Technological Challenges in Antibiotic Discovery and Development: A Workshop Summary

ISBN
978-0-309-29814-8

48 pages
8.5 X 11
PAPERBACK (2014)

Douglas Friedman and Joe Alper, Rapporteurs; Chemical Sciences Roundtable; Board on Chemical Sciences and Technology; Division on Earth and Life Studies; National Research Council

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A WORKSHOP SUMMARY

Douglas Friedman and Joe Alper, *Rapporteurs*

Chemical Sciences Roundtable

Board on Chemical Sciences and Technology

Division on Earth and Life Studies

NATIONAL RESEARCH COUNCIL
OF THE NATIONAL ACADEMIES

THE NATIONAL ACADEMIES PRESS
Washington, D.C.
www.nap.edu

THE NATIONAL ACADEMIES PRESS **500 Fifth Street, NW** **Washington, DC 20001**

This study was supported by the U.S. Department of Energy under Grant DE-FG02-07ER15872, the National Institutes of Health under Contract HHSN263201200074I (Task Order 25), and the National Science Foundation under Grant CHE-1231459.

International Standard Book Number-13: 978-0-309-29814-8
International Standard Book Number-10: 0-309-29814-8

Additional copies of this report are available from the National Academies Press, 500 Fifth Street, N.W., Lockbox 285, Washington, DC 20055; (800) 624-6242 or (202) 334-3313 (in the Washington metropolitan area); Internet, http://www.nap.edu.

Cover Art: Image of *Clostridium Difficule*. Courtesy of the Centers for Disease Control and Prevention.

Printed in the United States of America

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Preface

The Chemical Sciences Roundtable (CSR) was established in 1997 by the National Research Council. It provides a science-oriented apolitical forum for leaders in the chemical sciences to discuss chemistry-related issues affecting government, industry, and universities. Organized by the National Research Council's Board on Chemical Sciences and Technology, the CSR aims to strengthen the chemical sciences by fostering communication among the people and organizations—spanning industry, government, universities, and professional associations—involved with the chemical enterprise. One way it does this is by organizing workshops that address issues in chemical science and technology that require national or more widespread attention.

On September 23, 2013, the CSR held a one-day workshop on the technical challenges in antibiotic discovery and development that explored the current state of antibiotic discovery, examined the technology available to facilitate development, discussed the technical challenges present, identified novel approaches to antibiotic discovery, and discussed the incentives and disincentives industry faces in antibiotic development. The workshop featured both formal presentations and panel discussions among participants from academia, industry, and federal research agencies. The workshop program consisted of three themes:

- The challenges and approaches in overcoming antibiotic resistance;
- The challenges and approaches in screening for new chemical entities with antibiotic properties; and
- The challenges and approaches in delivering antibiotics to their intended site of action, particularly with regard to surmounting biophysical barriers.

This document summarizes the presentations and discussions that took place at the workshop. In accordance with the policies of the NRC, the workshop did not attempt to establish any conclusions or recommendations about needs and future directions, focusing instead on issues identified by the speakers and workshop participants. In addition, the organizing committee's role was limited to planning the workshop. The workshop summary has been prepared by workshop rapporteurs Douglas Friedman and Joe Alper as a factual summary of what occurred at the workshop.

Acknowledgment of Reviewers

This workshop summary has been reviewed in draft form by persons chosen for their diverse perspectives and technical expertise in accordance with procedures approved by the National Research Council's Report Review Committee. The purpose of this independent review is to provide candid and critical comments that will assist the institution in making the published workshop summary as sound as possible and to ensure that it meets institutional standards of objectivity, evidence, and responsiveness to the charge. The review comments and draft manuscript remain confidential to protect the integrity of the deliberative process. We wish to thank the following individuals for their review of this summary:

Carole Bewley, National Institute of Diabetes and Digestive and Kidney Diseases
Nicole Mahoney, The Pew Charitable Trusts
Melinda Moore, RAND Corporation
Douglas Weibel, University of Wisconsin, Madison

Although the reviewers listed above provided many constructive comments and suggestions, they did not see the final draft of the workshop summary before its release. The review of this summary was overseen by **Douglas Lauffenburger**, Massachusetts Institute of Technology. Appointed by the National Research Council, he was responsible for making certain that an independent examination of this workshop summary was carried out in accordance with institutional procedures and that all review comments were carefully considered. Responsibility for the final content of this workshop summary rests entirely with the authors and the institution.

Contents

Acronyms

BARDA	Biomedical Advanced Research and Development Authority
CDC	Centers for Disease Control and Prevention
CRE	carbapenem-resistant Enterobacteriaceae
CSR	Chemical Sciences Roundtable
FDA	U.S. Food and Drug Administration
GyrB	DNA gyrase B
KPC	*Klebsiella pneumoniae* carbapenemase
MIC	minimum inhibitory concentration
MRSA	methicillin-resistant *Staphylococcus aureus*
NDM	New Delhi metallo-β-lactamase
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
NRC	National Research Council
NSF	National Science Foundation
PBP2a	penicillin-binding protein 2A
PMMA	poly(methyl methacrylate)
TTSS	type three secretion system
VRE	vancomycin-resistant *Enterococcus*

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Introduction and Overview

"The Tao of antibiotic development is to balance three factors: target specificity or activity; druggable properties, which include the ability to synthesize, manufacture, and deliver the drug into the body; and pharmacokinetics, particularly off-target toxicity."
Rose Aurigemma

Antibiotic resistance is a serious and growing problem in modern medicine and it is emerging as a preeminent public health threat. Each year in the United States alone, at least two million people acquire serious infections with bacteria that are resistant to one or more antibiotics, and at least 23,000 people die annually as a direct result of these antibiotic-resistant infections (CDC 2013). In addition to the toll on human life, antibiotic-resistant infections add considerable and avoidable costs to the already overburdened U.S. health care system. Studies have estimated that, in the United States alone, antibiotic resistance adds $20 billion in excess direct health care costs, with additional costs to society for lost productivity as high as $35 billion a year. The overuse of antibiotics is the single most important factor leading to antibiotic resistance. According to the Centers for Disease Control and Prevention (CDC), "up to 50 percent of all the antibiotics prescribed for people are not needed or are not prescribed appropriately."[1]

At its September, 2012 meeting, the Chemical Sciences Roundtable (CSR) heard two presentations highlighting the need for new medications to combat the growing threat. These presentations also described some of the current challenges facing antibiotic development and some of the possible solutions to overcome those challenges. To better understand these important topics, the CSR held a workshop on September 23, 2013, in Washington, DC. The workshop explored the challenges and some approaches in overcoming antibiotic resistance, screening for new antibiotics, and delivering them to the sites of infection in the body. The workshop also conducted discussions about possible future endeavors that the field might take to develop the next generation of potent antimicrobial compounds capable of once again tilting the battle against microbial pathogens in favor of humans. In introductory remarks to the workshop, Carole Bewley, of the National Institute of Diabetes and Digestive and Kidney Diseases and a member of the workshop organizing committee, explained the purpose of the workshop in this way: "The goal here today is to give representatives from industry, government, and academia a broad view of the landscape of antibiotic development and the technological challenges and barriers to be overcome."

ORGANIZATION OF THE WORKSHOP SUMMARY

This workshop summary is organized into five chapters. This chapter recounts the overview of the antibiotic resistance problem presented by Rose Aurigemma of the National Institute of Allergy and Infectious Diseases (NIAID). Chapter 2 discusses some of the challenges researchers face in developing novel antimicrobial agents that overcome resistance. Lynn Silver, a consultant at LL Silver Consulting, LLC, discussed the need to select drug targets that are not subject to rapid resistance selection, and Shahriar Mobashery, of Notre Dame University, spoke about the mechanisms that bacteria use to neutralize many of the most potent antibiotics.

Discovering new antimicrobial agents requires screens to identify compounds that can serve as starting points for additional development; Chapter 3 discusses some of the challenges involved in developing suitable screens for compounds whose mechanism of action would make them unlikely to trigger resistance, bypass resistance mechanisms, or directly counter the pathways that produce resistance. Karen Shaw, of Cubist Pharmaceuticals, presented some advice on how to conduct screening and described some of the pitfalls based on knowledge accumulated in the pharmaceutical industry. Chaitan Khosla, of Stanford University,

[1] Press Release: Untreatable: Report by CDC details today's drug-resistant health threats. Centers for Disease Control and Prevention, September 16, 2013.

spoke about the approaches that he believes have a chance of reviving the discovery of new natural antibacterial agents. Chapter 4 focuses on challenges in drug delivery, with J. Rubén Morones-Ramírez of the Universidad Autonóma de Nuevo León discussing some of the different tacks that he has taken to define and deliver novel antimicrobial therapies and Mark Smeltzer of the University of Arkansas for Medical Sciences addressing the issue of overcoming the physical barrier created by biofilms. The final chapter recounts some of the key messages presented during the workshop.

Although not comprehensive, this summary provides the readers with an overview of several topics discussed at the workshop:

- The challenges in overcoming antibiotic resistance.
- The difficult task of identifying targets for drug development and screening chemical libraries for new chemical entities that can surmount resistance.
- The need to develop methods for bypassing biophysical barriers that impede delivery of antimicrobial agents to the sites of infection in the human body.
- The path forward to increase the generation of new antibiotics.

This summary does not contain any findings or recommendations related to these topics, as this was not part of the workshop's task. This summary describes presentations given at the workshop and the views expressed by workshop participants. As the workshop was limited in the time available to cover a very broad topic, it was therefore decided to cover only a subset of issues and questions facing the current state of antibiotic discovery and development in general terms. There clearly remain myriad specific issues and questions that were not discussed or presented during the workshop, and therefore are not included herein.

A VIEW FROM THE ANTIBIOTIC RESISTANCE BATTLEFIELD

To start her overview of the antibiotic resistance landscape, Rose Aurigemma, Section Chief for Drug Development at NIAID at the National Institutes of Health (NIH), stated the concern among infectious disease specialists that medicine may be on the verge of returning to a pre-antibiotic era in which there will be important human pathogens that no longer respond to any available antibiotic, and she noted the timeliness of the CDC's new report *Antibiotic Resistance Threats in the United States 2013*. She said that there are three organisms for which the situation is considered urgent—*Clostridium difficile*, carbapenem-resistant Enterobacteriaceae (CRE), and drug-resistant *Neisseria gonorrhoeae*—as well as a dozen organisms for which the threat of antibiotic resistance is currently considered to be serious (Figure 1-1).

Forty-three of 50 states have reported confirmed cases of CRE, as have countries throughout much of South America, Western Europe, and Asia. CRE results from the presence of various enzymes that cleave the lactam ring in carbapenem antibiotics and renders them ineffective. Given that carbapenems are currently an antibiotic of last resort, treatment overall becomes ineffective once resistance develops. The appearance of two specific enzymes in CRE—*Klebsiella pneumoniae* carbapenemase (KPC) and New Delhi metallo-β-lactamase (NDM)—are of particular concern.

Fluoroquinolone-resistant gonorrhea is a growing problem for which there is little awareness among the public, though NIAID has a significant research effort underway to combat this resistant organism. Because of the appearance of resistance, CDC changed its treatment recommendations for gonorrhea to move from fluoroquinolones to an intramuscular injection of cephalosporins such as cefixime and ceftriaxone. However, the incidence of resistance to those two antibiotics is also growing. "While [antibiotic-resistant gonorrhea] is not a deadly disease, it is a great public health concern," said Aurigemma.

Resistance develops, Aurigemma explained, as an evolutionary response to the selective pressure of antimicrobial drugs, particularly when patients do not complete a prescribed course of therapy. Other factors include overprescribing of drugs by physicians, the common practice of using broad-spectrum antibiotics when narrow-spectrum antibiotics would be preferred, and agricultural use in livestock. Common mechanisms of resistance include a change in drug target, such as when a bacterial enzyme undergoes a structural change to eliminate a drug binding site, or the appearance of a drug-metabolizing enzyme, as in the case of KPC and NDM. There can also be a change in drug access related to the appearance of membrane-bound efflux pumps that remove a drug from the organism as quickly as it enters or when microorganisms sequester themselves behind a biofilm barrier.

