TRANSLATIONAL SYSTEMS BIOLOGY

CONCEPTS AND PRACTICE FOR THE FUTURE OF BIOMEDICAL RESEARCH

Yoram Vodovotz & Gary An

TRANSLATIONAL SYSTEMS BIOLOGY

CONCEPTS AND PRACTICE FOR THE FUTURE OF BIOMEDICAL RESEARCH

Yoram Vodovotz
Department of Surgery and McGowan Institute for Regenerative Medicine, University of Pittsburgh, Pittsburgh, PA, USA

Gary An
Department of Surgery, University of Chicago, Chicago, IL, USA

ELSEVIER

AMSTERDAM • BOSTON • HEIDELBERG • LONDON • NEW YORK • OXFORD
PARIS • SAN DIEGO • SAN FRANCISCO • SINGAPORE • SYDNEY • TOKYO
Academic Press is an imprint of Elsevier

Academic Press is an imprint of Elsevier
32 Jamestown Road, London NW1 7BY, UK
525 B Street, Suite 1800, San Diego, CA 92101-4495, USA
225 Wyman Street, Waltham, MA 02451, USA
The Boulevard, Langford Lane, Kidlington, Oxford OX5 1GB, UK

Notices
Knowledge and best practice in this field are constantly changing. As new research and experience broaden our understanding, changes in research methods, professional practices, or medical treatment may become necessary.

Practitioners and researchers must always rely on their own experience and knowledge in evaluating and using any information, methods, compounds, or experiments described herein. In using such information or methods they should be mindful of their own safety and the safety of others, including parties for whom they have a professional responsibility.

To the fullest extent of the law, neither the Publisher nor the authors, contributors, or editors, assume any liability for any injury and/or damage to persons or property as a matter of products liability, negligence or otherwise, or from any use or operation of any methods, products, instructions, or ideas contained in the material herein.

ISBN: 978-0-12-397884-4

British Library Cataloguing-in-Publication Data
A catalogue record for this book is available from the British Library

Library of Congress Cataloging-in-Publication Data
A catalog record for this book is available from the Library of Congress

Typeset by MPS Limited, Chennai, India
www.adi-mps.com

Printed and bound in the United States of America

Contents

This page intentionally left blank

Preface

The more pity that fools may not speak wisely what wise men do foolishly. ***Touchstone, As You Like It, Act 1, Scene 2, Line 85***

Truth's a dog must to kennel; he must be whipp'd out… ***The Fool, King Lear, Act 1, Scene 4, Lines 109–110***

Foolery, sir, does walk about the orb, like the sun; it shines everywhere. ***Feste, Twelfth Night, Act 3, Scene 1, Lines 37–38***

The fool doth think he is wise, but the wise man knows himself to be a fool. ***Touchstone, As You Like It, Act 5, Scene 1, Lines 31–32***

Some of Shakespeare's most memorable characters are Fools. Whether in Comedy or Tragedy, the Fools in Shakespeare's plays serve an important role that mirrored their historical position. Whether by intellect, simplicity, or station, they embody the "other" in Shakespeare's worlds, standing outside the primary context of the play and thereby serving to provide an "honest" commentary on the proceedings, stripped of the social niceties and motivations that temper the comments of the other characters. Their position as Fools to a great degree protects them from the consequences of their statements and actions. In the historical (?) eras of Absolutism, Fools often provided the only means of vocalizing critiques of rulers and their societies; many scholars interpret their role as a vital social mechanism for funneling and focusing dissent in a nondisruptive and nonthreatening way. A Fool might make the comments regarding a Royal personage's peccadillos that are on the forefront of the court members' minds but about which are unable to note or speak of for fear of repercussion; however, spouting forth from the mouth of the Fool these exact same thoughts can safely be greeted with laughter (albeit a bit ostentatiously nervous and not too long or loud…).

Fools are intrinsically paradoxical. They are able to express the most cutting of critiques, but in so doing reinforce the stability of the societies they critique. They are outsiders, but often with the most intimate of relationships with their masters (i.e., Lear and his Fool, Viola and Feste, Rosalind and Touchstone). This paradoxical duality is even manifest in the most recognizable guise of the Fool: the harlequin costume of opposing and alternating color blocks. In Shakespeare's plays, his most notable Fools, such as Touchstone, Feste, and Lear's Fool, are often seen as mouthpieces for the plays' author. While this interpretation remains the subject of many an English Major's thesis and open to scholarly debate, there is something intrinsically consistent with the paradoxical nature of Fools in the belief that the author, who cannot be more intimately related to the piece he is writing, places his own truest words in the mouth of the plays' representative outsider. Regardless of whether they represent Shakespeare's opinions and beliefs, certainly these three Fools appear to possess a perspective more encompassing than the other characters in the plays they inhabit. Touchstone is the voice of logical reasoning and objective assessment among the refugees in the Forest of Arden; Lear's Fool provides the most comprehensive view and assessment of the madness and chaos following Lear's fateful decision; and Feste appears at sometimes an omniscient observer regarding the machinations taking place in the Duchy of Illyria.

Perhaps, the lesson to be taken from Shakespeare's Fools is that possession of the wider perspective, a grasping of the big picture, is what provides the most uncomfortable and disruptive conclusions, which, for the sake of the society, can only be spoken by Fools. It is truly ironic, then, that at the end of each of the plays noted above, there has been a dramatic disruption and reshuffling of the conditions at their respective beginnings, those same conditions that required the placement of disruptive comments in a Fool's mouth in an attempt to forestall the paradigmatic shifts that eventually would come to pass. Perhaps, then, the Fool is not such a fool after all.

We contend that the biomedical research community could potentially benefit from listening to a pair of Fools. Today, in biomedical research, paradoxes abound. We know more about the generative processes and mechanisms of disease than at any other point in history. The rate of this data acquisition shows no signs of slowing down, yet the introduction of new and more effective therapeutics for the diseases that most trouble us has never been less efficient. Many of those diseases that most trouble us—cancer, sepsis, obesity, autoimmune disorders—represent the hijacking of otherwise beneficial biological processes: cancer of growth and healing, sepsis of inflammation, obesity

of metabolism, and autoimmune disorders of immune protection. Even those therapeutics that have been life saving in the past (and still are in the present) have been revealed as producing a whole new set of problems that, in many ways, arise because of their initial success: antibiotics leading to increasingly lethal resistance, life support measures in the intensive care unit leading to the purgatory of chronic organ insufficiency and recurrent infections. Inside the biomedical community (for legitimate reasons we will discuss in this book), it is too easy to look only toward the patch of blue sky representing future technological and intellectual achievement. Yet, if one looks more widely (and not even much more widely, as the examples above can attest to) there are signs of the storm clouds building. This book is intended to help us turn around and look.

Contrary to genius, which is born, Fools might be made, through the correct and fortuitous mix of temperament, training, timing, and terrain. Two decades ago, the intellectual terrain of critical illness was ripe for disruption: clinical trials for the treatment of sepsis that, by all ostensible criteria for success, should have worked, did not. There was the beginning of an existential crisis in the biomedical research community that persists to this day in the Translational Dilemma. The traditional community would quickly retrench and retool, but for those with the right level of training (i.e., not too deeply embedded in the traditional academic paths) and the appropriate temperament (i.e., curious, contrarian, and stubborn about it), this was an opportune time to learn to become a Fool. About 15 years ago, working separately and initially without knowledge of each other's work, two very different people with very different backgrounds and for very different reasons began to question the state of biomedicine in the context of critical illness. Gary, a trauma surgeon and intensive care physician, saw a clinical dilemma, and being outside the academic research structure, was unburdened by knowledge and possessing of procedural naiveté. As such, he started on his own, on the fringe, with the naïve concept that if he, as someone without formal research training, could apply these new techniques to the challenges facing the translation of basic research knowledge, then surely just that demonstration would be enough to lead to adoption (after all, what could possibly go wrong!). Yoram, a researcher, a biochemist and immunologist, had experienced the academic biomedical research community at near its highest level, but with enough insight and perspective to recognize both its best and worst characteristics. He, too, pursued an unorthodox path, forming part of the leadership of an interdisciplinary team before that description had become *de riguer*, and enlisting the potential of industry by helping to start a biosimulation company. This odd couple had absolutely no reason to ever meet, to find common ground, to have compatible personalities, much less to collaborate. But, in 2002, they, as they say, "met cute" by total coincidence at lunch at the 25th Annual Meeting of the Shock Society. Conversation ensued, connections and common interests were identified, and a collaboration was born. Time and terrain contributed to foster the conditions that would enhance this collaboration, as at that 2002 meeting, Gary and Yoram were invited, along with their collaborators, to a workshop in Germany on complex systems approaches to critical illness. This led to the formation of the Society for Complex Acute Illness, which has and continues to serve as the focal point for the most dynamic discussions and developments in this still-growing field.

The simple fact that pursuing this line of investigation required the formation of a new scientific society points to the "outsider" nature of the endeavor. Yet while they remained outsiders, they kept hearing from many people that they were on the right track (perhaps the surreptitious support of the members of court that have an inkling that these Fools spoke some truth?). Self and system analysis ensued, and the germ of an idea grew out many late night discussions: what was it that they were doing that resonated with individual people but was distrusted, misunderstood, or ignored by so many others primarily at an organizational level? Were the problems, deficiencies, successes, and failures they observed specific to critical illness or a more general phenomenon?

Translational Systems Biology was the product of these discussions, disseminated since through multiple manuscripts on the topic, but facing a fair amount of resistance and indifference (after all, how dare the Fools try to be members of the court!). Hence the paradox of being the Fool, able to speak certain truths, but only given the freedom to do so because their voices serve to preserve the *status quo*. But, as seen in King Lear, Twelfth Night, and As You Like It, change will come. When the offer to write this book came along, we viewed this as perhaps a qualitative shift in how our message could be delivered, and hence, how it would be received. Few Fools are given this opportunity, to potentially have their words actually have real and tangible meaning. When exercising that opportunity, we realized that there needed to be a balance between tempering the traditional level of agitation that is the domain of Fools, but retaining the disruptive quality of the Foolery that first brought it attention. As such, readers should consider themselves warned that they may be rattled, that our chosen tone might bite, and that the images cast by the mirror provided might not be ones they would prefer to see. If, at some point, the reader might feel too accused, they should perhaps take at least some comfort in that, as all Fools are, the authors are part of the same system they are critiquing, and their reflections are present in that same mirror.

No one would subject themselves to that degree of uncomplimentary self-reflection unless it was for a hope that somehow doing so would make things better. It is all too easy to critique without providing an alternative solution.

We believe that we have avoided this trap: this is why we present Translational Systems Biology as a potential pathway to address what we interpret as the roadblocks and barriers to enhancing human health for the remainder of the twenty-first century and hopefully beyond. Consistent with our strategy of founding things on the fundamental principles of Science, we do not claim that this is a unique or even necessarily effective solution. Rather, we claim, that given a transparent analysis of the preponderance of the evidence, a problem with the structure and operations of the biomedical research community has been identified and we have proposed a *plausible* solution to that problem. If the result of this book is the establishment of new, competing views of how we as a research community can move forward, and these and our strategies are given the opportunity to be given the test of real-world implementation, then we can consider our endeavor a success. This, after all, is how Science progresses.

This book includes an *Acknowledgments* section, but we would like to take the last space of our *Preface* to give special thanks to those who most suffered the company of Fools, our families. Gary wishes to thank his lovely wife Melanie, who always wondered where this time consuming, semiprofessional hobby would lead, and his daughter Madeline, who may be cursed with too many similarities to her father; and Yoram wishes to thank his better half, Xing, who stood by his side and provided grounding, and his daughter Lena and his son Ethan, who grew up surrounded by the good and bad of Science.

This page intentionally left blank

Acknowledgments

The authors would like to acknowledge the contributions to this work of a large number of investigators, students, and postdoctoral fellows, with whom they have shared a career in science and without which this book could not have been written: Andrew Abboud, Khalid Almahmoud, John Alverdy, Derek Angus, Julia Arciero, Nabil Azhar, Steve Badylak, Derek Barclay, Arie Baratt, John Bartels, Binnie Betten, Bibiana Bielekova, Timothy R. Billiar, David Brienza, David Brown, Cliff Brubaker, Tim Buchman, Marius Buliga, Frederick D. Busche, David Carney, Steve Chang, Carson Chow, Scott Christley, Gilles Clermont, R. Chase Cockrell, Greg Constantine, Marie Csete, Judy Day, Edwin Dietch, Russell Delude, John Doyle, Joyeeta Dutta-Moscato, Bard Ermentrout, James Faeder, Jie Fan, Rena Feinman, Ira Fox, Ali Ghuma, Mitchell P. Fink, David Hackam, Pat Hebda, C. Anthony Hunt, Jelena Janjic, John Kellum, Moses Kim, Christine Kretz, Shilpa Krishnan, Swati Kulkarni, Rukmini Kumar, Claudio Lagoa, Ryan M. Levy, Nicole Li, Shirley Luckhart, Othman Malak, Qi Mi, Maxim Mikheev, John Murphy, Rajaie Namas, Rami Namas, Carl Nathan, Eddy Neugebauer, Gary Nieman, Juan Ochoa, David Okonkwo, Patricio Polanco, John Pollock, Ian Price, Jose M. Prince, Juan Carlos Puyana, Heinz Redl, Angela Reynolds, Beatrice Riviere, Glen Ropella, Matthew Rosengart, Jonathan Rubin, Alan Russell, David Sadowsky, Joydeep Sarkar, John Seal, Jason Sperry, Michael Sporn, Alexey Solovyev, Robert Squires, David L. Steed, Jordan Stern, Joshua Sullivan, David Swigon, Shlomo Ta'asan, Andres Torres, Jeffrey Upperman, Katherine Verdolini, Bill Wagner, Matt Wolf, Jinling Yin, Ivan Yotov, Akram Zaaqoq, Ruben Zamora, Cordelia Ziraldo, and Sven Zenker. The authors would also like to thank all of the students that participated over the years in the University of Pittsburgh's Systems Approach to Inflammation graduate course. Much of the inspiration we have received for our work over the years, and the inspiration and feedback we have gotten for the concepts embodied in this book, came from great friends who are not scientists: Michelangelo Celli, Renee Colbert, Ted Christie, Mike Farrell, Michael Greenberg, Ariel Kuperminc, Michael Martin, Meipo Fun-Martin, Scott Osterrieder, Barry Strubel, Clyde Takeguchi, and Nancy Wolper. The authors are especially grateful for the support for their work from the National Institutes of Health, the Department of Defense, the National Institute on Disability and Rehabilitation Research, the Commonwealth of Pennsylvania, the Pittsburgh Lifesciences Greenhouse, the Pittsburgh Tissue Engineering Initiative, and IBM, Inc.

