Biology is more theoretical than physics

Jeremy Gunawardena
Department of Systems Biology, Harvard Medical School, Boston, MA 02115

ABSTRACT **The word "theory" is used in at least two senses—to denote a body of widely accepted laws or principles, as in "Darwinian theory" or "quantum theory," and to suggest a speculative hypothesis, often relying on mathematical analysis, that has not been experimentally confirmed. It is often said that there is no place for the second kind of theory in biology and that biology is not theoretical but based on interpretation of data. Here, ideas from a previous essay are expanded upon to suggest, to the contrary, that the second kind of theory has always played a critical role and that biology, therefore, is a good deal more theoretical than physics.**

Monitoring Editor
Doug Kellogg
University of California, Santa Cruz

Received: Feb 5, 2013
Revised: Apr 1, 2013
Accepted: Apr 10, 2013

In a previous essay, I pointed out the curious case of the enzyme–substrate complex, which was widely used to understand enzymes before any enzyme–substrate complex was shown to exist (Gunawardena, 2012). Britton Chance, who brought these hypothetical entities into existence, was in no doubt that he was providing the first experimental confirmation of a theory (Gunawardena, 2012). In the intervening 30 years, biochemists happily used a theoretical entity because it was so useful and explained so much. Expediency overcame the kind of philosophical scruples that would make a physicist swoon.

Perhaps this is a marginal episode among enzymologists, which can be glossed over in favor of the party line that biology is not, of course, theoretical. I claim that this is far from the case. In fact, similar episodes have occurred throughout biology, involving some of its most important entities.

The receptor is a case in point. We now know many different types of receptor and have identified the corresponding gene families in various genomes (Ben-Shlomo *et al.*, 2003). Receptor theory, however, emerged in the pre–molecular era, in the work of Paul Ehrlich in immunology and John Newport Langley in physiology (Maehle *et al.*, 2002). It was the latter who saw, in the antagonistic interplay of alkaloid drugs, evidence for a "receptive substance" (Langley, 1905) to which the drugs could chemically bind. Further evidence came from one of Langley's students, at the time an undergraduate at Trinity College, Cambridge, who showed that the binding of a drug, D, to a receptive substance, R, would yield a steady-state fraction of bound receptors, DR, that varied with drug concentration, [D], as

$$\frac{[DR]}{R_{tot}} = \frac{[D]}{K + [D]} \qquad (1)$$

where K is a constant that depends on the binding (Hill, 1909). This formula reproduced the experimental data for nicotine acting on frog muscle, with contraction saturating hyperbolically at high concentrations of D. Formula 1 looks suspiciously like that of Michaelis and Menten, without the catalysis, and, indeed, the student anticipated their mathematical calculation (Colquhoun, 2006; Gunawardena, 2012). In a similar way to the enzyme–substrate complex, formula 1 provided evidence for the imagined, hypothetical receptive substance, R.

Langley's student was Archibald Vivian Hill, known better for the work on hemoglobin that won him a Nobel Prize and for his widely used Hill function. Why his first published paper faded from sight remains a mystery, but it was the seed from which quantitative pharmacology subsequently flowered (Colquhoun, 2006). In the hands of Alfred Clark and a succession of others, the mathematics of chemical binding to hypothetical receptive substances became the basis for understanding "the mode of action of drugs on cells" (Clark, 1933) and remains so to this day (Colquhoun, 2006; Limbird, 2004).

It took 30 years for the enzyme–substrate complex to become a chemical reality; the receptor took a good deal longer. When Raymond Ahlquist published his fundamental quantitative study in 1948 that delineated the α- and β-adrenergic receptors he was careful to say, "The adrenotropic receptors are those hypothetical structures or systems located in, or near, the muscle or gland cells affected by epinephrine" (Ahlquist, 1948). The report of a 1967 conference on the adrenergic receptors alluded to "the nebulous concept of the receptor" (Dresel, 1967). Receptors that respond to chemical stimuli (as opposed to the photoreceptor that responds

DOI: 10.1091/mbc.E12-03-0227
Address correspondence to: Jeremy Gunawardena (jeremy@hms.harvard.edu).

to light) were finally brought to light in the 1970s, but even as late as 1973, Ahlquist, of all people, remained skeptical of their material existence: "To me they are an abstract concept conceived to explain observed responses of tissues"[1] (Ahlquist, 1973). They had remained abstract for nearly three-quarters of a century while providing the intellectual basis for understanding physiology and drug action.

