

biotechnology

Bernard R. Glick

Terry L. Delovitch

Cheryl L. Patten

medical biotechnology

Bernard R. Glick

Department of Biology University of Waterloo Waterloo, Ontario, Canada

Terry L. Delovitch

Department of Microbiology and Immunology Western University

Cheryl L. Patten

Cheryl L. Patten

Biology Department

Iniversity of New Brunswick

Fredericton, New Brunswick, Canada



Copyright © 2014 American Society for Microbiology. All rights reserved. No part of this publication may be reproduced or transmitted in whole or in part or reused in any form or by any means, electronic or mechanical, including photocopying and recording, or by any information storage and retrieval system, without permission in writing from the publisher.

Disclaimer: To the best of the publisher's knowledge, this publication provides information concerning the subject matter covered that is accurate as of the date of publication. The publisher is not providing legal, medical, or other professional services. Any reference herein to any specific commercial products, procedures, or services by trade name, trademark, manufacturer, or otherwise does not constitute or imply endorsement, recommendation, or favored status by the American Society for Microbiology (ASM). The views and opinions of the author(s) expressed in this publication do not necessarily state or reflect those of ASM, and they shall not be used to advertise or endorse any product.

Library of Congress Cataloging-in-Publication Data

Glick, Bernard R., author.

Medical biotechnology / Bernard R. Glick, Department of Biology, University of Waterloo, Waterloo, Ontario, Canada; Terry L. Delovitch, Department of Microbiology and Immunology, Western University, London, Ontario, Canada; Cheryl L. Patten, Biology Department, University of New Brunswick, Fredericton, New Brunswick, Canada.

pages cm

Includes bibliographical references and index.

ISBN 978-1-55581-705-3 (hardcover)—ISBN 978-1-55581-889-0 (e-book) 1. Biotechnology. 2. Medical technology. I. Delovitch, T. L., author. II. Patten, Cheryl L., author. III. Title.

TP248.2.G57 2014 660.6—dc23 2013027259

10 9 8 7 6 5 4 3 2 1 Printed in the United States of America

Address editorial correspondence to ASM Press, 1752 N St. NW,
Washington, DC 20036-2904, USA
E-mail: books@asmusa.org
Send orders to ASM Press, P.O. Box 605, Herndon, VA 20172, USA
Phone: (800) 546-2416 or (703) 661-1593; Fax: (703) 661-1501
Online: http://www.asmscience.org

doi:10.1128/9781555818890

Cover and interior design: Susan Brown Schmidler Illustrations: Patrick Lane, ScEYEnce Studios

Image credits for cover and section openers

Cell image on cover: A dendritic cell infected with human immunodeficiency virus (HIV), showing projections called filopodia (stained red) with HIV particles (white) at their ends. Reproduced from the cover of *PLoS Pathogens*, June 2012. Courtesy of Anupriya Aggarwal and Stuart Turville (Kirby Institute, University of New South Wales).

DNA image on cover: majcot/Shutterstock

DNA image on chapter and section opener pages: Mopic/Shutterstock Section opener images: mouse, Sergey Galushko/Shutterstock; lab equipment, Vasiliy Koval/Shutterstock; vaccine, Nixx Photography/Shutterstock

Some figures and tables in this book are reprinted or modified from Glick et al., *Molecular Biotechnology: Principles and Applications of Recombinant DNA*, 4th ed. (ASM Press, Washington, DC, 2010).

medical biotechnology

To our spouses, Marcia Glick, Regina Delovitch, and Patrick Patten, for their omnipresent love and tolerance, support, wisdom, and humor

·

Preface

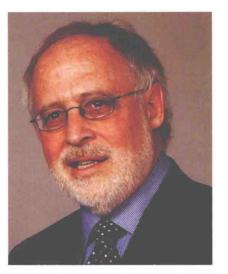
From the very beginning of the biotechnology revolution in the early 1970s, many scientists understood that this new technology would radically change the way that we think about health care. They understood early on, well before any products were commercialized, that medical science was about to undergo a major paradigm shift in which all of our previous assumptions and approaches would change dramatically. Forty years later, biotechnology has delivered on much of its early promise. Hundreds of new therapeutic agents, diagnostic tests, and vaccines have been developed and are currently available in the marketplace. Moreover, it is clear that we are presently just at the tip of a very large iceberg, with many more products in the pipeline. It is likely that, in the next 10 to 15 years, biotechnology will deliver not only new products to diagnose, prevent, and treat human disease but also entirely new approaches to treating a wide range of hitherto difficult-to-treat or untreatable diseases.

