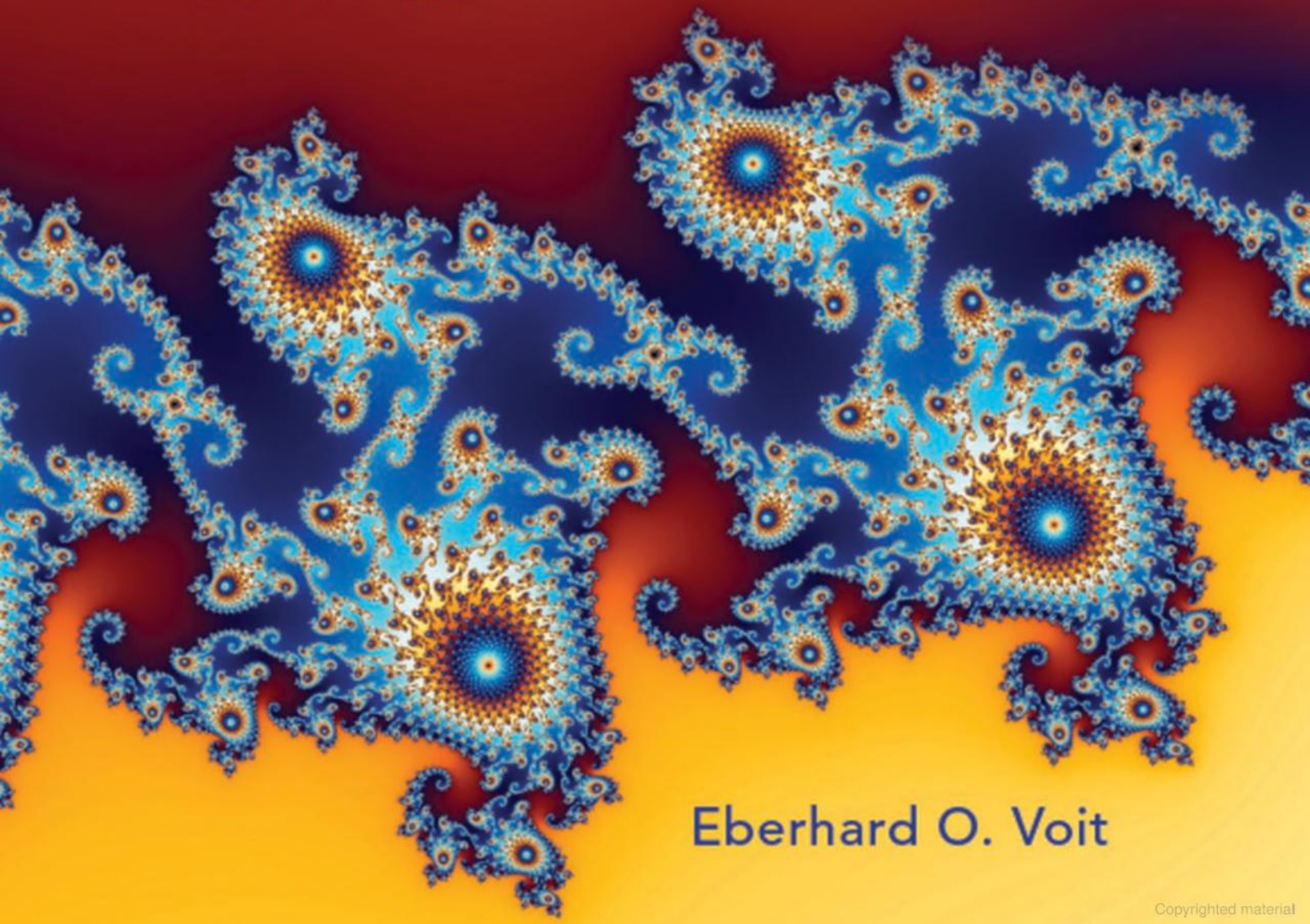
Garland Science

A FIRST COURSE IN SYSTEMS BIOLOGY

SECOND EDITION



Eberhard O. Voit

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Front cover image. The beautiful geometric shape of the fractal is called self-similar because it has the same appearance at smaller and smaller scales. It reminds us of fundamental design features like feedback loops that we encounter at many organizational levels of biological systems. Fractals are generated with nonlinear recursive models, and they are discussed with simpler examples in Chapter 4. (Courtesy of Wolfgang Beyer under Creative Commons Attribution-Share Alike 3.0 Unported license.)

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Biological Systems

1

When you have read this chapter, you should be able to:

- Describe the generic features of biological systems
- Explain the goals of systems biology
- Identify the complementary roles of reductionism and systems biology
- List those challenges of systems biology that cannot be solved with intuition alone
- Assemble a "to-do" list for the field of systems biology

When we think of biological systems, our minds may immediately wander to the Amazon rainforest, brimming with thousands of plants and animals that live with each other, compete with each other, and depend on each other. We might think of the incredible expanse of the world's oceans, of colorful fish swimming through coral reefs, nibbling on algae. Two-meter-high African termite mounds may come to mind, with their huge colonies of individuals that have their specific roles and whose lives are controlled by an intricate social structure (**Figure 1.1**). We may think of an algae-covered pond with tadpoles and minnows that are about to restart yet another life cycle.

These examples are indeed beautiful manifestations of some of the fascinating systems nature has evolved. However, we don't have to look that far to find biological systems. Much, much smaller systems are in our own bodies and even within our cells. Kidneys are waste-disposal systems. Mitochondria are energy-production systems. Ribosomes are intracellular machines that make proteins from amino acids. Bacteria are amazingly complicated biological systems. Viruses interact with cells in a well-controlled, systemic way. Even seemingly modest tasks often involve an amazingly large number of processes that form complicated control systems (**Figure 1.2**). The more we learn about the most basic processes of life, such as cell division or the production of a metabolite, the more we have to marvel the incredible complexity of the systems that facilitate these processes. In our daily lives, we usually take these systems for granted and assume that they function adequately, and it is only when, for example, disease strikes or algal blooms kill fish that we realize how complex biology really is and how damaging the failure of just a single component can be.

We and our ancestors have been aware of biological systems since the beginning of human existence. Human birth, development, health, disease, and death have long been recognized as interwoven with those of plants and animals, and with the environment. For our forebears, securing food required an understanding of seasonal changes in the ecological systems of their surroundings. Even the earliest forays into agriculture depended on detailed concepts and ideas of when and what to

Figure 1.1 Biological systems abound at all size scales. Here, a termite mound in Namibia is visible evidence of a complex social system. This system is part of a larger ecological system, and it is at once the host to many systems at smaller scales. (Courtesy of Lothar Herzog under the Creative Commons Attribution 2.0 Generic license.)

plant, how and where to plant it, how many seeds to eat or to save for sowing, and when to expect returns on the investment. Several thousand years ago, the Egyptians managed to ferment sugars to alcohol and used the mash to bake bread. Early pharmaceutical treatments of diseases certainly contained a good dose of superstition, and we are no longer convinced that rubbing on the spit of a toad during full moon will cure warts, but the beginnings of pharmaceutical science in antiquity and the Middle Ages also demonstrate a growing recognition that particular plant products can have significant and specific effects on the well-being or malfunctioning of the systems within the human body.

In spite of our long history of dealing with biological systems, our mastery of engineered systems far outstrips our capability to manipulate biological systems. We send spaceships successfully to faraway places and predict correctly when they will arrive and where they will land. We build skyscrapers exceeding by hundreds of

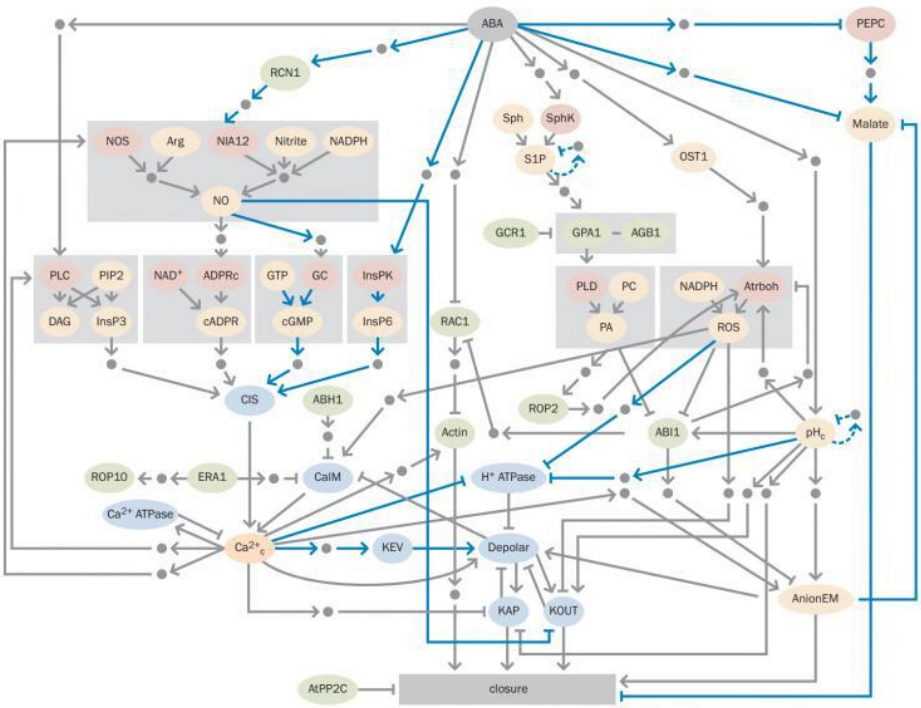

Figure 1.2 Diagram of a complicated system of molecules that coordinate the response of plants to drought. While the details are not important here, we can see that a key hormone, called abscisic acid (ABA), triggers a cascade of reactions that ultimately promote the closure of stomata and thereby reduce water evaporation [1]. Even a narrowly defined response like this closure process involves a complicated control system that contains a multitude of molecules and their interactions. In turn, this system is just one component within a much larger, physiological stress response system (cf. Figure 1.7). (From Saadatpour A, Albert I & Albert A. *J. Theor. Biol.* 266 [2010] 641–656. With permission from Elsevier.)

times the sizes of the biggest animals and plants. Our airplanes are faster, bigger, and more robust against turbulence than the most skillful birds. Yet, we cannot create new human cells or tissues from basic building blocks and we are seldom able to cure diseases except with rather primitive methods like cutting into the body or killing a lot of healthy tissue in the process, hoping that the body will heal itself afterwards. We can anticipate that our grandchildren will only shake their heads at such medieval-sounding, draconian measures. We have learned to create improved microorganisms, for instance for the bulk production of industrial alcohol or the generation of pure amino acids, but the methods for doing so rely on bacterial machinery that we do not fully understand and on artificially induced random mutations rather than targeted design strategies.

Before we discuss the roots of the many challenges associated with understanding and manipulating biological systems in a targeted fashion, and our problems predicting what biological systems will do under yet-untested conditions, we should ask whether the goal of a deeper understanding of biological systems is even worth the effort. The answer is a resounding "Yes!" In fact, it is impossible even to imagine the potential and scope of advances that might develop from biological systems analyses. Just as nobody during the eighteenth century could foresee the ramifications of the Industrial Revolution or of electricity, the Biological Revolution will usher in an entirely new world with incredible possibilities. Applications that are already emerging on the horizon are personalized medical treatments with minimal side effects, pills that will let the body regain control over a tumor that has run amok, prevention and treatment of neurodegenerative diseases, and the creation of spare organs from reprogrammed stem cells. A better understanding of ecological systems will yield pest- and drought-resistant food sources, as well as means for restoring polluted soil and water. It will help us understand why certain species are threatened and what could be done effectively to counteract their decline. Deeper insights into aquatic systems will lead to cleaner water and sustainable fisheries. Reprogrammed microbes or nonliving systems composed of biological components will dominate the production of chemical compounds from prescription drugs to large-scale industrial organics, and might create energy sources without equal. Modified viruses will become standard means for supplying cells with healthy proteins or replacement genes. The rewards of discovering and characterizing the general principles and the specifics of biological systems will truly be unlimited.

If it is possible to engineer very sophisticated machines and to predict exactly what they will do, why are biological systems so different and difficult? One crucial difference is that we have full control over engineered systems, but not over biological systems. As a society, we collectively know all details of all parts of engineered machines, because we made them. We know their properties and functions, and we can explain how and why some engineer put a machine together in a particular fashion. Furthermore, most engineered systems are modular, with each module being designed for a unique, specific task. While these modules interact with each other, they seldom have multiple roles in different parts of the system, in contrast to biology and medicine, where, for instance, the same lipids can be components of membranes and have complicated signaling functions, and where diseases are often not restricted to a single organ or tissue, but may affect the immune system and lead to changes in blood pressure and blood chemistry that secondarily cause kidney and heart problems. A chemical refinery looks overwhelmingly complicated to a layperson, but for an industrial engineer, every piece has a specific, well-defined role within the refinery, and every piece or module has properties that were optimized for this role. Moreover, should something go wrong, the machines and factories will have been equipped with sensors and warning signals pinpointing problems as soon as they arise and allowing corrective action.

In contrast to dealing with sophisticated, well-characterized engineered systems, the analysis of biological systems requires investigations in the opposite direction. This type of investigation resembles the task of looking at an unknown machine and predicting what it does (**Figure 1.3**). Adding to this challenge, all scientists collectively know only a fraction of the components of biological systems, and the specific roles and interactions between these components are often obscure and change over time. Even more than engineered systems, biological systems are full of sensors and signals that indicate smooth running or ensuing problems, but in most

Figure 1.3 Analyzing a biological system resembles the task of determining the function of a complicated machine that we have never seen before. Shown here as an example is the cesium fountain laser table of the United States Naval Observatory, which is used to measure time with extreme accuracy. This atomic clock is based on transitions in cesium, which have a frequency of 9,192,631,770 Hz and are used to define the second. See also [2].

cases our experiments cannot directly perceive and measure these signals and we can only indirectly deduce their existence and function. We observe organisms, cells, or intracellular structures as if from a large distance and must deduce from rather coarse observations how they might function or why they fail.