To counter these microbial responses, researchers are turning to the power of the 'omics'—genomics, proteomics, metabolomics, and the like—to identify new drug targets beyond the well-known resistance factors. Examples include regulators of bacterial growth and pathogenesis and transcriptional regulators involved in processes such as sporulation, toxin production, and adhesion. Antibiotic developers are also attempting to better understand the chemistry of getting drugs into bacteria, a process that largely remains a mystery. Aurigemma said that there is some worry that target-based screening approaches have been exhausted and that the available chemical libraries have already been plumbed for the best drugs. "We need new ideas, new approaches to screen for drugs, and perhaps that's where the 'omics' comes into play," she said. There is some excitement in the field around natural products, but fully exploiting nature's chemical libraries awaits improvements in both screening

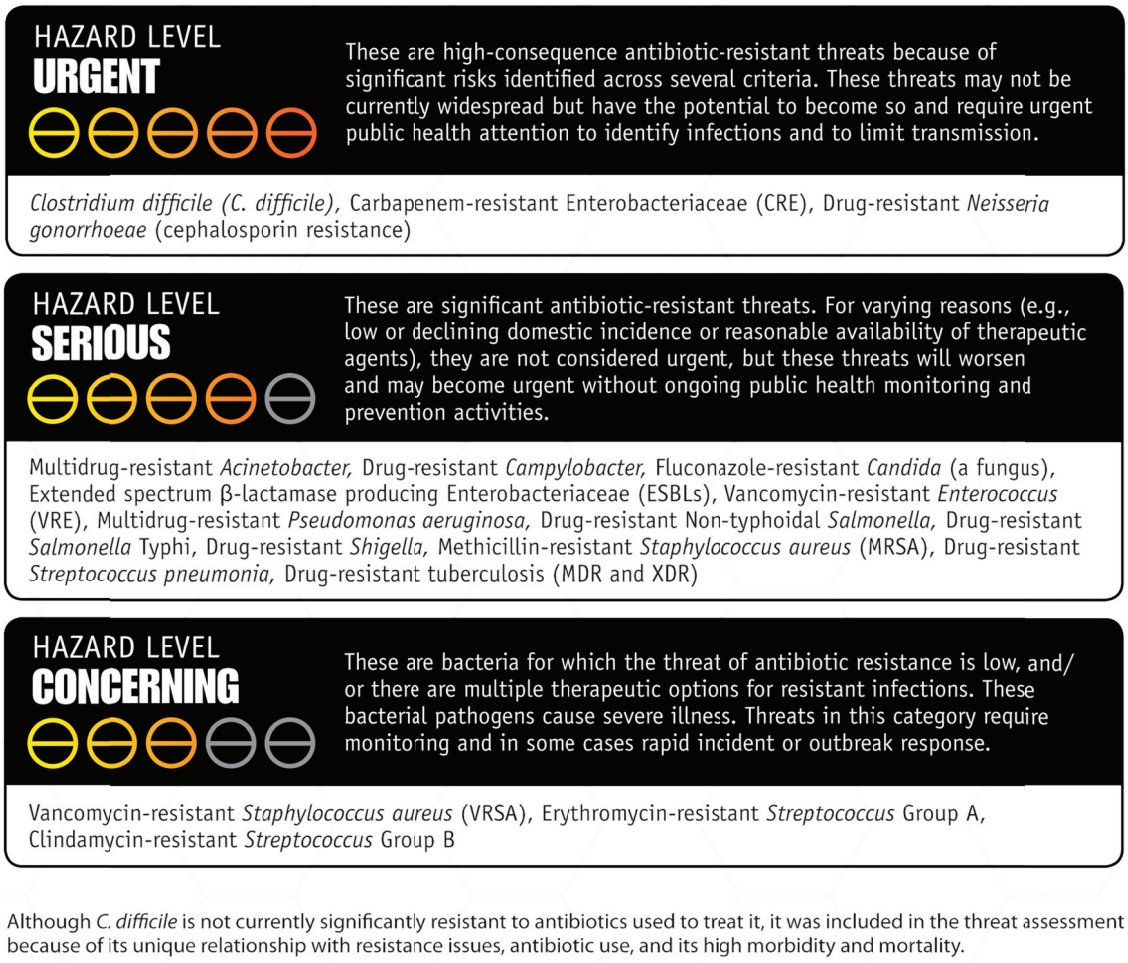

FIGURE 1-1 The growing threat of antibiotic resistance.
SOURCE: CDC (2013).

and synthetic chemistry technologies. "These compounds are often difficult to manufacture," she said, referring to potent naturally existing antimicrobial agents.

Aurigemma then discussed some nontechnical factors that are "getting in the way of good drugs." A major reason is skepticism in the financial markets of the value of antibiotics. Large pharmaceutical companies have largely exited antibacterial agent discovery, instead focusing their attention on chronic diseases. In theory, that should open the door to smaller companies that, as she put it, "are more likely to think out of the box and come up with novel ideas and take a risk in trying new paradigms." However, antibiotics traditionally fail early and often during development because of toxicity or formulation issues, reducing the appetite of the venture capital market to fund small companies during the discovery and early development phases of research. Federal support for early phase research and development is also in short supply these days, and uncertainty in the regulatory arena—the Food and Drug Administration (FDA) is developing new guidance for drug approvals and drug trials in order to meet this growing need for new antibiotics—further complicates the funding picture.

That said, Aurigemma noted that NIAID does have a comprehensive and sustainable research and development program feeding the pipeline with antimicrobial agents, including antibiotics. "Since pharma is not engaging in this research and small companies are limited in their resources, [NIAID] is trying to do as much as we can to support this effort," she said, adding that CDC, FDA, the Biomedical Advanced Research and Development Authority (BARDA), and the Defense Department are partners in supporting antibiotic development. In addition to supporting research, NIAID has also contracted with manufacturing facilities and has developed a network of clinical sites that can conduct clinical trials through phase II of the development process. One of NIAID's goals is to lower the risk of drug discovery and development by using a range of funding mechanisms, including grants, cooperative agreements, contracts, and

small business innovation research awards, to take a potential new agent through phase II trials, at which point it expects the commercial sector to take over a project.

Complicating the antibiotic world are recent discoveries about some of the "collateral damage" that can accompany antibiotic use. For example, recent work has uncovered a correlation between the level of use of antibiotics, particularly among children, and the incidence of obesity in various regions of the country. At this point, some researchers believe that this correlation may be tied to changes in the gut microbiome caused by antibiotics. Much work is still needed on this and other areas related to gut bacteria however, this example illustrates one hypothesis and why more work is needed.

The Tao of antibiotic development, Aurigemma explained, is to balance three factors: target specificity or activity; druggable properties, which include the ability to synthesize, manufacture, and deliver the drug into the body; and pharmacokinetics, particularly off-target toxicity. She said the latter factor is particularly challenging because antibiotic toxicity is often poorly understood. "And that is why many researchers focus on known antibiotic classes because they know the toxicities and they know what they are up against in terms of showing that a new antibiotic is safe or that any toxicities are within the class of known toxicities," she said. It is important to keep in mind that toxicity can arise from many mechanisms, including off-target binding and poor specificity.

After briefly reviewing the many known microbial targets for antibacterial development (Figure 1-2), Aurigemma noted that there are also host factors that could be targets for antimicrobial drugs. Intracellular pathogens, for example, could be susceptible to drugs that activate myeloid cells. It may be possible to identify the body's natural antibacterial peptides, known as defensins; block the inflammatory pathways that trigger sepsis; or block the host receptor for bacteria and other pathogenic organisms. Another promising approach is to harness bacteriophages, the natural killers of bacteria. Investigators are also developing monoclonal antibodies and vaccines that could prove helpful in treating or preventing infection by antibiotic-resistant organisms.

Aurigemma concluded her remarks by stating that the solution to antibiotic resistance lies not just with developing better antibiotics. "You want better detection. Stewardship of antibiotics is important. So, too, is better control to stop the spread of infection," adding that antibiotics have to be eliminated from animal feed, but acknowledging, too, that getting the food industry to agree to do so is going to be an uphill battle. "The fact that bacteria can mutate at such a rapid rate makes it challenging to keep ahead of them," she said in closing. "But I do think we are on the right track for developing the tools, and especially the public mindset, to make progress."

In response to a question about whether new diagnostic technologies that can rapidly identify specific strains with specific resistance patterns could lead to better use of narrow-spectrum antibiotics, Aurigemma said that this was indeed a promising area of research that could have a significant impact on how antibiotics are used. However, physician

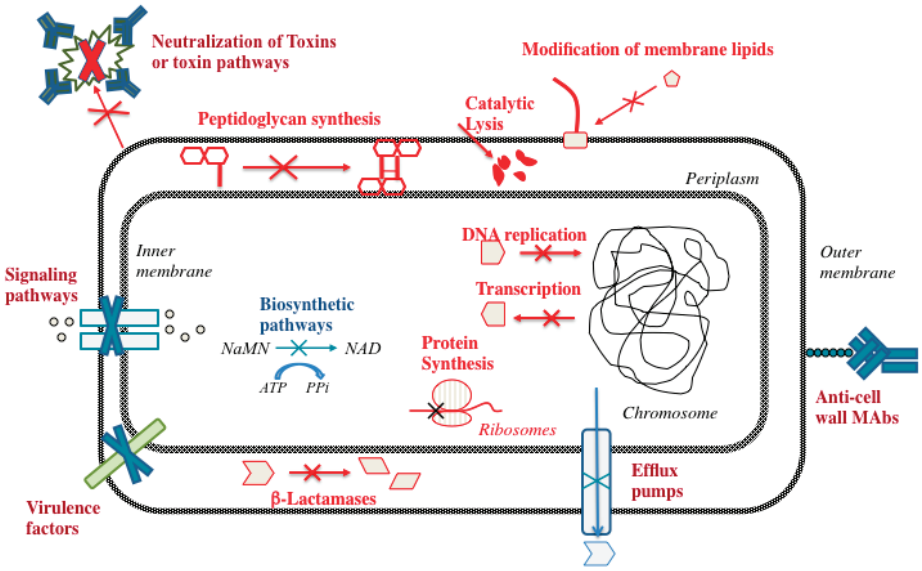

FIGURE 1-2 Many of the known targets under investigation for antibacterial development.
SOURCE: Rosemarie Aurigemma (2013).

education will play a critical role in determining whether that in fact plays out in real-world use.

Responding to another question, she noted that live biotherapeutics[2] are a promising avenue of research, particularly for *Clostridium difficile* infections. She also thought that combination therapy, where agents attack different microbial targets, could prove fruitful both in terms of therapeutic efficacy and avoiding the development of resistance. The challenge in developing combination therapies will be getting companies to work together on clinical development.

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[2] Live biotherapeutics refer to the use of bacteria or bacteriophage, as therapeutics for infection.

2

Challenges In Overcoming Antibiotic Resistance

"The last novel class [of antibiotics] to be licensed was discovered in 1987."
Lynn Silver

"If you are able to reverse the resistant phenotype, then you are rescuing drugs that have become obsolescent."
Shahriar Mobashery

The predominant focus of this workshop was to understand the barriers to developing new antibiotics that can overcome or bypass resistance to existing therapies. Two speakers addressed these challenges. Lynn Silver, a consultant at LL Silver Consulting, LLC, discussed the need to select drug targets that are not subject to rapid resistance selection, and Shahriar Mobashery, Professor of Life Sciences at Notre Dame University, spoke about the mechanisms that bacteria use to neutralize many of the most potent antibiotics.

SELECTING ANTIBACTERIAL TARGETS TO AVOID RESISTANCE SELECTION

What plagues the antibiotic field today, said Lynn Silver, is that the field is suffering from a discovery void, a gap of over 30 years when efforts to discover novel classes of antibiotics largely failed. "If you look at when things were discovered, we stopped discovering novel antibiotics in 1987," she stated, noting that every new antibiotic that has been approved for human use since then is a member of a chemical class discovered before 1987. For example, retapamulin, which was approved by the FDA in April 2007 and the European Medicines Agency in May 2007 for the treatment of bacterial skin infection, is a member of a class of compounds discovered in 1950. Newer antibiotics such as fourth-generation fluoroquinolones derive from nalidixic acid, which was discovered in 1962. By itself, that "innovation gap" would not be that big of an issue, but the problem is that resistance arose to most classes of antibiotics soon after their introduction, and there was little in the pipeline (a discovery void) in terms of antibiotics with novel mechanisms of action that could overcome resistance. One important exception has been vancomycin, which was discovered in 1953 and approved in 1958, but now even vancomycin-resistant strains of *Enterococcus* and *Staphylococcus* are becoming increasingly prevalent (Figure 2-1).

With each parry by bacteria, medicinal chemists developed new drugs that were not just molecules copied from others but derivatives of existing antibiotic classes with better pharmacological properties or modifications that overcame specific resistance mechanisms. For example, when cases of methicillin-resistant *Staphylococcus aureus* (MRSA) started appearing, medicinal chemists responded with compounds that block the action of the extended-spectrum β-lactamases that are responsible for resistance. Some of these compounds, which should also combat CRE, are now in clinical trials. "This is real science and it makes very good drugs," said Silver, "but the problem is that resistance keeps happening."

