of Borreliosis <i>Monica E. Embers, Stephen W. Barthold and Diego Cadavid</i>	
<b>Chapter16</b> .....	<b>499</b>
Genetic Manipulation of <i>Borrelia</i> <i>Patricia A. Rosa and Mollie W. Jewett</i>	
<b>Chapter 17</b> .....	<b>525</b>
Live Imaging <i>George Chaconas, Tara J. Moriarty, Jon Skare and Jenny A. Hyde</i>	
<b>Chapter 18</b> .....	<b>549</b>
Immune Response to <i>Borrelia</i> : Lessons from Lyme Disease Spirochetes <i>Linda K. Bockenstedt, R. Mark Wooten and Nicole Baumgarth</i>	
<b>Chapter 19</b> .....	<b>595</b>
Human and Veterinary Vaccines for Lyme Disease <i>Nathaniel S. O'Bier, Amanda L. Hatke, Andrew C. Camire and Richard T. Marconi</i>	
<b>Chapter 20</b> .....	<b>627</b>
Lyme Borreliosis in Domestic Animals <i>Stephen W. Barthold</i>	
<b>Chapter 21</b> .....	<b>649</b>
Epidemiology of Lyme Disease <i>Paul Mead and Amy Schwartz</i>	
<b>Chapter 22</b> .....	<b>665</b>
Lyme Disease Diagnostics <i>Michael R. Mosel, John Aucott, Steve E. Schutzer, Adriana Marques, Paul M. Arnaboldi, Raymond Dattwyler and Mark W. Eshoo</i>	
<b>Chapter 23</b> .....	<b>685</b>
The Widening Gyre: Controversies in Lyme Disease <i>Adriana Marques, Jacob E. Lemieux and Linden T. Hu</i>	
<b>Chapter 24</b> .....	<b>703</b>
Lyme Disease in Humans <i>Justin D. Radolf, Klemen Strle, Jacob E. Lemieux and Franc Strle</i>	
<b>Index</b> .....	<b>755</b>

## Preface

Remarkably, ten years have elapsed since the publication of ***Borrelia: Molecular Biology, Host Interaction and Pathogenesis***. By 2010, Lyme disease (Lyme borreliosis) had become the prototype of an ‘emerging infectious disease’. Once an obscure cause of arthritis in Southeastern Connecticut, in the span of three decades, Lyme disease was by then well recognized as the most prevalent vector-borne illness in the United States and Europe and a growing threat to global health. The first edition reflected two currents impacting the *Borrelia* field. One was the evolution of the study of pathogenic *Borrelia* from an arcane branch of microbiology to the mainstream of bacterial pathogenesis and clinical/translational research. The other was the advent of an unprecedented sociologic phenomenon – a coalition of self-proclaimed authorities accusing the biomedical community of conspiring to conceal the ‘real truths’ about the diagnosis and treatment of Lyme disease and the insidious nature of its etiologic agent. The book was the product of experts who pooled their energies and intellects to create the first state-of-the-art reference work covering the myriad, interlaced facets of the enzootic disorders caused by pathogenic *Borrelia*. The editors and authors had other intentions as well. We sought to showcase the extraordinary progress on a multitude of scientific and clinical fronts stemming from well-controlled, carefully executed, peer-reviewed research. And we conceived of the book as a vehicle for pushing back against the sophistry and shibboleths proffered to the public and legislators by our ‘Lyme literate’ detractors.

So, what, other than the title of the second edition, has changed during the last decade? Plenty. In reviewing the 24 chapters that comprise the volume, the editors have been impressed not just by the amount of new information generated by our colleagues but also its scope, technical virtuosity, and ingenuity. In fact, it should be evident to readers familiar with the first edition that the second edition is much more than an update; it is a reboot, and, in many instances, a re-evaluation of ideas and concepts prevalent at the time of the first edition. We now have much greater insight into the intricate

ecologic factors that sustain *Borrelia* species in the wild and the environmental forces driving their alarming expansion throughout the Northern Hemisphere. With the availability of next-generation sequencing, genomic sequences for numerous *Borrelia* strains and species, sophisticated methods for genetic manipulation, transcriptomics, and structural data for many virulence-related proteins, our understanding of borrelial evolutionary biology, physiology, pathogenicity, and immune evasion has increased exponentially. Genomics and genetics have brought us to the cusp of unraveling the great mystery of how different species and strains of Lyme disease spirochetes elicit such varied clinical outcomes; indeed, it has become increasingly clear that the answer lies to a large extent in the evolutionary and immunologic mismatch between the spirochete and its incidental, reservoir-incompetent human host. In 2010, experimentation with ticks was limited to a few laboratories. Now, many investigators study the tick phases of the enzootic cycle and our coverage of vector biology and *Borrelia*-tick interactions has expanded to provide more guidance for this experimental approach. In addition, live imaging of spirochetes was just coming online a decade ago: the second edition contains an expansive treatment of the options available to investigators who need to track live spirochetes and evaluate gene expression *in situ* in ticks and mice. The importance of motility and chemotaxis for borrelial invasiveness has long been appreciated; however, cryoelectron microscopy, in combination with genetics and structural biology, has provided stunning visual insights into the workings of the flagellar motor that enables these serpentine organisms to migrate from the bite site to distal tissues where they inflict damage. At the time of the first edition, the signaling molecule c-di-GMP and the transcription factor BosR had only just been discovered in *B. burgdorferi* and there was essentially no mention of the stringent response. In the ensuing ten years, genetic and biochemical dissection of the molecular mechanisms modulating gene expression as the spirochete traverses its enzootic cycle has revealed hitherto unrecognized complexities and, considering the small size of the

borrelial genome, a surprisingly sophisticated network of regulatory pathways. The composition of the field has changed as well. The author list contains the names of many gifted, enthusiastic early career investigators willing to take on the challenge of *Borrelia* for their life's work. Unfortunately, one thing has not changed – the Lyme Wars continue, led by individuals who espouse flawed studies purporting to show that Lyme disease spirochetes in humans adopt a persistent state impervious to antimicrobial therapy that causes chronic symptomatology. In this edition, three authorities offer their analysis of these and other data as well as their perspectives on the chronic Lyme disease controversy that continues to ensnare our field.

This book was completed in the shadow of COVID-19. It seems ironic that one newly emergent infectious disease would nearly derail our efforts to bring to fruition ten years of progress studying another. Everyone's research and personal lives were upended by the pandemic; some of the authors served on the front lines. We, the editors, wish to express our deepest gratitude to our colleagues for their perseverance and commitment.

**Justin D. Radolf and D. Scott Samuels**