This page intentionally left blank

SECTION I

INTRODUCTION AND OVERVIEW

This page intentionally left blank

CHAPTER

1.1

Interesting Times: The Translational Dilemma and the Need for Translational Systems Biology of Inflammation

It was the best of times, it was the worst of times, it was the age of wisdom, it was the age of foolishness, it was the epoch of belief, it was the epoch of incredulity, it was the season of Light, it was the season of Darkness, it was the spring of hope, it was the winter of despair… ***A Tale of Two Cities, Charles Dickens***

Consider the following scenarios:

You are driving home from a party and a drunk driver runs a red light, striking your car from the side, crushing the door and trapping you inside. The paramedics and firemen arrive quickly and cut you out, but you have lost a lot of blood and are in shock. They get you to the hospital, where you are found to have a broken leg, a broken pelvis, a collapsed and bruised lung, and are bleeding internally. You get a series of operations to stop the bleeding and fix the fractures *but end up in the Intensive Care Unit on a ventilator because your lungs were too badly damaged…*

You are recovering from a cycle of chemotherapy for your breast cancer, and your doctors are saying that you appear to be responding well, but a few days afterward you start having fevers and feeling very poorly. You call 911, and by the time the paramedics can get you to the hospital you have a very low blood pressure and are having trouble breathing. The Emergency Room doctors diagnose you with bacterial sepsis, because you are immunosuppressed from your chemotherapy. *They start intravenous fluids and antibiotics and transfer you to the Intensive Care Unit…*

It is flu season, and despite being careful you have come down with a bad cough. You stay home, drink fluids and have soup, but after about 4 days you start coughing up greenish-yellow phlegm and are sweating at night and have chills. Your family brings you to the hospital, where they say you have a pneumonia. You are admitted and placed on antibiotics, but over the next day your breathing becomes more difficult and your blood pressure starts to drop. *They transfer you to the Intensive Care Unit and tell you that then need to put you on a ventilator…*

You have been shoveling snow and have developed really bad chest pain. You call 911, and the paramedics take you to the emergency room where they diagnose you with a heart attack from occlusions in your heart's arteries. Based on where the blockage is, you need to have emergency heart bypass surgery. The operation goes fine, but afterward your kidneys no longer work so well and the wound on your leg where they took the vein graft is looking a bit red and maybe infected. After about 5 days you are having more trouble breathing and your *doctors say you need dialysis and transfer you back to the Intensive Care Unit…*

You skin your knee playing basketball at school. At first, everything seems fine, but after a couple of days you notice that it is getting more red and swollen. The redness starts creeping up your leg, you start having fevers and chills, and you feel dizzy and lightheaded. You go to the doctor, who diagnoses you with an infection of flesh-eating bacteria. She tells you that you need to be admitted to the hospital immediately. *By the time you get there, you are in shock from the infection, will need emergency surgery to fillet open your leg to get rid of the infected tissue and should expect to spend a considerable amount of time in the Intensive Care Unit…*

You are recovering from your broken hip, but because of the pain you have not been getting out of bed much. Your cough is worsening over the past few days, and despite trying to cough out the phlegm you are having more trouble breathing. You start to get some fevers, and the doctors diagnose you with a pneumonia and start you on

antibiotics. However, despite this treatment, a few days later your breathing gets so difficult *that they need to put you on a ventilator and transfer you to the Intensive Care Unit…*

Most of us do not spend much time thinking about acute inflammation or critical illness. Maybe we should. Regardless of the disease that scares you most, or of what statistics say people die from, in this day and age the final common pathway is nearly invariant: an encounter with the health-care system, and if you are sick enough, care in an Intensive Care Unit where you will be the beneficiary of the best life-saving technology that can be provided (at least as long as you live in a developed country). The disease process that puts you there, however, nearly always stems from the same source regardless of what started it: your body's inflammatory response to some initial insult that, if it becomes disordered, can lead to the rapid, progressive failure of multiple organs. Depending on myriad factors, you could either find yourself spending a long time in the hospital, after which time you may need further convalescence. Or you could die.

This is something of an ugly little secret, swept under the rug, lost in the shuffle as doctors and biomedical researchers focus on the individual diseases that drive toward this final common pathway. So why has a highly developed society like ours not yet solved the puzzle of critical illness? *We lay the blame squarely on the current state of biomedical research.*

Biomedical research today lives in a world that, in many ways, is strikingly similar to that of the French Revolution as described by Charles Dickens. It is a time of incredible promise, resulting from unprecedented advances in technology that has led to a previously inconceivable degree of characterization of biological systems. The window into the essential components and machinery of life has never been so wide and the resulting view so sharply defined. However, as this embarrassment of riches carries with it a wealth of expectations, so too is there a corresponding chasm of disappointment when those expectations are not met. How can this increased ability to peer into the workings of biological systems be translated into actionable knowledge that can be used to aid mankind? This is the *Translational Dilemma* that faces biomedical research: the ability to effectively translate basic mechanistic knowledge into clinically effective therapeutics, most apparent in attempts to understand and modulate "systems" processes/disorders, such as sepsis, cancer, and wound healing. Unfortunately, the Translational Dilemma appears to be cropping up more and more often, as, paradoxically, a greater understanding of the processes that lead to the transition from health are known, the more intractable trying to manipulate those processes seems to become. Thus, the current situation calls for a reassessment of the scientific process as an initial step toward identifying where and how the process can be augmented by technology. The US Food and Drug Administration report: "Innovation or Stagnation: Challenge and Opportunity on the Critical Path to New Medical Products" [1], clearly delineates the steadily increasing expenditure on Research and Development concurrent with a progressive decrease in delivery of medical products to market. In many ways, the biomedical community can be viewed as standing on a ledge in a canyon, able to see the upper rim above but faced with no path in that direction, and at the same time fearing the depths that lie below.

Nowhere is the Translational Dilemma more apparent than in the reductionist approaches to understanding and manipulating the acute inflammatory. Just for context, the developing world is a morass of acute and chronic infections, traumatic injuries due to lack of civilian safety infrastructure as well as military conflict, and nonhealing wounds due to multiple factors that include malnutrition and other man-made and natural causes [2,3]. We are perhaps more familiar on a daily basis with inflammation in the industrialized world. We have our share of infections, trauma, and wounds. In our case, these diseases are complicated by our profligate lifestyles, which lead to diabetes, and obesity. We live longer, but our lives are in some ways less healthy than ever before, and our long lives are fraught with aging-related diseases such as cancer, arthritis, and neurodegenerative diseases [4]. Our better (and more expensive) hospital system as compared to that of the developing world also means that many patients will spend at least some time in an intensive care unit due to organ failure that is related to, and likely at least in part driven by, maladaptive, whole-body inflammation.

In our own work, we have focused on one key aspect of inflammatory disease, namely acute inflammation following critical illness such as sepsis, trauma, and wound healing. So, we must first set briefly the stage with regard to what these diseases entail. Critical illness can result directly from trauma, hemorrhagic shock, and bacterial infection (sepsis). On its own, trauma/hemorrhage is a leading cause of death worldwide, often leading to inflammation-related late complications that include sepsis and multiple organ dysfunction syndrome/multiple organ failure (MODS/MOF) [5–7]. Sepsis alone is responsible for more than 215,000 deaths in the United States per year and an annual health-care cost of over $16 billion [8], while trauma/hemorrhage is the most common cause of death for young people in the United States, costing over $400 billion annually [9–11].

Acute inflammation plays a direct and driving role in the pathophysiology of these conditions, producing hyperinflammation initially, and the immunoparalysis at later phases. At a basic level of understanding, there have been

numerous advances in defining novel molecules, signaling and synthetic pathways, and gene regulatory networks contributing to inflammation. However, these advances were produced and remain in scientific silos that were unable to connect and integrate their accumulated knowledge, and therefore missed an essential, systems-level understanding of the inflammatory response. An unfortunate consequence of this fractured community is reflected in the dearth of available therapeutics for these deadly and costly diseases; as of the writing of this book, there is not a single approved therapeutic targeting any component of the inflammatory pathway for these diseases. This fragmentation is further reinforced by popular notions of inflammation, where it is invariably cast as a negative thing to be overcome. There is a poor recognition of the many individual-specific manifestations of inflammation, and a lack of understanding about the favorable and important roles that inflammation plays in our minute-to-minute adaptive responses to stress, injury, and infection. In short, inflammation and related phenomena are part of a complex biological/physiological/sociological system that has, to date, generally defied a unifying understanding.

It is now beyond doubt that inflammation, with its multiple manifestations at the molecular, cellular, tissue, organ, and whole-organism levels, drives outcomes, both positive and negative, following injury and infection, and can lead to diverse manifestations of chronic diseases such as rheumatoid arthritis, neurodegenerative diseases, the metabolic syndrome, and cancer. It is very important to mention the fact that inflammation is not in and of itself detrimental. Well-regulated, self-resolving inflammation is necessary for the appropriate communication and resolution of infection and trauma, and for maintenance of proper physiology and homeostasis. Though properly regulated inflammation allows for timely recognition and effective reaction to injury or infection, disorders of acute inflammation accompany trauma/hemorrhage, sepsis, the wound healing response, and many chronic degenerative processes. In these settings, inflammation of insufficient, disordered, or overabundant, and this mismatch between the underlying reason for initiating inflammation and the way that inflammation progresses can impair normal physiological functions. This paradox of a robust, evolutionarily conserved network of inflammation whose very structure may lead to disease [12] has resulted in its near ubiquitous involvement in those diseases that most dramatically manifest the Translational Dilemma. Indeed, most evidence suggests that either insufficient [13] or self-sustaining [14] inflammation drives the pathobiology of trauma/hemorrhage, sepsis, inadequate or exaggerated wound healing, and a host of disorders at the molecular, cellular, tissue, organ, and whole-organism levels. These complex interconnections generate—or manifest in, depending on your point of view—a series of nested, interacting and balanced negative and positive feedback loops (Figure 1.1.1).

It is therefore not entirely surprising that merely suppressing inflammation is an ineffective therapeutic strategy other than in extremely severe or very benign settings. As an alternative, we suggest that controlling and reprogramming inflammation may allow us to reap its benefits while minimizing its detrimental aspects. However, the paradigm under which most of science operates is reductionism, and reductionism has largely failed to provide a rational approach by which to accomplish this goal. In addition to the multiscale complexity inherent in its organizational structure, inflammation manifests very differently based on personalized factors. These factors include individual features of the initial inflammatory perturbation, the individual's demographic and disease histories (including genetic predispositions and setpoints/thresholds for inflammatory processes), and the impact of environment and clinical care.

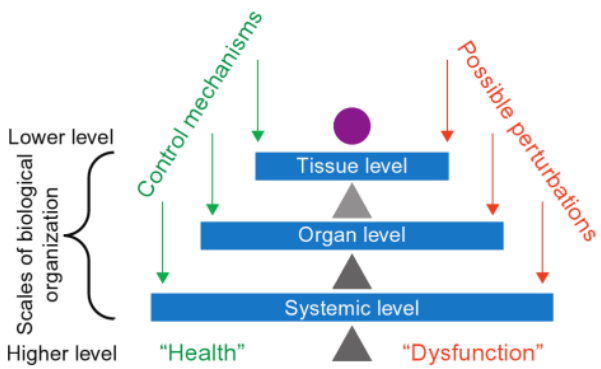

FIGURE 1.1.1 Multiscale control structure of inflammation. This figure demonstrates the tiered scales of biological organization. Control mechanisms (such as inflammation) attempt to balance insults/perturbations that threaten the health state (abstractly represented as the purple circle). Balance occurs at multiple tiers, and the multiscale nature of the control mechanisms allows for considerable robustness of the system to perturbations. Note that the control mechanisms themselves have a complex structure that can shift the balance as well. Source: *Reprinted from Ref. [15].*

Over a decade ago, there was recognition of the complex interplay between inflammation and physiology in critical illness and therefore also of the need to apply complex systems approaches such as computational modeling to unravel this complexity [16,17]. The use of mathematical and computational modeling of biological systems has become more common with the development of the systems biology and computational biology fields; the former generally modeling the behavior of intracellular signaling pathways and gene regulatory networks, the latter generally focusing on the development of correlation/pattern identification methods for large gene/protein data sets. We suggest that these types of analysis could be extended to decipher the multidimensional puzzle of inflammation and its consequences, and that, when geared toward practical applications, these methods hold the potential to transform the entire process of health-care delivery from preclinical studies, through clinical trial design and implementation, to personalized diagnosis and therapy, and ultimately to long-term care.

We and others have envisioned a rational, systems engineering-oriented, computationally based investigatory framework, *Translational Systems Biology*, that can integrate data derived from basic biology experiments with preclinical studies and clinical studies, and ultimately lead to the development of strategies for rational inflammation reprogramming [16,18]. Translational Systems Biology involves using dynamic mathematical modeling based on mechanistic information generated in early-stage and preclinical research to simulate higher-level behaviors at the organ and organism level, thus facilitating the translation of experimental data to the level of clinically relevant phenomena. Below, we provide the tenets and components of Translational Systems Biology. These concepts may seem a bit arcane at this point, but our presentation here sets the stage for their further explanation and discussion throughout this book.

PRIMARY GOAL: FACILITATE THE TRANSLATION OF BASIC BIOMEDICAL RESEARCH TO THE IMPLEMENTATION OF EFFECTIVE CLINICAL THERAPEUTICS

Primary Design Strategies:

1. Utilize dynamic computational modeling to capture mechanism.
2. Develop a framework that allows "useful failure" à la Popper.
3. Ensure that the framework is firmly grounded with respect to the history and philosophy of science.

Primary Methodological Strategies:

1. *Use dynamic computational modeling to accelerate the preclinical Scientific Cycle by enhancing hypothesis testing, which will improve efficiency in developing better drug candidates*. This includes the use and integration of methods applied to the other phases of the Scientific Cycle, i.e., high-throughput experimental platforms for real-world validation, generation of "omics" data sets representing enhanced observational capability, Big Data analysis to identify new correlations. Dynamic knowledge representation plays a big role here.
2. *Use simulations of clinical implementation via in silico clinical trials and personalized simulations to increase the efficiency of the terminal phase of the therapy development pipeline*. This focuses the targeted modeling goal on generating populations of simulations that can mirror how the biology manifests at the patient/clinical level, and looks to patient/epidemiological/clinical data as validation/verification metrics.
3. *Use the power of abstraction provided by dynamic computational models to identify core, conserved functions, and behaviors to bind together and bridge between different biological models and individual patients*. This will provide a formal and rationale guide to assess what aspects of mechanistic biology can be considered similar through the range of preclinical and clinical biological systems, and reduce the set of unknown factors that can be invoked to try and explain individual heterogeneity. Intrinsic to this goal is the need to dynamically model states of baseline health from whence disease states arise.