By and large, however, the discovery of novel compounds, especially in new classes toward new targets, that can be developed has so far failed, and Silver listed several possible reasons for this failure. One possibility is that research has focused on only a few bacterial targets, and to remedy this problem the drug discovery community has turned to genomics, crystallography, and bioinformatics to identify new targets. These approaches, however, have not been particularly successful. Another possibility is that medicinal chemists have not screened enough compounds, and in response to that possibility the field turned to high-throughput screening of large chemical libraries and natural product isolates. High-throughput screening has yielded few leads, and naturally occurring antibiotics have largely proven to be so difficult to isolate or have such poor pharmacological properties that the field has for the most part largely abandoned that avenue of discovery. The problem with all of these efforts, said Silver, is that antibiotic developers generally keep applying new technologies to address the discovery gap without understanding why these approaches were not succeeding in the first place.

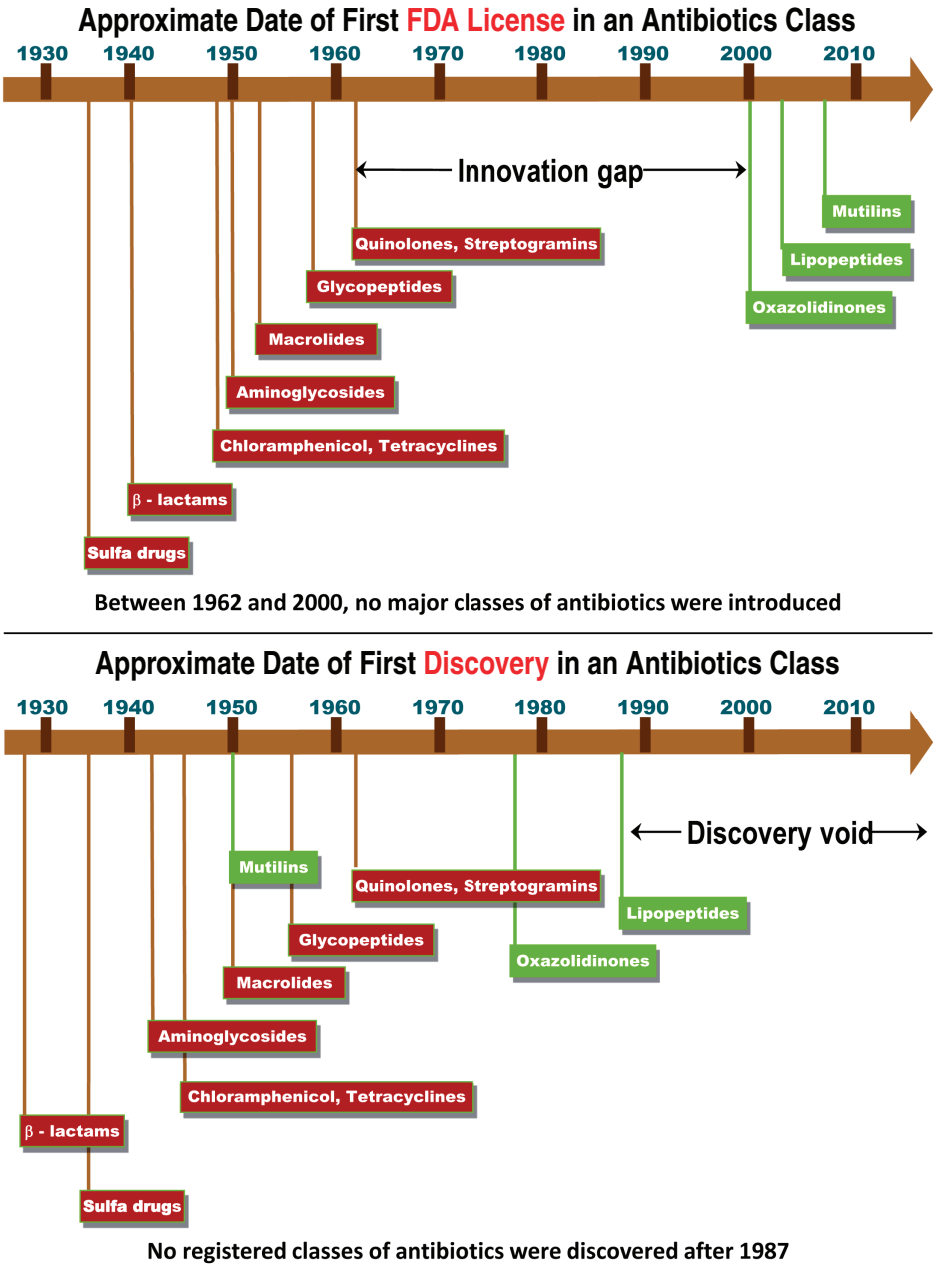

FIGURE 2-1 The innovation gap and discovery void in antibiotics discovery and development. A comparison of this first discovery in an antibiotic class and the first FDA licensure.
SOURCES: *Top*: Fischbach, M. A., and C. T. Walsh (2009). Antibiotics for emerging pathogens. *Science* 325(5944):1089-1093 with permission from AAAS. *Bottom*: Lynn L. Silver.

In her mind, there are two rate-limiting steps in antibiotic discovery. The first is the selection of targets that are not subject to rapid selection of resistance, and the second is the use of chemistries that are appropriate for antibacterial discovery. "Antibacterial agents have certain physiochemical parameters that are different from those of other human health drugs," said Silver, and as a result the chemical libraries that have been screened for antibacterial activity were unlikely to generate suitable leads for further development. In addition, there is no complete set of general rules or an agreed upon rational approach to getting drugs into gram-negative bacteria.

What makes a good antibacterial target? Silver explained that research has largely focused on attributes such as the

target not having a human homolog, that it is present in a range of bacteria, that it is essential, that it is druggable in the sense that it is possible to create molecules that interfere specifically with the activity of the target, and that the target has a low potential for cross-resistance with existing antibiotics. She believes, though, that this list should include two other largely ignored attributes: location of the target on or in bacteria and a low frequency of resistance to new compounds. She then noted that when she and her colleagues in industry looked at the targets of successful antibiotics, they found that these compounds fell into one of these categories. Either they had multiple targets or targets encoded by multiple genes, in which case high-level, target-based resistance to these compounds does not occur by single-step mutations, or they had single enzyme targets that were subject to single-step target-based resistance.

Based on this evaluation, Silver concluded that successful monotherapeutic antibacterials are not subject to single mutations for high-level resistance because they are multi-targeted, and that current drugs that inhibit single enzymes, because they are subject to single-step mutation, are generally used in combinations and when organismal load is low. This conclusion, she said, leads to the hypothesis that multiple targets are preferable for systemic monotherapy, and that the null hypothesis for single targeted agents is that they will select rapidly for resistance. In fact, she said, this is what has happened with tuberculosis and HIV, where single-agent therapy led to the rapid development of resistance but multi-drug therapy has proven to be highly successful. She also cited several examples of single-target monotherapies that entered clinical trials but failed because resistance developed rapidly. As an example, she said that one promising agent that targeted leucyl tRNA synthetase demonstrated excellent activity in vitro against a broad spectrum of gram-negative bacteria, but that resistance occurred in four of 34 patients after just one day of therapy during clinical trials. When isolated, these mutants were highly fit and grew as fast as wild-type bacteria. Silver noted that these findings were replicated in an in vitro system called the hollow fiber resistance model that mimics the pharmacokinetics of drug dosing and cell growth under conditions of cyclic dosage of antibiotics.

One way to move forward when the resistance frequency to new lead compounds is high is to optimize the chemistry, probably through an iterative approach, to reduce the resistance frequency. As an example of this approach, Silver cited a case in which researchers developed an analog of trimethoprim, which is an inhibitor of dihydrofolate reductase, which has higher affinity to its target. This compound demonstrated efficacy in clinical trials but has not yet been approved for human use. The one caveat to this approach is that as molecules are designed to have more than one interaction with specific enzymes, they may be effective against a smaller set of organisms, which could make the business case for the development of these agents less attractive.

Another path forward is to discover more multitargeted inhibitors, which Silver admitted is a more difficult approach. Potential targets in this class include multiple cell wall enzymes; DNA gyrase and topoisomerase IV; and other enzymes sharing active sites, such as DNA polymerases PolC and DnaE. Compounds that inhibit protein synthesis by binding to multiple targets in ribosomal RNA or that inhibit lipid formation might be good candidates, too, since these processes all involve multi-protein complexes. Compounds that damage the integrity of the bacterial cell wall, such as daptomycin or amphotericin B, have proven useful in treating resistant gram-positive bacteria and fungi, respectively, with little incidence of resistance. Silver noted that the field needs to explore additional pathways with similar active sites or ligands, such as the tRNA synthetases, the purine synthesis pathway, or cofactor synthesis pathways. In silico analyses of known ligand–target interactions combined with chemi- and bioinformatics could prove useful for identifying families of targets by the interaction with similar ligands.

Other targets for antibiotic development could fall into what she called the adjunctive category of therapies. These would include inhibitors and dispersers of biofilms, permeability enhancers, and efflux pump inhibitors. Virulence targets and antitoxins have been touted as resistance-proof since they do not kill the organism, but that idea awaits proof of concept. Metabolic targets are possible, but most metabolic targets identified so far have been single enzymes, and while regulatory targets are interesting scientifically, the general consensus within the antibiotic development community is that these pathways could be bypassed or be highly prone to resistance selection.

There are also a number of clinical approaches that could be taken, Silver explained. The simplest approach would be to dose patients with drug levels above the mutation prevention concentration, the concentration above which single-step mutations to resistance are not selected. However, dosing levels used today are generally geared toward efficacy, not resistance avoidance, and the mutation prevention concentration may be incompatible with toxicity and pharmacokinetic parameters. More promising are efforts to identify combinations of drugs that work by different or even synergistic mechanisms. A major challenge with this approach is to match pharmacokinetic properties so that the levels of each drug are high enough to overcome resistance. It may also be difficult, or even unethical, to conduct the necessary clinical trials that can demonstrate decreased resistance with combinations of drugs that by themselves result in resistance. Following a discussion of the myriad approaches to overcoming antibiotic resistance, Silver explained that there is some need for prioritization on which approaches are best suited for further development.

THE COMPLEX RESISTANCE MACHINERIES FOR β-LACTAM ANTIBIOTICS IN GRAM-NEGATIVE AND GRAM-POSITIVE BACTERIA

Two of the most challenging infections to treat are those caused by gram-positive MRSA and gram-negative carbapenem-resistant Enterobacteriaceae (CRE). In his presentation, Shahriar Mobashery discussed the work that his group has done in understanding the resistance mechanisms employed by these bacteria and developing approaches for overcoming resistance in those organisms. He noted that these resistance mechanisms are complex, multistep processes, but each step along the way toward manifestation of resistance offers the opportunity for intervention to reverse the resistant phenotype. "If you are able to reverse the resistant phenotype, then you are rescuing drugs that have become obsolescent," he explained.

Staphylococcus aureus was susceptible to β-lactam antibiotics such as penicillin well into the 1960s, when the β-lactamases were first discovered. Medicinal chemists countered that development by altering the structures of these drugs to overcome the catalytic function of those enzymes. However, within four years of the introduction of these second-generation penicillins, MRSA had not only appeared but had spread worldwide. Health officials expressed concern that vancomycin-resistant strains of MRSA would appear, and indeed they have. Mobashery said that by his count, 13 variants of vancomycin-resistant MRSA have been identified.

The mechanism that produces vancomycin resistance is complex, he explained, involving a set of genes whose origins were not from *Staphylococcus aureus*. The first such gene codes for a signal transduction protein, BlaR1, that binds β-lactams irreversibly as a result of a decarboxylation reaction triggered by β-lactam binding that in turn produces a conformational change in the protein that spans the bacterial cell membrane (Figure 2-2). This conformational change is observable using Fourier transform infrared spectroscopy. Once binding occurs, it activates a cytoplasmic protease

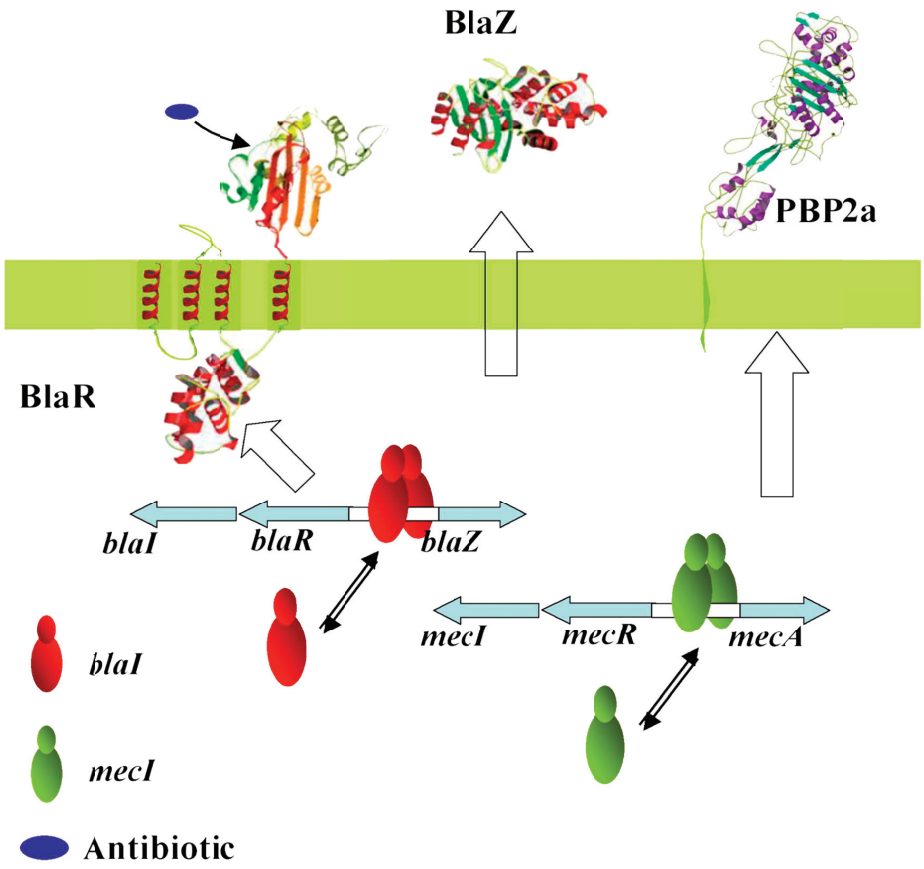

FIGURE 2-2 An example of antibiotic resistance. Sensing of β–lactam antibiotics requires a conformational change for transduction of information to the cytoplasmic side.
SOURCE: Shahriar Mobashery (2011).