We have written Medical Biotechnology with the premise that it might serve as a textbook for a wide range of courses intended for premedical and medical students, dental students, pharmacists, optometrists, nurses, nutritionists, genetic counselors, hospital administrators, and other individuals who are stakeholders in the understanding and advancement of biotechnology and its impact on the practice of modern medicine. The book is intended to be as jargon-free and as easy to read as possible. In some respects, our goal is to demystify the discipline of medical biotechnology. This is not a medical textbook per se. However, a discussion of some salient features of selected diseases is presented to illustrate the applications of many biotechniques and biochemical mechanisms. Thus, this book may be considered a biomedical road map that provides a fundamental understanding of many approaches being pursued by scientists to diagnose, prevent, and treat a wide range of ailments. Indeed, this presents a large challenge, and the future is difficult to predict. Nevertheless, we hope that this volume will provide a useful introduction to medical biotechnology for a wide range of individuals.

About the Authors



Bernard R. Glick is a professor of biology at the University of Waterloo in Waterloo, Ontario, Canada, where he received his PhD in biochemistry in 1974. His current research is focused on the biochemical and genetic mechanisms used by plant growth-promoting bacteria to facilitate plant growth. In addition to his nearly 300 research publications, Dr. Glick is a coauthor of the textbook Molecular Biotechnology: Principles and Applications of Recombinant DNA, published by ASM Press. According to Google Scholar, his work has been cited more than 15,000 times. In addition to having served two terms as chair of the Department of Biology at Waterloo, Dr. Glick has taught 10 courses in five countries on various aspects of biotechnology.



Terry L. Delovitch obtained his BSc in chemistry (1966) and PhD in chemistry/immunology (1971) at McGill University. He received postdoctoral training at the Massachusetts Institute of Technology and Stanford University and then joined the faculty of the University of Toronto. In 1994, he was appointed senior scientist and director of the Autoimmune Disease Group on Type 1 Diabetes at the Robarts Research Institute, Western University, London, Ontario, Canada. After a 45-year research career, he retired from Western in 2011. He has received several academic awards and published about 200 research papers, review articles, and book chapters. He is the former chief scientific advisor to the Juvenile Diabetes Research Foundation Canada and past president of the International Immunology of Diabetes Society, and he is a consultant or advisor for several biotechnology and pharmaceutical

companies, granting agencies, and journal editorial boards and a national allergy, asthma, and immunology research network. He and his wife, Regina, live in Toronto.



Cheryl L. Patten is an associate professor of microbiology and associate chair of the Department of Biology at the University of New Brunswick (UNB) in Fredericton, New Brunswick, Canada. Dr. Patten received her PhD from the University of Waterloo in 2001 and did postdoctoral work at McMaster University before joining the UNB faculty in 2004. Her research aims to understand how bacteria respond to the host environment at the biochemical and genetic levels. In particular, she is interested in secreted bacterial metabolites that may impact host health. As well as teaching introductory and advanced courses in microbiology, she enjoys introducing first-year science students to the wonders of biochemistry and molecular biology. She is a coauthor of another ASM Press textbook, Molecular Biotechnology: Principles and Applications of Recombinant DNA.

Contents

Preface xvii About the Authors xviii

SECTION

The Biology behind the Technology 1

Fundamental Technologies 3

Molecular Cloning 3

Preparation of DNA for Cloning 3

Insertion of Target DNA into a Plasmid Vector 7

Transformation and Selection of Cloned

DNA in a Bacterial Host 12

Cloning Eukaryotic Genes 15

Recombinational Cloning 19

Genomic Libraries 21

Amplification of DNA Using PCR 24

DNA Sequencing Technologies 28

Dideoxynucleotide Procedure 31

Pyrosequencing 33

Sequencing Using Reversible Chain Terminators 35

Sequencing by Ligation 36

Sequencing Whole Genomes 38

High-Throughput Next-Generation Sequencing Strategies 41



Genomics 42

Transcriptomics 46
Proteomics 51
Metabolomics 63
SUMMARY 67
REVIEW QUESTIONS 68
REFERENCES 69

2 Fundamental Concepts in Immunology 71

The Immune Response 71

Overview of Infection and Immunity 71

Functions of the Immune System 73

Innate Immunity 75 Adaptive Immunity 81

Cells of the Immune System: Cell-Mediated Immunity 88

Bone Marrow Precursor Cells 88

Myeloid Cells Mediate Innate Immunity 90

Lymphocytes 92

Antigen-Presenting Cells 96

Effector Cells 97

Tissues of the Immune System 97

Peripheral Lymphoid Organs 97 Lymphocyte Recirculation and Migration into Tissues 101 Antigen Recognition by T Cells 103

Humoral Immunity 114

Structure of Immunoglobulins 116 Functions of Immunoglobulins 118

Types of Antibodies: Applications 119

Polyclonal Antibodies 119
Antisera 120
Monoclonal Antibodies 120
Recombinant Antibodies 120

Immunological Techniques 122

Enzyme-Linked Immunosorbent Assay 123 Enzyme-Linked Immunospot Assay 125 Flow Cytometry 126 Mass Cytometry 128
Two-Photon Intravital Cell Imaging 129
SUMMARY 131
REVIEW QUESTIONS 132
REFERENCES 133