What Translational Systems Biology is not (which is not to say that the following are not laudable, or even necessary goals):

1. Translational Systems Biology is not using computational modeling to gain increasingly detailed information about biological systems.
2. Translational Systems Biology is not aiming to reproduce detail as the primary goal of modeling; level of detail included needs to be justified from a translational standpoint.
3. Translational Systems Biology is not aiming to develop the most quantitatively precise computational model of a preclinical, or subpatient level system.
4. Translational Systems Biology is not just the collection and computational analysis of extensive data sets in order to provide merely a broad and deep description of a system, even if those date sets span a wide range of scales of organization spanning the gene to socioenvironmental factors.

We emphasize that the implementation of a program of Translational Systems Biology does not preclude ongoing reductionist experimental investigations, the development and utilization of systems biology approaches to quantify fine molecular detail, or progress and utilization of Big Data-oriented computational biology work. All these approaches represent vital aspects within the Scientific Cycle, and as such have important roles to play in the current biomedical research environment. But, given our recognition that these strategies alone are not sufficient to meet the Translational Dilemma, we have crafted the description of Translational Systems Biology to limit overlap with those pursuits, and by so doing emphasize what is missing from all those approaches.

This book will introduce and demonstrate the Translational Systems Biology approach in three separate phases. The First Phase (represented by Section 2) describes the "Why?" behind the development of Translational Systems Biology. This section consists of primarily a historical, philosophical, and social survey of science, how it is used in biomedical research, and how those factors have led to the current biomedical research environment. The Second Phase (represented by Section 3) describes the "What?" in terms of how we propose to solve the issues identified at the end of the First Phase. This section introduces and provides a description of Translational Systems Biology and the primary intellectual strategies utilized in its pursuit. The Third Phase (represented by Section 4) described the "How?" in terms of the methodological approaches to implementing Translational Systems Biology. This section provides a more detailed survey of the specific methods and biological processes we have used and targeted in the development of Translational Systems Biology as applied to the study of inflammation. We will close with a discussion of how we suggest such a research program can come into being, given the sociopolitical and economic inertia present in the biomedical research community today.

Throughout this book, we will harken back to the opening quote from *A Tale of Two Cities* in relating the dual nature of much of what we will discuss; this is true not only for the sociohistorical aspects of the need for Translational Systems Biology, but also, interestingly, mirrored in inflammation itself. This means also that while we recognize that many of the barriers we need to overcome to move biomedical research forward are not placed by malice, negative products of good intentions are no less an impediment that those placed by intention. In fact, when the case is that such impediments arise out of the pursuit of what appear to be sensible reasons, it can make it that much more difficult to overcome these barriers. We hope that the reader will find us up to this challenge.

HOW TO APPROACH THIS BOOK

This book, like any book, has an intrinsically linear structure: it starts and progresses from page to page until you reach the end. But, just because a book has this particular form does not mean it has to be read in such a fashion. As the title suggests, this book provides a roadmap for how biomedical research can rise to meet it current challenges, but given the complexity of the nature of the Translational Dilemma, it also stands to reason that the solution is not a straight line. A wide array of issues are involved in the formulation of a strategy to address the Translational Dilemma, extending from the philosophical basis of science, to the procedural aspects of integrating *in silico* approaches with traditional experiment, to socio-operational issues involved with the current academic environment and the training of the next generation(s) of multidiscipline-capable scientists, as well as the economic and political incentives and disincentives that have prevented the large-scale application of the solutions we propose herein both in academia and industry. This web of history, rationale, cause, effect and solutions mean that readers of this book should feel free to jump from section to section, based on his or her interest at a particular time. Given its scope, this book incorporates sections written in a range of styles, and different sections may suit the reader's different moods at different times. Chapters in Section 2 primarily take the form of essays emphasizing historical and philosophical issues related to the state of biomedical science. These narratives are intentionally provocative, putting forth potentially controversial viewpoints and opinions in a tone and format usually not associated with scientific reports; our goal is to stimulate readers to try out alternative ways of thinking about the scientific endeavor (i.e., outside the infamous "box"), particularly as this pertains to the quest to improve human health. Some of these narrative sections include a suggested reading list for those interested in investigating the concepts raised in more depth. We indeed hope that these sections will stimulate the reader to question long-held beliefs. Chapters in Sections 3 and 4 take a form closer to traditional scientific review articles, presenting specific concepts and methods and providing examples from existing work in Translational Systems Biology as applied to the study of acute inflammation and critical illness. We consider the content of Sections 3 and 4 the objective evidence used to substantiate the claims and conclusions presented in the essays. As such, these sections contain lists of references consistent with scientific reports. To reiterate what we suggested above, readers are encouraged to take a nonlinear approach to this book, jumping around as their mood and intellectual inclination suggest. If, after reading this

book, we introduce some reasonable doubt about the how's and why's of the current *status quo* of the biomedical enterprise; if we stimulate an "a-ha" moment or just cause there to be a question; if we can change the dialog just a bit; or, if our book reaffirms the reader's belief that, despite everything we have said, the system is just fine as it is; then, we have accomplished our goal.

References

[1] U.S. Food and Drug Administration. Innovation or stagnation: challenge and opportunity on the critical path to new medical products; March 2004.
[2] Brennan RJ, Nandy R. Complex humanitarian emergencies: a major global health challenge. Emerg Med (Fremantle) 2001;13(2):147–56.
[3] Hewitson J, Brink J, Zilla P. The challenge of pediatric cardiac services in the developing world. Semin Thorac Cardiovasc Surg 2002;14(4):340–5.
[4] Bittles AH, Black ML. Evolution in health and medicine Sackler colloquium: consanguinity, human evolution, and complex diseases. Proc Natl Acad Sci USA 2010;107(Suppl. 1):1779–86.
[5] de Montmollin E, Annane D. Year in review 2010: critical care—multiple organ dysfunction and sepsis. Crit Care 2011;15(6):236.
[6] Gustot T. Multiple organ failure in sepsis: prognosis and role of systemic inflammatory response. Curr Opin Crit Care 2011;17(2):153–9.
[7] An G, Namas R, Vodovotz Y. Sepsis: from pattern to mechanism and back. Crit Rev Biomed Eng 2012;40:341–51.
[8] Angus DC, Linde-Zwirble WT, Lidicker J, Clermont G, Carcillo J, Pinsky MR. Epidemiology of severe sepsis in the United States: analysis of incidence, outcome, and associated costs of care. Crit Care Med 2001;29(7):1303–10.
[9] Namas R, Ghuma A, Hermus L, Zamora R, Okonkwo DO, Billiar TR, et al. The acute inflammatory response in trauma/hemorrhage and traumatic brain injury: current state and emerging prospects. Libyan J Med 2009;4:97–103.
[10] Patton GC, Coffey C, Sawyer SM, Viner RM, Haller DM, Bose K, et al. Global patterns of mortality in young people: a systematic analysis of population health data. Lancet 2009;374(9693):881–92.
[11] World Health Organization. Young people: health risks and solutions; 2011. Fact Sheet No. 345 (Updated May 2014 http://www.who.int/mediacentre/factsheets/fs345/en/).
[12] Nathan C. Points of control in inflammation. Nature 2002;420(6917):846–52.
[13] Namas R, Ghuma A, Torres A, Polanco P, Gomez H, Barclay D, et al. An adequately robust early TNF-α response is a hallmark of survival following trauma/hemorrhage. PLoS One 2009;4(12):e8406.
[14] Neunaber C, Zeckey C, Andruszkow H, Frink M, Mommsen P, Krettek C, et al. Immunomodulation in polytrauma and polymicrobial sepsis—where do we stand? Recent Pat Inflamm Allergy Drug Discov 2011;5(1):17–25.
[15] An G, Nieman G, Vodovotz Y. Computational and systems biology in trauma and sepsis: current state and future perspectives. Int J Burns Trauma 2012;2(1):1–10. [PMID: 22928162.]
[16] An G, Vodovotz Y, editors. Complex systems and computational biology approaches to acute inflammation. New York, NY: Springer; 2013. http://dx.doi.org/10.1007/978-1-4614-8008-2.
[17] Vodovotz Y, Billiar TR. *In silico* modeling: methods and applications to trauma and sepsis. Crit Care Med 2013;41:2008–14.
[18] Vodovotz Y, Csete M, Bartels J, Chang S, An G. Translational systems biology of inflammation. PLoS Comput Biol 2008;4:1–6.

SECTION II

THE CURRENT LANDSCAPE: WHERE IT CAME FROM, HOW WE GOT HERE, AND WHAT IS WRONG

This page intentionally left blank

CHAPTER

2.1

A Brief History of the Philosophical Basis of the Scientific Endeavor: How We Know What We Know, and How to Know More

If I have seen further it is by standing on the sholders [sic] of Giants.—**Sir Isaac Newton**

Knowledge is in the end based on acknowledgement.—**Ludwig Wittgenstein**

The game of science is, in principle, without end. He who decides one day that scientific statements do not call for any further test, and that they can be regarded as finally verified, retires from the game.—**Karl Popper**

What is science? What does it mean to think scientifically? What does it mean to "do science?" This is a book about identifying the foundations needed to answer those questions: identifying the basis for what we believe, why we believe it, and how that translates into how we behave. We propose in this book that today's biomedical science is broken, and that it has reached this critical point in part because of the trends of scientific history. Furthermore, we suggest that biomedical research also has the ability to transcend its current crisis based on principles arising out of that same history. We hope to make our point by tracing a systematic path from the foundations of scientific thought to answering questions about how we should be addressing the challenges to the biomedical research in the twenty-first century, and beyond. In our view, the rationale for Translational Systems Biology represents the results of applying the scientific process to process of science. To make our case, we must go the beginning.

What follows here is a very brief survey and explicitly noncomprehensive and selective tour through the history of the philosophical basis of science. For readers interested in a more in-depth examination of many of the individuals and concepts introduced in this chapter, at the end of this chapter we have provided a list of Suggested Additional Readings, consisting of the source materials as well as summarizing books that we have found beneficial in our own reading. To a great degree, the events and anecdotes that we use to relate our story reflect the nature of *our* story as well (we get very meta in this book). After all, every story requires some choice on the part of the storyteller about what to leave in and what to leave out. For a writer, that is just good writing; we believe that it is also essential to being a good scientist. We will see though the examples below, and in other stories throughout this book, that perhaps the greatest challenge to science is absolutism masquerading as prevailing dogma. Despite the fact that science is grounded in skepticism, all too often the prevailing scientific community of the day forgets that they, too, need to be subjected to the same scrutiny that they promote within their respective scientific disciplines. This in of itself is telling, and suggests that, at a fundamental level, the nature of humans and the societies they form need to be subjected to constant and persistent vigilance, and primed for course correction.

In the beginning, to paraphrase the Bible, there was religion. Without getting into issues about the specifics of each religion, the fact that religion is a ubiquitous component of every human society points to its role in serving a basic human need. That need is the drive to provide an explanation for the vast and varied world about us; in short, it is a desire to find some order to the world, a way to explain to ourselves why things are the way they are. It can be argued that this desire and need to *generalize* from disparate observations has been the primary outlet for humanity's

intellectual and reasoning capabilities throughout history. In many ways, the concept of intelligence can be linked to the capability to formulate useful generalizations and extrapolate lessons learned from one particular situation to other, recognizably similar situations. The power and influence of the quest for order (and more specifically, substantiate, reinforce, and justify our existing concepts of order) will show its impacts, both positive and negative, throughout our story. Humans are storytellers: the manifestation of that tendency, as applied to understanding the world around us, is to generalize our knowledge by creating stories about why things are the way they are. It is important at this point to stop for a moment and recognize the implications of such basic human drives: human beings want order in their world, they seek explanations, they construct stories, and they become invested in their stories. We will return to these themes throughout this book.

Some say science has supplanted religion as the primary means by which humans understand the world and their place in it. All too often this statement is associated with an implication of an "either/or" condition related to science and religion. Unfortunately, this false dichotomy has its origins in misunderstanding what each domain represents. Rather than thinking about religion and science as competitors, it is more constructive to recognize how each of them addresses the human desire for order by appealing to different aspects of our psyche. We propose that the fundamental difference between the two is that while religion requires faith, science requires doubt. Put in another way, religion says there is a final answer beyond which no more can be known and we must just accept its existence, not only despite not being able to prove it, but perhaps *because* it cannot be proven. In contrast, science says that there are always more questions, since doubt always remains and there is always something more to be known; once the possibility of proof/disproof disappears, so, too, does the domain of science. The scientific process can then be characterized as how humans deal with addressing their doubt (at a particular point), and recognizing that addressing that doubt just raises another set of doubts. Given our list of aspects of human nature, what is the root and history of doubt? Analysis of doubt must begin with an understanding of how we know what we know, and that entails a basic understanding of the philosophical discipline of epistemology: how much can we believe in, and what we can experience through our senses and interpret with our reason?

MODELS IN THE CAVE

One of the earliest and best-known stories about the limits of our senses in interpreting the natural world involves Plato's Cave. In this parable, the perception of reality of the prisoners in the cave is limited to the shadows projected on the cave wall by various light sources behind them. We will dispense with, for the moment, the discussion about the consequences to the single prisoner who turns around, but rather use the first portion of this tale as a representation of how we interact with the world. The parable assumes that there is a reality that is "hidden" to our most basic perceptions of what is projected on the wall of the cave; enlightenment then arises as we gradually turn around. But rather than necessarily focusing on what we find when we turn around, in terms of characterizing a process we wish instead to look at *how* we turn around: what do we see when we turn around a little bit that makes us want to turn around a little more. We propose that the process of turning around and how that turning results in various changes in the shadows is analogous to the use of increasingly artificial objects, i.e., models, to shed progressively more clarity on the true nature of the objects being projected. Viewed in this fashion, this famous parable provides an early description to how we view the world through models. By changing the projections available to our senses through the use of specific artificial objects (i.e., models), these models may cast specific types of shadows, and the comparison between the different shadows can lead us to make some inferences about the source of the light. Thus, even at this early stage, we can see that the greater the number of perspectives we can generate, the more comprehensive picture we can derive.