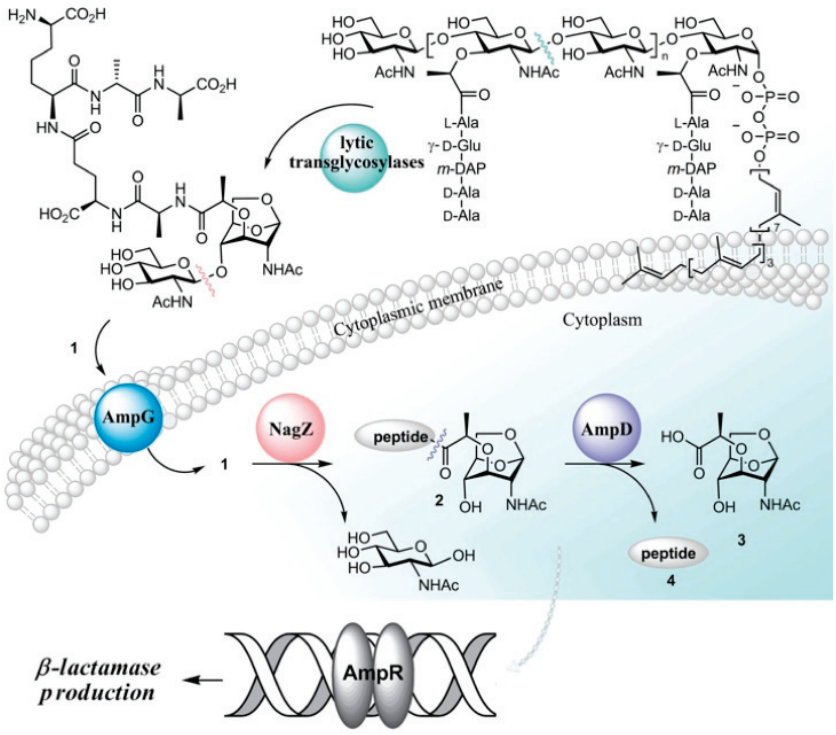

FIGURE 2-3 The link between β-lactamase production and cell wall recycling is mediated by several enzymes, including ligase AmpD. SOURCE: Shahriar Mobashery (2011).

domain that degrades several gene repressors. Upon degradation of these repressors, full-blown resistance develops within minutes to tens of minutes of exposure as a result of the BlaZ gene expressing β-lactamase and the MecA gene expressing the penicillin-binding protein 2A (PBP2a). Work in Mobashery's laboratory has shown that this is a reversible process when antibiotic is no longer present, suggesting that PBP2a could be an interesting target for drugs that could block the development of resistance.

One of the functions of PBP2a is to crosslink peptides in the bacterial cell wall, a critical function for viability of the cell wall, and in nonresistant organisms the β-lactam antibiotics are able to inactivate this enzyme through an irreversible acylation reaction. From the results of x-ray crystallography experiments, it appears that allosteric control is involved in the activity of this protein and that it is possible to impact that control with certain cephalosporins. Indeed, ceftaroline, a fifth-generation cephalosporin that is active against MRSA, appears to overcome resistance by binding to mutated PBP2a at the allosteric site. Binding to the allosteric site induces a conformational change in this enzyme that opens its active site and enables a second molecule of ceftaroline molecule to bind there and inhibit PBP2a's ability to act as a transpeptidase in cell wall synthesis.

Briefly turning to the subject of gram-negative bacteria, Mobashery discussed his group's work synthesizing a number of small carbohydrates that mimic small glycans released when the bacterial cell wall is damaged. These glycans serve as signaling compounds that activate lytic transglycosylases that are involved in the development of resistance in gram-negative bacteria (Figure 2-3). Activation of one enzyme in particular, ligase AmpD, triggers the production of a β-lactamase involved in resistance. His group has been conducting crystallographic studies in an attempt to better understand the function of the AmpD protein and to determine if there are sites in the protein that might be good targets for drug development.

DISCUSSION

In response to a question about the use of nanotechnology to develop novel antibiotics that might overcome resistance, Silver said that this is an avenue worth pursuing, but that she worried about toxicity. She noted that nanoparticles may help with getting compounds into bacteria, particularly gram-negative species. Mobashery said his concern with nanoparticles was with how they are cleared from and metabolized by the human body.

Silver asked Mobashery if it might be worth looking at existing libraries of natural products to see if any of them could induce the type of conformational changes his research has identified. He thought this could be a productive exercise and that it may be possible to find compounds that are not β-lactams that would bind to the allosteric site and synergize with a β-lactam.

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3
Challenges in Screening

"The problem is that most antimicrobial agents don't conform to [Lipinski's] rules."
Karen Shaw

"There are three, and only three, methods that I can think of that have a chance of reviving the discovery of new natural antibacterial natural products."
Chaitan Khosla

Two speakers addressed the challenges of identifying potential new antibiotics capable of overcoming resistance. Karen Shaw, Vice President of Biology at Cubist Pharmaceuticals, presented some advice on how to conduct screening and described some of the pitfalls based on knowledge accumulated in the pharmaceutical industry. Chaitan Khosla, Professor of Chemistry and Chemical Engineering at Stanford University and Director of the Stanford Institute of Chemical Biology, spoke about the approaches that he believes have a chance of reviving the discovery of new natural antibacterial agents.

CHALLENGES IN DISCOVERING NEW ANTIBIOTICS THROUGH SCREENING

In the 1990s, genomics promised a wealth of new targets for antibiotic discovery, but that promise has gone unfilled, said Karen Shaw. "The bottom line is that genomics has not revealed new targets that gave us new drugs." The 1990s also saw the failure of screening and optimization paradigms for both synthetic molecule collections and natural product libraries, she said. To illustrate the failure of both approaches, she recounted the results of a seven-year effort at GlaxoSmithKline that examined 300 genes that were conserved among bacteria, did not have a human homolog, and were essential to bacterial survival. The company conducted 70 high-throughput screening campaigns, producing 16 hits that resulted in five leads, two of which were optimized and none of which progressed to human clinical trials. "So why aren't we finding new inhibitors?" asked Shaw.

One answer is that big pharmaceutical libraries were designed largely to follow Lipinsky's rules[1] that have guided the search for drugs in other therapeutic areas. "The problem is that most antimicrobial agents don't conform to these rules," said Shaw. Even with the advent of new tools such as genomics, researchers in numerous laboratories kept finding the same compounds repeatedly, as was the case with the compound actinonin, a peptide deformylase inhibitor, that was discovered simultaneously by many laboratories. Efforts to use combinatorial chemistry to create new libraries did not generate significant new leads, a failure that Shaw pinned on the fact that the design of the combinatorial libraries being tested was usually driven by the needs of other therapeutic areas. Natural product screening produced many positive hits, but there was a problem prioritizing which compounds to pursue and how to synthesize what were often complex molecular structures.

Shaw noted that too often, screens for antimicrobial activity and enzyme activity are not concordant. "Those two things are often not linked, more often than one would like to see," said Shaw. Off-target toxicity is often the real source of antimicrobial activity, even with compounds generated in chemical optimization programs. Cytotoxicity assays can also provide false positives or even rule out potential compounds too early if mammalian cell toxicity results from a functional group that would have had the potential to be eliminated in optimization work. On the other hand, molecules that appear "clean," that is, they are not toxic to mammalian cells, might be nonspecifically absorbed by proteins such as albumin and therefore at lower effective concentrations in the assay medium.

[1] Lipinsky's rules are a set of five criteria for the design of orally available drugs.

Another problem in screening arises from the reliance on model microbial organisms. "We've known for quite a long time, especially in the genomics era, that testing in one species is not sufficient," said Shaw. She added that some species have bypass pathways that are absent in other species, while there are duplications of genes in some species and not others. She reiterated Silver's earlier remark that the field must start focusing on dual targeting to avoid and surmount resistance.

Cell penetrance is an issue in gram-negative bacteria given that any compound has to get past two different cell membranes to gain entry to the cell. Shaw noted that one strategy for addressing that issue is to create a charged, water-soluble molecule that can pass through the outer membrane via embedded porins. Then, once the compound reaches the periplasm between the two membranes it assumes an uncharged, hydrophobic state that enables it to pass through the cytoplasmic membrane and bypass resistance-associated efflux pumps. This ability to change charge given the different environmental conditions is what makes the fluoroquinolones effective against gram-negative bacteria, she explained.

Natural products have been the subject of intense screening over many decades, yielding large numbers of active compounds but few novel ones with suitable biological mechanisms. Most of the hits identified in natural products screens, said Shaw, are generally toxic. "Potency is not a key parameter, mechanism is," she said. Too often, investigators pursue compounds with the best results in terms of minimum inhibitory concentration (MIC), but these compounds tend to be generally toxic. "What matters is having a molecule in hand, even if it's a weak hit, and a target that it binds to," Shaw explained, since that then gives medicinal chemists a molecule that they can try to optimize to improve its bacterial toxicity.

This last idea, she said, brings up an important philosophical question: "Are you looking for a drug, or are you looking for a hit? If you're after a hit, you start with the most susceptible organism you can find with the idea that you'll identify many compounds that you can then sort through later," she said. "If you want to find a drug, you start out with the most resistant organism and screen your library with the hope that you'll find a molecule that you need to modify a little bit."

Shaw then discussed synergy screening to look for molecules that inhibit β-lactamase or that are cell wall synergists. The key is to conduct large-scale searches for targets that have the potential to interact in vivo. Another approach, one that is quite old, is to screen pathways to identify inhibitors of bacterial macromolecule synthesis. More recently, protein-driven screens have looked at enzyme activity, binding properties, and the ability to inhibit protein–protein interactions. RNA targets have also become a focus of assays in the era of genomics.

Discussing the work that her group at Trius Therapeutics (which was purchased by her current employer, Cubist Pharmaceuticals, in July 2013) conducted to screen marine natural products, she said the philosophy driving the project was to identify a validated hit-target pair that could then become the focus of structure-based drug design to optimize target affinity, specificity, antibacterial activity, and in vivo properties. Based on the results of screening around 10,000 mixtures isolated from marine sources, it turned out to be easier to find compounds that were active versus gram-positive organisms and rare to find gram-negative activity against wild-type strains. The only way that she and her colleagues identified compounds active against gram-negative bacteria was to use an *E. coli* permeability mutant, and most of the hits identified using this mutant were also effective against gram-positive organisms.

After describing some of the details of how she and her colleagues screened the resulting hits for macromolecular synthesis inhibition activity, she noted that this type of assay is capable of distinguishing inhibition of pathways specific for DNA synthesis, RNA synthesis, protein synthesis, and cell wall assembly. Compounds that inhibited multiple pathways were discarded, while those that were selective for one pathway became the subject of further study. She added that it was possible to screen crude extracts using these assays rather than needing to isolate the individual components of the extracts (Figure 3-1). Using this approach, her group was able to screen 2,000 fractions in roughly nine months, which she characterized as good throughput, and follow those initial results with resistance studies to quickly prioritize potential hits for further study. For nucleic acid hits, subsequent screens looking at specific mechanisms of action, such as inhibitors of DNA polymerase or ligase, and at general nucleic acid binding or DNA intercalation were able to quickly and efficiently separate good leads from bad.

