Why We Need Systems Biology

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Abstract

In this review, we address the justification for creating a new scientific discipline, and suggest possible goals for systems biology. We argue that systems biology is distinct from genomics and bioinformatics, as well as older disciplines such as medicine, evolutionary biology, and traditional molecular biology. Systems biology has the potential to address questions about the quantitative behavior and design of biological systems, which are not the central focus of other areas of biology. We discuss spontaneously arising biological oscillators as an example of a phenomenon that requires a systems-biological explanation. We also argue that there is, from evolutionary biology, an emerging view of organisms as 'poorly oiled machines,' and that systems biology is needed to understand and predict the behavior of organisms that have not been rationally constructed.

Introduction

Do we need yet another biological discipline? We already have molecular biology, genomics, bioinformatics, cell biology, ecology, medicine, physiology, anatomy, genetics, to say nothing of specialized disciplines such as neurobiology, microbiology,

entomology, plant biology, and marine biology. What are the characteristics of systems biology that differentiate it from an already crowded area?

One candidate definition of systems biology is 'an approach to biology with a big picture viewpoint' – systems biology looks a the many components that define a biological system, as well as considering the 'purposes' or 'design' of the system. The problem is that this is actually a definition of biology. This definition might be most meaningful to biochemists and molecular biologists who for many years focused on single proteins and genes and then felt unsatisfied. In particular, this definition is not useful to the ecologists, physiologists, clinicians, and evolutionary biologists who have had a big-picture approach to biology for decades and who may wonder what all the fuss is about.

Another definition is 'an approach to biology that seeks to understand and predict the quantitative features of a multicomponent biological system.' This is perhaps more useful. Most molecular biologists would confess that they are not good at quantitative explanations or predictions. This definition implies and lends credence to a mode of research that was previously thought to be unimportant by people who didn't practice it, and provides a rubric for those who do. This focus on quantitation is useful because it indicates that new types of observations should be made – observations that a biologist might never be motivated to make, or might ignore if they were made in passing.

Why is quantitation important?

Let us make a historical point. Molecular biology itself was started by physicists with a highly quantitative bent. In spite of this, molecular biology has developed into a

largely qualitative science. Calculus and even algebra are almost never used. Models are constructed based on simple two-dimensional drawings and are usually phrased as 'either-or' – based on what is known, two alternative models are envisaged that together make up the world of possible models. The two models should make different predictions that can be evaluated in a qualitative manner.

Consider two famous experiments of early molecular biology: the Delbruck-Luria fluctuation experiment (Luria and Delbruck, 1943) and the Pardee, Jacob, and Monod experiment ("PaJaMo" Pardee et al., 1959). In the first case, the issue was whether bacteria were capable of mutation like higher organisms, or whether variation in bacterial phenotype was due to interaction with the environment. The authors used simple mathematical models representing each hypothesis, performed an experiment, and chose the hypothesis that fit the data. In the PaJaMo experiment, the issue was whether or not the inducibility of beta-galactosidase enzyme activity was a feature of the gene encoding beta-galactosidase. There were only two possibilities, which made qualitatively different predictions. A quantitative model wasn't necessary.

A survey of about half a century of molecular biology since PaJaMo indicates that when molecular biologists have tested hypotheses, we have done so almost entirely in the PaJaMo mode and not in the Delbruck-Luria mode. It simply hasn't been necessary to use sophisticated mathematical models to understand how life works.

If that is the case, then why do we now need a quantitative approach? Consider the following rationales.

1. We have terabytes of genomic data and can't possibly analyze it using the human brain, so we need computers and mathematical models to help do our thinking for us.

2. In the "observe-explain-predict-control-design" framework of scientific progress, molecular biology has succeeded at the 'explain' phase, and now needs to move to the 'predict' phase. Specifically, we need to make quantitative predictions to verify that our understanding of biology is complete – that we aren't missing something.

3. Many molecular-biological systems can be explained in a qualitative way, and practitioners have done so. The time has now come to explain those odd cases that were previously set aside during the harvest of low-hanging fruit. For example, biological oscillators may require a quantitative view that is missing from the usual practice of molecular biology.

4. Traditional molecular biology can usually explain how things work, but cannot generally explain why a system works in one way and not another. Answering questions of design requires a quantitative analysis.

5. Biology itself is undergoing a quiet revolution as it shifts from viewing natural selection as taking place at the organismal level to taking place at the gene level. The collection of genes in a genome is no longer viewed as a well-oiled machine so much as a transient alliance of sequences that work together (or pretend to) only as long as they are in the same organism, with the opportunity for cheating. A quantitative systems biology approach would calculate how an organism will behave, since optimal function might not be expected.