A critical social aspect of Plato's lesson is that it is extremely difficult to achieve the insight afforded by turning around if your peers are satisfied with their current condition (i.e., they ask you "why keep turning?") Plato goes on to add that if, even in the face of this discouragement, one were able to turn around and obtain enlightenment, an attempt to return to the cave and communicate this information is likely/certainly to be welcomed with suspicion and hostility. We would argue that in order to convince the rest of the prisoners to allow additional turning, the turner would be well served to provide some tangible benefit to the group from their endeavor. Thus, the pursuit becomes something more than just an intellectual/philosophical exercise; we have added the *applied* aspect of insight and science to the equation. We would argue that this translation of insight into practice and its feedback to the general population is a critical component of the successful integration of science into its parent society. Furthermore, in order for this applied strategy to come to pass, the turner needs to recognize when he has turned enough to be able to sufficiently operate and interact in his world; this is the difference between a pure pursuer of knowledge,

and those with a more practical bent. For those of us in the latter group (which, as biomedical researchers, is where we should be), we must identify circumstances in which we can say we have turned around enough: "This set of shadows is good enough for me to live my life and help my fellows." Of course, success brings it dangers as well. Perhaps the implementation of a particular insight could prove to be almost "too" beneficial for the overall group; there is belief that all that can be known has become known. This is the pathway to dogma, a manifestation of the seductive danger that we have done enough turning, become satisfied, and are unable to realize that there is more light to be seen. These are the circumstances in which we struggle within a particular paradigm to find useful solutions, not yet recognizing the overall futility of such a pursuit. It is not easy to look beyond our current success to the point of future failure, but this is, in fact, what science requires.

EARTH AT THE CENTER: A REASONABLE MISTAKE, AND AN UNREASONABLE PERPETUATION

One of the most famous examples of the danger of being wedded to a limiting paradigm is the Ptolemic Geocentric Cosmos. The story of Ptolemy's Epicycles is well known: a view of the Universe predicated upon the concept (ultimately proven wrong) that everything rotated around the Earth, i.e., geocentricism. The epicycles referred to the presumed curly-cue movement of certain astronomical bodies as they rotated around the earth; a behavior required in order to explain how some bodies, such as Mars, appeared to move backward and forward in their progression across the night sky. Rather than rehash just how wrong this model was, and the socio-religio-political forces that supported and perpetuated this concept, we would rather look at the history of this cosmology as it affected the evolution of science, particularly in terms of how the scientific community of the time responded to the increasing acquisition of data and observations about the night sky. A major, unfortunate consequence of the eventual failure of Ptolemy's Epicycles is that it has cast a negative light on its originator. Ptolemy was, in fact, a genius in his or any time, producing critically important treatises on optics, music, and geography in addition to his work on astronomy. He provided a vital link from the Greco-Roman advances in science through the flowering of Persian and Islamic thought into the European Middle Ages. In fact, his attempts to bring mathematical order to the observed world constitute a vital step in the search for nonsupernatural/metaphysical unifying explanations for real-world phenomena. Paradoxically, to a great degree, it was his acknowledged genius that proved to be a hindrance when it came to assessing his conceptual model of the world and cosmos; as the centuries passed, his concepts formed an unchallengeable "truth" for subsequent would-be explorers of the natural world. It was within this context of accepted "truth" that subsequent astronomical observations were interpreted: the data could only be correct if it corresponded to the expectations of the general structure of the Ptolemic paradigm. In fact, as the formulae of the epicycles were tweaked over the centuries to fit the existing data, the predictive capacity of this astronomical viewpoint became stronger and stronger, so much so that had significantly greater predictive power when compared to the original Copernican heliocentric view. Today, this discrepancy is usually mentioned as a preamble to discussions of the contribution of Kepler and elliptical orbits to astronomy and physics, but doing so misses important aspects of the original collision between the geocentric and heliocentric viewpoints. First was the obvious point that the Ptolemic Universe was pretty good at predicting things! This suggests that given enough data for fitting, a resulting model can become very efficient indeed in predicting future behavior. This, in fact, is a pragmatic rationale for today's Big Data science; the predictive power of a model can be independent from the model's representation of how the underlying data were generated. Therefore, sometimes prediction is good enough. However, this notion inevitably leads to the next question: when is prediction not good enough? What happens when we want our models to do more than just tell us what is going to happen, but not why? Going back to Plato's Cave, what if we want to turn around a little more? This brings up the second aspect of the transition from Ptolemic geocentricism to the Copernican viewpoint: providing the potential for expansion of a descriptive model not only in terms of the scale of data (i.e., more and more of the same metrics), but also in terms of the scope of data able to be explained. In short, the true power of the heliocentric view was an increase in the generality of the proposed explanation; it allowed for expansion beyond astronomy into more general physical processes now interpretable through the grand work of Newton and the introduction of *theory* to the scientific endeavor.

THE SCIENTIFIC METHOD OF FRANCIS BACON

Francis Bacon (1561–1626) is often credited with being the father of the Scientific Method. As first put forth in his *Novum Organon* or "New Method" (1620), he described the use of *induction* and the steady collection of evidence

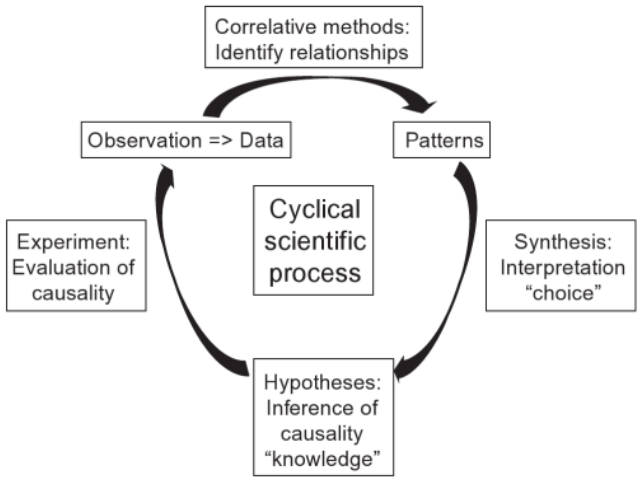

FIGURE 2.1.1 The Scientific Cycle. The iterative cycle of observation, interpretation (hypothesis generation), and experiment, originally described by Francis Bacon and the means by which scientific knowledge is generated.

as a means of establishing a description of the natural world (we will talk about induction in a little more depth shortly). Intrinsic to this process is the ranking of evidence both for and against a particular explanation, and the recognition that this is an iterative process and that some modicum of "truth" would be arrived at in a steady and incremental fashion. The steps in this process have been termed the *Scientific Method*, and the iterative nature of the implementation of this process is called the *Scientific Cycle* (Figure 2.1.1). The description of the Scientific Cycle is rightly considered to be the origin point of modern science, and we do not think it an overstatement to say that the adoption of the Scientific Cycle is responsible for the sum total of technological achievement since the seventeenth century. We will return again and again to the fundamental role of the components of the Scientific Cycle throughout this book in order to provide a foundational basis for the strategies we propose.

However, Bacon realized that, in the real world, one could not count on the perfect execution and outcome of any given process; living at the dawn of the Enlightenment, he was well aware of the frailties in the human condition. Therefore, in addition to the description of an iterative cycle of observation, categorization of evidence and refinement of hypotheses, Bacon also identified specific hindrances to the effect execution of this process, which he termed "Idols of the Mind." This list of his is striking in its timelessness:

1. Idols of the Tribe (*Idola tribus*): This refers to the human tendency to look for more order and regularity in systems than may truly exist, and leads to the tendency for people to follow their preconceived ideas about things.
2. Idols of the Cave (*Idola specus*): This refers to the tendency for an individual's personal likes, dislikes, and desired viewpoints and outcomes to affect their reasoning.
3. Idols of the Marketplace (*Idola fori*): This is due to ambiguities that arise from the use of words in science that have a different meaning than their common usage.
4. Idols of the Theater (*Idola theatri*): This is the following of academic dogma and letting those known facts preclude asking questions about the world.

Sound familiar? Herein lie the roots of *cognitive bias*; a term unfortunately often applied to those that we wish to criticize, but less frequently turned upon ourselves and our beliefs. The robustness of Bacon's overall process has been clearly demonstrated in the scientific advances over the past 500 years, as improvements in methodology, and specifically statistics, have attempted to address some of his "Idols." However, as we will see, history has shown that these idols continue to bedevil scientific communities today.

NEWTON: THE (JUSTIFIABLE) ORIGINS OF PHYSICS ENVY

Among the great thinkers of the Enlightenment it is difficult to find an individual more impactful on science than Sir Isaac Newton (1642–1727). Known primarily for his invention (concurrent with Gottfried Leibnitz) of

calculus and his authorship of the *Principia*, which introduced the Laws of Motion and tied them to mathematical representations, he also pioneered investigation of optics as well as advanced analytical geometry. He also contributed to, if not drove, the societal shift to the concept of a mechanistic universe operating under Natural Law. Deism, the concept of a disengaged watchmaker god, came to dominate thought among Enlightenment thinkers, including some of the most influential of the United States' Founding Fathers, namely Benjamin Franklin and Thomas Jefferson, who perhaps not coincidentally also happened to make significant scientific contributions. The fact that Newton was also involved in occultism and eschatological Biblical interpretation gives insight into both the range of his interests as well as the socioreligious environment in which he lived. If Bacon can be considered the originator of the Scientific Method, it was Newton who, through his accomplishments, would establish the paradigm in which that Method would be applied. Here we will focus primarily on the impact of the *Principia*, which we simply state as introducing the "how" into investigation of the natural world. To summarize briefly, in the *Principia* Newton provides an explanation of *how* the observations of the heliocentric astronomy of Kepler could be generated through interactions of the physical world, and expressed that explanation in fundamental laws that could be mathematically represented, including with the newly developed calculus. This is the origin of physics. Prior to Newton, observations could be correlated together, and some order could be brought to those correlations through mathematical expression. However, there was no explicit description of how properties of the physical world could actually generate the observed correlations. By introducing *mechanism* into the scientific endeavor, Newton not only contributed to the societal shift away from supernatural explanations of the world but also provided a pathway toward the rational development of technology through design as opposed to prototyping. After Newton, one could now move beyond generating only descriptions of the physical world to being able to ask questions about how the physical world operates. Though Bacon's Scientific Method preceded Newton's career, today we invariably cast the cycle of observation, inductive hypothesis generation, and collection of substantiating evidence in the context of Newton's mechanistic world, wherein the step of induction consists both of establishing a correlative pattern within the observations as well as positing causal mechanisms that can provide means of generating those correlations.

The content of *Principia* also introduces the concept of *theory* in science. A theory is a statement of fundamental mechanistic principles that goes beyond merely explaining how a set of behaviors *could* be so; rather, a theory defines why a set of behaviors *must* be so. Theory, therefore, should be thought of a means of limiting and constraining the range of possible behaviors of a system; a theory states that, in fact, *anything is not possible*. For instance, based on Newton's Theory of Gravitation, things could not fall up from the surface of the Earth; rather, they must inevitably fall down. By providing a mathematical basis for his Laws, Newton further established the power of formalism as a means of substantiating the general application of theory. By abstracting the components of a system, the mathematical expression of a theory could be agnostic to the specific circumstances of that system, as long as the components of the real world could be reliably represented by the math. In this fashion, the application of theory suits our desire to generalize our knowledge, and the power of the abstraction allows us to apply that theory to circumstances not directly involved in the theory's development. Inherent in this process is the recognition that, given the iterative cycle, there must be some reason to believe that a potential theory can operate in this fashion. It is from here that the standard of *prediction* as a means of establishing trust arises.

The fact that his Theory was applicable at so basic a level, i.e., the *physical* nature of things, along with the unquestioned elegance of its relationship to calculus, lent credence to the impression that Newton had achieved an explanation of the Natural World as its most basic and fundamental level. The inevitable consequence of this impression is that a search for "truth" in the physical world would invariably lead to a question of physics, and that physics, as a discipline, represented the ideal paradigm for meshing real-world observation with the explanatory power of formal representation through mathematics. This is the origin of what we term "physics envy" among other scientific disciplines; a condition that has only been reinforced through the successes of General Relativity and Quantum Mechanics. There is a prevailing impression, particularly in biology, that our investigations should aspire to physics-level characterization of the systems we study. This is doubly so if the biological system we are studying is related to a disease; our lack of a cure for this disease is often related to our lack of a physics-level understanding of the underlying biological processes. Implicit in this aspiration/envy is that the goal of investigation should invariably involve a progressive reduction of our system into simpler and simpler components. This *reductionist* paradigm has its origins in Newton, and has dominated science up until the end of the twentieth century. We shall see later how, despite its unquestionable success, we now appear to have reached a boundary in the effectiveness of reductionism, and that transcending that boundary requires overcoming Bacon's Idols.

THE PROBLEM OF INDUCTION: HUME'S EMPIRICISM

The Baconian Method is foremost a method that relies on inductive reasoning, i.e., the generalization of one observed situation or condition to represent the greater set of situations. Newton now brought mechanistic causality as part of the goal of the inductive process. In its iterative form, cyclical induction is demonstrably useful. However, can induction lead to an absolute truth, or are we just aiming for some sufficient level of trust? This question was addressed by the Scottish Enlightenment philosopher David Hume (1711–1776), who focused on the problem of induction as it pertains to a wide range of human behaviors, but for our purposes will be focused on the establishment of causality. Hume divided the process of coming to a conclusion about an observed phenomenon into either a process of "reason," which we would today consider deductive logic, versus "induction," which is the generalization of one observation to a class of phenomena. Expanding upon the mechanistic Natural Law of Newton, Hume argued for an explicitly causal universe, where every observed phenomenon was generated by some causal, mechanistic event. In other words, Hume denied the role of supernatural forces as actors in the interactions observed in the real world (again, moving beyond Newton's occultist and mystical tendencies). The accumulation of knowledge about the real world can then be represented by a collection of causal mechanisms pertaining to observed phenomena. However, it became quickly apparent that an initial condition would not necessarily result in a unique outcome, i.e., multiple possible outcomes (or mechanisms) might occur. This lack of a specific outcome removed it from the domain of formal deductive proof, which provides a unique conclusion. By demonstrating that deductive logic cannot be used to derive a result merely by knowing a cause, Hume asserted that reason alone was not a means of generating new knowledge, and therefore that induction was required to establish causality. While Bacon and Newton had demonstrated the process and success of a strategy involving induction, Hume proved that such a strategy must be applied. However, now there was a problem: how reliable was induction? The Problem of Induction refers to the question as to whether an inferred individual example of a phenomenon can lead to generalized statement about the entire class of phenomenon. Hume realized that in order for induction to lead to knowledge it required two conditions:

1. The individual example must represent all cases of the specific phenomenon. The classic example of this is the observation that most swans are white, therefore, it is highly likely that all swans are white (which is incorrect, as some swans are black).
2. The conditions under which the inferred in the past will continue to hold in the future. This is the Principle of Universality. To paraphrase: that which has been true in the past will also be true in the future.