One interesting profile that emerged during this effort was what Shaw called the "flatline" profile. This profile was seen with compounds that had antimicrobial activity but no inhibition of any of the macromolecular pathways. Mixtures displaying this profile often led to new targets that could be the subject of further study.

Shaw then turned to the subject of antisense assays, which look for nucleic acid sequences that selectively attenuate gene expression. For essential genes, attenuation can result in growth inhibition. These assays are run by introducing plasmids that produce antisense RNA sequences under the control of a regulatable promoter. The point of this approach is that moderate levels of antisense induction can be used to titrate growth rate and that cells become hypersensitized to any further insult that is specific to the attenuated target. Her group's experience with this type of assay showed that screening for growth inhibition is only the first step and that not all antisense clones are equally susceptible to drugs act-

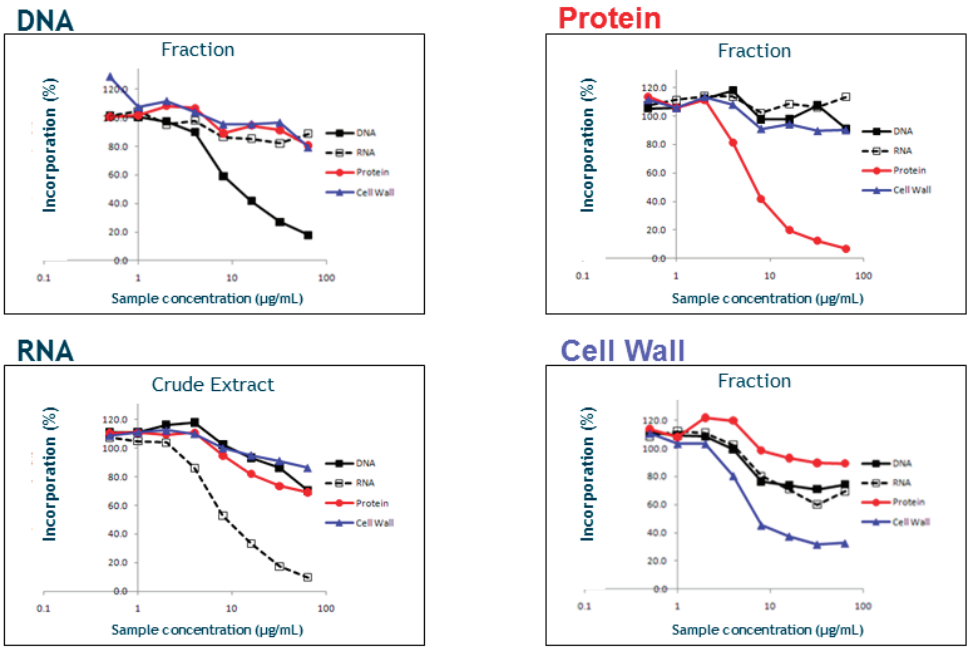

FIGURE 3-1 An example of macromolecular synthesis inhibition assays using fractions and crude extracts.
SOURCE: Karen Shaw (2013).

ing on the target of interest, for reasons that are still unclear. In addition, it turns out that not all targets can be assayed using antisense approaches, again for reasons that are not clear.

After briefly describing how these assays are run, she showed how researchers at Merck used this approach to identify both known and new inhibitors of synthases FabH and FabF from libraries of natural products. In another set of experiments, Shaw and her colleagues ran an antisense assay looking at cell wall targets. These experiments demonstrated synergy between known cell wall inhibitors and inhibition of several early steps in cell wall biosynthesis. She noted that a benefit of conducting antisense screening is that it is a focused approach that provides strains optimized for target-specific hypersensitivity. Another benefit is that antisense screening quickly identifies antibacterial agents that act by defined mechanisms. Antisense assays can enable screening for on-target activity during structure-based drug design optimization and they can identify compounds that act synergistically with those targets. For example, antisense assays showed that Mur ligase pathway enzyme inhibitors are likely to be synergistic with penicillin binding protein inhibitors.

Summarizing the results of screening 22,000 crude samples and fractions from marine sources, Shaw said that about seven percent demonstrated antimicrobial activity. Macromolecular synthesis inhibition eliminated 80 percent of those hits as being nonspecific inhibitors. About half of the remaining samples were eliminated through cross-resistance studies, DNA intercalation assays, and other rapid screens. Those samples remaining then needed to go through fermentation, fractionation, and structural elucidation, which she said are always going to be the rate limiting steps in natural products screening. In the end, only a very few compounds were judged worthy of entering a structure-based drug design program.

Before discussing new approaches and sources of compounds, Shaw said that one of the lessons learned from screening efforts is that an enzyme hit might not translate to how the cell is being killed and that improvements in antimicrobial activity are often not associated with target-based inhibition. "It is important to prove that initially and consistently through optimization," she said. Hits are a plentiful, so the real issue is prioritizing which hits are worth pursuing. Hits from combination screens, such as those looking for β-lactamase inhibitors, need to be confirmed to be working via a multiples mechanisms, and it is important to do resistance studies early and often. Finally, it is important to get the biology right and to beware of functional and nonfunctional homologs in different species.

Another new approach that she encountered recently uses mass spectrometry to create "molecular fingerprints" that can then be used to track molecular networking across an entire organism. The challenge with this approach, which is being pursued by a company called Siernas, is data analysis, but it is being used to identify novel compounds that then can be put through antibacterial screens. In one analysis of a marine sponge, this approach yielded 20 pure compounds for further testing.

Shaw concluded her talk by briefly reviewing the work that her team did looking for inhibitors of topoisomerase IV,

also known as ParE, and DNA gyrase B (GyrB) that would not display cross-resistance with fluoroquinolone antibiotics. This project had its origins in the 1950s with the discovery of novobiocin, a natural product that showed some promise against gram-positive bacteria but that was not dual targeting and was judged to be prone to resistance. Using crystal structures, Shaw and her colleagues were able to map the sequence diversity of GyrB and ParE in gram-negative and gram-positive bacteria and use that information to develop highly potent, broad spectrum antibiotics that are undergoing further development. She noted that of the four compounds that initially were most promising, macromolecular synthesis assays quickly showed that two of the compounds were working by nonspecific mechanisms.

NEW WAYS OF LOOKING AT OLD ANTIBIOTICS AND THEIR TARGETS

"There are three, and only three, methods that I can think of that have a chance of reviving the discovery of new natural antibacterial natural products," said Chaitan Khosla. The first, highlighted in the presentations by Silver and Shaw, is the tried and true method of conventional activity-based screening, though today driven by new methodologies. The second involves mining orphan secondary metabolic pathways, and the third engineers known antibiotic pathways to make new molecules. To illustrate these approaches, Khosla discussed how they are being used with a class of antibiotics derived from an assembly-line process involving polyketide synthases (Figure 3-2). The best-known member of this family of antibiotics is erythromycin, but it also includes the 14- to 16-membered macrolide antibiotics, rifamycin, mupirocin, tiacumicin, and the streptogamins. He noted, too, that of all the antibiotic synthetic pathways explored so far, the polyketide assembly line is perhaps the most mature in terms of foundational mechanistic knowledge and the availability of associated technologies for engineering this pathway.

As an example of the first approach, screening against new targets using modern technologies, Khosla discussed the type three secretion system (TTSS) that many gram-negative bacteria use to inject bacterial effector proteins directly into the host cell cytoplasm, bypassing the cell's membrane-bound defense mechanisms. The TTSS has emerged as a potential target only within the past decade, he explained, in part because of the idea that it is not essential for bacterial survival or reproduction and therefore may not be subject to the selective pressures that generate resistance, though he added that this idea has not yet been tested rigorously. When the TTSS was first characterized, medicinal chemists screened large numbers of existing medicinal chemistry libraries with little success until the discovery in 2008 by a group in Japan of a new type of antibiotic that "doesn't resemble anything that we've seen before through traditional screening because they were specifically screening for compounds that block this new target," said Khosla. Working with the group in Japan, Khosla and his colleagues have now characterized the assembly-line process that creates this new class of antibiotics, known as the guadinomines.

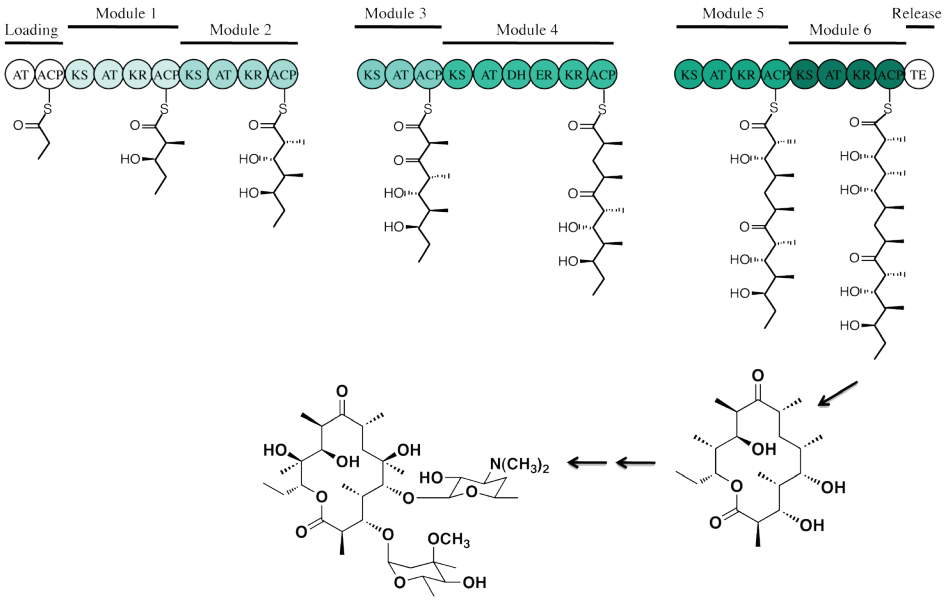

FIGURE 3-2 An example of mining orphan metabolic pathways. Assembly-line polyketide antibiotic biosynthesis.
SOURCE: Chaitan Khosla (2013).

He explained that this is an important advance given the difficulty in producing these molecules synthetically or isolating them from natural sources in quantities sufficient for further development.

Other examples of new classes of molecules discovered by looking at novel targets using modern screening technologies include kibdelomycin, which was identified from soil samples by a team at Merck using the type of antisense-induced sensitivity screen that Shaw discussed. Khosla noted that making this molecule is likely beyond the capabilities of synthetic chemists and that his group is working with Merck to characterize the biosynthetic pathway used to make it and various derivatives. Other approaches include conducting single-molecule biophysical studies of important targets, such as DNA gyrase, to identify key molecular features involved in target binding, and using reconstituted lipid metabolism systems to look for inhibitors of yet another aspect of macromolecule synthesis. "These kinds of approaches can be very resource-efficient ways to discover new species-specific [narrow-spectrum] antibiotics," said Khosla.

Turning to the second method of identifying new antibiotics—mining orphan secondary metabolic pathways—Khosla explained that his group has used whole-genome sequencing and other technologies to identify close to 900 distinct assembly-line polyketide synthases, of which fewer than 20 percent have well-characterized substrates and products. To his knowledge, there is no ongoing large-scale effort to mine this family of polyketide assembly lines despite the fact that more than a dozen commercially important antibiotics come from the 20 percent of assembly lines that are well characterized and that make known molecules. His group has been developing techniques for refactoring these assembly-line pathways in heterologous hosts to produce novel compounds from glucose and propionic acid. In one project using such a system, Khosla's team and collaborators from Kosan Biosciences were able to produce commercial quantities of the anticancer agent epothilone D within three years after this compound's discovery.

Given the success of this and a few other similar projects, Khosla said that industry is interested in this approach but that there are a number of challenges that have to be overcome for it to gain wider use. Assembling the DNA constructs needed to create one of these assembly lines is feasible, but still too costly. "This will cease to be a relevant problem once we get to the stage where we can assemble DNA at less than five cents a base," he said. A number of research groups, including his, are developing promising methods of expressing the very large proteins that make these assembly lines, another challenge facing the field, but more work is needed to identify methods of tailoring enzymes to create novel structures and to address the supply of all but the simplest precursor molecules to feed into these assembly lines. There is also the need for continued development of analytical methods to more rapidly elucidate molecular structures, though using heterologous systems simplifies this problem if one knows the precursors and enzymes involved that limit the structural possibilities for the products. This challenge may become simpler still with the successful development of reconstituted in vitro systems rather than heterologous systems using whole organisms.

Looking to the future, Khosla said that it will one day be possible to engineer these assembly lines to make entirely new molecules, the third approach to antibiotic discovery. His group and others have developed a number of methods of engineering assembly-line polyketide biosynthetic pathways, but more work is needed to truly realize the promise of this approach. The main challenge to address is conceptual rather than technical, he explained. "We do not yet understand fully how these assembly lines work, and we certainly do not yet understand the structural basis for the modularity of these assembly lines," he said. "Until we do, it is premature to predict where this approach will take us."