What exactly is it that makes biological systems so difficult to grasp? It is certainly not just size. **Figure 1.4** shows two networks. One shows the vast highway system of the continental United States, which covers several million miles of major

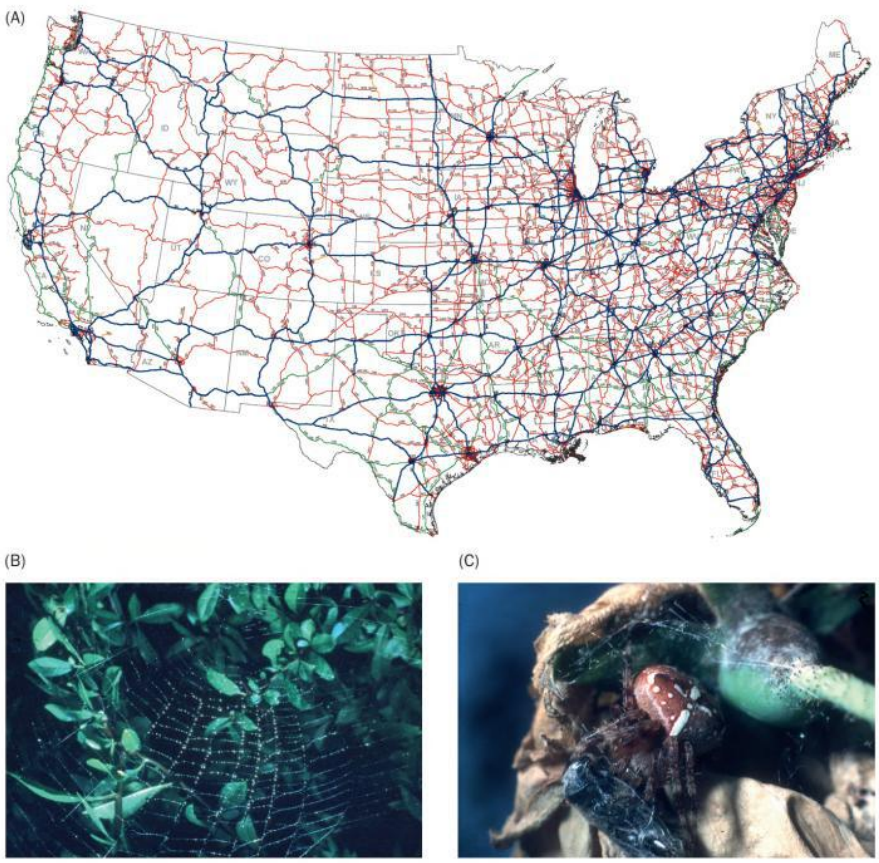

Figure 1.4 The size of a network or system is not necessarily correlated with its complexity. (A) The network of major highways in the continental United States covers over 3 million square miles. Nonetheless, its functionality is easy to grasp, and problems with a particular road are readily ameliorated with detours. (B) The web of the European diadem spider (*Araneus diadematus*) (C) is comparatively small, but the functional details of this little network are complex. Some lines are made of silk proteins that have the tensile strength of steel but can also be eaten and recycled by the spider; other lines are adhesive due to a multipurpose glue that may be sticky or rubbery depending on the situation; yet others are guide and signal lines that allow the spider to move about and sense prey. The creation of the web depends on different types of spinneret glands, whose development and function require the complex molecular machinery of the spider, and it is not yet clear how the instructions for the complicated construction, repair, and use of the web are encoded and inherited from one generation to the next. ((A) From the United States Department of Transportation.)

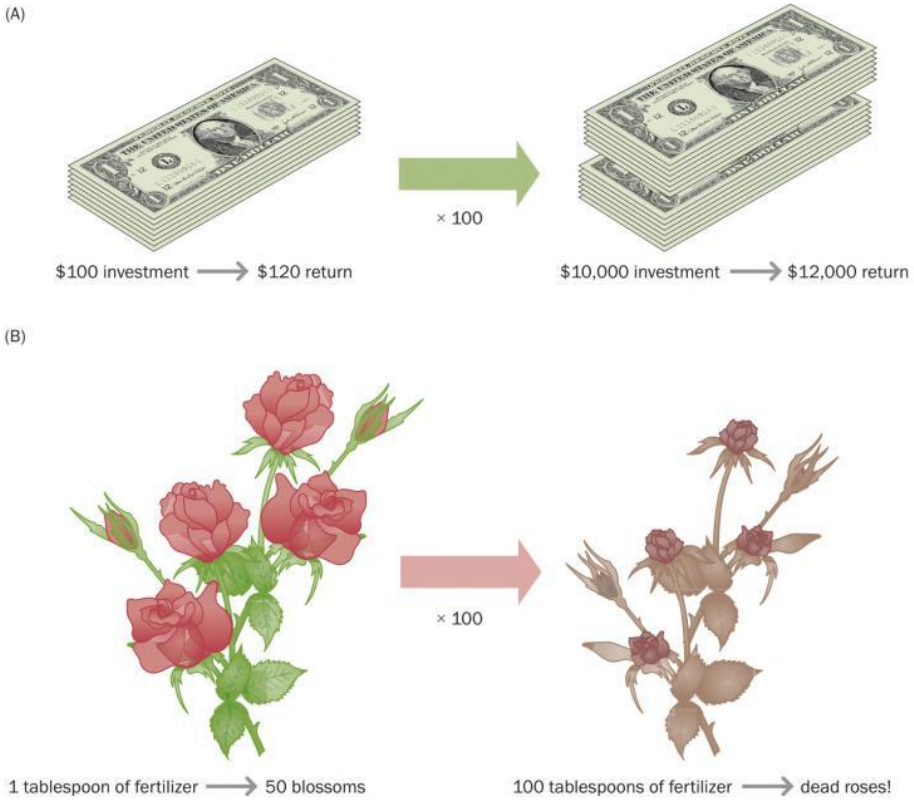

Figure 1.5 Biological phenomena are often difficult to understand, because our minds are trained to think linearly. (A) The return on an investment grows (or decreases) linearly with the amount invested. (B) In biology, more is not necessarily better. Biological responses often scale within a modest range, but lead to an entirely different response if the input is increased a lot.

highways. It is a very large system, but it is not difficult to understand its function or malfunction: if a highway is blocked, it does not take much ingenuity to figure out how to circumvent the obstacle. The other network is a comparably tiny system: the web of a diadem spider. While we can observe the process and pattern with which Ms. Spider spins her web, we do not know which neurons in her brain are responsible for different phases of the complicated web production process and how she is able to produce the right chemicals for the spider silk, which in itself is a marvel of material science, let alone how she manages to survive, multiply, and maybe even devour her husband.

Biological systems often consist of large numbers of components, but they pose an additional, formidable challenge to any analysis, because the processes that govern them are not linear. This is a problem, because we are trained to think in linear ways: if an investment of $100 leads to a return of $120, then an investment of $10,000 leads to a return of $12,000. Biology is different. If we fertilize our roses with 1 tablespoon of fertilizer and the rose bushes produce 50 blossoms, a little bit more fertilizer may increase the number of blossoms, but 100 tablespoons of fertilizer will not produce 5000 blossoms but almost certainly kill the plants (**Figure 1.5**). Just a small amount of additional sun exposure turns a tan into sunburn. Now imagine that thousands of components, many of which we do not know, respond in such a fashion, where a small input does not evoke any response, more input evokes a physiological response, and a little bit more input causes the component to fail or exhibit a totally different "stress" response. We will return to this issue later in this and other chapters with specific examples.

REDUCTIONISM AND SYSTEMS BIOLOGY

So the situation is complicated. But because we humans are a curious species, our forebears did not give up on biological analysis and instead did what was doable, namely collecting information on whatever could be measured with the best current methods (**Figure 1.6**). By now, this long-term effort has resulted in an amazing list of biological parts and their roles. Initially, this list contained new plant and animal

Figure 1.6 Collecting information is the first step in most systems analyses. The eighteenth-century British explorer Captain James Cook sailed the Pacific Ocean and catalogued many plants and animal species that had never been seen before in Europe.

species, along with descriptions of their leaves, berries, and roots, or their body shapes, legs, and color patterns. These external descriptions were valuable, but did not provide specific clues on how plants and animals function, why they live, and why they die. Thus, the next logical step was to look inside—even if this required stealing bodies from the cemetery under a full moon! Cutting bodies open revealed an entirely new research frontier. What were all those distinct body parts and what did they do? What were organs, muscles, and tendons composed of? Not surprisingly, this line of investigation eventually led to the grand-challenge quest of discovering and measuring *all* parts of a body, the parts of the parts (. . . of the parts), as well as their roles in the normal physiology or pathology of cells, organs, and organisms. The implicit assumption of this reductionist approach was that knowing the building blocks of life would lead us to a comprehensive understanding of how life works.

If we fast-forward to the twenty-first century, have we succeeded and assembled a complete parts catalog? Do we know the building blocks of life? The answer is a combination of yes's and no's. The catalog is most certainly not complete, even for relatively simple organisms. Yet, we have discovered and characterized genes, proteins, and metabolites as the major building blocks. Scientists were jubilant when the sequencing of the human genome in the early years of this millennium was declared complete: we had identified the ultimate building blocks, our entire blueprint. It turned out to consist of roughly three billion nucleotide pairs of DNA.

The sequencing of the human genome was without any doubt an incredible achievement. Alas, there is much more to a human body than genes. So, the race for building blocks extended to proteins and metabolites, toward individual gene variations and an assortment of molecules and processes affecting gene expression, which changes in response to external and internal stimuli, during the day, and throughout our lifetimes. As a direct consequence of these ongoing efforts, our parts list continues to grow at a rapid pace: A parts catalog that started with a few organs now contains over 20,000 human genes, many more genes from other organisms, and hundreds of thousands of proteins and metabolites along with their variants. In addition to merely looking at parts in isolation, we have begun to realize that most biological components are affected and regulated by a variety of other components. The expression of a gene may depend on several transcription factors, metabolites, and a variety of small RNAs, as well as molecular, epigenetic attachments to its DNA sequence. It is reasonable to expect that the list of processes within the body is much larger than the number of components on our parts list. Biologists will not have to worry about job security any time soon!

The large number of components and processes alone, however, is not the only obstacle to understanding how cells and organisms function. After all, modern computers can execute gazillions of operations within a second. Our billions of telephones worldwide are functionally connected. We can make very accurate

predictions regarding a gas in a container, even if trillions of molecules are involved. If we increase the pressure on the gas without changing the volume of the container, we know that the temperature will rise, and we can predict by how much. Not so with a cell or organism. What will happen to it if the environmental temperature goes up? Nothing much may happen, the rise in temperature may trigger a host of physiological response processes that compensate for the new conditions, or the organism may die. The outcome depends on a variety of factors that collectively constitute a complex stress response system (**Figure 1.7**). Of course, the comparison to a gas is not

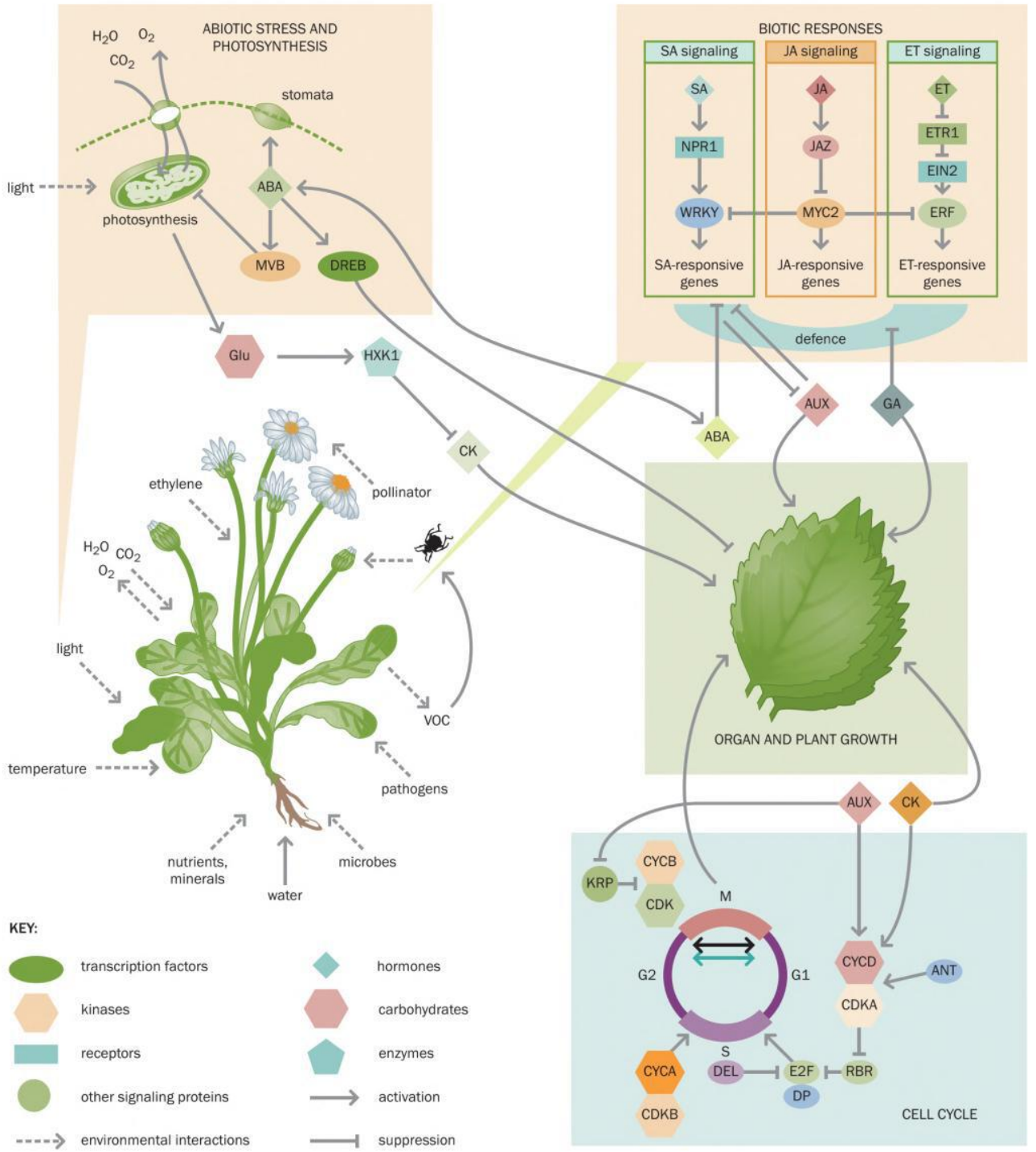

Figure 1.7 Stress responses are coordinated by systems at different levels of organization (cf. Figure 1.2). At the physiological level, the stress response system in plants includes changes at the cellular, organ, and whole-plant levels and also affects interactions of the plant with other species. (From Keurentjes JJB, Angenent GC, Dicke M, et al. *Trends Plant Sci.* 16 [2011] 183–190. With permission from Elsevier.)

quite fair, because, in addition to their large number, the components of a cell are not all the same, which drastically complicates matters. Furthermore, as mentioned earlier, the processes with which the components interact are nonlinear, and this permits an enormous repertoire of distinctly different behaviors with which an organism can respond to a perturbation.