Hume demonstrated that the Problem of Induction could not be solved using deductive logic: Condition 2 failed by being subject to circular reasoning, i.e., assuming the truth of the Principle of Universality, which could not be proved independent of that assumption. Therefore, inferred statements could never be "proven" to be true; rather they could only be substantiated to some degree of confidence by the accumulation of observed empirical evidence. This concept now ties back to the Scientific Cycle of Bacon and Newton. Since the outcome of induction could not be proven, answering questions about the natural world no longer became a search for philosophical absolute truth, but rather a perpetual task of weighing evidence and finding a "best" (for now) explanation. By removing the certainty and inevitability of deductive proof from evaluation of causality, Hume offered the promise of induction-based empirical science as the pathway to knowledge through practice and endeavor as opposed to proof.

LOGIC AND ITS TRUE/FALSE PROMISE: LOGICAL POSITIVISM, GODEL AND POPPER

Hume's empiricism appeared to be borne out by the flowering of scientific knowledge seen during the late and post-Enlightenment period. Most strikingly, the success of empiricism-guided scientific inquiry manifested in applied and translational effects on society as a whole. By the end of the nineteenth century, advances in physics and chemistry found tangible expression through the steam engine, industrialization of chemical processing, advances in metallurgy, the early electrical grid, the telegraph, the early telephone, and the wireless. The world had changed from one limited by muscle power, physical senses, and the cycles of the sun to one that harnessed energy from the basic actions of the physical world, expanded what could be seen and communicated, and extended human activity to the full 24 hours of the day and across all the four seasons. It is no surprise, therefore, that optimism regarding the possibilities of technology would rule the day. From a philosophical standpoint, this optimism found expression in the school of Logical Positivism. Hume, through his approach to addressing the problem of induction

and establishing causality through empiricism, is often considered the forerunner of Logical Positivism. This school of philosophy, which found its apotheosis in the first half of the twentieth century following the publication of Ludwig Wittgenstein's *Tractatus Logico-Philosophicus* in 1921, sought to provide an overarching logical framework for epistemology and put forth strict criteria for what could be considered knowledge and legitimate topics for science. They asserted that the only knowledge suitable to be discussed as such was that which could be empirically verified. It posed the formal elegance of mathematics and logic as the only way to express scientific principles, and Wittgenstein's *Tractatus* laid out the characteristics of such an ideal scientific language. It placed unquantifiable concepts that reflected explanatory interpretation of data, such as mechanisms, theories and principles, as being outside the realm of science, and therefore subject to discussion only though intrinsically ambiguous and imprecise natural language. Only that which could be measured and observed, i.e., data, could be verified and considered to be true. At first, this strict criterion for what could and could not be considered "science" might seem opposed to the prevailing optimism we just noted above. It would seem counterintuitive that the Logical Positivists would want to limit the scope of what could be considered for scientific discourse. However, we would argue that the seemingly unbounded success of empirical science gave the Logical Positivists the freedom to believe that they could limit those aspects of the world subject to formal characterization. The success of empiricism in the nineteenth and early twentieth century gave them the confidence that even after the implementation of such stringent criteria that science would find a way. This optimism concurrently manifested in the German mathematician David Hilbert's (1882–1943) attempt to provide a foundational description of mathematics through logic.

Alas, it was not meant to be. The Incompleteness Theorems of Kurt Godel (1906–1978) proved that Hilbert's overall goal of unifying all mathematics was impossible, and ever since then investigations in mathematics, logic and, by extension computer science, have been divided into those aspects of those disciplines that can be completely knowable versus those that cannot. Unfortunately, the work of Godel and others also demonstrated that the vast majority of conditions fell into the category of those things that cannot be completely knowable. In a somewhat similar fashion, the philosopher Karl Popper (1902–1994) undermined the agenda of the Logical Positivists by demonstrating the logical inconsistency of their program. By returning to the Problem of Induction, Popper proved that, despite the operational effectiveness of empiricism in individual cases, by setting the goal of science as the *verification* of inferred relationships of empirical observations, this could not be done without making the circular argument that the Principle of Universality was true. Also, he pointed out that the Logical Positivist approach could not yield uniquely true statements, as it was subjected to the fallacy of affirming the consequent: if p leads to q, and q is true, then p is true. As such, you could never completely verify that something was actually and perpetually true. Alternatively, Popper suggested that the goal of empirical investigation should be to deny the consequent, i.e., try to prove that a particular statement was false, which was now a logically tractable task (through *molus tollens*). By putting forth this reorientation in the goal of science, Popper shifted the frame of skepticism to the overall goal of the inductive process, seeking to falsify false statements rather than to try to justify potentially correct ones. This actually returned to the root of science in terms of employing doubt. Rather than trying to abolish doubt, Popper suggested that we can only confirm it, and move on. We consider this a "meta" application of the Scientific Method, where the Method is applied in a self-reflective, recursive fashion to analyze itself.

CHARLES PEIRCE SUGGESTS TAKING A GUESS: THE ABDUCTIVE APPROACH

From the onset, with Bacon's description of the Scientific Method, the process of inference and induction has been a primary source of epistemological investigation and discussion. Intrinsic to Bacon's inductive reasoning is that it incorporates both the observation and its interpretation as a *hypothesis*. As we have seen, the Newton's introduction of mechanism into the process of induction led to a prevailing conflation of hypothesis with mechanism. The American Pragmatist school of philosophy, and in particular one of its earliest proponents, Charles Sanders Peirce (1839–1914), further decomposed the process of induction and hypothesis generation to ask "where do our explanations/hypotheses come from?" Peirce realized that the step of hypothesis formation was not performed in a historical vacuum. Rather, inferences that lead to a putative hypothesis involved the contextualization of the question within a framework of existing knowledge. In short, when faced with a new observation, the scientist would draw upon a previously known list of rules/mechanisms/relationships in order to explain the current topic of investigation. This provided, if not a specific answer, but at least a formal process to address the fallacy of Affirming the Consequent introduced earlier in discussing the Logical Positivists: that given that p leads to q, and if q is true, then p cannot be said to be uniquely true. *Abductive* reasoning removes the mandatory uniqueness criteria for this logical statement: it now states that, given prior knowledge, p can be abducted as a possible means of producing

q, where the range of possible p's is limited by prior knowledge and the researcher's experience. Peirce called this process, scientifically, "guessing." From a pragmatic and operational standpoint, abductive reasoning provides a means of expanding knowledge from one known context to one that is deemed "similar."

This type of logic does have a formal syntax and set of operations, but we are not concerned with these here; rather, we point to the importance of abduction in terms of how deeply ingrained it is (even if it is rarely explicitly recognized as so) into the current scientific process. In some ways, it is much like the "crazy uncle in the attic": the scientific community understands that guessing is a necessary aspect to the scientific endeavor, but, on its face, somehow does not seem "rigorous" enough to constitute science; i.e., we scientists do not want to think about how much we do is reliant on guessing. However, as we have noted above in terms of roadblocks to advancing science, we need to deal with what we really do and not just want we would like to think we do, and this requires us to be honest about the intrinsic components of what we do. Of course, it is not all bad news, because accepting abductive reasoning means that we continue to rely on experiment and the accumulation of evidence, but now with the essential realization that the groundwork of assumptions we operate upon is a shifting and mutable landscape.

THE MAPPING PROBLEM: BACK TO PLATO?

The introduction of Abduction and the concept of Popperian falsification reinforce the importance of *experiment*. Experiment is not explicitly noted in the Bacon's original description of the Scientific Method, but quickly became an accepted part of the iterative cycle. The separation of the inductive process into its component parts makes experiment an explicit component of the Baconian induction. We will leave discussion about what constitutes a "good experiment" to later, and will focus now on what actually constitutes an experimental science (as opposed to merely observational science; more on this later). We assert that an experiment uses a model that is a cartoon of the real-world system the experiment is attempting to study; such a model represents an abstraction in which features deemed important are emphasized at the expense of realistic detail. Thus, by definition, an experimental model is "less" than the real thing; moreover, the very fact of its usefulness is to a great degree dependent upon how it is specifically "less" than the real thing. We would argue that any model sufficiently useful to answer specific questions about a system represents a model incapable of representing all the important properties of the system. The relationship between the model and the real thing is called a *mapping.* In mathematical terms, a *map* is an operation linking members of one set to another set. Here, the vernacular noun "map" provides an ideal example of the concept. A physical two-dimensional map, be it a road map, a topological map, or a population density map, represents the visualization of data about the real world in some reduced form (even beyond the compression of three dimensions to two) that emphasizes the data type chosen. A description of the mapping relationship between a model and its real-world referent is then a necessary piece of information in being able to contextualize the information obtained from the experiment. To follow Peirce, knowing the mapping of the model allows one to identify the appropriate prior knowledge that can be used to support the "guess." It should also be evident that not only can models be mapped to reality, but they can (and should) also be mapped to each other.

Now, recognizing that we obtain much of our information about the world around us through inferences made via our experiences with multiple maps/abstractions of the real world, we find ourselves back in Plato's Cave. The shadows on the wall are projections of reality made by various reduced objects (Plato's "artificial objects"), but now, armed with the fore knowledge of their limitations, rather than obscuring the reality behind us, they can provide more insight into how we turn around, and informing us that we are turning in the correct direction. The shadows can serve as guides, and provide tangibly useful knowledge in the process. But despite their usefulness, we must remember that they are themselves potential distractions from what we are ultimately trying to understand.

So, what have we hoped to gain by taking this little tour? Each one of the stories above presents an important and relevant lesson that will run as recurrent themes throughout this book. Plato tells us that we perceive the world through shadows of reality, i.e., models. He also warns us that those bringing insight might not be received with all too favorable enthusiasm. The lessons of Ptolemic Geocentrism are that even genius needs be challenged to overcome both the inertia of prior belief as well as the seductive allure of predictive correlation. Bacon not only brought us the key description of our process, the Scientific Method, but also recognized that there existed "Idols" that perpetually need to be torn down. Newton demonstrated the power of mechanism and abstract representation, and irrevocably integrated both of those processes into the Scientific Cycle. He also paved the way for the establishment of reductionism as the existing paradigm for scientific investigation. Hume closed the door on ontological truth with his skepticism and limits on induction, but opened a pathway for science that is reliant upon empirical testing. The Logical Positivists showed what can happen if you push too far to what appears to be a logical conclusion at the

cost of forgetting the foundations of your belief, something Popper pointed out as he pulled us back to a tractable viewpoint that could actually be implemented. Peirce introduced a third logical way in addition to deduction and induction, namely the abductive process that underpins the practice of science today. Together, Peirce and Popper offer two tangible operating principles for the practice of science: (i) educated guesses supported by experiments and (ii) a goal of viewing experiments as falsifying endeavors. These two principles can be unified into an overarching strategy to point a way forward that rests on firm philosophical foundations.

Having provided, through this brief overview, the basis for how science should be performed, we now turn in the next section on the very special case of biology.

Suggested Additional Readings

Source Material (In order of presentation in the chapter):
Plato, *The Republic*
Francis Bacon, *Novum Organum, or The New Organum*
Isaac Newton, *The Principia: Mathematical Principles of Natural Philosophy*
David Hume, *An Enquiry Concerning Human Understanding*
David Hume, *A Treatise of Human Nature*
Ludwig Wittgenstein, *Tractatus Logico-Philosophicus*
Kurt Godel, *On Formally Undecidable Propositions in Principia Mathematica and Related Systems*
Karl Popper, *Conjectures and Refutations: The Growth of Scientific Knowledge*
Karl Popper, *The Logic of Scientific Discovery*
Ludwig Wittgenstein, *Philosophical Investigations*
Charles Peirce, *Reasoning and the Logic of Things: The Cambridge Conferences Lectures of 1898*

Popular Books about the Source Material

As we noted at the beginning of this chapter, our survey of the history and philosophy of science is admittedly highly selective and provided with the explicit purpose of setting the intellectual stage for our description of Translational Systems Biology. As such, our survey is not intended to be a comprehensive report on the philosophy of science and its development: there are several excellent volumes that can provide the reader that information. We list below several such books that fit that description, as well as several other books that focus on specific aspects of the survey we have provided.

Wittgenstein's Poker: The Story of a Ten-Minute Argument Between Two Great Philosophers by *David Edmonds* and *John Eidinow*
Beyond Wittgenstein's Poker: New Light on Popper and Wittgenstein by *Peter Munz*
Gödel's Theorem: An Incomplete Guide to Its Use and Abuse by *Torkel Franzén*
Gödel, Escher and Bach: An Eternal Golden Braid by Douglas Hofstater
Masterpieces of World Philosophy by Frank MacGill
The Discoverers by Frank Boorstin
From Dawn to Decadence: 500 Years of Western Cultural Life 1500 to the Present by Jacques Barzun

This page intentionally left blank

CHAPTER

2.2

A Brief History of Biomedical Research up to the Molecular Biology Revolution

Animals are classified as follows:
1. *those that belong to the Emperor,*
2. *embalmed ones,*
3. *those that are trained,*
4. *suckling pigs,*
5. *mermaids,*
6. *fabulous ones,*
7. *stray dogs,*
8. *those included in the present classification,*
9. *those that tremble as if they were mad,*
10. *innumerable ones,*
11. *those drawn with a very fine camelhair brush,*
12. *others,*
13. *those that have just broken a flower vase,*
14. *those that from a long way off look like flies.*

Celestial Emporium of Benevolent Knowledge—***Jorge Luis Borges***' *fictional taxonomy of animals from his 1942 short story The Analytical Language of John Wilkins.*

Cast in the overall context of scientific endeavors, biology occupies contradictory positions as perhaps the most basic, most investigated, and yet least rigorously developed discipline. One of the most basic observations of the world is that there are things that are alive and that there are things that are not. The fact that this seemingly sharp dividing line for characterizing the world has been a constantly moving and increasingly nebulous line further complicates the placement of biology in relation to the physical sciences of physics and chemistry.

Biology is the study of living things. Encapsulated in that simple definition are two overarching processes that influence biological study even in the present day: observation and categorization. First, one observes that there are things that appear to be alive. It then immediately follows that you start making a list of the observed characteristics that cause a thing to be either living or not. You then observe that there are differences among the living things, that some groups are more similar to each other, while others are considerably different. Lists of characteristics are made (sometimes not substantially less fanciful than the one that precedes this chapter), categories are created and defined, and organisms are placed into these categories. So basic is this process of categorization that, in Genesis, God specifically tasks Adam with naming the animals, making him the first biologist listed in the Bible. From the beginning of biology, nearly all discourse has involved discussion, disagreement, and debate about the composition of these lists of characteristics, the resulting categories, and the placement of individual types. Interestingly enough, the fact that defining the most basic characteristic of biology, i.e., "What is alive?" remains an open topic of inquiry points to the practical challenges of this task. Controversy at even this most fundamental level reinforces the notion that the permutations of categorization, even if proceeding in a more methodical fashion than Borges' taxonomy, are

endless. In fact, perhaps the main reason Borges' list, with its seemingly random construction, strikes us as being so odd is it violates the basic human urge to find order in the world.