6. On the practical side, it is no longer enough to qualitatively explain things. The responsibilities of molecular biology have expanded to include, for example, the quantitative aspects of disease states and treatments.

Let us consider these in order.

(1) We need systems biology to digest mass quantities of data

This first position is really a semantic confusion. The fields of genomics and bioinformatics have developed to deal with the immense amounts of data that have come from genome sequencing, genome-scale expression analysis, and so on. If terms like genomics, bioinformatics, and systems biology are to mean anything at all, they should have clear definitions and they should preferably mean different things. We therefore envision that systems biology primarily addresses small to large, but not immense, data sets, and tries to exhaustively describe relationships within such data sets. Genomics and bioinformatics are responsible for data sets that are so large that the human mind cannot comprehend them without prior computer intervention.

(2) Observe, explain, predict, control, design

The second position is a sociological description of how science often progresses, rather than an argument that it should progress this way in some particular case. It is often useful to control biological systems. We control farm animals and plants, diseases, forests, pets, and so on. Much of this has been done without any detailed understanding or ability to predict the quantitative behavior of biological systems; trial and error has

worked well. In contrast, in the efforts of physicists (and engineers, etc.) to control the physical world (e.g. in building machines), detailed mechanistic understanding has been feasible and useful. Much of the cultural clash between biologists and physicists derives from this different history. The argument that prediction and quantitative understanding will revolutionize biology is based on the historical experience of the physically-minded, and not on logical argument.

(3) We haven't explained everything, such as oscillators

Biological oscillations are a poster child for quantitative systems biology. The qualitative understanding of an oscillating system – why it oscillates vs. reaching a steady state - simply cannot be achieved without a quantitative analysis. Here we make a few observations about biological oscillations that may facilitate future research. Of course, biological oscillations should not be the sole focus of quantitative systems biology. For example, noise management, chaotic phenomena, signal processing and a variety of other quantitative phenomena will also be of interest.

Two types of oscillators are found in biological systems.   The first is the class of oscillators that appear to benefit their organism, such as the heart beat, breathing, the day-night cycle, the cell cycle, the oscillation of osteoblast/osteoclast activity in bone maintenance, the menstrual cycle and other reproductive cycles, and the rhythmic appearance of insects such as the 17-year locust.

The second class of oscillators include phenomena such as manic-depression, relapsing-remitting multiple sclerosis, atrial and ventricular fibrillation, the oscillation of NADH levels in yeast (Wolf et al., 2000), and cyclic neutropenia (e.g. Migliaccio et

al.1990). These oscillations are harmful or at best neutral for the organism. Such oscillations may be an emergent but unselected phenomenon, resulting from the inherent properties of a complex system that has been perturbed by mutation, drug treatment, or autoimmune damage.

Oscillatory phenomena for simple physical systems are easily described by an ordinary differential equation:

$$F \; = \; F_{spring} \; + \; F_{friction}$$ (Kleppner and Kolenkow, *An Introduction to Mechanics* p. 414)

$$= \; - \; kx \; - \; bv$$

$$= \; - \; kx \; - \; bx'$$

or

$$mx'' = \quad - \; kx \; - \; bx'$$

(For those molecular biologists in the readership for whom physics is a distant memory, recall that force = mass times acceleration, that acceleration is the second derivative of position [x], and that velocity [v] is the first derivative of position. In this equation, k is the spring constant and b is the friction constant.)

The point about this equation is that it can predict a few modes of behavior: stable oscillation, damped oscillation, and movement to equilibrium. In physical phenomena such as a swinging pendulum, the number of actual physical variables (such as the length of the pendulum, the weight, friction, and initial displacement) is usually small. A pendulum is also generally free of stochastic influences – for example, the number of oxygen and nitrogen molecules impinging on a swinging pendulum is large, so that we can summarize their effects by a single friction factor.

Biological systems such as oscillators differ in a number of ways from seemingly analogous physical systems. First, there are often a large number of variables and equations that need to be handled to predict the behavior of a system. Second, the role of stochastic forces can be large – in contrast to the number of air molecules impinging on a pendulum, the number of copies of a gene in a cell is usually one or two, and the stochastic binding of a transcription factor will have a large impact on the cell's behavior. Third, the observed values for variables and behaviors will generally occupy a small fraction of the space of possible values, because natural selection will have eliminated many possibilities. Fourth, time delays play a significant role in biology in a way that is usually absent in simple physical systems, such as the damped oscillator.

The Lotka-Volterra equations (recently reviewed by Bayliss and Choquenot, 2002), which are designed to model predator-prey interactions, may be broadly useful in understanding biological oscillations. In the examples below, there are no literal predators and prey, but different molecular species may effectively play these roles by inhibiting or promoting their own and each other's synthesis.