A similarly abstract entity to the receptor was the ion channel. We can trace its origin back to Hodgkin and Huxley's seminal work in the early 1950s on the squid giant axon, which culminated in their famous mathematical model (Hodgkin and Huxley, 1952). The Hodgkin–Huxley model is often said to explain the action potential, whose complex temporal behavior it beautifully reproduces, but it can do so only by assuming hypothetical entities that would later become the voltage-gated sodium and potassium channels. Hodgkin and Huxley were careful not to speculate on how these entities worked, saying only that "Details of the mechanism will probably not be settled for some time" (Hodgkin and Huxley, 1952). The entities were suggested to be protein channels in the plasma membrane in the 1960s; they were eavesdropped upon with patch clamps in the 1970s; and they were finally cloned in the 1980s (Catterall, 2012). By then, the channel concept had been widely used for 30 years to understand the nerve impulse in several excitable tissues.

Coincidentally, Hodgkin and Huxley were also at Trinity College, Cambridge. I remember Andrew Huxley, then Master of the College in succession to Alan Hodgkin, telling us awed young mathematicians how he heroically integrated the fearsome equations of the Hodgkin–Huxley model with a mechanical hand calculator. It took him, he said, only a few weeks.

The most dramatic example of a biological entity whose chemical nature remained mysterious while mathematical calculations allowed it to be widely used, is, of course, the gene. Mendel's algebra was rediscovered in 1900, and in the hands of the Morgan school, the "theory of the gene" (Morgan, 1926) became enormously successful. In parallel with this, the mathematical population genetics of Fisher, Haldane and Wright showed how continuously varying traits were consistent with discrete genes and laid the foundations for the neo-Darwinian synthesis of genetics and evolution (Dobzhansky, 1937). But what, exactly, were genes? They were evidently related to the chromosomes found by the microscopists—Morgan's Nobel Prize carefully cites his "discoveries concerning the chromosome in heredity" and does not mention genes—but, well into the 1940s, it was the protein component of the chromosomes, not the nucleic acid, that was believed to be hereditarily relevant: "Knowing what we now know from X-ray and related studies of the fibrous proteins … it is but natural to assume … that they form the long scroll on which is written the pattern of life" (Astbury and Bell, 1938). Even when the significance of DNA as genetic material became clearer, the mechanism of genetic self-reproduction remained mysterious at least until the time of Watson and Crick. Morgan was quite firm on the matter: "Frankly, these are questions with which the working geneticist has not much concern himself … There is no consensus of opinion as to what the genes are—whether they are real or purely fictitious" (Morgan, 1965). Only mathematics can keep you on the straight and narrow in the midst of such ontological uncertainty. One of the most important and productive concepts in modern biology was, for ever such a long time, a mathematical fiction.

[1]I am indebted to Bob Lefkowitz's 2012 Nobel lecture (Lefkowitz, 2012) for this wonderful quote.

There are two further instances in which genes of a particular type were exploited on the basis of calculation before their chemical identity was confirmed. The first is Luria and Delbrück's demonstration, based on their famous fluctuation theorem, that phage resistance in bacteria arose through mutation and selection (Luria and Delbrück, 1943), which created the field of bacterial genetics. The second is Alfred Knudson's prediction, based on statistical analysis of retinoblastoma, of tumor-suppressor genes (anti-oncogenes) and his two-hit hypothesis, which was "the guiding principle in our search for tumor suppressor genes in colorectal cancer over the past 15 years" (Baker *et al.*, 2003) and led to the prevailing view of carcinogenesis as a cascade of somatic mutations (Knudson, 2001).

In each of these examples—enzyme–substrate complex, receptor, ion channel, gene, tumor suppressor—a material entity was hypothesized to exist. Mathematical reasoning was used to show that certain assumptions about the entity led to conclusions that explained experimental findings, thereby providing evidence for the unseen entities. Mathematical arguments were more compelling as explanations than informal stories because of their logical necessity. If you accept the assumptions of a mathematical argument, you are required to accept its conclusions. If Socrates is a man and all men are mortal, you cannot deny that Socrates is mortal. At heart, mathematical reasoning is no more than such Aristotelian syllogisms dressed up in modern garb. (Of course, there is the small issue that the argument must be correct in the first place, but let us not chase that rabbit here.)