Over the past 15 years, research in his laboratory has shown that protein–protein interactions through the assembly lines are critical to moving reactive intermediates through the assembly line in a directional manner. His group is now exploring methods of intentionally designing new pathways using protein–protein interaction principles, but he characterized this work as "slow science" that requires a design, build, and test paradigm.

DISCUSSION

One point raised during the discussion period was that when organisms are screened for the purpose of antibiotic discovery, they are often grown under benign conditions rather than in circumstances in which they might turn on orphan pathway or metabolic defense mechanisms and produce novel compounds. Shaw and others in the audience noted that it is becoming more common to add either various stimulatory compounds, such as lipopolysaccharide, or other organisms to the culturing system, but this has yet to be done in a sufficiently organized way to determine if that approach makes a difference in terms of the antibacterial compounds the organisms produce. Regarding this last point, Silver argued that bacteria have been waging war against each other for millions of years and that it is unlikely that experiments of this sort would yield anything that has not been identified already in natural product screens from soil and other bacterial environments.

Khosla noted during the discussion period that some of the products of the polyketide assembly lines are further modified by oxidases, oxygenases, and transferases. In most cases, the biochemistry and genomic locations of these adjunct "tailoring" enzymes have been described in the literature. In general, the genes coding for these enzymes are clustered with the genes coding for the assembly lines.

While tailoring may be necessary to produce a molecule with maximum antibacterial activity, the unmodified molecules typically have enough activity to show up as hits in screening assays. He added, too, that with the new in vitro systems it is possible to examine intermediate compounds that are rarely seen in the natural or heterologous systems for biological activity.

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4

Challenges In Drug Delivery

"In most cases, the most complex part of developing a therapeutic is to design its appropriate delivery system."
J. Rubén Morones-Ramírez

"You can kill any bacterial cell on the planet with any antibiotic on the planet, but the real question is whether you can kill any bacteria on the planet with any antibiotic on the planet without killing the patient."
Mark Smeltzer

In the final session of the workshop, two speakers discussed some additional challenges facing the antibiotics discovery enterprise and possible approaches for addressing those challenges. J. Rubén Morones-Ramírez, Professor of Chemistry at the Universidad Autónoma de Nuevo León, spoke about some of the different tacks that he and his colleagues have taken to design and deliver novel antimicrobial therapies. Mark Smeltzer, Professor of Microbiology and Immunology at the University of Arkansas for Medical Sciences, addressed the issue of biofilms and described methods for getting antibiotics past these physical barriers.

NOVEL APPROACHES TO ANTIMICROBIAL THERAPEUTICS DESIGN

The first approach that J. Rubén Morones-Ramírez discussed in his presentation involved the use of synergistic antibiotic cocktails that include nanoparticulate silver as an active component. Silver has long been known to have broad-spectrum antimicrobial properties, but there was little mechanistic understanding of this property. Using transmission electron microscopy, Morones-Ramírez was able to show that silver nanoparticles bind to the bacterial cell membrane, inducing significant morphological changes. Binding was largely to the sulfur and phosphate groups on the cell membrane glycans and it interfered with the bacterial respiratory system. Further study with nanoparticles of different size showed that only nanoparticles in the 4- to 5-nanometer diameter range interacted strongly with the bacterial membrane, which he pointed out correlates with the fact that the larger surface-to-volume ratio of smaller nanoparticles makes them more active catalytically.

Using energy-dispersive x-ray spectroscopy, Morones-Ramírez and his collaborators were able to identify silver nanoparticles inside the bacteria, demonstrating that they are able to not only interact with the cell membrane, but penetrate it as well. They also found that nanoparticles were able to interact with bacteriophage that happened to be infecting some of the bacteria. This prompted a study looking at the effects of silver nanoparticles on HIV infection that showed that silver nanoparticles were able to bind to the gp120 protein on the HIV capsid (Figure 4-1) and thereby interfere with the entry mechanism that the virus uses to enter cells.

Wishing to pursue the mechanistic aspects of the antimicrobial properties of silver nanoparticles, Morones-Ramírez employed some of the tools of systems biology. At a blood concentration of 30 micromolar, which preliminary studies showed was nontoxic to mice, silver nanoparticles were able to reduce gram-negative bacterial load by about 3,000-fold. Metabolic network analysis using microarray expression data showed that nanoparticulate silver was upregulating iron metabolism, triggering a membrane stress response and disrupting metabolic regulation. Followup gene knockout studies supports the hypothesis that silver nanoparticles disrupt the integrity of the outer and inner membrane of gram-negative bacteria, triggering misregulation in the Krebs (or TCA) cycle and electron transport chain and the breakdown of iron-sulfur clusters, producing an increase in reactive oxygen species that ultimately causes cell death .

From this mechanistic understanding came the prediction that since many antibiotics have at least some effect on the intracellular concentration of reactive oxygen species, it may be possible to potentiate that effect with agents that could shift the intracellular oxidation state in bacteria. In fact, adding silver increased the antimicrobial effect of a wide range of antibiotics and even rendered active antibiotics that were normally ineffective against gram-negative bacteria. In addition, Morones-Ramírez and his collaborators were able to take advantage of the fact that nanoparticulate silver impacts the permeability of the gram-negative bacterial

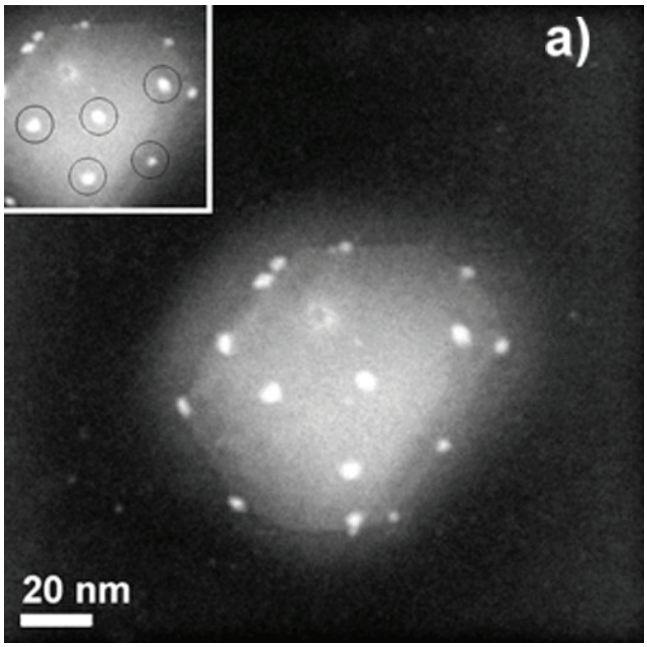

FIGURE 4-1 The interaction of silver nanoparticles (bright white spots) with HIV-1.
SOURCE: Elechiguerra (2005).

membrane system to render *E. coli* susceptible to vancomycin, which normally has no effect on gram-negative bacteria. Subsequent experiments showed that the combination of vancomycin and silver nanoparticles was able to cure mice with gram-negative bacterial peritonitis and gram-negative bacterial urinary tract infections. Additional experiments showed the same effect when persistent gram-negative infections associated with biofilms were treated with silver nanoparticles and gentamicin, ampicillin, or ofloxacin. He and his collaborators are now exploring the effects of other transition metals on antibiotic potency.

Morones-Ramírez then discussed the microbial competition project his group is conducting in an attempt to harness microbial ecosystems as sources of novel antimicrobial agents. One set of experiments in which the co-cultured *E. coli* and *Candida albicans* showed that *E. coli* produces a novel antifungal agent that enables it to have a growth advantage over *Candida*. Though efforts to isolate and identify this antifungal compound have eluded him so far, he believes that the concept of co-culturing microorganisms to look for new antimicrobial compounds is sound and he plans to pursue this idea to search for antibiotics that would be effective against resistant strains.

Noting that the most difficult part of a drug discovery effort often involves solving a drug delivery problem, Morones-Ramírez concluded his talk with a description of the antibiotic delivery system his group is developing using biocompatible microbial exopolymers. When stressed by exposure to toxic metals, some microorganisms respond by producing a protective polymer coating. Working with a group at the University of New Orleans, Morones-Ramírez and his team have used these exopolymers as stabilizers to control silver nanoparticle production and create a polymer-encapsulated nanoparticle assembly that exhibits potent antimicrobial properties. He also briefly described work just beginning in his laboratory on a light-activated[1] polymeric nanoparticle delivery system for some infections.

OVERCOMING ANTIBIOTIC CHALLENGES IN BIOFILM-ASSOCIATED INFECTIONS

In the workshop's final presentation, Mark Smeltzer discussed his group's efforts to develop antibiotics to treat chronic osteomyelitis, a limb-threatening infection associated with biofilm formation that can develop after bone injuries and joint replacement surgery. He explained that he first became interested in this problem when a patient with chronic osteomyelitis who was about to undergo his sixth revision surgery demanded amputation instead. What struck Smeltzer about this patient's infection was that it was caused by a *Staphylococcus aureus* isolate that was only resistant to penicillin and was not MRSA, yet it was still persisting in the face of repeated local treatments with antibiotic-eluting polymer beads packed into the wound after each surgery.

Osteomyelitis is a good example of the broader issue of biofilm formation as a mechanism of antibiotic resistance. There are many additional examples of biofilm-related infections; however, this discussion focuses on the *Staphylococcus aureus* biofilm as an exemplar.

As a bacteriologist who specialized in *Staphylococcus aureus*, Smeltzer was well aware of the ability of this bacterium to form a biofilm, which he characterized as multiple layers of bacteria growing within a glycocalyx, a polysaccharide mixture secreted by the growing bacteria. The effect of the biofilm, he explained, is that it reduces antibiotic delivery to the underlying organisms and creates an intrinsically resistance phenotype without other genetic or biochemical changes, in large part because organisms growing within a biofilm are metabolically inert. To illustrate this point, he discussed the results of an experiment in which a catheter colonized with biofilm-embedded *Staphylococcus aureus* was exposed to vancomycin, linezolid, and daptomycin, at concentrations 5, 10, and 20 times the breakpoint minimum inhibitory concentration of each antibiotic. "After three days of direct exposure, changing the antibiotic every day, we still had not cleared the catheter. That's the problem with a biofilm," he explained (Figure 4-2).

One approach to addressing this problem is to improve local antibiotic delivery. Current practice is to mix daptomycin with poly(methyl methacrylate) (PMMA), also known in

[1] Light-activated polymer delivery systems have been use for topical and subsurface infections, as well as in surgery.

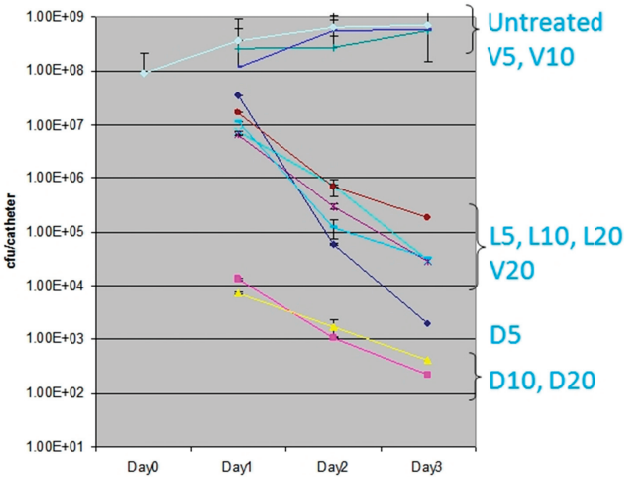

FIGURE 4-2 The intrinsic resistance of *Staphylococcus aureus* biofilms. D, L, and V refer to daptomycin, linezolid, and vancomycin, respectively, at 5, 10, and 20 times the minimum inhibitory concentration.
SOURCE: Weiss (2009a).

the biomedical community as bone cement. Bone cement, as its name implies, is not porous and there is little diffusion of antibiotics out of this material. Nonetheless, orthopedic surgeons routinely load some amount of daptomycin in pellets made from this material in the operating room and implant the pellets into the wound. Smeltzer noted that if the amount of daptomycin that his studies show are needed to be effective, it would require almost $20,000 worth of antibiotic alone. As a solution, his team added the polysaccharide xylitol and daptomycin to PMMA, producing a porous structure. This porous structure released levels of daptomycin that remained 10 times higher than the breakpoint minimum inhibitory concentration for over a week. Tests in animals with osteomyelitis showed that the xylitol plus daptomycin combination was highly effective at eliminating the *Staphylococcus aureus* infection.

Another approach that Smeltzer and collaborator Paul Dunman at the University of Rochester have taken is to develop new antibiotics with a focus on efficacy in biofilm-associated infections. The target of this effort is ribonuclease RnpA, which has low homology to mammalian proteins, is conserved across bacterial pathogens, and is involved in two essential cellular processes. Conventional screening identified a number of hits, and these were then tested against *Staphylococcus aureus* biofilms. Two compounds from this second screen showed significant activity, though these compounds were associated with unacceptable cytotoxicity in a human hepatic cell line assay. Nonetheless, these compounds may serve as the starting point for further medicinal chemistry derivatization.