EVEN SIMPLE SYSTEMS CAN CONFUSE US

It is easy to demonstrate how quickly our intuition can be overwhelmed by a few nonlinearities within a system. As an example, let's look at a simple chain of processes and compare it with a slightly more complicated chain that includes regulation [3]. The simple case merely consists of a chain of reactions, which is fed by an external input (**Figure 1.8**). It does not really matter what *X*, *Y*, and *Z* represent, but, for the sake of discussion, imagine a metabolic pathway such as glycolysis, where the input, glucose, is converted into glucose 6-phosphate, fructose 1,6-bisphosphate, and pyruvate, which is used for other purposes that are not of interest here. For illustrative purposes, let's explicitly account for an enzyme *E* that catalyzes the conversion of *X* into *Y*.

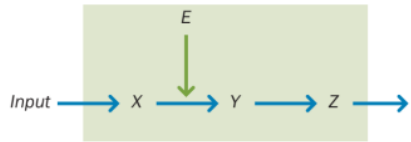

Figure 1.8 The human brain handles linear chains of causes and events very well. In this simple pathway, an external input is converted sequentially into *X*, *Y*, and *Z*, which leaves the system. The conversion of *X* into *Y* is catalyzed by an enzyme *E*. It is easy to imagine that any increase in *Input* will cause the levels of *X*, *Y*, and *Z* to rise.

We will learn in the following chapters how one can formulate a model of such a pathway system as a set of differential equations. And while the details are not important here, it does not hurt to show such a model, which might read

$$\begin{aligned} \dot{X} &= Input - aEX^{0.5}, \\ \dot{Y} &= aEX^{0.5} - bY^{0.5}, \\ \dot{Z} &= bY^{0.5} - cZ^{0.5}. \end{aligned} \tag{1.1}$$

Here, *X*, *Y*, and *Z* are concentrations, *E* is the enzyme activity, and *a*, *b*, and *c* are rate constants that respectively represent how fast *X* is converted into *Y*, how fast *Y* is converted into *Z*, and how quickly material from the metabolite pool *Z* leaves the system. The dotted quantities on the left of the equal signs are differentials that describe the change in each variable over time, but we need not worry about them at this point. In fact, we hardly have to analyze these equations mathematically to get an idea of what will happen if we change the input, because intuition tells us that any increase in *Input* should lead to a corresponding rise in the concentrations of the intermediates *X*, *Y*, and *Z*, whereas a decrease in *Input* should result in smaller values of *X*, *Y*, and *Z*. The increases or decreases in *X*, *Y*, and *Z* will not necessarily be exactly of the same extent as the change in *Input*, but the direction of the change should be the same. The mathematical solution of the system in (1.1) confirms this intuition. For instance, if we reduce *Input* from 1 to 0.75, the levels of *X*, *Y*, and *Z* decrease, one after another, from their initial value of 1 to 0.5625 (**Figure 1.9**).

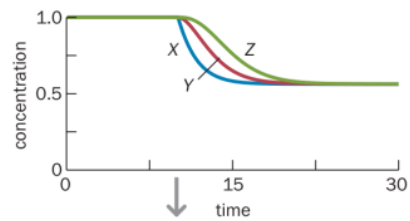

Figure 1.9 Simulations with the system in (1.1) confirm our intuition: *X*, *Y*, and *Z* reflect changes in *Input*. For instance, reducing *Input* in (1.1) to 75% at time 10 (arrow) leads to permanent decreases in *X*, *Y*, and *Z*.

Now suppose that *Z* is a signaling molecule, such as a hormone or a phospholipid, that activates a transcription factor *TF* that facilitates the up-regulation of a gene *G* that codes for the enzyme catalyzing the conversion of *X* into *Y* (**Figure 1.10**). The simple linear pathway is now part of a functional loop. The organization of this loop is easy to grasp, but what is its effect? Intuition might lead us to believe that the positive-feedback loop should increase the level of enzyme *E*, which would result in more *Y*, more *Z*, and even more *E*, which would result in even more *Y* and *Z*. Would the concentrations in the system grow without end? Can we be sure about this prediction? Would an unending expansion be reasonable? What will happen if we increase or decrease the input as before?

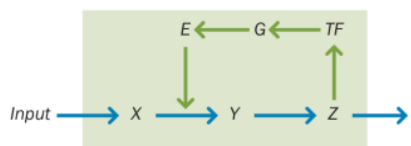

Figure 1.10 Even simple systems may not allow us to make reliable predictions regarding their responses to stimuli. Here, the linear pathway from Figure 1.8 is embedded into a functional loop consisting of a transcription factor *TF* and a gene *G* that codes for enzyme *E*. As described in the text, the responses to changes in *Input* are no longer obvious.

The overall answer will be surprising: the information given so far does not allow us to predict particular responses with any degree of reliability. Instead, the answer depends on the numerical specifications of the system. This is bad news for the unaided human mind, because we are simply not able to assess the numerical consequences of slight changes in a system, even if we can easily grasp the logic of a system as in Figure 1.10.

To get a feel for the system, one can compute a few examples with an expanded model that accounts for the new variables (for details, see [3]). Here, the results are more important than the technical details. If the effect of *Z* on *TF* is weak, the

response to a decrease in *Input* is essentially the same as in Figure 1.9. This is not too surprising, because the systems in this case are very similar. However, if the effect of *Z* on *TF* is stronger, the concentrations in the system start to oscillate, and after a while these oscillations dampen away (**Figure 1.11A**). This behavior was not easy to predict. Interestingly, if the effect is further increased, the system enters a stable oscillation pattern that does not cease unless the system input is changed again (Figure 1.11B).

The hand-waving explanation of these results is that the increased enzyme activity leads to a depletion of *X*. A reduced level of *X* leads to lower levels of *Y* and *Z*, which in turn lead to a reduced effect on *TF*, *G*, and ultimately *E*. Depending on the numerical characteristics, the ups and downs in *X* may not be noticeable, they may be damped and disappear, or they may persist until another change is introduced. Intriguingly, even if we know that these alternative responses are possible, the unaided human mind is not equipped to integrate the numerical features of the model in such a way that we can predict which system response will ensue for a specific setting of parameters. A computational model, in contrast, reveals the answer in a fraction of a second.

The specific details of the example are not as important as the take-home message: If a system contains regulatory signals that form functional loops, we can no longer rely on our intuition for making reliable predictions. Alas, essentially all realistic systems in biology are regulated—and not just with one, but with many control loops. This leads to the direct and sobering deduction that intuition is not sufficient and that we instead need to utilize computational models to figure out how even small systems work and why they might show distinctly different responses or even fail, depending on the conditions under which they operate.

The previous sections have taught us that biological systems contain large numbers of different types of components that interact in potentially complicated ways and are controlled by regulatory signals. What else is special about biological systems? Many answers could be given, some of which are discussed throughout this book. For instance, two biological components are seldom 100% the same. They vary from one organism to the next and change over time. Sometimes these variations are inconsequential, at other times they lead to early aging and disease. In fact, most

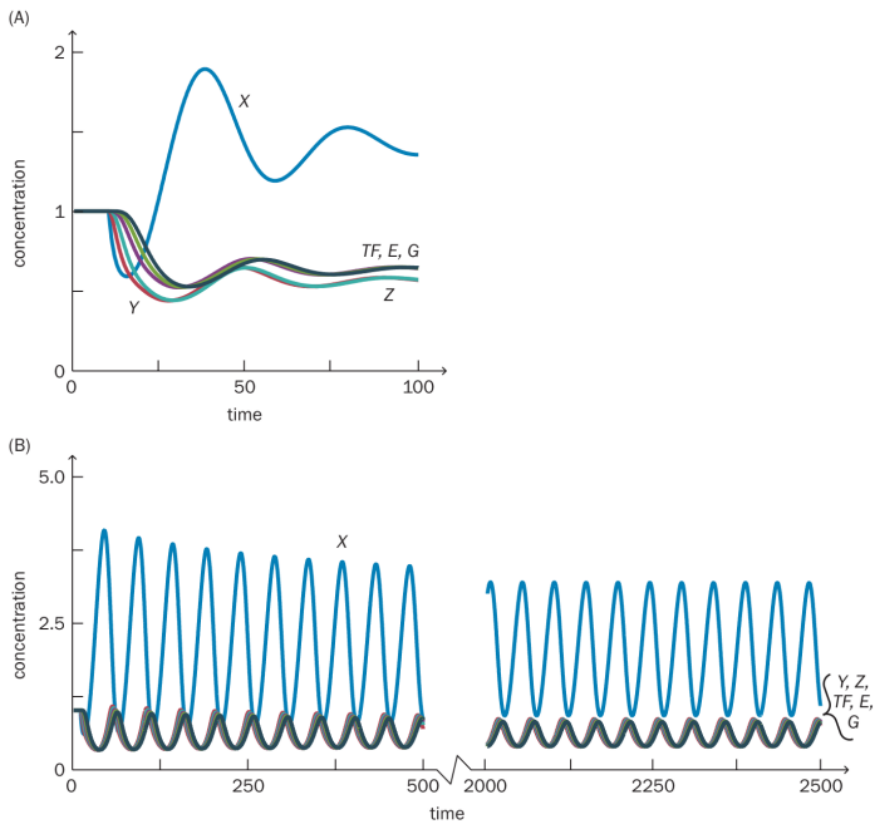

Figure 1.11 Simulation results demonstrate that the looped system in Figure 1.10 may exhibit drastically different responses. If the effect of *Z* on *TF* is very small, the response is essentially like that in Figure 1.9 (results not shown). (A) If the effect of *Z* on *TF* is relatively small, the functional feedback loop causes the system to go through damped oscillations before assuming a new stable state. (B) For stronger effects of *Z* on *TF*, the system response is a persistent oscillation.

diseases do not have a single cause, but are the consequence of an unfortunate combination of slight alterations in many components. Another feature that complicates intuition is the delay in many responses to stimuli. Such delays may be of the order of seconds, hours, or years, but they require the analyst to study not merely the present state of a biological system but also its history. For instance, recovery from a severe infection depends greatly on the preconditioning of the organism, which is the collective result of earlier infections and the body's responses [4].

Finally, it should be mentioned that different parts of biological systems may simultaneously operate at different scales, with respect to both time and space. These scales make some aspects of their analysis easier and some harder. Let's begin with the temporal scale. We know that biology at the most basic level is governed by physical and chemical processes. These occur on timescales of the order of milliseconds, if not faster. Biochemical processes usually run on a scale of seconds to minutes. Under favorable conditions, bacteria divide every 20–30 minutes. Our human lifespan extends to maybe 120 years, evolution can happen at the genetic level with lightning speed, for instance, when radiation causes a mutation, while the emergence of an entirely new species may take thousands or even millions of years. On one hand, the drastically different timescales make analyses complicated, because we simply cannot account for rapid changes in all molecules of an organism over an extended period of time. As an example, it is impossible to study aging by monitoring an organism's molecular state every second or minute. On the other hand, the differences in timescales justify a very valuable modeling "trick" [5, Chapter 5]. If we are interested in understanding some biochemical process, such as the generation of energy in the form of adenosine triphosphate (ATP) by means of the conversion of glucose into pyruvate, we can assume that developmental and evolutionary changes are so slow in comparison that they do not change during ATP production. Similarly, if we study the phylogenetic family tree of species, the biochemical processes in an individual organism are comparatively so fast that their details become irrelevant. Thus, by focusing on just the most relevant timescale and ignoring much faster and much slower processes, any modeling effort is dramatically simplified.

Biology also happens on many spatial scales. All processes have a molecular component, and their size scale is therefore of the order of ångströms and nanometers. If we consider a cell as the basic unit of life, we are dealing with a spatial scale of micrometers to millimeters, with some exceptions such as cotton "fiber" cells reaching the length of a few centimeters [6] and the afferent axons of nerve cells in giraffes, reaching from toe to neck, extending to 5 meters [7, p. 14]. The sizes of typical cells are dwarfed by higher plants and animals and by ecosystems such as our oceans, which may cover thousands of square kilometers. As with the different temporal scales, and using analogous arguments, models of biological systems often focus on one or two spatial scales at a time [5]. Nonetheless, such simplifications are not always applicable, and some processes, such as aging and algal blooms, may require the simultaneous consideration of several temporal and spatial scales. Such multiscale assessments are often very complicated and constitute a challenging frontier of current research (see Chapter 15).

WHY NOW?

Many of the features of biological systems have been known for quite a while, and, similarly, many concepts and methods of systems biology have their roots in its well-established parent disciplines, including physiology, molecular biology, biochemistry, mathematics, engineering, and computer science [8–11]. In fact, it has been suggested that the nineteenth-century scientist Claude Bernard might be considered the first systems biologist, since he proclaimed that the "application of mathematics to natural phenomena is the aim of all science, because the expression of the laws of phenomena should always be mathematical" [12, 13]. A century later, Ludwig von Bertalanffy reviewed in a book his three decades of attempting to convince biologists of the systemic nature of living organisms [14, 15]. At the same time, Mihajlo Mesarović used the term "Systems Biology" and declared that "real advance ... will come about only when biologists start asking questions which are based on systems-theoretic concepts" [16]. The same year, a book review in *Science*

envisioned "... a field of systems biology with its own identity and in its own right" [17]. A few years later, Michael Savageau proposed an agenda for studying biological systems with mathematical and computational means [5].

In spite of these efforts, systems biology did not enter the mainstream for several more decades. Biology kept its distance from mathematics, computer science, and engineering, primarily because biological phenomena were seen as too complicated for rigorous mathematical analysis and mathematics was considered applicable only to very small systems of little biological relevance. The engineering of biological systems from scratch was impossible, and the budding field of computer science contributed to biology not much more than rudimentary data management.

So, why has systems biology all of the sudden moved to the fore? Any good detective will know the answer: motive and opportunity. The motive lies in the realization that reductionist thinking and experimentation alone are not sufficient if complex systems are involved. Reductionist experiments are very good in generating detailed information regarding specific components or processes of a system, but they often lack the ability to characterize, explain, or predict **emergent properties** that cannot be found in the parts of the system but only in their web of interactions. For instance, the emergence of oscillations in the example system represented by the equations in (1.1) cannot be credited to a single component of the system but is a function of its overall organization. Although we had complete knowledge of all details of the model pathway, it was very difficult to foresee its capacity either to saturate or oscillate in a damped or stable fashion. Biology is full of such examples.

A few years ago, Hirotada Mori's laboratory completed the assembly of a complete catalogue of single mutants in the bacterium *Escherichia coli* [18]. Yet, the scientific community is still not able to foresee which genes the bacterium will up- or down-regulate in response to new environmental conditions. Another very challenging example of emergent system properties is the central nervous system. Even though we understand quite well how action potentials are generated and propagated in individual neurons, we do not know how information flows, how memory works, and how diseases affect the normal functioning of the brain. It is not even clear how information in the brain is represented (see also Chapter 15). Thus, while reductionist biology has been extremely successful and will without any doubt continue to be the major driving force for future discovery, many biologists have come to recognize that the detailed pieces of information resulting from this approach need to be complemented with new methods of system integration and reconstruction [19].