The entities mentioned above provided a conceptual framework for interpreting data and designing new experiments—for reasoning about reality—that allowed biology to move forward despite the hypotheses not being universally accepted and the entities remaining, in the words of those who worked on them, hypothetical, nebulous, abstract, or fictitious. Biology turns out, on this reading, to be a good deal more theoretical than physics. However contrary this may be to the party line, we really should not be so surprised. Biology, after all, is a good deal harder than physics. If scientific research is stumbling around in a dark cellar looking for a black cat, then biology is doing so without knowing there is a cat there until one accidentally falls over it. Theory can sometimes conjure up the cat before the accident. Those biologists who have exploited that capability—Delbrück, Fisher, Haldane, Hill, Hodgkin, Huxley, Knudson, Luria, Michaelis, Mendel, Menten, Morgan, and Wright, among the few mentioned here—have lit the cellar for others to follow. The value of theory is often claimed to lie in making predictions or in fitting models to data, both of which are no doubt commendable, but, as the examples here reveal, theory has played a far more valuable role by showing us how to think about entities that lie beyond our grasp. It has helped biologists to see in the dark.

But perhaps this is now history rather than science. Are we not in the era of systems biology, in which we know all the entities and have enumerated the parts lists of organisms? What role does theory play now? Well, leaving aside the fact that we continue to fall over new entities—the RNA world has provided several of late—systems biology must still unravel the mechanisms through which molecular entities give rise to physiology. Theory can also illuminate mechanisms.

Mechanisms, however, are more elusive than entities. Once an entity is confirmed to exist, whatever led to that discovery, whether mathematical reasoning or inspired hunch, becomes irrelevant. Existence can no longer be denied. But whether a proposed mechanism is believed to be the correct explanation of some aspect of biology is contingent upon our state of ignorance. How a mechanism works depends on the context in which we think it might be

operating. Nowhere is this tension between mechanism and context, between component and system, more acute than in embryological development, whose analysis has stimulated an intricate dialogue between theory and experiment extending over many rounds of apparent success and subsequent failure (Roth, 2011). Such interplay requires careful analysis that is beyond the scope of this short essay.

Nevertheless, the following remarks suggest that theory may play a different role in systems biology as compared with theory in the pre–systems era discussed above or, indeed, in physics and engineering. A theory, or, more concretely, a model, of a mechanism is not a description of reality; it is a description of our assumptions about reality, as Michaelis and Menten already showed us (Gunawardena, 2012). Models, therefore, have to evolve with our knowledge: they are always wrong but sometimes useful. This is as true for the experimentalist's informal model, the cartoon in the last figure, as for the theorist's formal model in the Supplemental Information. What the former lacks in deductive capability it makes up for in greater flexibility to shifting assumptions, which is perhaps why it remains valuable. Informality and formality may need to coexist, in ways inconceivable in physics and engineering.

Such issues prompt the question as to whether the theory we have is adequate for the task at hand. The struggles with embryological development have prompted more than one biologist to suggest otherwise (Roth, 2011). Indeed, there is a loose end, or perhaps a slow-burning fuse, which I point out with some trepidation. Our current strategy is founded on the view that *the properties of the components determine those of the system*. We call this reductionism, and it has been the high point of scientific progress, especially in biology. Let us entertain, however, another view, that *the properties of the components depend on the system of which they are a part*. We encounter this all the time, but we prefer not to say so explicitly. For instance, we prefer to say that "a protein is determined by its gene sequence." Really? If the intracellular pH or ionic balance is not right, the protein will not assemble correctly. If the packaging machinery in the endoplasmic reticulum does not put on the right modifications or the chaperone machinery is unable to fold up the polypeptide, what emerges will have quite different properties. The *frq* gene, a central component of the circadian clock, shows nonoptimal codon usage in *Neurospora*. If the codons are optimized, the resulting polypeptide exhibits altered structure, stability, and phosphorylation pattern, and the clock no longer works (Zhou *et al.*, 2013). Yet, it is the same amino acid sequence. The phrase "a protein is determined by its gene sequence" is shorthand for saying that, actually, a protein is not determined by its gene sequence but also needs a functioning cell, of the right type. The protein depends on the system of which it is a part. To an experimentalist, the reality behind the shorthand is well understood; it must be remembered when designing any experiment. We might pause to worry about how the language we use constrains the way we think, but the question is, why do we confess the primary role of the system in our private day-to-day work while, at the same time, publicly insisting that reductionism works?