Having touched upon the manifestation of this desire for order as a driving force in our discussion about the history of science in general, we will now examine the role of categorization in the development of biology as a science. As with our survey of the philosophy of science, we adopt a highly selective review of some of the principle points in the history of biology, with the specific goal of trying to see the origins of the hallmarks of the current biomedical research environment.

The study of living things can be seen to have three distinct threads. Clearly there is considerable crossover among these threads, but as we will see, they actually represent distinct disciplinary paths. The first, which we have touched upon above, is the task of categorization, which can generally be considered as the creation of taxonomies. The second task involves investigating how living things function; this field can generally be labeled as physiology. The third task attempts to define the source of living things: this is the study of origins and evolution.

The intersection between biology and medicine requires reconciling these three threads. Medicine as a discipline has, for much of its existence, focused on the second thread: the characterization of function. This makes perfect sense, since the goal of medicine is to restore a person to their normal function and is therefore inherently linked to physiology. Because function implies mechanisms, the study of physiology necessarily involves understanding dynamics; this casts the goal and study of biology clearly in the Newtonian concept of a mechanistic universe. But the investigation of mechanism cannot occur without having the categories of structure to build upon. Being part of biology, the science of medicine is dependent upon the basic categorization of biological properties and structures to provide guidance as to what should be done, but what is to be done is highly dependent upon some concept of what is wrong, i.e., identifying the dys*function*. This dual nature of medicine is still evident today in the two general categories of subjects present in the medical curriculum: anatomy (concerning description of structures) and physiology (concerning description of function). We suggest that there is a cyclical relationship between these two tasks, in which one perspective represents the cutting edge of progress for a period of time, and then the situation is reversed. We further propose that this cycle takes place at different levels of organization that mirror the prevailing general biological research of the day, initially starting at the organism level, and then becoming more basic with advances in technology. As such, the level of physiology one can describe is tied to the current state of structural categorization; thus, the categorization must precede the description of function (obviously).

The need to describe function is where the importance of dynamics comes in, as dynamic characterization is inherent in the description of causal mechanisms, and characterization of mechanism is the precondition for developing interventions. We would argue that it is the need to characterize dynamics that separates the First Biological Thread of developing taxonomies from the Second Biological Thread of studying physiology. We live in an era of technologies that can provide structural and component description of biological systems at increasingly higher resolution. Paradoxically, we suggest that this abundance of riches has created an imbalance between the ability and desire to categorize versus the need to describe function. The aforementioned two historical threads must be rebalanced in order to begin to address the current challenges in biomedical research. Moreover, we will also see that the Third Biological Thread, evolution, will also need to be integrated into the biomedical research paradigm, and we will propose how this can be accomplished. Again, we look to the history of these processes to identify the historical context of the current paradigms in biological and biomedical research. Having done this, we hope to delineate the preexisting factors that hinder us from accomplishing the goal of rebalancing the relationship between structure and function.

As with our discussion on the philosophy of science, we turn first to the Greeks for insight into the origins of our Western tradition. Looking at the works of Aristotle, the Father of Natural Science, and those of Hippocrates, the Father of Western Medicine, provides insight into how early this division arose. First and foremost, Aristotle's primary lasting impact in biology is through his systematic and orderly approach to categorizing living things. He based his classification on his observations of physical attributes, not only in terms of their external manifestations, but also employed dissection to gain insight into the common forms that lay beneath the skin. In so doing, he set the precedent for looking for "deeper" structures underlying what is evident only to the naked eye. His investigations into how living organisms functioned were reflections of the structures he identified, and served primarily to help him create his categories both of organisms (for instance those with closed circulatory systems versus those with open circulatory systems) and of organs (with their imputed roles). Less admirably, Aristotle also put forth that there was a progression from simpler organisms to more complex ones, the Great Chain of Being, with more complex organisms representing a transition toward more perfect forms, a process that culminating in humans. On the positive side, this can be seen as a description of proto-evolution, and the roots of phylogenetic trees. Unfortunately, on the negative side, Aristotle's concept of "progress" underlying biological complexity continues to bedevil the

understanding of evolution and its outcomes, and has often been invoked by decidedly anti-intellectual, antisocial, and antiscientific pseudomovements such as eugenics, Creationism, and Intelligent Design.

To a great degree in a concurrent and parallel fashion, Hippocrates utilized the same principles of observation and categorization to characterize human disease. Various symptoms and physical maladies were seen to occur together, lead to subsequent conditions, and sometimes respond to certain interventions. The similarity in the cycle of scientific/biological investigation and the process of diagnosis should not be surprising; both represent manifestations of the desire to acquire knowledge in a systematic fashion, and lends credence to the fundamental nature of Bacon's characterization of the Scientific Method nearly 2000 years later after the Greek pioneers. One significant difference, however, is the inherent medical goal of needing to characterize function. A diagnosis of disease is not enough; rather, there should also be a strategy for doing something about it. This points to a fundamental difference in the endpoint goals between biological and biomedical science: medical research is intrinsically applied knowledge, in which the explicit task of medicine is to attempt to improve human health. Therefore, biomedical research can be considered inherently "translational," as the goal is to translate biological knowledge into medical interventions.

Taken together, the Greek experience is interesting in the apparent early parallel developments of these two fields. Such is the effect of the Greek historical legacy that this circumstance, by and large, persisted for centuries. Certainly, the Greeks realized that humans were also biological creatures, but the embedded concept of human "specialness" (still an ongoing socioreligious issue today), and the explicitly applied nature of medical investigation, helped create a divergence between the trajectories of general biological research versus medical investigations. General biological investigation was dominated for a large portion of its history by the fields of zoology and botany, which focused on the discovery and categorization of organisms. Medical investigations (if we can call them that), at least essentially up to the nineteenth century, attempted to characterize function by operating mainly through trial and error, without a process of understanding how to acquire knowledge of fundamental principles of disease. We can see the legacy of both of these paths today in the gulf between basic science and clinical medicine.

Basic science research has followed the legacy of general biological science, with a root emphasis on categorization, identification of structure, and description. The general historical trend has been one in which biology has advanced by being able to execute those tasks at ever-finer degrees of resolution (see Aristotle's dissections, for instance). Prior to the invention of the microscope, classification of species in zoology and botany comprised the bulk of biological investigation. Expertise was characterized by the degree of detail provided in description, identifying the subtle differences that separated this species from that species. Attempts to understand the structural order underlying the diversity of life focused on detailed description of what was shared and what was different between organisms. The invention of the microscope and the discovery of the microscopic world brought with it an opportunity to describe and characterize at an increased level of resolution, with the same skills and processes for defining detailed descriptive lists now translated into the field of Histology. Thus, this emphasis on descriptive characterization can be seen as embedded historically in the practice of biology: where once naturalists counted feathers, noted patterns of scales, marked the shape of leaves, and listed the configuration of toes, today geneticists produce lists of base sequences and single nucleotide polymorphisms. The key point here is that for the vast majority of the history of biology, the emphasis was on identifying what made things unique and distinct. Thus, expertise became focused on the encyclopedic characterization of what made things different from each other, rather than emphasizing commonalities among them.

Why do we think this is significant? We believe the result of this particular historical thread is that it has caused biology to emphasize treating its subjects as special, unique cases, with the focus being placed on the differences seen across biological systems. The result of this mindset is that it has led to a conceptual paradigm that resists attempts to find unifying principles among biological systems. Is this the only influence on biological thought? No, certainly not; above we have already noted the existence of the two other historical threads of function and origins. But we assert that the primary nature in biological science of the urge to describe and categorize places a significantly impactful (though likely subconscious) bias toward what the basic science research community considers expertise in their particular field. This is exactly how Bacon's Idols of the Theater would manifest.

As we have noted above, the primacy of description and categorization is not unearned: these tasks must precede the investigation of Second Biological Thread (function) and the Third Biological Thread (origins). Certainly, both the Second and Third Threads advanced (and advanced knowledge) in the millennia following Aristotle and Hippocrates. However, the history of medical "science" prior to the nineteenth century is notable for what we today would perceive as superstition, magical thinking, and barbarism. To us, the invocation of diseases arising from disturbances in various bodily "humors" (blood, yellow bile, black bile, phlegm) themselves tied to four "universal" elements (air, fire, earth, and water, respectively) strikes us as fanciful. To design therapies based on this understanding of disease, i.e., bleeding a patient to treat a fever (heat = air + fire = blood + yellow bile), to us

seem ignorantly barbaric. It certainly did not help that for a significant period of Western European history, certain diseases were seen as the physical manifestation of moral shortcomings, a symptom of the human tendency to fall back on religion if corresponding knowledge did not exist or was not accessible. However, we think it would be unfair to cast too much aspersion to these practices given our relatively privileged cultural and historical perspective. Within the context of their time, these practitioners of early (and, may we say, prescientific?) medicine were operating using their reason to the best of their ability given the intellectual context in which they lived. To a great degree, it is possible to draw a rough analogy between the state of pre-nineteenth century medicine and the era of Ptolemic Geocentrism: both traditions heavily relied upon a primary authority drawn from a perceived Golden Age of the Greeks, and interpreted and invoked observed functions and behavior within that given preexisting mindset.

It is a reasonable assumption that the general level and capability of human intellect has remained unchanged since the origin of the species, manifest as observational capacity, creativity, curiosity, and interpretation, all controlled of course for the context of existing societal knowledge. Therefore, we should be aware that future societies, armed with a greater knowledge base, could just as easily look back upon our time as a primitive era. (For a whimsical example, in the movie "Star Trek IV: The Voyage Home," when Dr. McCoy is engaged as part of the operation to rescue Chekov from the "barbarities of twentieth century Medicine," he tosses off several *bon mots* reflecting his disdain for what we today consider state of the art.) With this in mind, we should turn our questioning minds as to how we, as a community of researchers, are able to avoid the intellectual and procedural traps that kept some very smart people doing some pretty silly (in retrospect) things.

To draw our analogy between biology and astronomy a bit further, one could characterize the difficulty in transforming biological science via the insights of the Enlightenment as there being no equivalent of Newton in the biological sphere. As we have seen, Newton brought mechanism to the physical world; such a transformation did not occur at the same time in biology. This is actually the root of the "physics envy" we discussed previously: since then, we biologists have been wishing for our own Newton and an equivalent Laws of Biological Behavior (or Fundamental Laws of Biology, a program run by the U.S. Defense Advanced Research Programs Agency [1]) to match his Laws of Planetary Motion.

One by-product of this desire is the presence of intermittent crossovers between the physical sciences and biology. A notable early example of this intersection is the interest in casting electricity as a motive biological force. Research that led to the understanding that nerves and muscles acted as conductors for electrical activity struck the imaginations of the time, and found its literary expression in Mary Shelley's *Frankenstein*.

From a positive standpoint, the recognition that living systems operated on mechanical principles led to substantial advances in physiology. Many organ systems could find their analogies in mechanical systems, the heart as a pump, the lungs as bellows, the kidneys as filters. The application of the physics of those systems led to unprecedented understanding about how biology actually functioned. And, critically, this understanding of biological function become manifest in a set of medical practices that continue to dominate today, in terms of cardiopulmonary bypass, mechanical ventilation, hemodialysis, prosthetic limbs, and artificial joints. While these are all biological replacements, they for the most part are based on a physical–mechanical interpretation of the relevant biology, and are correspondingly suited to characterization using concepts and methods from the physical sciences.

But what about fundamental driving forces specific for biology, independent (at least at first pass) from the realms of physics and chemistry? Or was deconstruction down to the level of physics description necessary to find driving principles for life? A negative manifestation of this desire to find something "special" about biology was the persistence of the concept of *vitalism*. This concept harkened back to our earliest division of the world into those things that were alive and those things that were not. Since alive things could be made un-alive, and life could spring from inert matter, it was natural to assume that there was "something" that, when added or removed, could affect this change. Today (as we will see) we know enough about the physical basis of biological systems to have (hopefully) cast aside the need to invoke mysterious supranatural forces (though, for a cautionary tale, see Chapter 2.5 on Complexity). Also, parenthetically, we see some kernels of persistence of this concept of "something undefinable" being present as we have moved away from focusing on what is alive to what makes us human, i.e., the properties that define our mind.

Fortunately, there were other significant findings about fundamental properties of biological systems that represented, at that time, a distinct departure from the physical sciences, notably the concept of Genetics and the description of Evolution. The stories of the lives and careers of Gregor Mendel (1822–1884) and Charles Darwin (1809–1882) and their respective discoveries have filled volumes much thicker than this one, so we will not recapitulate either of their lives in any detail. Rather, we wish to emphasize what each of these discoveries meant within the context of biological investigation, and how each provided what can be considered a novel approach toward examining biological systems. Both Genetics and Evolution represented the application of a process that could generate observed

phenomena known for centuries: breeding of animals and plants for specific characteristics extended back to the origins of agriculture and domestication, and zoologists and botanists knew that there were clear patterns in the categories of organisms they had cataloged. The introduction of what could be considered fundamental processes that transcended specific examples within biology, genetics, and evolution began to provide biology with something akin to the Laws of Motion and the Laws of Thermodynamics. Keeping in mind the desired effect of a theory to limit the range of possibilities within the system in which the theory is applied, the effect of genetics was to place constraints on the characteristics that could be passed from one generation to the next. Similarly, the Theory of Evolution placed limits on how organisms could come to be, and how communities of organisms could be structured. It is further evidence of their impact that while these theories arose independently, they complemented each other very well.

What was still to be elucidated was a means to link the fundamental laws of biology with the processes governed by physics and chemistry. Specifically, what was the physical object that provided the function described in genetics and evolution? To answer this question, we turn to a monograph written in 1944 by the physicist Edwin Schrödinger titled *What is Life?* In this book, collected from a series of lectures given at Trinity College in Dublin, Schrödinger (he of the indeterminately alive quantum mechanical cat, quite the meme in its day) sought to address the question: "How can the events in space and time which take place within the spatial boundary of a living organism be accounted for by physics and chemistry?" To approach this question, Schrödinger synthesized prevailing thought about what the carrier of genetic information could be, and provided a logical argument linking the properties necessary for this object to carry out its function, and how such an object not only could but would necessarily arise out of the laws of physics and thermodynamics to produce life. He described that this carrier of genetic information needed to be an "aperiodic crystal" that carried the genetic information in its chemical bonds; his description of the properties of the carrier of genetic information are credited by James Watson and Francis Crick as having inspired their investigations that eventually led their discovery that DNA was such a crystal. The age of molecular biology was born.