The opportunity for systems biology is the result of the recent confluence and **synergism** of three scientific frontiers. The first is of course the rapid and vast accumulation of detailed biological information at the physiological, cellular, molecular, and submolecular levels. These targeted investigations of specific phenomena are accompanied by large-scale, high-throughput studies that were entirely infeasible just a couple of decades ago. They include quantification of genome-wide expression patterns, simultaneous identification of large arrays of expressed proteins, comprehensive profiling of cellular metabolites, characterization of networks of molecular interactions, global assessments of immune systems, and functional scans of nervous systems and the human brain. These exciting techniques are generating unprecedented amounts of high-quality data that are awaiting systemic interpretation and integration (**Figure 1.12**).

The second frontier is the result of ingenuity and innovation in engineering, chemistry, and material sciences, which have begun to provide us with a growing array of technologies for probing, sensing, imaging, and measuring biological systems that are at once very detailed, extremely specific, and usable *in vivo*. Many tools supporting these methods are in the process of being miniaturized, in some cases down to the nanoscale of molecules, which allows diagnoses with minute amounts of biological materials and one day maybe biopsies of individual, living cells. Devices at this scale will allow the insertion of sensing and disease treatment devices into the human body in an essentially noninvasive and harmless fashion [20–22]. Bioengineering and robotics are beginning to render it possible to measure hundreds or thousands of biomarkers from a single drop of blood. It is even becoming feasible to use molecular structures, prefabricated by nature, for new purposes in medicine, drug delivery, and biotechnology (**Figure 1.13**).

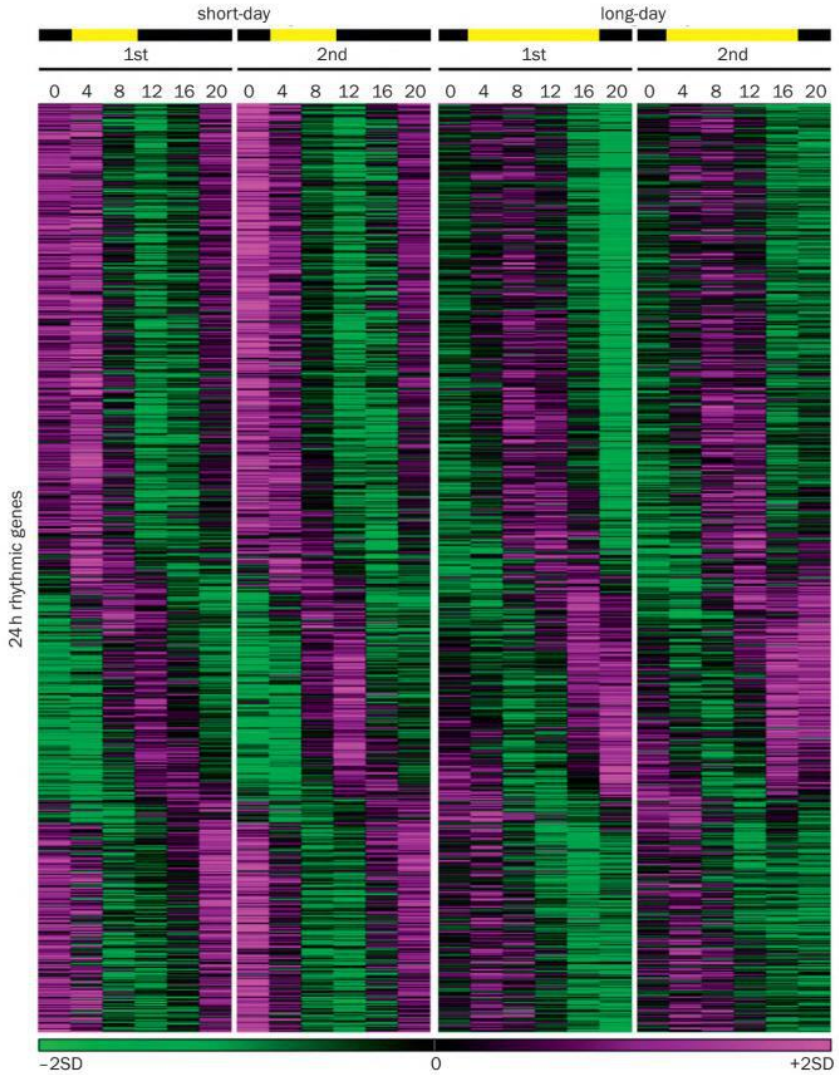

Figure 1.12 Modern high-throughput methods of molecular biology offer data in unprecedented quantity and quality. As an example, the heat map shown here represents a genome-wide expression profile of 24-hour-rhythmic genes in the mouse under chronic short-day (left two panels) and long-day (right two panels) conditions. (From Masumoto KM, Ukai-Tadenuma M, Kasukawa T, et al. *Curr. Biol.* 20 [2010] 2199–2206. With permission from Elsevier.)

The third frontier is the co-evolution of mathematical, physical, and computational techniques that are more powerful and accessible to a much wider audience than ever before. Imagine that only a few decades ago computer scientists used punch cards that were read by optical card readers (**Figure 1.14**)! Now, there are even specific computing environments, including Mathematica® and MATLAB®, as well as different types of customized mark-up languages (**XML**), such as the systems biology mark-up language **SBML** [23] and the mark-up language AGML, which was developed specifically for analyzing two-dimensional gels in proteomics [24].

Before today's much more effective computer science techniques were available, it was not even possible to keep track of the many components of biological systems, let alone analyze them. But over the past few decades, a solid theoretical and numerical foundation has been established for computational methods specifically tailored for the investigation of dynamic and adaptive systems in biology and medicine. These techniques are now at the verge of making it possible to represent and analyze large, organizationally complex systems and to study their emergent properties in a rigorous fashion. Methods of machine learning, numerical mathematics, and bioinformatics permit the efficient mining and analysis of the most useful data from within an overwhelming amount of data that are not pertinent for a given task. Algorithmic advances permit the simulation and optimization of very large biological flux distribution networks. Computer-aided approximation approaches yield ever-finer insights into the dynamics of complex nonlinear systems, such as the control of blood flow in healthy and diseased hearts. New mathematical, physical, and computational methods are beginning to make it possible to

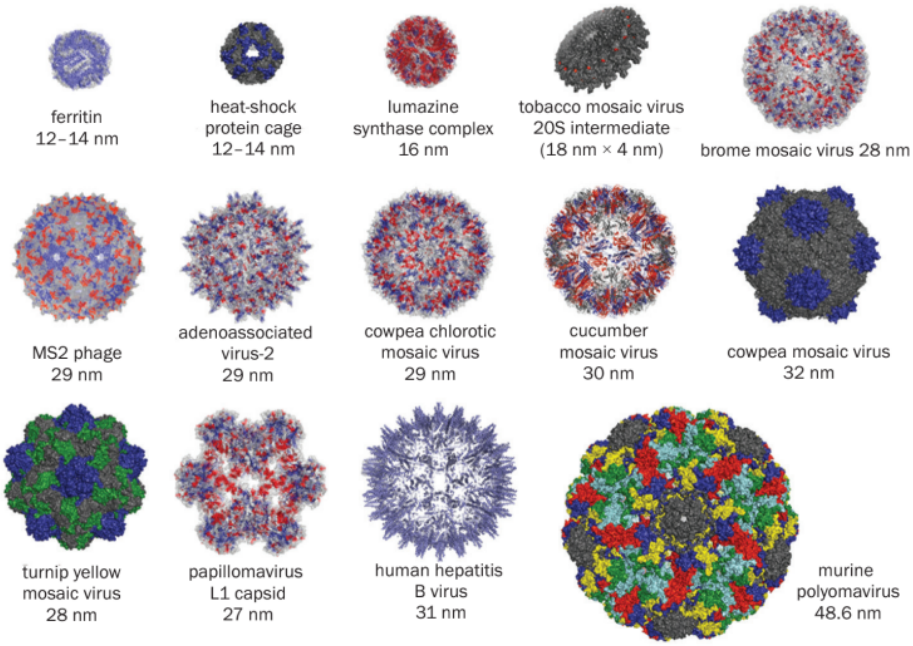

Figure 1.13 "Protein cages" are particles that have applications in bionanotechnology and nanomedicine. These particles are very interesting biological building blocks because they self-assemble into a variety of different shapes. The features of these bionanoparticles can be genetically manipulated and fine-tuned for biomedical purposes, such as drug delivery, gene therapy, tumor imaging, and vaccine development. (From Lee LA & Wang Q. *Nanomedicine* 2 [2006] 137–149. With permission from Elsevier.)

Figure 1.14 Advances in computer power, accessibility, and user-friendliness over the past 40 years have been tremendous. Not too long ago, computer code had to be fed manually into the computer with punch cards. (Courtesy of Mutatis mutandis under the Creative Commons Attribution-Share Alike 3.0 Unported license.)

predict the folding of proteins and the binding between target sites and ligands. These predictions, in turn, suggest insights into specific molecular interactions and promise the potential of targeted drug interventions that minimize toxic side effects.

Motive and opportunity have met to make systems biology attractive and feasible. It has become evident that the relevant disciplines complement each other in unique ways and that the synergism among them will revolutionize biology, medicine, and a host of other fields, including biotechnology, environmental science, food production, and drug development.

COMMUNICATING SYSTEMS BIOLOGY

It is not a trivial task to talk succinctly about 25,000 genes and their expression state or about the many processes occurring simultaneously in response to a signal that a cell receives at its outer surface. Our minds are ill equipped to characterize numerical relationships, let alone discuss complicated mathematical functions, especially if these depend on many variables. If we did not have numbers, we would even have problems describing everyday features such as temperature. Of course, we can say that it is cold or hot, and we have a dozen or so adjectives in between. But if we need more accuracy, common language ceases to be sufficient. Discerning 37.0°C from 38.4°C is not easy without a numerical scale, but it is necessary to have the tools to

describe the difference, for instance, because the former reflects normal body temperature, while the latter is a sign of fever.

We may willingly or grudgingly accept the fact that we need mathematics, which comes with its own terminology, but communication is a two-way process. If we start talking about eigenvalues and Hopf bifurcations, we are almost guaranteed to lose mainstream biologists, let alone laypeople. This is a real problem, because our results must be conveyed to biologists, who are providing us data, and to the public that pays for our research and has a right to reap the fruit of the enormous investment of resources going into science [25]. The only true solution to this challenge is the bilingual education and nurturing of systems biologists who can translate biological phenomena into math and computer code and who can explain what it really means for the biological system if the real part of an eigenvalue is positive [19].

Communication is not trivial even within biology itself, because specialization has progressed so far that different fields such as molecular biology, immunology, and nanomedicine have developed their own terminology and jargon. Let's look at this issue in the form of a parable from Indian folklore that describes six blind men exploring an elephant (**Figure 1.15**). This story is quite old and usually ends in utter confusion, but it is useful to analyze it further than is usually done. The story has it that each of the blind men touched a different part of an elephant and came to a different conclusion concerning the object of his research. The man touching the side thought he was touching a wall, the one feeling the leg concluded he was touching a tree. The elephant's trunk gave the impression of a snake, the tusk that of a pointed scimitar, the tail felt like a rope, and the ear appeared to be like a large leaf or fan. It is not difficult to see the analogy to a complex biological system like the onset of Alzheimer's disease. The first scientist found "the Alzheimer gene," the second discovered "a strong association between the disease and former head injuries," another scientist detected "problems with fatty acid metabolism in the brain," and yet another suggested that "aluminum in cookware might be the culprit." As in the case of the elephant, the scientists were right, to some degree.

Let's analyze the elephant story a little further. The first problem among the six blind men might have been the homogeneous pool of researchers. Including a female or a child might have provided additional clues. Also, we have to feel sorry for the Indian men for being blind. However, they were apparently not mute or deaf, so that a little discussion among them might have gone a long way. While all six were blind, it is furthermore fair to assume that they had friends with working vision, who could have set them straight. They could have used not just their hands but also their other senses,

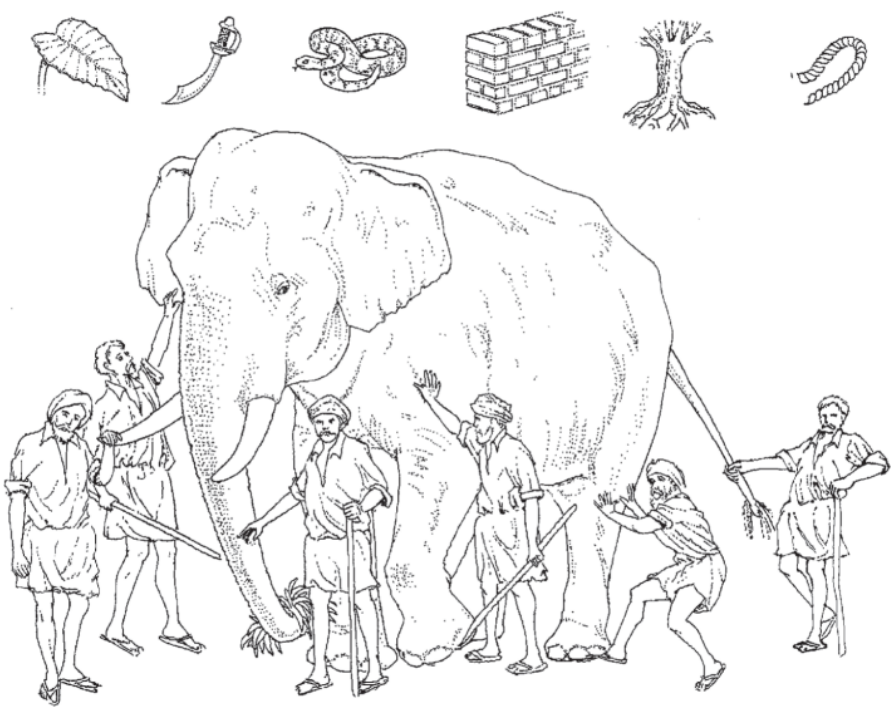

Figure 1.15 Information about isolated parts of a system alone does not always reveal the true nature of the system. An old story of six blind Indian men trying to determine what they touch is a parable for the dangers of scientific silos and the lack of good communication.

such as smell. Do tree trunks really smell like elephant feet? Finally, they apparently stayed in their one spot, thereby greatly limiting their experience base.