In the final portion of his presentation, Smeltzer discussed studies aimed at finding targets that would impact biofilm formation, which would eliminate some of the issues with treating biofilm-based infections in the first place. He noted that there is a substantial body of literature on this subject, but much of it is contradictory. His group chose to focus on the transcriptional regulator SarA and was able to show that SarA-negative *Staphylococcus aureus* mutants do not form biofilms. Further study showed that protease production rose substantially in this SarA-negative mutant and that some combination of four extracellular proteases were inhibiting biofilm formation by the mutant.

These findings raise the possibility of treating osteomyelitis with a combination of an antibiotic and a suitable protease, but delivery becomes the problem. "If you can't get vancomycin out of bone cement, you're not going to get a protease out either," explained Smeltzer. He noted that his group plans on exploring various biocompatible and biodegradable polymers, such as chitosan, as delivery vehicles that could be formed into implantable pellets and used to release proteases locally at the site of infection. Alternatively, it may be possible to identify a small molecule that would repress SarA activity and increase expression of protease genes by the bacteria themselves, which in essence is similar to the approach taken to develop inhibitors of β-lactamases to restore antibiotic susceptibility. So far, Smeltzer's group has identified several hits and has shown that one of them has a marked effect on SarA expression and biofilm formation in two different strains of *Staphylococcus aureus* isolated from patients with osteomyelitis.

DISCUSSION

In response to a question about the toxicity of silver nanoparticles, Morones-Ramírez said that silver is toxic to mammals, but at much higher concentrations than for bacteria. He noted that mammalian cells appear to have a mechanism absent from bacteria for eliminating silver at the concentrations that are toxic to bacterial cells.

Smeltzer said in response to a question about biofilms in conditions other than osteomyelitis that there are bead-based delivery options that would be applicable to soft tissue infections, though probably not for the lungs in the case of tuberculosis. He also acknowledged that the SarA inhibitors are likely to be specific for *Staphylococcus aureus* infections, but that most biofilm-forming organisms would have some other molecular Achilles heel that could be attacked. He added that his group is exploring a nanoparticle delivery system that could be irradiated with a laser to also deliver heat to the site of infection, which could amplify the effects of antibiotic therapy.

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5

General Observations

Throughout the workshop, and during a final discussion session, speakers and workshop participants made general observations about the issues associated with antibiotic development to counter resistance. These observations are gathered in this final chapter to capture some of the broad themes emerging from the workshop. These themes should not be seen as consensus conclusions of the workshop and are associated with the individual or individuals who made the observation.

- There is a need for faster methods of isolating potential antibiotics, determining their structure, and characterizing their mechanisms of action. (Aurigemma, Silver, Mobashery)
- There is not enough interaction among the different constituencies that need to be involved in the discovery, development, and use of antibiotics: between clinicians and researchers, between medicinal chemists and biologists, between small companies developing antibiotics and large companies that take them through approval to the market. (Smeltzer, Morones-Ramirez, Silver, Aurigemma, Shaw)
- The mechanisms by which antibiotics enter cells and bypass biophysical barriers need to be better understood. (Silver, Smeltzer)
- The solution to antibiotic resistance lies not just with developing new molecules, but also with better stewardship of current agents as well as improved detection and control to more quickly stop the spread of infectious organisms. Training for medical professionals should be improved. Antibiotics should also be eliminated from animal feed. (Aurigemma)
- There is a need to develop novel screening technologies to identify new targets, with awareness of prior efforts, for drug development. To a large extent, there have been no new targets for antibiotic development discovered since the late 1980s. (Silver, Shaw, Khosla)
- Since the physiochemical properties of antimicrobial agents do not follow the same rules as other classes of pharmaceutical agents, there is a need to develop synthetic and natural product libraries specifically for antibiotic development. (Shaw)
- The search for new antimicrobial agents should focus on molecules that bind to multiple targets or that work in combination with other drugs that target independent microbial processes. (Silver)
- Mining and engineering secondary metabolic pathways can be a productive route to identifying antimicrobial agents with novel molecular structures and mechanisms of action. (Khosla)

Appendix A

Statement of Task

An ad hoc committee will plan and conduct a public workshop in September 2013 in Washington, DC. This one day workshop will explore the current state of antibiotic development, examine the technology available to facilitate development, the technical challenges present, identify approaches to antibiotic discovery, and discuss the incentives and disincentives industry faces in antibiotic development.

The workshop will be held in the context of the need to enable antibiotic development in light of the decreasing availability of new antimicrobial agents.

The committee will develop the workshop agenda, select and invite speakers and other participants, and moderate the discussions. The focus of the workshop will be on three main goals:

1. Identify and examine the state of antibiotic research and development;
2. Discuss the technical difficulties in antibiotic discovery; and
3. Highlight recent successful approaches for the development of novel antimicrobials.

Appendix B

Agenda

Technological Challenges in Antibiotic Discovery and Development

A Workshop by the Chemical Sciences Roundtable

September 23, 2013—Washington, DC

8:00 – 8:15 Introduction to Workshop and Topic

Session 1: Overview
Chair: Carole Bewley, CSR Member

8:15 – 9:15 Resistance is Futile: A View from the AR Battlefield
Rosemary Aurigemma, Section Chief, Drug Development, NIAID, NIH

9:15 – 9:45 Break

Session 2: Challenges in Overcoming Antibiotic Resistance
Chair: Ken Moloy, CSR Member

9:45 – 10:30 Selecting Antibacterial Targets to avoid Resistance Selection
Lynn Silver, Consultant, formerly with Merck

10:30 – 11:15 The Complex Resistance Machineries for β-Lactam Antibiotics in Gram-Negative and Gram-Positive Bacteria
Shahriar Mobashery, Department of Chemistry and Biochemistry, University of Notre Dame

11:15 – 11:45 Discussion with Speakers

11:45 – 12:45 Lunch

Session 3: Challenges in Screening
Chair: John Kozarich, CSR Member

12:45 – 1:30 Challenges in Discovering New Antibiotics Through Screening
Karen Shaw, Sr. Vice President, Biology, Trius Therapeutics

1:30 – 2:15 New Ways of Looking at Old Antibiotics and Their Targets
Chaitan Khosla, Department of Chemical Engineering, Stanford University

2:15 – 2:45 Discussion with Speakers

2:45 – 3:15 Break

Session 4: Challenges in Drug Delivery
Chair: Luis Martinez, CSR Member

3:15 – 4:00 Novel Approaches to Antimicrobial Therapeutic Design: A Story of Cocktails and In Vivo Nanomachines
J. Ruben Morones-Ramírez, Enbiotix and Universidad Autónoma de Nuevo León

4:00 – 4:45 Overcoming Antibiotic Challenges in Biofilm-Associated Infection
Mark Smeltzer, University of Arkansas Medical Center

4:45 – 5:15 Discussion with Speakers

Closing Discussion: The Path Forward
Chair: John Kozarich, CSR Member

5:15 – 6:00 Overall panel discussion with all speakers

6:00 Workshop Adjourns

Appendix C

Biographical Information

ORGANIZING COMMITTEE MEMBERS

Carole A. Bewley, National Institute of Diabetes and Digestive and Kidney Diseases, is a Senior Investigator at the National Institutes of Health, and Chief of the Natural Products Chemistry Section in the Laboratory of Bioorganic Chemistry, NIDDK. She received her Ph.D. in Oceanography and Marine Natural Products Chemistry from Scripps Institution of Oceanography, UCSD, and was a Cancer Research Institute Postdoctoral Fellow in protein NMR. Her current research program focuses on bioactive marine natural products, protein-carbohydrate recognition, and HIV entry. Dr. Bewley has received the National Institutes of Health Director's Award, is an editorial board member of *Current Medicinal Chemistry–Anti-Infectives*, and is a chartered member of Synthetic and Biological Chemistry (CSR/NIH) and Molecular Libraries (NIH Roadmap) study sections. She has been an active member of the American Chemical Society for 15 years, serves on Editorial Advisory Boards and as an expert reviewer for multiple ACS journals, and is a member of the Long Range Planning Committee, Division of MedChem for the ACS.

John W. Kozarich, ActivX Biosciences, Inc., is Chief Executive Officer and President of ActivX Biosciences, Inc. He is also the Chief Scientific Advisor of Kyorin Pharmaceutical Co., Ltd., Adjunct Professor at the Scripps Research Institute, and Chairman of the Board of Ligand Pharmaceuticals, Inc. Dr. Kozarich has over 20 years experience in academic and pharmaceutical research. Most recently, he was Vice President at Merck Research Laboratories, where he was responsible for programs including antimicrobial drug discovery, enzymology, 5a-reductase biology, lipid biochemistry, nuclear receptors, ion channels and structural biology. He has been involved in a number of Merck drug programs, including Propecia, Type-1 5a-reductase inhibitor, and MRSA carbapenams. He also has had primary responsibility for a number of Merck collaborations with biotechnology companies, such as Aurora Biosciences, Cubist, and KaroBio. In addition, he has played a major role in Merck's acquisition of SIBIA and in the development of its new Boston Research Center. Previously, Dr. Kozarich held faculty positions at the University of Maryland, College Park, and Yale University School of Medicine. He also served as Vice President, Research and Development at Alkermes, a biotechnology company that develops products based on sophisticated drug delivery technologies. Dr. Kozarich is internationally known for his work on enzyme mechanisms and on the chemistry of DNA cleaving antitumor drugs. He was an American Cancer Society Faculty Research Awardee and in 1988 received the Pfizer Award in Enzyme Chemistry of the American Chemical Society for his unique and broad research contributions. He has also served on numerous government and academic committees. Dr. Kozarich has authored over 125 primary scientific publications and holds three patents.

Luis E. Martínez, Trinity University, is the Director for Innovation and Entrepreneurship and Adjunct Professor of Chemistry at Trinity University in San Antonio, Texas. Dr. Martínez's research interests include the discovery, development, and application of unique, transition metal-mediated, solid-phase synthetic methods for the high-throughput synthesis of pharmacologically active small molecules and the concurrent assessment of the chemical genetics of the resulting compound libraries in infectious disease, immune response, oxidative stress and cell cycle control. Dr. Martínez's experience spans both academia and business. Prior to his position with UTEP, Dr. Martínez served as a Senior Account Executive with Feinstein-Kean Healthcare, an

Ogilvy PR Worldwide Company. Dr. Martínez has also been involved with scientific workforce diversity and American competitiveness, broadening participation in research and the recruitment and retention university minority faculty and students in science for over a decade. He has been actively involved with SACNAS (Society for the Advancement of Chicanos and Native Americans in Science) and has served as a member of the SACNAS Board of Directors for eight years. In addition to his current service on the SACNAS Board, he also currently sits on the ACS Minority Affairs Committee. Dr. Martínez received his B.S. in Chemistry with honors in 1991 from Trinity University (San Antonio, TX) and his Ph.D. in Organic Chemistry from Harvard University in 1997.

Kenneth G. Moloy, DuPont Central Research and Development, is a Research Fellow at DuPont Central Research and Development. He received a Ph.D. in Inorganic Chemistry from Northwestern University in 1984 and a B.S. in Chemistry from Indiana University in 1980. Following graduate school he joined Union Carbide's Technical Center in South Charleston, WV, working in long range R&D. In 1995 he moved to the DuPont Experimental Station in Wilmington, DE. Dr. Moloy's expertise lies in the areas of organometallic chemistry, catalysis, organic chemistry, and process chemistry. Dr. Moloy has chaired the Gordon Research Conference on Organometallic Chemistry and also the Organometallic Subdivision of the ACS Division of Inorganic Chemistry. Dr. Moloy recently participated on a NAS committee to revise *Prudent Practices in the Laboratory*, which was published in 2011.

SPEAKERS

Rosemarie Aurigemma, Ph.D. is Chief of the Drug Development Section in the Office of Biodefense Research Resources and Translational Research at NIAID where she leads a team of professionals in the management of a portfolio of contracts to support preclinical and advanced development of novel therapies for biothreat agents, public health pathogens and emerging infectious diseases. In this role, Dr. Aurigemma also serves as co-chair of the Public Health Emergency Medical Countermeasure Enterprise (PHEMCE) Biologics Working Group to establish policies and practices for meeting medical countermeasure needs as required by the Assistant Secretary of Preparedness and Response (ASPR, DHHS). Prior to joining NIAID in 2009, Dr. Aurigemma managed a portfolio of grants and contracts within the Developmental Therapeutics Program at the National Cancer Institute's Division of Cancer Treatment and Diagnosis to usher novel cancer therapies from bench to phase II clinical trials. Her earlier experience was in drug discovery and development and clinical development in the biotechnology industry. Dr. Aurigemma holds a Ph.D. in Microbiology from Colorado State University and a B.S. in Biology from Cornell University.