Reference

[1] Beard J. 50 Year of Bridging the Gap: DARPA's Bio-Revolution. Defense Advanced Research Project Agency, ed.

Suggested Additional Readings

The Origin of Species by Charles Darwin
Mendel's Principles of Heredity by William Bateson
Life Acending: Ten Great Inventions of Evolution by Nick Lane
The Epic History of Biology by Anthony Serafini
The Accidental Species: Misuderstandings of Human Evolution by Henry Gee
What Is Life? by Edwin Schrödinger

This page intentionally left blank

CHAPTER

2.3

Biomedical Research Since the Molecular Revolution: An Embarrassment of Riches

In that Empire, the Art of Cartography attained such Perfection that the map of a single Province occupied the entirety of a City, and the map of the Empire, the entirety of a Province. In time, those Unconscionable Maps no longer satisfied, and the Cartographers Guilds struck a Map of the Empire whose size was that of the Empire, and which coincided point for point with it. The following Generations, who were not so fond of the Study of Cartography as their Forebears had been, **saw that the vast Map was Useless...—*Jorge Luis Borges*, *"On Exactitude in science"***

If the eighteenth century was dominated by chemistry and the nineteenth and early twentieth centuries by physics, then certainly the latter part of the twentieth century was the time for biology. The discovery of DNA spawned the molecular biology revolution. The seismic shift in the orientation of biology that resulted cannot be understated, and has, in fact, been expounded upon in many excellent books. Herein, we will concentrate on the both the positive and—unfortunately for the current state of biomedical research—negative aspects of this and related developments. Let us begin with the positive. For one, as Schrödinger had foreseen, the descriptive nature of biology could now be grounded in fundamental laws and processes derived from the physical sciences. The power of this approach is that biologists could finally ask "how?" The age-old goal of being able to tie description with function, all while satisfying the Newtonian goal of reductionism, for the first time seemed possible: we were now finally able to speak legitimately of understanding the "machinery of life."

In the pursuit of gaining insight into this machinery, the burgeoning field of molecular-oriented biology (which continues to dominate the general field of biology today) sought to meet two of the core aspects of the Newtonian paradigm, drawn from what was considered, subconsciously if not explicitly, the "parent" science of physics. These two aspects are: (i) the concept of fundamental laws and (ii) the manifestation of those laws as mechanisms. As we have seen in our survey of the history of science, the focus on establishing fundamental principles as seen in the Newtonian paradigm has its roots in the basic human urge of finding order in the universe. The position of physics as the most fundamental science is predicated upon the fact that physical processes underlie all of the phenomena observed in the material world. The laws of physics provide constraints on what is possible, and they percolate up into the types of chemical reactions that are allowed, and what form and output those reactions can take in various circumstances. Inherent in this mindset is that all material phenomena can somehow be *reduced* into physical processes, which in turn can be characterized by the laws of physics. This is the root of the *reductionist* paradigm, which presupposes that the pathway to understanding can come from the understanding of the underlying processes at increasingly detailed levels of resolution, perhaps all the way down to the domain of particle physics. Even if the end point of the investigation does not progress all the way down to the subatomic world, the primary exploratory process consists of studying a system by breaking it down into its constituent components, studying them, and then reconstructing the aggregate behavior. We will see below how the deconstruction process is integral to the execution of the Scientific Method but will also later show at what point this strategy breaks down in the reconstruction phase. It is this latter aspect of the molecular biology revolution to which we ascribe a fair bit of the blame for the current morass in which biomedical research finds itself.

Another inherent concept of fundamental laws is that they are shared across the entire range of phenomena to which they are applied. Here, biology runs into a bit of a paradox, based on its historical emphasis on characterizing differences between organisms: we know that organisms A and B are different, but they are also quite similar in some ways. The question, then, is in what way are they similar? Given the historical emphasis in biology on categorization via morphology, similarity has generally been characterized in terms of structural characteristics and by placement within the phylogenetic tree. For example, using humans as a reference point, primates are more similar to humans than rodents, rodents are more similar to humans than fruit flies, fruit flies are more similar to humans than nematodes, nematodes are more similar to humans than yeast, yeast are more similar to humans than bacteria, and bacteria are more similar to humans than very small stones. The ability to assess and potentially defend similarity becomes critical with the development and growing emphasis on experimental biology, which is predicated upon the use of biological proxies to gain insight into potentially shared mechanisms and characteristics. It is at this point that we need to introduce the concept of using models as part of the scientific process.

Central in the pursuit of mechanistic understanding is the use of the experimental method, which we have seen as initially promoted by Bacon: is only through the use of the Scientific Method that causal hypotheses (= mechanistic knowledge) can be derived. Since the hypotheses generated in the execution of the Scientific Method are the product of induction, the empiricism of Hume tells us that these hypotheses cannot ever be proven definitively, but rather only can aspire to become trusted by the collection of a "definitive" degree of evidence. The task of establishing the criteria for what could be considered "a definitive degree" became the province of statistics; we discuss statistics and statistical/data-driven modeling in Chapters 4.1 and 4.2. What at first may have seemed a relatively straightforward question: "Does this outcome/phenomenon happen more often than would be expected from random events?" has since morphed into a mathematical discipline in of itself. The power of statistics to identify correlations is undeniable, but all too often it is forgotten that the output of any statistical model can only identify an association or correlation pattern among data sets: the *reason* for that pattern being present requires the additional step of performing abduction, à la Pierce. In short, the outcome of a statistical test cannot tell you a mechanism, it can only allow you to impute or abduce one, which must then be subjected to future testing. To say "correlation is not causality" is a scientific truism, but unfortunately, like many truisms, has become so pervasive that its fundamental importance can become lost as newer, more complex and more enticing technologies arise. We will see how this is the case when we talk about the limitations of Big Data and data-driven modeling.

In terms of its impact on the development of experimental biology, the important role of statistics in determining what could be considered a "significant" experimental result necessarily fed back as constraints on the design of the experiment; i.e., the design parameters of an experiment became focused on whether it could be a statistically significant experiment. This introduces the concept of a "good" experiment. The general scientific community has a strong consensus as to what constitutes a good experiment: controlled, predetermined conditions that limit unintended variability within the experiment, a control group representing the base condition, a single perturbation/variable that separates the control group from the perturbed group, and enough samples of each experimental group such that a predetermined degree of statistical significance can be obtained. These are the minimal criteria to create a valid, interpretable experiment. Note, however, that the relationship between the experimental platform and the intended target of investigation is *not* part of this characterization. Rather, the emphasis on what constitutes a "good experiment" is only concerned with the operations of the experiment as an endeavor in of itself, not necessarily on what that experiment means in relation to the subject being studied. In fact, this type of discussion is all too often considered "speculative" and somehow nonscientific (strange, given the driving impetus for science to explain phenomena). The emphasis is placed overwhelmingly on the quality and interpretability of specific experiment and its specific results. On one hand, this is completely understandable and necessary: if the "atomic" process in the Scientific Method is the experiment, it is certainly critically important to know that the first-order conclusions drawn from that experiment are as correct as possible. However, our goal is to reemphasize that the experiment is ostensibly being performed with the purpose of studying something else, and so we provide a reminder that the experiment at hand is only a means to an end. The fact that we compelled to make this reminder draws from our (admittedly subjective) experiences in scientific conferences and as reviewers for papers and grants: all too often the critiques and discussions focus entirely on methodological issues related to the execution of the specific experiments at hand, rather than the big picture that the experiment is supposed to elucidate. Again, we recognize the importance of critique at this level, but the overwhelming emphasis on experimental procedure overshadows the even more basic question: "Does this experiment, even if performed perfectly, tell you what you think its telling you about the system you are trying to study?" The infrequency of this question and the associated discussion substantially restricts the utility of any conclusions drawn from the experiment and, at best, limits the impact of the experiment within the context of an overall (and by this we mean the overall research community knowledge base)

research strategy. At worst, the lack of "speculation" about the higher-order conclusions and insights that can be drawn from the specific experiments carried out within a single study generates intellectual cul-de-sacs in which significant time, effort, and resources can be spent. (Note: In later chapters we will demonstrate that, given the structure and incentives of the current biomedical research environment, these cul-de-sacs are not actually an "at worst" scenario, but rather a desirable target for a sustained professional career. This produces an analogy that corresponds to achieving a nice, pastoral ideal, but unfortunately at the expense of advancing human health.)

A substantial part of the problem is that this step of synthesis and contextualization is extremely poorly formalized; there are no traditional formal processes for the synthesis of knowledge that correlate to those developed for experimental execution and interpretation, and as a result the step involving synthesis, integration, and contextualization of knowledge have largely been left to the intuition of the researcher. We will see that the approach of Translational Systems Biology is to target precisely this step of knowledge integration and synthesis within the larger Scientific Cycle.

But before we go there, we wish to point out another effect of this restricted view of what constitutes the goal of experimental biology. If we are following the Newtonian paradigm, which says that we can apply reductionism to study biology and that there are sufficient similarities to justify using proxy biological objects (i.e., models), and that there are design constraints imposed by the statistical standards of evaluating that experiment, then a great deal of emphasis must be placed on the creation of these biological proxy objects. This now becomes an engineering task: how can I engineer this biological system in order to meet the design constraints and features noted above? The nontriviality of this task is immediately evident in a survey of publications generated and careers built and sustained that focus on the development of different cell culture lines, genetically modified cells and animals, and the methods for molecular manipulation necessary to generate them. We recognize that this is an absolutely necessary endeavor: it is only through the generation of these biological objects that experimental biology can be realized. However, we also wish to point out what we perceive to be an unintended consequence of the emphasis on the creation of experimentally tractable models: the more they are engineered in order to meet the constraints required for a "good experiment," their ability to serve as actual proxies for the system actually being studied can become more and more impaired. In short, the engineering of biological models produces highly artificial objects that may not reproduce the important and significant characteristics of the system they were initially intended to help study. Again, we wish to emphasize that we realize the importance of this practice, but at the same time note that there are multiple assumptions inherent to the use of biological models that must be recognized.

More importantly, what can be done about this disconnect between reductionist experiments and the need to put those experiments in a larger context? What methods can be utilized to be able to leverage the knowledge generated by the use of these artificial systems? We will propose and describe later in this book how computational modeling can be used to accomplish this task, an approach that carries with it not a little irony, since computational models are often heavily critiqued for not mapping to the real world. Any yet, this mapping is often overlooked when dealing with clearly artificial biological models. To paraphrase G.E. Box, while all computational models *might* be wrong, all biological models are *certainly* wrong; the task is to find out how to make them usefully wrong.

There are certainly noncomputational means by which to identify the relationship between a model and its referent: this is the mapping process we had previously described in our survey of the history of science. Unfortunately, this process is not well formalized in biological and biomedical research; however, mapping is practiced on an intuitive and *ad hoc* level frequently, and sometimes in a tremendously constructive manner (several good examples from the field of inflammation can be found here: [1–3]). We have already seen the rudimentary level of mapping in our example of what organisms are more similar to humans than others; the main question is how are they more similar, and how can that be used to interpret an experiment. In addition to mapping the selected model organisms to the reference system of interest, it is also necessary to map the intended outputs/observables produced from the experiment: do they mean the same thing to the model organism as they do to the intended reference system (for sake of argument, let us say that is a human being; see the recent controversy regarding this point in the field of acute inflammation [4]). It should be noted that all this mapping is done at the level of phenotypic description: in order to be clear, we use the Merriam-Webster's definition of phenotype: "the observable properties of an organism that result from the interaction of the genotype and the environment." Note that this spans the range of observable metrics from number of limbs, to physiological vital signs, to circulating and tissue mediators. Note that this also includes cellular gene expression patterns, which represents the organism's response to environmental stimuli based on its germ line genomic structure (which represents its genotype). The key point here is to recognize that the output of most "omics" analysis represents a snap shot of the state of the organism/cell that may be dynamically changing over time. This goes back to perhaps earlier distinction, where the genotype represented the behavioral potential of an organism and the "programming" that was passed on through procreation, while phenotype

represents the multiple forms that organism might take during its lifetime as generated by the intersection of its genetic potential and environmental factors. In order to interpret the output of a biological model, it is necessary to be as explicit as possible in determining what the mapping is between our phenotype of choice. For instance, we know the conversion from murine blood pressure to human blood pressure; therefore, we can say with some degree of certainty that one level in a mouse corresponds physiologically to another in a human.

Note that up to this point we have not addressed anything specifically related to technological advances; we have only discussed the fundamental aspects of what happens when you think biology can be decomposed into physics and chemistry and decide that is the way biology will be done. We mention this because the allure of molecular biology is so great, and it is so easy to become enamored with the latest method aimed at looking deeper and smaller and finer, that it is unreasonably easy to lose perspective of the inherent assumptions and prejudices associated with the endeavor itself. Let's face it: we love our methods. Humans are tool makers at their core, and the amount of inventiveness and intellectual capital expended at making a better mouse trap (or imaging technology, or cell line, or genetically modified animal, or network inference algorithm, or modeling language, *ad infinitum*…) makes us want to justify that task. Coupled with a reductionist paradigm, the drive for method development can take a codependent life of its own, thereby limiting the beneficial aspects of both method-making creativity and the power of reductionist science.

One need not look any further than the history of the study of DNA. It took some time after the identification of DNA as the carrier of genetic information for experimental biology engineering to develop the necessary tools to study it. First of all, the recognition that DNA was what carried the genetic code identified biology's reductionist target: as physics had the atom, biology has the gene. Second, just as physics had developed its particle accelerators in order to take the atom apart into its constituent components, so too would biology need to develop technologies to take apart the gene into the pieces and structure of DNA in order to gain insight into how it all worked. Moreover, this pursuit for more and more detailed characterization was bolstered by what was perceived as a fundamental concept (and so semi-appropriately titled) "The Central Dogma of Molecular Biology," first stated by Francis Crick in 1958. The Central Dogma can be summarized as: Genetic information flows from DNA→RNA→Protein→Function. While the limitations of the Central Dogma are now recognized, it was the primary conceptual model that drove research in molecular biology, overwhelmingly for its first half century, and still significantly in the years since. The rationale for the investigatory process developed under the directive of the Central Dogma can be described thusly: since DNA sequences determine function, if we can find out all the DNA sequences, then we can characterize all function. Implicit to this task was the rationale that if the ATCG "code" could be "broken," then the "secrets of life" would be unveiled. Experimental method engineering concerning DNA therefore focused on developing methods of finding the particular sequences of DNA within genes. Suffice it to say that the successes in developing means of base location, identification, recombinant DNA technologies, and the automation of those technologies laid the groundwork for what was thought of at the time as the Holy Grail of biomedical research, the Human Genome Project. It was believed that if we could completely map the human genome then it would provide the basis for answering the puzzles of human biology and disease. Again, the code-breaking analogy was heavily in play, with the supposition that once the cipher key was found then the messages within the code would be unveiled.