It is again easy to translate these issues into biology, especially when we think of purely reductionist strategies. Instead of a homogeneous pool of biologists analyzing biological systems, it is without doubt more effective to have a multidisciplinary team including different varieties of biologists, but also physicists, engineers, mathematicians, chemists, and smart people trained in the liberal arts or economics. Instead of only focusing on the one aspect right in front of our nose, communication with others provides context for singular findings. We don't know whether the Indian men spoke the same language, but we know that even if biologists, computer scientists, and physicists all use English to communicate, their technical languages and their views of the scientific world are often very different, so that communication may initially be superficial and ineffective. That is where multidisciplinary groups must engage in learning new terminologies and languages and include interdisciplinary translators. Just as the Indian men should have called upon their seeing friends, investigators need to call in experts who master techniques that have not been applied to the biological problem at hand. Finally, it behooves the trunk analyzer to take a few steps and touch the tusk and the side. Established scientific disciplines have in the past often become silos. Sometimes without even knowing it, researchers have kept themselves inside these silos, unable or unwilling to break out and to see the many other silos around, as well as a whole lot of space between them.

Systems biology does not ask the six blind men to abandon their methods and instead to run circles around the elephant. By focusing on one aspect, the reductionist "elephantologists" are poised to become true experts on their one chosen body part and to know everything there is to know about it. Without these experts, systems biology would have no data to work on. Instead, what systems biology suggests is a widening of the mindset and at least rudimentary knowledge of a second language, such as math. It also suggests the addition of other researchers, assisting the "trunkologist" and the "tuskologist" by developing new tools of analysis, by telling them in their language what others have found, by closing the gap between trunks and tusks and tails.

One strategy for accomplishing this synergism is to collect the diverse pieces of data and contextual information obtained by the six blind men and to merge them into a conceptual model. What kind of "thing" could consist of parts that feel like a snake, tree trunks, large walls, two scimitars, two fans, and a rope? How could an aberrant gene, former head injuries, and altered brain metabolism functionally interact to result in Alzheimer's disease? Well-trained systems biologists should be able to develop strategies for merging heterogeneous information into formal models that permit the generation of testable hypotheses, such as "tree-trunk-like things are connected by wall-like things." These hypotheses may be wrong, but they can nevertheless be very valuable, because they focus the scientific process on new, specific experiments that either confirm or refute the hypothesis. An experiment could be to walk along the "wall" as far as possible. Is there a "tree trunk" on the end? Are there "tree trunks" to the left and to the right? Is there a "pointed scimitar" at one or both ends? Is the "snake" connected to a "wall" or to a "tree trunk"? Does the "wall" reach the ground? Each answer to one of these questions constrains further and further what the unknown "thing" could possibly look like, and this is the reason that refuted hypotheses are often as valuable or even more valuable than confirmed hypotheses. "The wall does indeed not reach the ground!" Then, how is it supported? By "tree trunks"?

The story tells us that effective communication can solve a lot of complex questions. In systems biology, such communication is not always easy, and it requires not only mastering the terminology of several parent disciplines but also internalizing the mindset of biologists and clinicians on the one hand and of mathematicians, computer scientists, and engineers on the other. So, let's learn about biology. Let's study laboratory data and information and explore the mindset of biologists. Let's study graphs and networks with methods from computer science. Let's see how mathematicians approach a biological system, struggle with assumptions, make simplifications, and obtain solutions that are at first incomprehensible to the non-mathematician but do have real meaning once they are translated into the language of biology.

THE TASK BEFORE US

We have discussed the need to understand biological systems. But what does that really mean? Generically, it means that we should be able to explain how biological systems work and why they are constructed in the fashion as we observe them and not in a different one. Second, we should be able to make reliable predictions of responses of biological systems under yet-untested conditions. And third, we should be able to introduce targeted manipulations into biological systems that change their responses to our specifications.

This level of understanding is a tall order, and we will need many years to achieve it even for a narrowly focused domain within the huge realm of biological systems. An important component of the task is the conversion of actual biological systems into computational models, because this conversion, if it is valid, allows us almost unlimited and comparatively cheap analyses. The resulting models of biological systems come in two types. The first focuses on specific systems and includes all pertinent functional and numerical details—one might think of the analogy to a flight simulator. The second type of model is intended to help us understand the fundamental, generic features of the organization of biological systems; here one might think of elementary geometry, which offers us valuable insights into spatial features of the world by dealing with ideal triangles and circles that do not really exist in nature. The two model types point to two opposite ends of a large spectrum. The former models will be large and complex, while the latter will be as reduced and simple as feasible. In practice, many models will fall between these two extremes.

To pave the way toward these goals, this book is organized in three sections. The first of these introduces in four chapters a set of modeling tools for converting biological phenomena into mathematical and computational analog and for diagnosing, refining, and analyzing them. The second section describes in five chapters the molecular inventories that populate biological systems, and the five chapters in the third section are devoted to representative case studies and a look into the future.

The modeling approaches parallel two fundamental properties of biological systems, namely their static structure and their dynamics, that is, their changes over time. For static analyses, we will characterize and rationalize how nature put particular systems together and which parts are directly or loosely connected with each other. We will see that there are distinct types of connections and interactions. One important difference is that some connections allow the flow of material from a source to a target, while others serve the sole purpose of signaling the state of the system. In the latter case, no material changes location. Like a billboard that is not changed whether hundreds of people look at it or nobody at all, a signaling component may not be changed when it sends a signal. It is also to be expected that some connections are crucial, while others are of secondary importance. Finally, there is the very challenging question of how we can even determine the structure of a system. What types of data do we need to infer the structure of a system, and how reliable is such an inference?

The dynamics of a system is of the utmost importance, because all biological systems change over time. Organisms traverse a life cycle during which they undergo tremendous changes. Even a lowly yeast cell fills up with scars where it has given birth to daughter cells, and once its surface is full, the cell slides into the sunset of life. We can easily see these changes under a microscope, but an incomparably larger number of changes remain hidden from our sight. Gene expression patterns, amounts of proteins, profiles of metabolites, all of these change dramatically between birth and death. In addition to normal changes throughout its lifetime, every organism responds to fast and slow changes in the environment and adapts rather quickly to new situations. Today we may observe and characterize the gene expression network in a bacterium, but tomorrow it may already have changed in response to some environmental pressures. Indeed, bacteria evolve so quickly that the commonly used term "wild type" no longer has much meaning. Even more than static aspects, dynamic aspects of biological systems mandate the use of computational models. These models help us reveal how fascinating living systems are, with respect both to their overall efficiency and to the ingenuity with which dynamic responses and adaptations are coordinated.

Whether static or dynamic, some model designs and analyses will be performed in the bottom-up and others in the top-down direction. However, since we seldom

start at the very bottom, that is, with individual atoms, or at the very top with models of complete organisms as they interact with their environment, most modeling strategies in systems biology are in truth "middle-out," to use Nobel Laureate Sydney Brenner's expression (cited in [26]). They begin somewhere in between the extremes, maybe with pathways or with cells. Over time, they may be incorporated into larger models, or they may become more and more refined in detail.

The second section of the book addresses the molecular inventories of biological systems. Paralleling the biological organization of organisms, one chapter is devoted to gene systems, one to proteins, and one to metabolites. A further chapter discusses signal transduction systems, and the final chapter of this section describes features of populations. Clearly, all these chapters are woefully incomplete and should not be thought of as substitutes for real biology books. Their purpose is merely to provide brief overviews of the main classes of biological components that are the foundation of all modeling strategies.

The third section contains case studies that in one way or another highlight aspects of biological systems that are in some sense representative. One chapter describes the coordinated stress response system in yeast, which operates simultaneously at the gene, protein, and metabolite levels. Another chapter provides a presentation of how very different models can be useful to focus attention on selected aspects of a multiscale system, the heart. A third chapter indicates how systems biology can contribute to medicine and drug development. The fourth chapter illuminates aspects of the natural design of biological systems and of the artificial design of synthetic biological systems. Finally, the last chapter discusses emerging and future trends in systems biology.

EXERCISES

1.1. Search the Internet, as well as different dictionaries, for definitions of a "system." Extract commonalities among these definitions and formulate your own definition.

1.2. Search the Internet for definitions of "systems biology." Extract commonalities among these definitions and formulate your own definition.

1.3. List 10 systems within the human body.

1.4. Exactly what features make the system in Figure 1.10 so much more complicated than the system in Figure 1.8?

1.5. Imagine that Figure 1.10 represents a system that has become faulty owing to disease. Describe the consequences of its complexity for any medical treatment strategy.

1.6. In Figure 1.2, are there control paths along which ABA either activates or inhibits closure of stomata? If so, list at least one path each. If both paths exist in parallel, discuss what you expect the effect of an increase in ABA to be on stomata closure?

1.7. Imagine a control system like that in Figure 1.2, but much simpler. Specifically, suppose there is only one activating and one inhibiting path in parallel. Suppose further that the activating path reacts much faster than the inhibiting path. What would be the consequence with respect to the effect of an increase in input (ABA) on output (stomata closure)? How could a difference in speed be implemented in a natural cell? Does it matter how strong or weak the activation and inhibition are? Discuss!

1.8. We have discussed that it is often difficult to infer the structure of a biological system from data. Is it possible that two different systems produce exactly the same input–output data? If you think it is impossible, discuss and defend your conclusion. If you think the answer is affirmative, construct a conceptual example.

1.9. List and discuss features supporting the claim that reductionism alone is not sufficient for understanding biological systems.

1.10. List those challenges of systems biology that cannot be solved with intuition alone.

1.11. Discuss why it is important to create terminology and tools for communicating systems biology.

1.12. Assemble a "to-do" list for the future of systems biology.

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Introduction to Mathematical Modeling

2

When you have read this chapter, you should be able to:

- Understand the challenges of mathematical modeling
- Describe the modeling process in generic terms
- Know some of the important types of mathematical models in systems biology
- Identify the ingredients needed to design a model
- Set up simple models of different types
- Perform basic diagnostics and exploratory simulations
- Implement changes in parameters and in the model structure
- Use a model for the exploration and manipulation of scenarios

At the core of any computational analysis in systems biology is a mathematical **model**. A model is an artificial construct in the language of mathematics that represents a process or phenomenon in biology. **Modeling** is the process of creating such a construct and squeezing new insights out of it. Mathematical models in systems biology can take many forms. Some are small and simple, others large and complicated. A model can be intuitive or very abstract. It may be mathematically elegant in its streamlined simplicity or account for very many details with a large number of equations and specifications that require sophisticated computer **simulations** for their analysis. The key commonality among all good models in biology is their ability to offer us insights into processes or systems that we would not be able to gain otherwise. Good models can make sense of a large number of isolated facts and observations. They explain why natural systems have evolved into the particular organizational and regulatory structures that we observe. They allow us to make **predictions** and **extrapolations** about experimentally untested situations and future trends. They can lead to the formulation of new hypotheses. Good models have the potential to guide new experiments and suggest specific recipes for manipulating biological systems to our advantage. Models can tell us how cell cycles are controlled and why plaques appear in certain sections of our arteries and not in others. Future models may help us understand how the brain works and prescribe specific interventions in tumor formation that will be infinitely more subtle and less crude than our current methods of radio- or chemotherapy.

Modeling in systems biology is really a special way of looking at the world. Whether we just conceptualize a situation in the form of components and interactions, or whether we actually set up equations and analyze them, we begin to see how complicated phenomena can be conceptually simplified and dissected into manageable submodels or **modules**, and we develop a feel for how such modules interact with each other and how they might respond to changes. Be aware! Modeling may become a life-changing adventure.

An analogy for a mathematical model is a geographical map. This map is obviously very different from reality, and many details are missing. What do the houses look like in some remote village? Are the lakes clean enough for swimming? Are the people friendly? Then again, it is exactly this simplification of the complicated real world that makes the map useful. Without being distracted by secondary details, the map allows us to look at the network of major roads throughout an entire country while sitting at home on our living room floor. The map provides us with distances and highway types that let us estimate travel times. Altitudes on the map indicate whether the area we want to drive through is hilly and could be cold even during the summer months. Once we learn how to interpret the map, we can infer what the countryside might look like. Empty spaces, like the Badlands in South Dakota, suggest that living in the area might be difficult or undesirable for humans. The density of cities and villages provides us with some idea of how easy it might be to find lodging, food, or gas. Clusters of cities along the ocean probably imply that living there is desirable. Mathematical models have the same generic properties: they reflect some aspects quite well, but barely touch on others or ignore them altogether. They allow us to infer certain properties of reality but not others. The inclusion or exclusion of certain features in models has a direct, important implication for the modeling process. Namely, the key task in designing a suitable model is a crisp focus on the aspects of primary interest and a representation of these aspects that retains their most important properties. At the same time, extraneous or distracting information must be minimized, and the model must be limited in complexity to permit analysis with reasonable efficiency.

Modeling is a mathematical and computational endeavor that in some sense resembles the fine arts (**Figure 2.1**). Basic math problems in calculus or linear algebra have the correct solutions in the back of the book, or at least somebody,

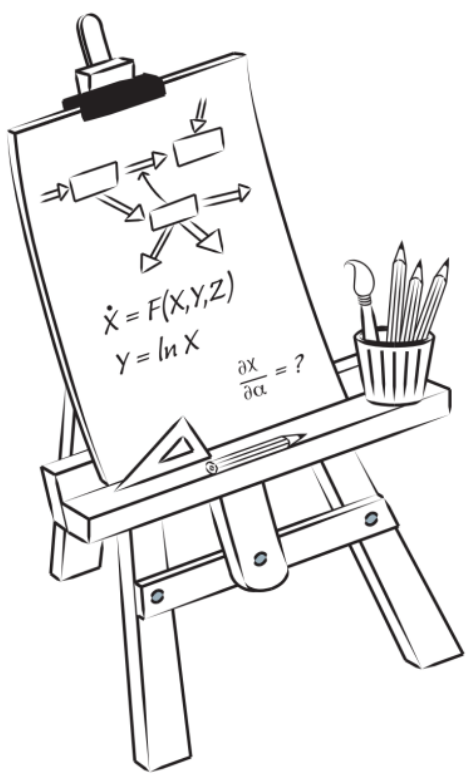

Figure 2.1 Modeling resembles a fine art. Modeling requires both mastery of techniques and creativity.

somewhere, knows these unique, correct solutions. In contrast to this rigor and crispness, many aspects of modeling allow a lot more flexibility. They permit different approaches, and often there are multiple, quite distinct solutions that are all valuable and correct in their own right. Who is to say that an oil painting is better than a watercolor? Or that an opera has higher artistic value than a symphony? Similarly, a mathematical model has aspects and properties in different dimensions and its value is not always easy to judge. For instance, is a very complicated, detailed model better than a simple, yet elegant model?

The answers to these questions depend critically on the goals and purposes of the model, and it is not just beneficial but indeed mandatory to specify the reasons for setting up a model and to ask a lot of questions before we embark on actually designing it. These questions must clarify the potential utilization of the model, its desired accuracy, the degree of complexity we are willing to accept, and many other details that we will touch on in this chapter. Indeed, it is prudent to invest quite a bit of time and exploration in pondering these aspects before one begins selecting a specific model structure and defining variables, parameters, and other model features, because technical challenges often distract from the big picture.