3 The Genetic Basis of Disease 135

Chromosomal Disorders and Gene Mapping 135

Chromosomes and Chromosome Abnormalities 135 Human Genome Mapping 146

Genome-Wide Association Studies 159

Single-Gene Disorders 164

Mode of Inheritance 164

Thalassemia 166

Sickle-Cell Anemia 167

Hemophilia 168

Cystic Fibrosis 169

Tay-Sachs Disease 170

Fragile X Syndrome 171

Huntington Disease 173

Polygenic Disorders and Gene Clustering 174

GWAS Strategies To Map Genes for Polygenic Disease 176

Breast Cancer 178

Alzheimer Disease 182

Type 1 Diabetes 185

Cardiovascular Disease 191

Mitochondrial Disorders 193

Disorders 193

Genetics 194

Mitochondrial Homeostasis and Parkinson Disease 195

Prevalence 199

Diagnosis and Prognosis 200

Treatment 201

SUMMARY 201

REVIEW QUESTIONS 202

REFERENCES 203

4 Immune Pathogenesis 207

Models of Immune System Lesions 207

Immunological Tolerance 207

Failure of Immune Tolerance and Development of Autoimmune Disease 218

Immune Surveillance against Tumors 224

Immune Evasion by Tumors 229

Inflammation and Immune Hypersensitivity Disorders 229

Types of Inflammation and Associated Immune Hypersensitivity Reactions 230

Immediate Hypersensitivity 230

Therapy for Immediate Hypersensitivity 234

Antibody- and Antigen-Antibody Complex-Induced Disease 236

T-Cell-Mediated Diseases 239

Immunodeficiency Disorders and Defects in Development of the Immune System 241

Primary Immunodeficiencies 242

Secondary Immunodeficiencies 245

AIDS 245

SUMMARY 252

REVIEW OUESTIONS 253

REFERENCES 254

5 Microbial Pathogenesis 257

Introduction 257

Bacterial Infections 258

Attachment to Host Cells 258

Invasion and Dissemination 261

Evasion of Host Defenses and Proliferation 266

Damage to Host Tissues 272

Identification of Bacterial Virulence Factors 279

Evolution of Bacterial Pathogens 285

Treatment of Bacterial Infections 290

Viral Infections 293

Attachment and Entry 295

Viral Gene Expression and Replication 300

Virus Assembly and Release 311 General Patterns of Viral Infections of Humans 315 Targets for Treatment of Viral Infections 320 SUMMARY 323 **REVIEW OUESTIONS 324** REFERENCES 325

SECTION II

Production of Therapeutic Agents 327

Manipulating Gene Expression in Prokaryotes 330

Modulation of Gene Expression 329

Promoters 331

Translational Regulation 334

Codon Usage 335

Protein Stability 336

Fusion Proteins 338

Metabolic Load 341

Chromosomal Integration 343

Increasing Secretion 346

Overcoming Oxygen Limitation 349

Reducing Acetate 350

Protein Folding 352

Heterologous Protein Production in Eukaryotic Cells 354

Eukaryotic Expression Systems 354

Saccharomyces cerevisiae Expression Systems 356

Other Yeast Expression Systems 360

Baculovirus-Insect Cell Expression Systems 362

Mammalian Cell Expression Systems 368

Directed Mutagenesis 374

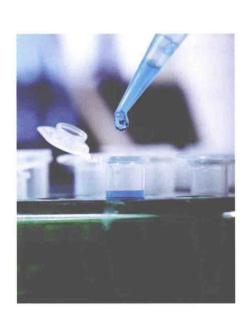
Oligonucleotide-Directed Mutagenesis with M13 DNA 374

Oligonucleotide-Directed Mutagenesis with Plasmid DNA 377

PCR-Amplified Oligonucleotide-Directed Mutagenesis 377

Error-Prone PCR 379

Random Mutagenesis 381



DNA Shuffling 383
Examples of Modified Proteins 384
SUMMARY 386
REVIEW QUESTIONS 388
REFERENCES 389

7 Genetic Engineering of Plants 393

Plant Transformation with the Ti Plasmid of A. tumefaciens 396

Physical Transfer of Genes to Plants 401

Chloroplast Engineering 403

Transient Gene Expression 405

Molecular Pharming 408

Therapeutic Agents 408

Antibodies 411

Edible Vaccines 412
SUMMARY 418
REVIEW QUESTIONS 418
REFERENCES 419

SECTION III

Diagnosing and Treating Human Disease 421

8 Molecular Diagnostics 423

Immunological Approaches To Detect Protein Biomarkers of Disease 424

Enzyme-Linked Immunosorbent Assays 424
Measuring Disease-Associated Proteins by Sandwich ELISA 428
Diagnosing Autoimmune Diseases by an Indirect ELISA 429
Immunoassays for Infectious Disease 430
Protein Arrays To Detect Polygenic Diseases 432
Immunoassays for Protein Conformation-Specific Disorders 435

DNA-Based Approaches to Disease Diagnosis 437Hybridization Probes 437