While this work was taking place, other researchers generated sets of experimental tools to characterize the molecular pathways that defined biology. These tools took the form of the artificial biological objects we noted above, cell lines, modified animals and the interventions used to perturb them, as well as the assays needed to look at the presence and levels of the molecules and pathways that drove their behavior. These investigations necessarily took the reductionist approach and followed the tenets of good experiments: single pathways were isolated, individual molecules within those pathways were identified, and their responses to different manipulations were evaluated. This type of characterization was a godsend to the pharmacology community, as it provided a reproducible process that could be used, at large scale, to engineer potential manipulations to various conditions. The preclinical research pipeline became essentially standardized, with a defined set of experimental models that through which a particular compound would be tested up until the point of conducting a clinical trial. There were incremental advances as new methods came online, but the overall structure of this research and development process remains essentially unchanged even today (which, unfortunately, is part of the problem) [5]. The unlocking of the Human Genome was sure to provide the final piece of information that would make the remainder of the drug development endeavor just a matter of being able to do enough experiments in order to solve a particular problem: finding cures would become something limited by resources, not knowledge. Here, we see the convergence of the Newtonian promise of molecular biology. We have our fundamental law: the Central Dogma. We have our process in which to characterize a disease process: reductionist experiments. We have the manifestation of what based on the Central Dogma should define a human being: the Human Genome. What could possibly go wrong?

It goes without saying that, of course, we have the benefit of retrospection as we list what went wrong. The inevitable advantage of hindsight is that we know what happened; we have information that very smart people in the past did not have. We have already seen this phenomenon when we discussed biomedical research in its prescientific phase: knowing how it comes out can make anyone *seem* like a genius or a critic (or both). So "yes," the issues we will note below now seem self-evident and totally obvious to us today. But our goal is to suggest that knowledge of the past, particularly the fundamental mental processes at play, can provide us with some strategy of how to avoid similar issues today. And, we are not the first to note the potential difficulty, if not futility, of the endeavor to gain biologically meaningful knowledge for the sum total of "omic" data [6,7].

Once the technology to sequence DNA reached a point where we were collecting more and more sequences, it was becoming apparent that the Central Dogma seemed to have some cracks. Much of the genome, in fact most of it, did not appear to actually code for proteins. Initially as a result of the assumption of the Central Dogma, this DNA was initially assumed to be "nonfunctional." The subsequent realization that these noncoding regions performed regulatory functions helped point out the limitations of the Central Dogma. Additionally, there appeared to be multiple types of RNA that, while they did not get translated into protein, also had significant function. Furthermore, many, if not most, proteins required posttranslational modifications in order to carry out their biological activity. Finally, it was also apparent that genes could have their function permanently modified without affecting their actual sequence via epigenetic changes. What had previously been thought as the only way to generate biological function, i.e., through the transcription of DNA to RNA and then translation of RNA to protein, now was just one of many possible means of converting the genetic code into biological activity. This realization added several additional layers of complexity into the interpretation of the human genetic code. Rather than breaking a code with a cipher, it now became a task akin to translating a work of prose or poetry from one language into another.

The bigger problem was with the reductionist paradigm. The overall complexity of the pathway structure in biological systems was well recognized, and there was a pervasive belief that the soundness of experimental process would be able to parse out significant effects from nonsignificant ones. To a certain degree, biomedical research counted on their reference phenotypes to coalesce the multiple interactions present into statistically significant conclusions: to make an analogy, it did not matter what was going on under the hood if the car still got you where you wanted to go. This was fine, as long as the preclinical research translated to clinical efficacy. But, unfortunately, starting in the late 1980s, the success in translating what were considered to be near certainly effective candidates started to fall off, and in dramatic ways. Potential drugs directed at sepsis, at cancer, at immunological diseases, at cardiovascular diseases, started not to perform as expected in clinical trials. In the area of sepsis (our area of expertise), the failures of a series of molecular interventions directed against what were perceived as principle mechanistic drivers of the sepsis syndrome led to what could be considered an existential crisis within the sepsis research community. These drugs should have worked, by all accepted metrics of preclinical efficacy; yet they did not. This is the classic manifestation of the Translational Dilemma: the inability to translate basic mechanistic biomedical knowledge into an effective therapeutic.

The mainstream research community cycled back to their basic strategies and focused on two main issues. The first was that they had insufficient information: the characterization of the target disease process was too nonspecific in terms of biological mechanism. The "disease" previously thought to be a single entity in actuality represented a multiplicity of potentially nonequivalent processes; therefore, understanding what to target with which drug required finer granularity in the definition of the disease. This realization led first to biomarker panels, then to DNA microarray (transcriptomic) analysis, and now various forms of "omics" characterization. Where previously a disease might be, at most, defined by a few alterations in biomarkers, now thousands of data points are used to characterize the disease "phenotype." This is the pathway to Big Data.

The second issue was that perhaps the biological proxies we used in the preclinical investigations did not actually represent what we thought they did in terms of being able to reproduce the essential aspects of the target disease process [4]. This has proven to be a more difficult challenge to address, since the fundamental limitations in terms of the design parameters for experimental platforms remain, i.e., reproducibility, reductionism, and strength of experimental signal. Here also the adoption of finer-grained phenotype description has been proposed as a means of trying to link experimental models to each other and to human conditions, though this strategy is fraught with its own challenges. While there may be some similarities in terms of organ level or systemic phenotype, and certain shared properties and functions between relatively analogous pathway modules, there are going to be specific differences between species in terms of their absolute configurations.

An example of this is the recent controversy over the mapping between the mouse and human response to trauma and sepsis [4,8]. On one hand, of course a mouse is not a man, therefore, from a detailed descriptive level one, could

not expect to directly translate the alterations in one species to the alterations in the other. At some fundamental level, however, there must be some way to identify what aspects of the responses are similar, and can therefore allow them to be compared. What is pretty certain by now, however, is that constructing a detailed parts list is not the way to do it.

Unfortunately, we see all these attempts to address the Translational Dilemma as having their roots in the tradition of biology to emphasize description. The heterogeneity of biological systems is a given; the tradition of biology suggests that greater insight can be obtained by describing that heterogeneity at ever greater detail. This is the paradigm that is sweeping the biomedical research community now, the manifestation of the current general societal fascination with Big Data, and seen in the emphasis on genome-wide association studies (GWAS), molecular profiling of tumors and patients, transcriptomics, metabolomics, metagenomics, metatranscriptomics, and whatever new "omics" becomes the latest trend. The promise of Big Data is that this methodology will provide a means of untangling the multiplicity of effects present in biology and allow an answer to emerge.

We suggest caution. We will discuss the issues related to and a place for Big Data in a later chapter, but suffice it to say, that given our penchant to look at fundamentals, we note that it should be immediately evident that the correlations offered by Big Data are only one component in the application of the Scientific Method to the Translational Dilemma. Remember our truism: correlation is not causality, and the goal of intervention requires an understanding of mechanistic causality. The step to move beyond Big Data is recognizing that we must vastly expand how we do the experiments we need to do in order to evaluate mechanism. Figure 2.3.1 depicts how the Scientific Cycle appears now in a high-throughput, Big Data environment (compare with Figure 2.1.1); there is a clear bottleneck in the iterative process at the point that requires hypotheses to be tested. The need to address the scientific step of hypothesis testing and evaluation arises out of our root-cause analysis and diagnostic approach to the Translational Dilemma, but the persistent and future consequences of this imbalance should be readily evident in this time and now. We should not have to wait until what should be recognized as an unrealistic promise of an approach (i.e., Big Data supplanting the Scientific Cycle) has been demonstrated to be so; we have the opportunity to act to implement a research strategy now that we can anticipate we will need in the future. We recognize, however, that there are multiple barriers to acting in such a manner, but we hope that the information in this book will move us away from functioning in a perpetual near-crisis mode, particularly with our health is at stake.

We've been introduced to the Translational Dilemma, and to this point we've focused on how we obtain the knowledge to decide how to potentially develop new ways of treating patients and enhancing human health, and some of the issues related to that pathway. But we've also been introduced to the translational barrier presented by

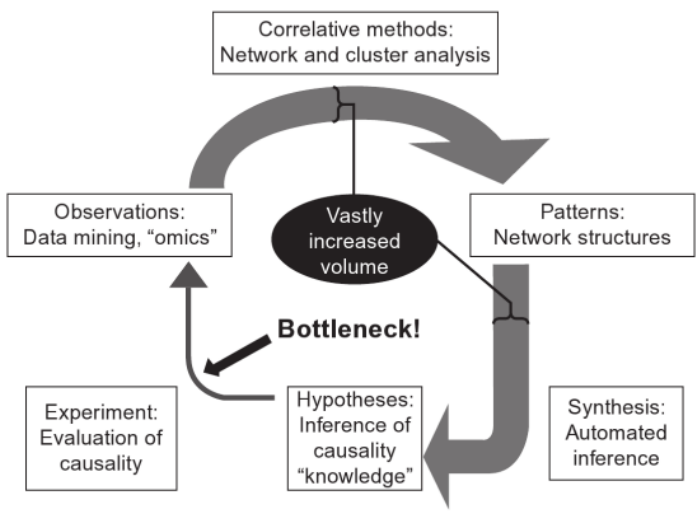

FIGURE 2.3.1 Imbalanced scientific cycle in a high data environment. The current state of the Scientific Cycle following advances in the extraction of experimental data (high-throughput "omics" methods), the development of correlative methods of data analysis (clustering algorithms, machine learning), and the generation of potential hypotheses (automatic inference). In essence, the process of acquiring data and identifying patterns in these data has become parallelized, leading to an exponential increase in the number of potential hypotheses to be tested. However, the process of experimental testing remains a time-consuming, serial process, limited in terms of resources, person-hours, and the time scale of biological processes (i.e., cells have to grow and animals need to respond to experimental stimuli). This imbalance has led to a bottleneck at the critical step of hypothesis evaluation, and currently manifests as the Translational Dilemma.

the clinical trial; now we turn our attention to examining, in a critical fashion, this last step between a good idea and an effective therapeutic.

References

[1] Nathan C. Points of control in inflammation. Nature 2002;420(6917):846–52.
[2] Nathan C, Sporn M. Cytokines in context. J Cell Biol 1991;113:981.
[3] Tracey KJ. The inflammatory reflex. Nature 2002;420(6917):853–9.
[4] Seok J, Warren HS, Cuenca AG, Mindrinos MN, Baker HV, Xu W, et al. Genomic responses in mouse models poorly mimic human inflammatory diseases. Proc Natl Acad Sci USA 2013;110(9):3507–12.
[5] An G, Bartels J, Vodovotz Y. *In silico* augmentation of the drug development pipeline: examples from the study of acute inflammation. Drug Dev Res 2011;72:1–14.
[6] Csete ME, Doyle JC. Reverse engineering of biological complexity. Science 2002;295(5560):1664–9.
[7] Mesarovic MD, Sreenath SN, Keene JD. Search for organising principles: understanding in systems biology. Syst Biol (Stevenage) 2004;1(1):19–27.
[8] Osuchowski MF, Remick DG, Lederer JA, Lang CH, Aasen AO, Aibiki M, et al. Abandon the mouse research ship? Not just yet! Shock 2014;41(6):463–75.

This page intentionally left blank

CHAPTER

2.4

Randomized Clinical Trials: A Bridge Too Far?

The desired final outcome of all the research and development discussed in the prior sections is proof that the proposed intervention will actually work by improving the health outcome in a real human population taking the drug. The importance and significance of this step cannot be overstated: the evaluation of a therapy in a clinically relevant population is the final hurdle that will determine whether the drug is approved by regulatory agencies (e.g., the U.S. Food and Drug Administration), which in turn will determine whether the drug goes to production, and set into motion an intense, expensive, and influential marketing campaign to get doctors to use and/or prescribe the drug. The entire process leading up to the clinical trial is—or should be—based on the Scientific Method, and so it would seem logical to suppose that the Scientific Method would be used as the cornerstone of this final evaluation. Based on the criteria set forth in Bacon's Scientific Method, that final evaluation takes the form of a randomized, prospective clinical trial powered to determine the efficacy of the drug. Let us take a brief look at the components and justification thereof that make up what is considered the "Gold Standard" of clinical evidence.

Keeping in mind that we are still essentially operating in the Baconian/Newtonian world of evaluating a single variable (i.e., the therapy), the underlying rationale for a Randomized Prospective Clinical Trial is very familiar: it is the same rationale that underlies what is traditionally considered to be a "good experiment." Therapeutic drug and devices reach their ultimate end user—the patient—via a multistep process. This process culminates in regulatory approval (e.g., the U.S. Food and Drug Administration). This process generally consists of years/decades of basic research to identify candidate therapeutic targets, followed by sequential studies to demonstrate safety and some acceptable degree of efficacy (e.g., dosage or timing that results in greatest therapeutic benefit with least harm) in both experimental animals and humans. The final step is a pivotal (Phase III) clinical trial, which is randomized (i.e., subjects that meet predecided inclusion and exclusion criteria are recruited into either a placebo or treatment arm in a random fashion) and double-blinded (i.e., neither the clinician nor the patient knows *a priori* the study arm in which the patient is enrolled) [1–5]. The enrollment into this Phase III trial is usually not individualized in any fashion beyond the set inclusion and exclusion criteria (and, of course, the withdrawal of a patient from the study if certain predecided adverse events occur). This process is considered the *sine qua non* of clinical translation, and it has indeed resulted in numerous drugs and devices available to physicians to treat diseases.

Briefly described, a Phase III trial incorporates the following features. The study population is divided into two equally characterized groups; one group serves as the control group, representing the absence of the intervention, while the other group receives the intervention. The potential for cognitive bias (having its origins in Bacon's Idols of the Mind) is recognized, and steps are taken to try to reduce their effect. These steps include *randomization*, which the placement of an individual into control or treatment groups using some random means, *blinding*, which is the process by which the investigators do not know which group a particular individual is assigned, and being *prospective*, meaning that the data are being collected from the initiation of the study forward and therefore data analysis cannot be influenced by any prior knowledge about what outcome is desired or sought. At the end of the experiment, a statistical analysis is performed to determine if any resulting difference between the two groups is greater than would be expected by random events, and if so then the intervention is deemed to be beneficial. Note that this is the simplest description for the ideal circumstance of a clinical study. We will see shortly that there are