As in the fine arts, the creation of a good model requires two ingredients: technical expertise and creativity. The technical aspects can and must be learned through the acquisition of a solid repertoire of math and computer science skills. To make theoretical and numerical analyses meaningful, one also has to dig sufficiently deeply into biology in order to understand the background and terminology of the phenomenon of interest and to interpret the model results in a correct and sensible manner. As with the fine arts, the creative aspect of the process is more difficult to learn and teach. For instance, it requires more experience than one might expect to ask the right questions and reformulate them in such a way that they become amenable to mathematical and computational analysis. For a novice, all data may look equally valuable, but they really are not. In fact, it takes time and experience to develop a feel for what is doable with a particular set of data and what is probably out of reach. It is also important to learn that some data are simply not well suited for mathematical modeling. This insight is sometimes disappointing, but it can save a lot of effort and frustration.

The shaping and refining of specific questions mandates that we learn how to match the biological problem at hand with appropriate mathematical and computational techniques. This matching requires knowledge of what techniques are available, and it also means that the modeler must make an effort to understand how biologists, mathematicians, and computer scientists are trained to think. The differences in approach are quite pronounced in these three disciplines. Mathematicians are encouraged to simplify and abstract, and there is hardly anything more appealing than a tough problem that can be scribbled on the back of a napkin. Computer scientists look at problems algorithmically, that is, by dissecting them into very many, very tiny steps. Contrast that with biologists, who have learned to marvel at the complexity and intricacies of even the smallest biological item, and you can imagine the mental and conceptual tensions that may develop during the modeling process.

The formation of good questions requires decisions about what is really important in the model, which features can be ignored, and what inaccuracies we are willing to tolerate when we simplify or omit details. It is this complex of decisions on the inclusion and exclusion of components, facts, and processes that constitutes the artistic aspect of modeling. As in the fine arts, a good model may give an outsider the impression "Oh sure, I could have done that," because it is well designed and does not reveal the hard work and failed attempts that went into it. A good degree of proficiency with respect to these decision processes can be learned, but, like everything else, it requires practice with simple and then increasingly complex modeling tasks. This learning by doing can be exhilarating in success and depressing in failure, and while we have every right to enjoy our successes, it is often the failures that in the end are more valuable, because they point to aspects we do not truly understand.

The computational side of modeling draws from two classes of methods: mathematical analysis and computer simulation. Most practical applications use a combination of the two, because both have their own strengths. A purely mathematical analysis leads to general solutions that are always true, if the given assumptions and prerequisites are satisfied. This is different from even a large set of computer

simulations, which can never confer the same degree of certainty and generality. For instance, we can mathematically prove that every real number has a real inverse (4 has 0.25, −0.1 has −10), with the notable exception of 0. But if we were to execute a mega-simulation on a computer, randomly picking a real number and checking whether its inverse is real, we would come to the conclusion that indeed all real numbers have a real inverse. We would miss zero, because the probability of randomly picking zero from an infinite range of numbers is nil. Thus, in principle, mathematics should always be preferred. However, in practical terms, the mathematics needed to analyze biological systems becomes very complicated very quickly, and even relatively simple-looking tasks may not have an explicit analytical solution and therefore require computational analysis.

Independent of the specific biological subject area and the ultimate choice of a particular mathematical and computational framework, the generic modeling process consists of several phases. Each phase is quite distinct both in input requirements and in techniques. Broadly categorized, the phases are shown in **Figure 2.2**, together with a coarse flow chart of the modeling process. Further details are presented in **Table 2.1**. The process starts with conceptual questions about the biological problem, becomes more and more technical, and gradually returns to the biological phenomenon under study. It is very important to devote sufficient effort to each of these phases, especially the early ones, because they ultimately determine whether the modeling effort has a good chance of succeeding.

One of the early choices addresses the explanatory character of the model. One may choose a comparatively simple regression model, which correlates two or more quantities to each other, without, however, suggesting a rationale for the correlation. As an example, one may find that cardiovascular disease is often associated with high blood pressure, but a regression model does not offer an explanation. As an alternative, one could develop a much more complex mechanistic model of cardiovascular disease, in which blood pressure is a component. If formulated validly, the model would explain the mechanistic connection, that is, the network of causes and effects leading from high blood pressure to the disease. Even if we disregard the difficulties in formulating a comprehensive mechanistic model, the choice of model type is not black and white. Regression models do not provide an explanation, but they often make correct, reliable predictions. An explanatory model may also be quite simple, but if it accounts for a large number of processes, it often becomes so

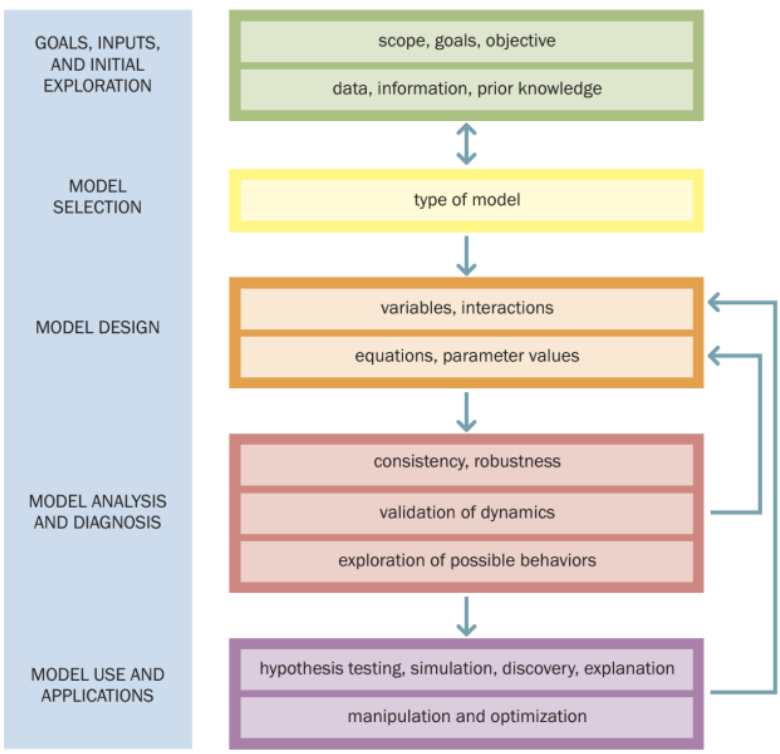

Figure 2.2 Flow chart of the modeling process. The modeling process consists of distinct phases, but is often iterative and involves refinements in model design and repeated diagnosis. See also Table 2.1. (Adapted from Voit EO, Qi Z & Miller GW. *Pharmacopsychiatry* 41(Suppl 1) [2008] S78–S84. With permission from Thieme Medical Publishers.)

TABLE 2.1: ISSUES TO PONDER DURING THE MODELING PROCESS

Goals, Inputs, and Initial Exploration

- Scope of the model, including goals, objectives, and possible applications
- Data needs and availability (types, quantity, quality)
- Other available information (nonquantitative heuristics, qualitative input from experts)
- Expected feasibility of the model
- Relevance and degree of interest within the scientific community

Model Selection

- Model structure
  - Explanatory (mechanistic) versus correlative (black box)
  - Static versus dynamic
  - Continuous versus discrete
  - Deterministic versus stochastic
  - Spatially distributed versus spatially homogeneous
  - Open versus closed
  - Most appropriate, feasible approximations

Model Design

- Model diagram and lists of components
  - Dependent variables
  - Independent variables
  - Processes and interactions
  - Signals and process modulations
  - Parameters
- Design of symbolic equations
- Parameter estimation
  - Bottom-up
  - Top-down

Model Analysis and Diagnosis

- Internal consistency (for example, conservation of mass)
- External consistency (for example, closeness to data and expert opinion)
- Reasonableness of steady state(s)
- Stability analysis
- Sensitivity and robustness analysis
- Structural and numerical boundaries and limitations
- Range of behaviors that can or cannot be modeled for (example, oscillations, multiple steady states)

Model Use and Applications

- Confirmation, validation of hypotheses
- Explanation of causes and effects; causes of failure, counterintuitive responses
- Best case, worst case, most likely scenarios
- Manipulation and optimization (for example, treatment of disease; avoidance of undesired states or dynamics; yield optimization in biotechnology)
- Discovery of design principles

complicated and requires so many assumptions that it may no longer describe or predict new scenarios with high reliability. In reality, most models are intermediates—explanatory in some aspects and correlative in others.

In the following sections, we discuss each phase of the modeling process and illustrate it with a variety of small, didactic "sandbox models" as well as a famous, very old model describing the spread of infectious diseases. The process begins with an exploration of the available information and the selection of a model that can utilize this information and ultimately yield new insights (see Figure 2.2 and

Table 2.1). Often, one will go back and forth between these two aspects before a suitable model design is chosen. Once an appropriate model type is determined, the actual model design phase begins. Components of the model are identified and characterized. The resulting model is diagnosed, and, should troublesome aspects emerge, one returns to the model design. A model that fares well in the diagnostic phase is used for explanations, simulations, and manipulations. It often turns out that model features are not optimal after all, requiring a return to the model design phase.

GOALS, INPUTS, AND INITIAL EXPLORATION

The initial phase of model development consists of defining the purpose of the model and of surveying and screening input data and contextual information. This setting of goals, combined with an exploration of available information and the context for the model, is arguably the most crucial of all phases. This assessment may sound counterintuitive, because one might think that the most effort would go into some complicated math. However, if insufficient consideration is given to this initial exploration, the modeling process may derail right away, and one may not even notice that something is not quite the way it was intended until the final phases of the modeling process.

The main task of the input and exploration phase is to establish the specific purposes and goals of the model, and to feel out whether the model may have a chance to achieve its goals, given the available data and information. "Feeling out" does not sound like rigorous mathematical terminology, but actually describes the task quite well. It is often here where experience is needed the most: right at the beginning of the effort!

2.1 Questions of Scale

The first important aspect of the exploration phase is to ask what are the most appropriate scales for the model, with respect to both time and space, and also with respect to the organizational level (**Figure 2.3**). These scales are typically tied to each other, because processes at the organizational level of a cell occur on a small spatial scale and usually run faster than processes at the scales of organisms or ecological systems. Suppose we are interested in trees and want to develop a mathematical model describing their **dynamics**. While that might sound like a fine idea, it is far too unspecific, because trees may be studied in many different ways. To model the growth of a forest or a tree plantation, the scale of organization is typically the individual tree, the spatial scale is in hectares or square kilometers, and the corresponding timescale is most likely in years. Contrast this with the photosynthesis in a leaf or needle, which truly drives tree growth. That organizational

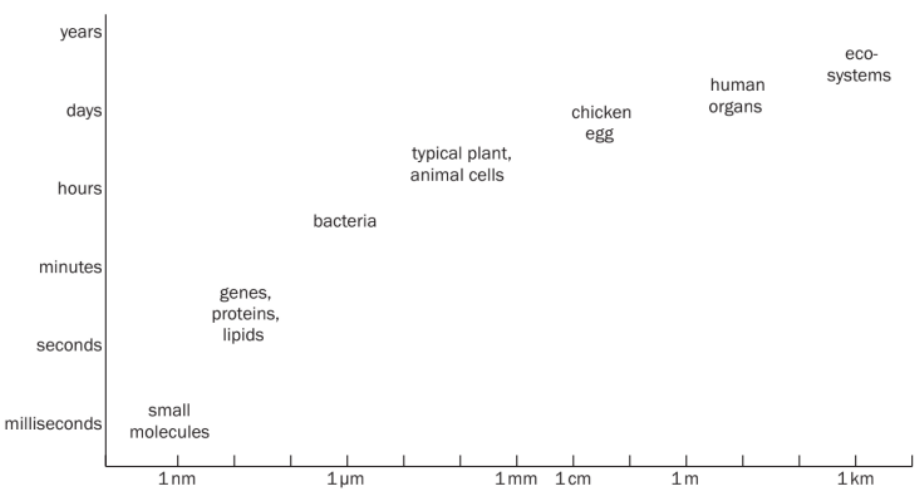

Figure 2.3 Scales in biology span several orders of magnitude in size and time. This plot shows typical sizes and time ranges. Of course, human organs and ecosystems contain small molecules and genes, so that they really span multiple size- and time-scales, which makes multiscale modeling challenging and fascinating.

level is biochemical or physiological, and the corresponding timescale is that of seconds or minutes. It is easy to imagine other time and organizational scales between or outside these two scenarios.

While multiscale modeling is one of the challenging frontiers of systems biology (see Chapter 15), the attempt to accommodate too many different scales at once is very difficult and, at least at the beginning of model design, dangerous. Although there are exceptions (see for instance Chapter 12), it is usually not really feasible to account in the same model for the entire spectrum of processes, from the details of photosynthesis or wood formation to the long-term growth of forests. Nonetheless, the real strength of mathematical models lies in their ability to explain phenomena at one level through the analysis and integration of processes at a lower level. Thus, a good model, even of small size, may span two or maybe three levels of biological organization. For instance, it may describe the formation of a leaf in terms of genetic and biochemical processes. A model may also skip some levels. For instance, we could try to associate long-term tree growth and final size with alterations in gene expression. In this case, one might be able to translate the effects of gene modulations into the tree's growth rate, but a model of this type would not capture all the biochemical and physiological causes and effects that occur between gene expression and the total amount of wood in the forest after 50 years.

The determination of suitable scales depends very significantly on the available data that are needed to construct, support, and validate the model. If only yearly biomass measurements of the tree stand are available, we should not even attempt to model the biochemistry that deep down controls tree growth. Conversely, if the model uses light conductance as main input data, good as they may be, this input information would hardly support a model of the growth of trees in the forest over many years.

As a different example, suppose it is our task to improve the yield of a fermentation process in which yeast cells produce alcohol. Typical experimental techniques for this task are selections of mutants and changes in the growth medium. Thus, if we intend to use a model to guide and optimize this process, the model must clearly account for gene activities and their biochemical consequences, as well as for concentrations of nutrients in the medium and their uptake by cells.

2.2 Data Availability

The quantity of available data provides important clues for exploring options of model selection and design. If a complicated model with many **variables** and parameters is supported only by very scarce data, the model may capture these data very nicely, but as soon as we try to apply it to a slightly different scenario, such as wider spacing of the trees or a richer fertilization regimen, the model predictions may become unreliable or inaccurate. This over-fitting problem (that is, using a model that is too complicated for the given data) always looms when only one or a few datasets are available. A similar problem arises if the data show a lot of **variability**, or noise, as it is often called in the field of data analysis. In this case, many models may yield a good representation of the particular data, but predictions regarding new data may be unreliable. Counterintuitive as it may sound, it is often easier to model bad data than good data, because bad data grant us a lot more latitude and potentially let us get away with an inferior model. Of course, this inferior model may turn round and bite us later when the model is expected to fit new data and simply doesn't!