Chaitan Khosla, Professor in the Departments of Chemistry and Chemical Engineering at Stanford University, and Director of the Stanford Institute for Chemical Biology, received his Ph.D. in 1990 at Caltech. After completing postdoctoral studies at the John Innes Centre in the UK, he joined Stanford University in 1992. His research on polyketide synthases has enabled fundamentally new approaches for the engineering of antibiotics. More recently, he has also investigated celiac sprue pathogenesis with the goal of developing therapies for this widespread but overlooked disease. He has co-authored over 300 publications and 70 U.S. patents, and is the recipient of several awards and honors including the Eli Lilly Award in Biological Chemistry and the Pure Chemistry Award from the American Chemical Society; the Allan P. Colburn Award and the Professional Progress Award from the American Institute of Chemical Engineers; and the Alan T. Waterman Award from the National Science Foundation. He is an elected member of the American Academy for Arts and Science and the National Academy of Engineering. Over the past two decades, he has co-founded three biotechnology companies and the non-profit Celiac Sprue Research Foundation.

Shahriar Mobashery is the Navari Family Professor in Life Sciences at the Department of Chemistry and Biochemistry at the University of Notre Dame. He received dual bachelor's degrees in biological sciences and in chemistry from the University of Southern California (1981). and a Ph.D. in chemistry from the University of Chicago (1985). After postdoctoral studies at the Rockefeller University (1986-1988), he joined Wayne State University as an Assistant Professor in 1989, where he was promoted to Professor in 1997. He assumed the Navari Family Chair at the University of Notre Dame in 2003. Professor Mobashery heads a multidisciplinary research lab. His research interests center on machineries for biosynthesis and recycling of the bacterial cell wall, discoveries of novel antibiotics and elucidation of mechanisms of antibiotic resistance. He is also interested in understanding progression of a number of diseases of the extracellular matrix, including stroke, traumatic brain injury, diabetic wound healing, and cancer metastasis, among others. This mechanistic knowledge is used in pharmacological intervention of these diseases.

José Rubén Morones-Ramírez joined the faculty of his alma mater, the Universidad Autónoma de Nuevo León (UANL), in August 2012 as a full time professor in the School of Chemistry for the Chemical Engineering Department. He is a member (candidate level) of the National Mexican Science and Technology Counsel and currently coordinates the UANL new university-wide program in

Systems and Synthetic Biology, where he has founded the NanoBiotechnology Research Group and where he is the Principal Investigator. He earned his B.S. in Chemical Engineering from the Universidad Autónoma de Nuevo León in Mexico and obtained his Ph.D. in Chemical Engineering from the University of Texas at Austin. Dr. Morones-Ramírez completed a 4 year post-doc with Prof. James Collins at the Howard Hughes Medical Institute, Boston University and the Wyss Institute at Harvard University. Dr. Morones-Ramírez's scientific interests involve doing translational research inspired in the fields of Nanotechnology and Systems and Synthetic Biology to advance the development and design of therapeutics, materials, alternative and clean energy, and contribute to increase the world's food and water supplies. In his scientific career he has published 13 scientific peer reviewed research articles with a combined total of more than 2,000 scientific citations; he has produced two patents and has been involved in the foundation of different startup biotechnology companies such as Chrysalis (focused on the development of Bioplastics), Enbiotix (focused on the development of novel ways to potentiate antibiotics) and Biopristine (focused on the synthesis of antimicrobial textiles using silver nanoparticles). He has been awarded the Bruce and Sharon Thornton Commercial Potential Award and the Malcolm Milburn Endowed Award for Entrepreneurs. Through the founding of Biopristine, he won first place and seed money at the both the Austin and the Global Idea to Product Competition. He is passionate about sharing his excitement for science (particularly the field of nanobiotechnology and synthetic biology) in Latin America and has accomplished this through different science articles written in Latin American journals and local university newspapers about the current international status of the fields. In 2010, as recognition of his labor, he obtained the 2nd place as best scientific media journalist by AgroBioMexico. Dr. Morones-Ramírez is also the lead faculty advisor of the undergraduate student teams NanoUANL, which will be participating in the BioMod 2013 Competition at the Wyss Institute at Harvard, and Team UANL, which will be participating in the prestigious iGEM competition in 2013. He was a member of the organizing committee of the 2012 and 2013 International Green Engineering and Chemistry Meeting, in Monterrey, Mexico. He is also the lead faculty organizer of the first International meeting on Genomic Biotechnology 2013 (AseBioGen 2013) held in Monterrey, Mexico, October 2013. He is a member of the Materials Research Society, the ASM, the AIChE, the ACS, the BMES and the IMIQ.

Karen Joy Shaw established the Microbiology Department at Trius Therapeutics (which was purchased by Cubist Pharmaceuticals in July 2013) and developed strategies for differentiating Tedizolid (a novel oxazolidinone antibiotic which has completed Phase 3 clinical trials) from competitors through resistance studies. In addition, a successful DNA gyrase/topoisomerase IV structure based drug design program led to the creation of a novel class of broad spectrum antimicrobial agents. Her leadership of microbiological, enzymological, and mechanism-of-action studies was instrumental in ensuring appropriate SAR assumptions. Prior to joining Trius, Dr. Shaw was Team Leader, Infectious diseases at Johnson & Johnson Pharmaceutical Research & Development (1999-2005) where she developed bacterial microarray technology for *E. coli* and *S. aureus.* She and her team utilized this technology to determine antibacterial mechanism of action and analyze bacterial pathogenesis. In addition, she implemented several anti-infective projects and identified a viable lead series with in vivo efficacy and a novel mechanism-of-action. As a research fellow at Schering-Plough Research Institute (1984-1999) she initiated the use of genomic approaches for the discovery of novel antibacterial and antifungal agents. Dr. Shaw holds a B.S. in Biology from Brooklyn College, a Ph.D. in genetics from the University of Connecticut, and completed her postdoctoral fellowship at Washington University School of Medicine. Dr. Shaw's research interests are the discovery and development of novel antibacterial agents in addition to the epidemiology of bacterial resistance mechanisms.

Lynn L. Silver is currently an independent consultant at LL Silver Consulting, LLC, advising industry and academic clients in the area of antibacterial discovery and early development. Previously, at Merck Research laboratories, from 1982 to 2003, she conducted research and supervised groups involved in discovery efforts for new antibacterials in both natural products and chemical collections, support of chemical synthetic projects on improved antibacterials, pre-clinical evaluation of antibacterial drug candidates and the study of antibacterial resistance. Her expertise includes broad knowledge of antibacterial agents, screen design and execution, microbiological evaluation of hits and leads, and studies of mechanism of action and resistance. She was involved in the discovery of the first inhibitors of LpxC, the natural product inhibitor of FabF, platensimycin, and the MRSA carbapenems. She was a member of several project teams coordinating the advancement of drugs through the regulatory process, including INVANZ®.

Dr. Silver received her doctorate at Tufts University in Molecular Biology and Microbiology in 1974 and did postdoctoral work on bacterial DNA replication at the Université de Genève, and on DNA replication biochemistry of bacteriophage T4 at NIH. Throughout her career, she has authored significant research papers and reviews in the field of bacterial genetics, physiology, and biochemistry, as well as discovery and analysis of antibacterial agents, targets, and resistance. She is a member of the Editorial Board of *Antimicrobial Agents and Chemotherapy* (1997-2014), an ASM Branch Lecturer (2007-2009), a member of Scientific Advisory Boards of a number of biotechnology companies,

a standing member of the NIH DDR study section, and has spoken at and chaired numerous meetings focused on antibacterial discovery.

Mark Stephen Smeltzer, Ph.D., is a native Arkansan who was born in El Dorado and grew up in nearby Norphlet. His family moved to Halstead, Kansas in 1996 when Dr. Smeltzer was in the 4th grade. He subsequently obtained his undergraduate degree in biology and chemistry from Washburn University in Topeka before accepting a research technician position at the Kansas State University College of Veterinary Medicine. This led to an MS degree and ultimately to a Ph.D. in 1990. He completed a postdoctoral fellowship with Dr. John J. Iandolo, a KSU Distinguished Professor and well known researcher studying *Staphylococcus aureus* infection. He then joined the faculty at the University of Arkansas for Medical Sciences (UAMS) in 1993, largely because of the recruiting efforts of Dr. Carl Nelson, who at the time was chair of the Department of Orthopaedic Surgery. Dr. Nelson's clinical specialty was hip and knee replacement surgery, and he had a strong interest in overcoming the complication of infection in these procedures, the primary cause of which is *S. aureus*.

Dr. Smeltzer is currently a Professor in the Department of Microbiology and Immunology and in the Department of Orthopaedic Surgery. He has been a microbial pathogenesis investigator for more than 25 years and has maintained an interest in orthopaedic infections caused by *S. aureus*. Together with colleagues at UAMS and around the country, he has taken a broad approach to this work that includes efforts to improve methods for early diagnosis, optimize methods for local antibiotic delivery in the treatment of infection, and define the mechanistic basis for *S. aureus* biofilm formation and bone destruction. He has also begun to explore novel approaches to the detection and treatment of these infections including the possibility of using antibody-directed nanotechnology as a means of eradicating the offending bacteria irrespective of their metabolic or even antibiotic resistance status.

Dr. Smeltzer's laboratory has been continuously funded by the National Institutes of Health and other granting agencies since 1996, and he is currently principal investigator on two R01 grants, one R56, and two grants from the Congressionally Directed Medical Research Program. He has been the recipient of numerous research awards, including the New Investigator Award from the Orthopaedic Research Society, the Randall Award as the Outstanding Young Investigator from the South Central Branch of the American Society for Microbiology, and election as an ASM Distinguished Lecturer. He has received numerous teaching awards including the Red Sash, Gold Sash, and Golden Apple. Dr. Smeltzer also currently directs the Center for Microbial Pathogenesis and Host Inflammatory Responses (CMPHIR), where he helps junior investigators develop their careers as independent scientists, integrate their efforts with other investigators on campus in a clinically-relevant and synergistic manner, facilitate interactions with mentoring faculty and Center leadership, and remove to the greatest extent possible any administrative and technical barriers to their success.

Appendix D

Workshop Attendees

Chemical Sciences Roundtable Members

William F. Carroll, Jr., Occidental Chemical Corporation
Jennifer S. Curtis, University of Florida
Michael R. Berman, Air Force Office of Scientific Research
Carole Bewley, National Institute of Diabetes and Digestive and Kidney Diseases
Donna G. Blackmond, Scripps Research Institute
Richard R. Cavanagh, National Institute of Standards and Technology
Miguel Garcia-Garibay, University of California, Los Angeles
Jacquelyn Gervay-Hague, National Science Foundation
John Kozarich, ActivX Biosciences, Inc.
Luis E. Martínez, Trinity University
Kenneth G. Moloy, DuPont Company Experimental Station
Michael E. Rogers, National Institute of General Medical Sciences

Speakers

Rosemarie Aurigemma, NIAID/NIH
Chaitan Khosla, Stanford University
Shahriar Mobashery, University of Notre Dame
José R. Morones-Ramírez, Universidad Autónoma de Nuevo León, Nuevo León, Mexico
Karen Shaw, Trius Therapeutics
Lynn Silver, LL Silver Consulting, LLC
Mark Smeltzer, University of Arkansas for Medical Sciences

Participants

Heather Alger, The Pew Charitable Trusts
Joseph Alper, Life Science and Nanotechnology Consulting
Oleg Barski, NIGMS/NIH
Helena Boshoff, NIH
Edward Cox, FDA CDER
Miles Fabian, NIGMS/NIH
Barbara Gerratana, NIGMS/NIH
Shannon Greene, American Society for Microbiology

Kirk Gustafson, National Cancer Institute
Flora Katz, Fogarty International Center, NIH
Eric Kuenstner, NIH
Joe Larsen, HHS/BARDA
Su-Lin Lee, Postdoc
Nicole Mahoney, The Pew Charitable Trusts
Pamela Marino, NIGMS/NIH
Belhu Metaferia
Marguerite J. Miller, NIDDK/NIH
Bryan Mott, NCATS/NIH
Julia Oh, NIH
Joshua Rosenthal
Sara Ruiz, USAMRIID
Marian Wachtel, NIAID/NIH
Xiaoning Wang, LBC/NIDDK/NIH
Dan Xi, NCI/NIH

NRC Staff

Douglas Friedman, Program Officer, Board on Chemical Sciences and Technology
India Hook-Barnard, Senior Program Officer, Board on Life Sciences
Kathryn Hughes, Senior Program Officer, Board on Chemical Sciences and Technology
Elizabeth Finkelman, Administrative Assistant, Board on Chemical Sciences and Technology