In part, it is because we lack an adequate theoretical framework in which both views of component and system are valid on an equal footing. When we do not know how to reason, we do not. We also like a quiet life. We prefer private schizophrenia to public outrage. There is so much we can do now, so much data to be gathered, so many reductionist models to be built, why spoil the party with explosive philosophical distractions? Expediency seems the best option, with freaks like *frq* being marginalized. But the loose end remains; the fuse continues to smolder. Eventually, we will need a new kind of mathematical theory to reconcile component and system. Theory's greatest contribution to biology may yet lie in the future.

ACKNOWLEDGMENTS

I thank two anonymous reviewers for their thoughtful comments and Alfonso Martinez-Arias for pointing out the reference to Roth (2011).

REFERENCES

Ahlquist R (1948). A study of the adrenotropic receptors. Am J Physiol 153, 586–600.

Ahlquist R (1973). Adrenergic receptors: a personal and practical view. Perspect Biol Med 17, 119–122.

Astbury WT, Bell FO (1938). Some recent developments in the X-ray study of proteins and related structures. Cold Spring Harbor Symp Quant Biol 6, 109–121.

Baker S, Kinzler KW, Vogelstein B (2003). Knudson's hypothesis and the TP53 revolution. Genes Chromosomes Cancer 38, 329.

Ben-Shlomo I, Hsu SY, Rauch R, Kowalski HW, Hsueh AJW (2003). Signaling receptome: a genomic and evolutionary perspective of plasma membrane receptors involved in signal transduction. Sci STKE 187, re9.

Catterall WA (2012). Voltage-gated sodium channels at 60: structure, function and pathophysiology. J Physiol 590, 2577–2589.

Clark AJ (1933). The Mode of Action of Drugs on Cells, London: Edward Arnold.

Colquhoun D (2006). The quantitative analysis of drug-receptor interactions: a short history. Trends Pharmacol Sci 27, 149–157.

Dobzhansky T (1937). Genetics and the Origin of Species, New York: Columbia University Press.

Dresel PE (1967). Report on the discussion of session I: general concepts of receptors. Ann NY Acad Sci 139, 645–648.

Gunawardena J (2012). Some lessons about models from Michaelis and Menten. Mol Biol Cell 23, 517–519.

Hill AV (1909). The mode of action of nicotine and curari determined by the form of the contraction curve and the method of temperature coefficients. J Physiol 39, 361–373.

Hodgkin AL, Huxley AF (1952). A quantitative description of membrane current and its application to conduction and excitation in nerve. J Physiol 117, 500–544.

Knudson AG (2001). Two genetic hits (more or less) to cancer. Nat Rev Cancer 1, 157–162.

Langley JN (1905). On the reactions of cells and of nerve-endings to certain poisons, chiefly as regards the reaction of striated muscle to nicotine and curari. J Physiol 33, 374–413.

Lefkowitz RJ (2012). A brief history of G-protein coupled receptors. Nobel Lecture slides. Available at: www.nobelprize.org/nobel_prizes/chemistry/laureates/2012/lefkowitz-lecture_slides.pdf (accessed 14 May 2013).

Limbird LE (2004). The receptor concept: a continuing evolution. Mol Interv 4, 326–336.

Luria SE, Delbrück M (1943). Mutations of bacteria from virus sensitivity to virus resistance. Genetics 28, 491–511.

Maehle A-H, Prüll C-R, Halliwell RF (2002). The emergence of the drug receptor theory. Nat Rev Drug Discov 1, 637–641.

Morgan TH (1926). The Theory of the Gene, New Haven, CT: Yale University Press.

Morgan TH (1965). The relation of genetics to physiology and medicine. In: Nobel Lectures, Physiology or Medicine 1922–1941, Amsterdam: Elsevier.

Roth S (2011). Mathematics and biology: a Kantian view on the history of pattern formation theory. Dev Genes Evol 221, 255–279.

Zhou M, Guo J, Cha J, Chae M, Chen S, Barral JM, Sachs MS, Liu Y (2013). Non-optimal codon usage affects expression, structure and function of clock protein FRQ. Nature 495, 111–115.