While crisp, numerical data are the gold standard for mathematical modeling, we should not scoff at qualitative data or information that contains a fair amount of uncertainty. For instance, if we are told that experiments show some output increasing over time (even though the exact degree is not given), this piece of information can provide a valuable constraint that the model must satisfy. Especially in a clinical setting, this type of qualitative and semiquantitative information is prevalent, and the more of it that is available, the more any model of the phenomenon is constrained. Expressed differently, this information can be extremely helpful for weeding out models that could otherwise appear plausible. In a study of Parkinson's disease, we asked clinicians for concentrations of dopamine and other brain metabolites associated with the disease. While they could not give us exact values, they

provided us with rough, relative estimates of concentrations and metabolic fluxes through the system, and this information, combined with literature data, allowed us to set up a model that made surprisingly accurate predictions [1].

Finally, the exploration phase should ascertain whether experimental biologists or clinicians are really interested in the project. If so, their interest will help drive the project forward, and their direct insights and contacts with other experts can be invaluable, because much biological knowledge is not published, especially if it is semiquantitative or not yet one hundred percent supported by data and controls. It is, of course, possible to base models solely on information found in the literature, but if no experts are at hand, many pitfalls loom.

MODEL SELECTION AND DESIGN

To illustrate the process of model design, let's consider one specific example that we will carry through this chapter and revisit again in Chapter 4. This example concerns an infectious disease, such as tuberculosis or influenza, that easily spreads throughout a human population (**Figure 2.4**). Down the road, we would like to determine whether it is beneficial to vaccinate all or some individuals, whether infected persons should be quarantined, or whether the disease will eventually go away by itself because people recover and acquire immunity. Immediately, many questions come to mind. Do people die from the disease? If the death rate is small, can we ignore it in the model? How does the disease get started in the first place? For how long are people sick? How infectious are they? Are there individuals who show no symptoms yet are infectious? Do all people in our target population have potential contact with all other people? Do we need to account for air travel? Should we attempt to characterize waves of infection that start at the point of the first contact with an infected person and move through certain geographical areas or the entire population? Does our disease model have to be accurate enough to predict whether a specific person will get sick, or is our target the average infection level within a community or the nation? Clearly, questions are unlimited in number and type, and some of them may be important to ponder now while others are probably less relevant, at least at the beginning.

For our purposes of studying a very complex phenomenon with as simple a model as possible, let's suppose the following:

- The infectious disease is spread by human-to-human contact.
- Although bacteria or viruses are the true vectors of the disease, we ignore them.
- Individuals may acquire and lose immunity.
- The primary questions are how fast the disease progresses, whether it disappears without intervention due to immunity, and whether vaccination or quarantine would be effective.

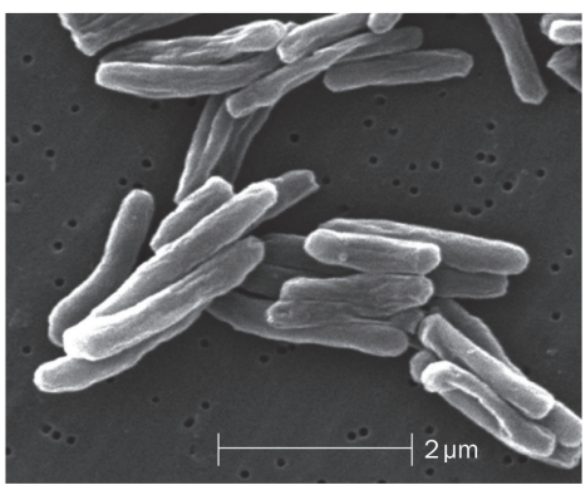

Figure 2.4 Mathematical modeling can be a powerful tool for studying the spread of bacterial diseases. Shown here is the bacterium *Mycobacterium tuberculosis*, which causes tuberculosis, one of the ongoing scourges of humankind.

- We do not intend to account for the spatial distribution of cases of infection throughout some geographical area.

2.3 Model Structure

Once we have decided what the model is supposed to accomplish, we need to determine its structure. Some decision points are given in Table 2.1. None of these are really associated with different degrees of sophistication, usefulness, or quality of the model. We are simply required to make decisions, again based on the data and the purposes of the model.

The first decision targets the question of how much detail the model is expected to explain. As mentioned before, the two extremes in this dimension are **correlative** and **explanatory models**; most models have aspects of both. A correlative model simply and directly relates one quantity to another. For instance, data may show that the infectious disease is particularly serious among the elderly; specifically, the number of infected individuals per 100,000 may increase with age (**Figure 2.5**). A **linear** or **nonlinear** regression could even quantify this relationship and allow us to make predictions about the average disease risk for a person of a certain age. However, even if the prediction were quite reliable, the model would not give us a hint as to why older people are more likely to become infected. The model does not account for health status, genetic predisposition, or lifestyle. Sometimes a prediction would be wrong, but the model would not be able to explain why.

An explanatory model, by contrast, relates an observation or outcome of an experiment to biological processes and mechanisms that drive the phenomenon under investigation. To illustrate, imagine an explanatory model of cancer. Cancer is associated with cells that are not dying when they should. Cell death normally occurs through a process called apoptosis, whose control involves the tumor suppressor protein p53, which in turn acts as a transcription factor for a number of pertinent genes. The p53 protein becomes activated under many stress conditions, such as ultraviolet radiation or oxidative stress. Pulling the pieces of information together allows us to compose a conceptual or mathematical model in the form of a chain of causes and effects: Suzie gets baked on the beach. She is exposed to enough ultraviolet radiation to activate the p53 proteins in her skin. The proteins change the normal activation of some genes. The genes cause some cells to escape apoptosis and proliferate out of control. Suzie is diagnosed with melanoma. The model explains to some degree (although not completely, of course) the development of cancer. This explanatory potential does not come for free: explanatory, mechanistic models are usually much more complicated than correlative models and require vastly more input in the form of data and assumptions. While explanatory models offer more insight, we should not discard correlative models offhand. They are much easier to construct and often make more reliable predictions than explanatory models, especially when assumptions are uncertain and data are scarce.

The second decision refers to the question of whether something associated with the phenomenon of interest changes over time. If the data show trends changing with time, we will probably prefer a dynamical model, which allows us to quantify these changes. By contrast, in a static model, such as a regression model, only the dependencies of one variable on others are explored. **Box 2.1** contrasts static

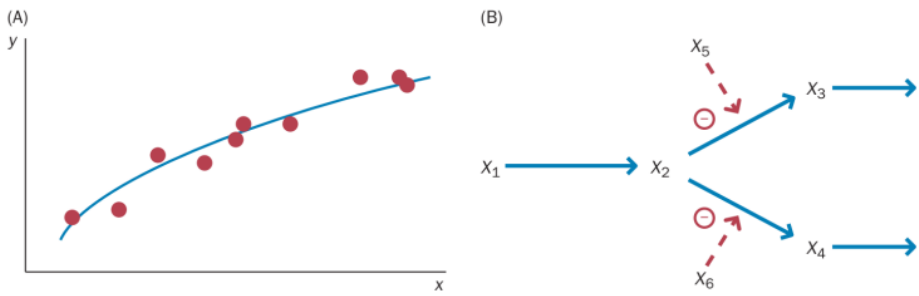

Figure 2.5 Correlative and explanatory models serve different purposes. The correlation between x and y in (A), whether linear or nonlinear, permits robust predictions of a value of y, given a new value of x, although the model does not explain the connection between x and y. An example could be disease prevalence (y) and age (x) among the elderly. The simple explanatory model in (B) could describe a metabolic pathway with two inhibitors (X_5 and X_6). It not only captures the (positive) correlation between X_3 and X_6, but also provides an explanation for why X_3 increases if the inhibitor X_6 of the other branch of the pathway is increased.

BOX 2.1: STATIC AND DYNAMIC MODELS

Suppose it is our task to compute the volume of an *Escherichia coli* cell. Studying a photograph of an *E. coli* culture (**Figure 1**), we see that each bacterium looks more or less like a hot dog with variable length and flattened ends, and since the volume doesn't change if the bacterium curves up, we can just study the shape when it is stretched out. Thus, a simple static model could be a cylinder with two "caps" at the end. The caps look a little flatter than hemispheres and we decide to represent them mathematically by halved ellipsoids that have the same axis radius in two directions (a circular cross section) and a shorter axis radius in the third direction (**Figure 2**). The formula for the volume of a whole ellipsoid is $\frac{4}{3}\pi r_1 r_2 r_3$, where r_1, r_2, r_3 are the axis radii. Thus, if the "straight" part of the cell is x µm long and has a diameter of y µm, and if each cap has a height of z µm, we can formulate a static model for the model of an *E. coli* cell (**Figure 3**) as

$$V(x,y,z) = \underbrace{x\pi\left(\frac{y}{2}\right)^2}_{\text{cylinder}} + \underbrace{2\left(\frac{4}{3}\pi\frac{y}{2}\frac{y}{2}\frac{z}{2}\right)}_{\text{two half-ellipsoildal caps}} \tag{1}$$

$$= \left(\frac{x}{4} + \frac{z}{3}\right)\pi y^2. \tag{2}$$

Our static model allows us to pick any length, width, and cap height and to compute the corresponding volume of the bacterium.

Let's now suppose the bacterium has just divided and is growing. The size parameters x, y, and z become variables that depend on time t, and we may write them as $x(t)$, $y(t)$, and $z(t)$. It may even happen that these variables do not develop independently from each other, but that the bacterium maintains some degree of proportionality in its shape, which would mathematically connect $x(t)$, $y(t)$, and $z(t)$ through constraint relationships. The main consequence is that the volume is no longer static, but a function of time. The model has become a dynamic or **dynamical** model.

In the typical understanding of dynamical models, one expects that the current state of the object or system will influence its further development. In the case of a bacterium, the growth in volume very often depends on how big the bacterium already is. If it is small, it grows faster, and as it reaches its final size before it divides, growth slows down. This "self-dependence" makes the mathematics more complicated. Specifically, one formulates the change in volume over time as a function of the current volume. The change in volume over time is given in mathematical terminology as its time derivative dV/dt. Thus, we come to the conclusion that volume-dependent growth should be formulated as

$$\frac{dV}{dt} = \text{some function of volume } V \text{ at time } t \tag{3}$$

$$= f\big(V(t)\big) = f(V). \tag{4}$$

This type of formulation, in which the derivative of a quantity (dV/dt) is expressed as a function of the quantity (V) itself, constitutes an ordinary differential equation, which is also lovingly called a diff. eq., o.d.e., or ODE. Very many models in systems biology are based on differential equations. The function f is not limited to a dependence on V; in fact, it most often contains other variables as well. For example, f could depend on the temperature and the substrate concentration in the medium, which will certainly affect the speed of growth, and on a lot of "internal" variables that describe the uptake mechanisms with which the bacterium internalizes the substrate. Looking more closely, many biochemical and physiological processes are involved in the conversion of substrate into bacterial volume and growth, so that a very detailed dynamical model can quickly become quite complicated. Setting up, analyzing, and interpreting these types of dynamical models is at the core of computational systems biology.

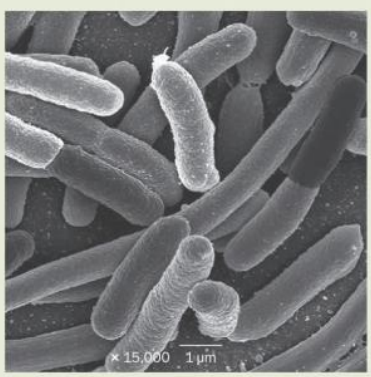

Figure 1 The shape of *E. coli* bacteria resembles cylinders with caps. The shape may be modeled approximately with the geometrical shapes shown in Figures 2 and 3.

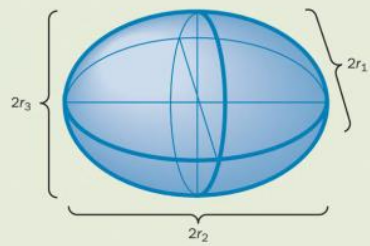

Figure 2 To model the shape of a bacterium (see Figure 1), we need a cylinder and "caps" at both ends. The caps can be modeled with flat ellipsoids. Here, such an ellipsoid is shown with two longer radii of the same length $r_1 = r_2$ and one shorter radius r_3.

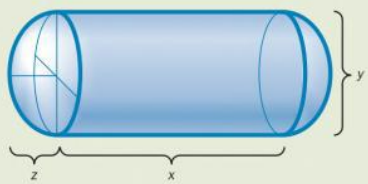

Figure 3 Static model of the volume of *E. coli*. The model is inspired by the actual cell shape (Figure 1) and composed of a cylinder with caps at both ends (Figure 2).

It is no secret that biological data always contain variability, uncertainties, measurement inaccuracies, and other types of noise. Thus, as a final point to ponder when choosing a model: should we—must we—always use a model that somehow captures this randomness? Not necessarily. If we are primarily interested in average model responses, a deterministic model will generally do just fine. However, if we need to explore all possible outcomes, the worst and best cases, or the likelihood of specific outcomes from a system with a lot of uncertainty, we may be best advised to choose a stochastic model.

Because this is an important and subtle issue, let's study similarities and differences between deterministic and stochastic models in more detail. As we discussed, each trial in a stochastic system is unpredictable, but we can make very accurate predictions about average or long-term behaviors. By comparison, deterministic models present us with situations where everything is exactly and unambiguously defined. Yet, there are strange cases where the behavior of the system over time is so complicated that we cannot forecast it. A quite simple, but very instructive example is the blue sky catastrophe, which is discussed in **Box 2.2**. This system is completely determined and does not contain any noise or stochastic features. All information about the system is fixed in its definitions and settings, and yet the system is able to generate chaotic time courses that are impossible to anticipate. Chapter 4 discusses more cases of this nature.

As an example of a stochastic process, suppose we irradiate a culture of microorganisms with ultraviolet light (see, for example, [4]) in order to introduce random mutations. This method of random mutagenesis is often used to create strains that out-compete the wild type in a specific situation of interest. For instance, we may want to find a strain with improved tolerance to acid in its environment. We know from genetics that most mutations are neutral or decrease rather than increase fitness. Nonetheless, one mutation in a million or a billion may improve the microorganism's survival or growth rate, and this particular mutation will rise to the top and multiply in a properly designed random mutagenesis experiment. For simplicity, let's suppose that the mutations occur randomly throughout the genome with a rate of between three and four mutations per kilobase (1000 base pairs of DNA) [5].

A typical question in this scenario is: What is the probability of actually finding three or four mutations in a given DNA segment that is one kilobase long? Our first inclination might be to claim (wrongly) that the probability is 1, because isn't that what we just assumed? Not quite. We assumed that the *average* number of mutations is between three and four, and need to realize that it could happen that there are only one or two mutations, or five or six, owing to the randomness of the mutation process. Models from probability theory allow us to assess situations of this type. For instance, we can forecast the probability of encountering one, two, or three mutations in a given piece of DNA. Or we can make statements like "with p percent probability, we will find at least four mutations." Would it be possible to find 200 random mutations within 1000 base pairs? In a true stochastic process, this outcome is indeed theoretically possible, although the probability is extremely low.

Even in this clear-cut scenario with precise conditions and well-defined questions, different models are available. For instance, we could use the binomial model or the Poisson model. Both are applicable in principle. The binomial model would be better suited for small numbers (here, short segments of DNA), while it would be very cumbersome for large numbers (long segments of DNA). In fact, a pocket calculator would not be able to manage the numbers that would have to be computed for 1000 kilobases. The Poisson model uses a clever approximation, which is not very accurate for small numbers (short segments of DNA), but is incomparably easier to use and misses the correct results for large numbers (long segments of DNA) by only very small errors. **Boxes 2.3** and **2.4** discuss these two models as typical representatives of simple stochastic models.

2.4 System Components

Biological systems models contain different classes of components, which we discuss in this section. The most prominent components are variables, which represent biological entities of interest, such as genes, cells, or individuals. Because biological

BOX 2.3: THE BINOMIAL PROCESS: A TYPICAL STOCHASTIC MODEL

In the text, we asked: What is the probability of finding a certain number of mutations in a DNA segment, if one typically observes three or four mutations per 1000 base pairs? Let's do some math to find out. We formulate the problem as a binomial process, for which we need two ingredients, namely the average mutation rate, which we assume to be 3.5 (instead of "3 or 4") mutations per kilobase, and the number of nucleotides within the DNA segment of interest. From this information, we can compute how likely it is to find 3, 4, 1, 20, or however many mutations within some stretch of DNA.

In the language of probability theory, a mutation in our experiment is called a success and its (average) probability is usually termed p; in our case, p is 3.5 in 1000, that is, 0.0035. A failure means that the base is not mutated; its probability is called q. Because nothing else can happen in our experiment (either a base is mutated or it is not; let's ignore deletions and insertions), we can immediately infer $p + q = 1$. Put into words: "for a given nucleotide, the probability of a mutation plus the probability of no mutation together are a sure bet, because nothing else is allowed to happen."

In order to deal with more manageable numbers, let's first assume that the DNA piece contains only $n = 10$ bases. Then the probability P to find k mutations is given as

$$P(k;n,p) = \frac{n!}{k!(n-k)!} p^k (1-p)^{n-k}, \tag{1}$$

according to the binomial formula of probability theory, which we will not discuss here further. In this formulation, the term $n!$ (pronounced "n factorial") is shorthand for the product $1{\cdot}2{\cdot}3{\cdot}\cdots{\cdot}n$. The semicolon within the function indicates that k is the independent variable, while n and p are constant parameters, which may, however, be changed from one example to the next. So, what is the probability of finding exactly one mutation in the 10-nucleotide segment? The answer results from substituting values for the parameters and for k, namely,

$$P(1;10,0.0035) = \frac{10!}{1!9!} 0.0035^1 (1-0.0035)^9 = 0.0339. \tag{2}$$

That is not a very big probability, so the result is rather unlikely: roughly 3%. What about two mutations? The answer is

$$P(2;10,0.0035) = \frac{10!}{2!8!} 0.0035^2 (1-0.0035)^8 = 0.000537, \tag{3}$$

which is even smaller. It is not difficult to see that the probabilities become smaller and smaller for higher numbers of mutations. So, what's the most likely event? It is that there is no mutation at all. This probability is given by the binomial **probability distribution** (1) just like the others, namely,

$$P(0;10,0.0035) = \frac{10!}{0!10!} 0.0035^0 (1-0.0035)^{10} = 0.9655. \tag{4}$$

In over 96% of all 10-nucleotide DNA segments, we expect to find no mutation at all!

From these three results, we can estimate the chance of finding more than two mutations. Because all probabilities taken together must sum to 1, the probability of more than two mutations is the same as the probability of *not* having zero, one, or two mutations. Thus, we obtain

$$P(k > 2) = 1 - 0.9655 - 0.0339 - 0.000537 \approx 0.00002, \tag{5}$$

which is 2 in 100,000!

If we increase the length of the DNA piece, we should intuitively expect the probabilities of finding mutations to increase. Using the same formula, the probability of exactly one mutation in a 1000-nucleotide segment can be written as

$$P(1;1000,0.0035) = \frac{1000!}{1!999!} 0.0035^1 (1-0.0035)^{999} \tag{6}$$

This is a perfectly fine formulation, but our pocket calculator goes on strike. 1000! is a huge number. To ameliorate the situation, we can play some tricks. For instance, we see from the definition of factorials ($n! = 1{\cdot}2{\cdot}3{\cdot}\cdots{\cdot}n$) that the first 999 terms in 1000! and 999! are exactly the same and cancel out from the fraction, leaving us simply with 1000 for the first term. With that, we can compute the probability of 1 mutation as 0.1054 and the probability of no mutations at all as 0.0300 (0! is defined as 1). Indeed, in contrast to the scenario with 10 bases above, we are now about three times more likely to encounter one mutation than no mutation at all. Is it imaginable that we would find 10, 20, or 30 mutations? The probability of finding 10 mutations is about 0.00226. Formulate the corresponding probabilities for 20 and 30. It is not hard to do, but even with the tricks above we run into problems computing the numerical results from the formulae. Box 2.4 offers a solution!

chemical reaction, which is the number of molecules the reaction can process within a given amount of time. The particular values of the parameters depend on the system and its environment. They are constant during a given computer experiment, but may assume different values for the next experiment. Finally, there are (universal) constants, such as π, e, and Avogadro's number, which never change.

In order to design a specific formulation of a model, we begin with a diagram and several lists, which are often developed in parallel and help us with our bookkeeping. The diagram contains all entities of the biological system that are of interest and indicates their relationships. These entities are represented by variables and drawn as nodes. Connections between nodes, called edges, represent the flow of material from one node to another. For instance, a protein in a signaling cascade may be unphosphorylated or phosphorylated in one or two positions. Within a short timespan, the total amount of the protein is constant, but the distribution among the three forms changes in response to some signal. Thus, a diagram of this small protein phosphorylation system might look like **Figure 2.7**.

In contrast to the flow of material, a diagram may also contain the flow of information. For instance, the end product of a pathway may signal—through **feedback** inhibition—that no further substrate should be used. This signal is distinctly

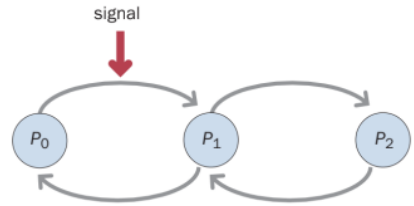

Figure 2.7 Different states of a protein in a signaling cascade. The protein may be unphosphorylated (P_0) or singly (P_1) or doubly (P_2) phosphorylated. The total amount of the protein remains the same, but, during signal transduction, material flows among the pools, driven by kinases and phosphatases, which are not shown here (see Chapter 9).

experiment and could therefore be considered independent. This replacement would make the model simpler, but might preclude analyses of other model settings where the variable does change. It actually happens more frequently that independent variables are replaced by dependent variables in later model extensions. A reason could be that the extended system affects the dynamics of a formerly independent variable. Second, if an independent variable is constant throughout an experiment, we could replace it with a parameter. However, it is often beneficial to keep parameters and independent variables separate. First, variables and parameters have a different biological meaning, which may help us with the estimation of appropriate values for them. And second, the computational effort of including an independent variable or a parameter is the same.

The third list is actually closer to a table or spreadsheet. It shows which of the (dependent or independent) variables have a direct effect on any of the processes in the system. As in the example of Figure 2.8, an effect may be positive (enlarging or activating) or negative (diminishing or inhibiting). The list accounts for all edges (flow of material between pools), as well as all signals that affect any of the edges.

The final list contains parameters that contribute to the external or internal conditions of the system, such as pH, temperature, and other physical and chemical determinants, which will ultimately require numerical specification.

Throughout the model design process, we will add to these lists quantitative information about pool sizes, magnitudes of fluxes, strengths of signals, and normal values and ranges of parameters.

The lists are the basis for establishing a diagram of the system. For the design of this diagram, it is beneficial to begin by representing each variable with one of the core modules in **Figure 2.9**. In the simpler case (Figure. 2.9A), each variable is displayed as a box with one process entering and one exiting. In the end, there may be more or fewer arrows, but using one influx and one efflux is a good default, since it reminds us later to include these processes when we set up equations. Indeed, a dependent variable tends to deplete without influx and to keep accumulating without efflux. Of course there are situations where depletion or accumulation is the desired behavior, and, if so, we can secondarily remove the corresponding process. However, these situations are quite rare. We may also use the default in Figure 2.9(B), which reminds us that many processes are regulated by other variables and that the variable in question may send out signals that could affect the influxes or effluxes of other variables. Independent variables typically do not have an influx, but they have an efflux if they feed material into the system. Once all variables are defined, they need to be functionally connected: the efflux of one variable may become the influx of another, and the signal sent by one variable may affect the influxes or effluxes of other variables.

Let's illustrate this construction of a diagram with the example of a population that is experiencing the outbreak of an infection. The model is intended to answer some of the questions mentioned earlier. Following some old ideas of Kermack and McKendrick [7], we use a similar terminology and keep things as simple as possible; one should, however, note that thousands of variations on this model have been proposed since Kermack and McKendrick's days. We begin by defining just three dependent variables, namely, the number of individuals susceptible to the disease (S), the number of infected individuals (I), and the number of individuals that are "removed" (R) from the two pools, because they have acquired immunity. We suppose that all individuals could be in contact with each other, at least in principle, and assume that a certain percentage of the immune individuals (R) lose their immunity and become susceptible again. We also allow for the possibility that individuals are born or immigrate and that individuals may die while infected. A diagram summarizing this population dynamics is shown in **Figure 2.10**. Processes

Figure 2.9 Default core modules. When designing a diagram of a system, it is beneficial to augment the display of each variable with an influx and efflux (A) and possibly with modification signals that affect the variable or are sent out by the variable (B).

Toggle switch (genetics)
A phenomenon or **model** in which a gene is either fully expressed or silent, but does not allow intermediates. (Chapter 14)

Toll-like receptor (TLR)
A member of a class of **signaling** proteins that recognize a variety of foreign macromolecules, especially those on the cell walls of bacteria, and trigger the initiation of an immune response. See also **Receptor** and **Transmembrane protein**. (Chapter 7)

Training set
A portion of a dataset used by a **machine learning algorithm** to distinguish different classes of outputs and to establish classification rules. See also **Validation**. (Chapter 5)

Trajectory
The collective change in the **state** of a **system** over time; usually between a **stimulus** and some later state, such as a steady state. (Chapter 10)

Transcription
The process of creating a matching messenger RNA (mRNA) from a DNA sequence. (Chapter 6)

Transcription factor
A protein affecting the expression of a specific gene by binding to the corresponding DNA. (Chapter 6)

Transcriptome
The totality of RNAs transcribed from the **genome** of an organism. See also **-ome**. (Chapter 6)

Transfer RNA (tRNA)
One of several specific RNAs that facilitate the attachment of the correct amino acid to a growing **peptide** or protein, while it is being translated from mRNA. This **translation** process occurs within a ribosome. (Chapter 6)

Transient
A collective term for behaviors of a **system** between the time of a stimulus and the time the system reaches a **steady state**, a **stable oscillation**, or some other **attractor** or point of interest. (Chapter 4)

Translation
The process of generating a protein or **peptide** whose **amino acid** sequence corresponds to the **genetic code** of an mRNA. The process occurs within a ribosome and uses **transfer RNA**. See also **Post-translational modifications**. (Chapter 6)

Transmembrane protein
A member of a large class of proteins that span the membrane of a cell and thereby allow communication between the outside and inside of the cell. Transmembrane proteins are involved in **signal transduction** and a large number of diseases. See also **Gap junction** and **G-protein-coupled receptor.** (Chapter 7)

Transposon
A stretch of DNA that can move from one location in a **genome** to another. (Chapter 6)

Trial (stochastic process)
One of many evaluations of a **stochastic model**. (Chapter 2)

Two-component (signaling) (TCS) system
While not exclusively limited to this definition, usually a specific type of **signaling** mechanism, with which microorganisms sense their environment. See also **Receptor** and **Signal transduction**. (Chapter 9)

Two-dimensional gel electrophoresis
An experimental method for separating proteins by their size and electrical charge. (Chapter 7)

Ubiquitin
A small protein that, when attached to other proteins, tags them for disassembly in the **proteasome** and later recycling of the resulting **peptides** and **amino acids**. The tagging process is called ubiquitination. (Chapter 7)

Uncertainty
Degree to which an experimental result is not exactly known, owing to experimental inaccuracies or other challenges; see also **Variability**. Advanced measurement techniques might reduce uncertainty, but not variability. (Chapter 5)

Validation
The process of testing (and confirming) the appropriateness of a **model** with data that had not been used for the construction of the model. See also **Training set**, **Identifiability**, and **Sloppiness**. (Chapter 2)

Variability
Degree to which an experimental result is affected by differences among individual cells, organisms, or other items under investigation; see also **Uncertainty**. Advanced measurement techniques might reduce uncertainty, but not variability. (Chapter 2)

Variable
A symbol representing a component of a **system**, which may or may not change over time. See also **Dependent** and **Independent variable**. (Chapter 2)

Vector
1. In mathematics, a convenient arrangement of variables or numbers in a one-dimensional array. Often used in conjunction with a **matrix**. Many aspects within the fields of linear algebra, **multivariate** calculus, and statistics are based on vectors and matrices. (Chapter 4)
2. In genetic engineering, an artificially constructed virus or plasmid used to introduce foreign DNA into a bacterium or host cell. (Chapter 14)

Ventricle
One of the two larger chambers of the heart. See also **Atrium**. (Chapter 12)

Vertex (pl. Vertices)
Synonym of **node**. (Chapter 3)

Voltage-gated
Property of a membrane channel to open and close in response to the membrane potential. (Chapter 12)

Western blot
A technique for measuring gene expression based on produced protein. See also **Northern blot**. (Chapter 6)

XML
Abbreviation for eXtensible Mark-up Language. Computer language with a defined vocabulary that permits the structuring of data and facilitates translation into different languages and software packages. See also **Ontology** and **SBML**. (Chapters 1 and 15)

X-ray crystallography
A technique for determining the structure of a protein. The protein must first be crystallized. An X-ray shot through the crystal scatters in a characteristic fashion that allows the deduction of the molecular structure of the protein. (Chapter 7)

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