An example: Glycolytic oscillations in yeast. Under certain conditions, populations of yeast cells will show oscillations in the levels of $NADH/NAD^+$ ratio, which occur on the order of 1 cycle/sec. The oscillations are thought to be due to oscillations in portions of the glycolytic pathway, and presumably the concentrations of ATP and other metabolites are also oscillating with the same period as NADH. Between cells, the oscillations are synchronized by acetaldehyde, which diffuses in and out of cells (Richard et al., 2000).

The NADH/NAD$^+$ oscillations were first observed in yeast cultures that had been arrested in the glucose-ethanol transition and then treated with cyanide and glucose. The oscillations appear not to be a phenomenon that results from natural selection, but are simply an emergent property of a complex system. Various groups (e.g. Wolf et al., [2000]) have proposed models to explain this phenomenon.

Future research in quantitative systems biology could address the following questions relating to the distinctive characteristics of biological vs. physical systems.

1. What is the best way to manage the large number of equations and variables? Do we simply rely on computers? How does a human achieve an intuitive understanding of a system with seemingly simple behavior that overlies seemingly complex mechanisms? What will it mean to 'understand' a large data set if much of the data is analyzed by a computer and not by a human brain?

2. What are the design features of biological systems that relate to stochastic phenomena resulting from the use of a small number of molecules? How are stochastic effects minimized? What fraction of regulatory effects in, for example, signal transduction pathways have the purpose of minimizing stochastic phenomena? Do biological systems ever purposefully amplify molecule-driven stochastic phenomena?

3. How does evolution explore the space of parameters that govern biological phenomena? For example, how does an oscillatory phenomenon evolve from a non-oscillatory phenomenon? How common are oscillatory phenomena that have no apparent selective value? Are there situations where unwanted oscillatory

phenomena arise and evolution addresses them by generating additional regulatory mechanisms? (Sometimes it is easier to add a vibration damper than to eliminate the cause of vibration.)

(4) "How does it work?" vs. "Why does it work this way?"- Systems biology and the design issue

Molecular biology has historically focused on how things work. Systems biology can ask why biological systems work the way that they do. The question of biological design, except in the most obvious cases, is considered to be either not of interest, someone else's problem (e.g. evolutionary biologists), or just plain unanswerable (e.g. lost in the mists of early evolution). In particular, if a question about why something works the way it does can be answered in a way that can be qualitatively tested, that is considered reasonable, but a question requiring a quantitative answer is often left aside.

Two examples of biological systems design derive from the study of prokaryotic repressors. The lactose repressor and lambda repressor proteins are both made in small amounts – about 40 copies of the lac repressor tetramer per cell and about 100 copies of lambda repressor monomer per cell. The lac repressor gene (*lacI*) has a very weak promoter (Calos 1978) and is thought to be transcribed stochastically, once every few cell divisions, and this mRNA is efficiently translated so that a burst of lac repressor is occasionally made and is then diluted (also stochastically) during the following cell divisions.

In contrast, the lambda repressor gene (*cI*) is transcribed at a high rate from a reasonably strong promoter, making a larger amount of mRNA that could yield a large

amount of protein if efficiently transcribed (Meyer et al., 1978). However, this mRNA begins with the start codon for the lambda repressor protein and completely lacks a Shine-Dalgarno sequence, so it is very poorly translated (reviewed in Ptashne et al., 1980). The result is that there is much less stochastic variation in the level of lambda repressor in a cell.

These differences could be due to evolutionary accidents. In each case, the system needs a small amount of repressor, and there are two different ways of doing this. Each example may seem energetically inefficient: the Lac system makes beta-galactosidase and lactose permease even when they are not needed, and the lambda system may make a higher level of mRNA than may be needed. We might imagine that these energetic inefficiencies are insignificant compared to the rest of the energy usage of the whole cell, and that natural selection does not act on this level of energetic efficiency.

The alternative explanation is that these designs reflect the particular distinctive needs of each system. The Lac case is rationalized as follows. A quirk of lactose repressor is that lactose itself is not the natural inducer, but instead the inducer is allolactose, a sugar that is not normally abundant in nature but that is produced from lactose by a side reaction of beta-galactosidase. Thus, if an E. coli cell finds itself in a lactose-rich environment, the Lac operon is not induced unless a bit of beta-galactosidase is first present to create allolactose. The stochastic induction of the Lac operon in the absence of an inducer allows the production of enough beta-galactosidase that, if lactose is present, the system can be fully induced. (Induction of permease may also help in this process.) The result is that the Lac operon is induced in an all-or-none manner in a subset of cells (Novick and Weiner, 1957; Yildirim et al, 2004).

The lambda system operates with completely different requirements. Lambda is a lysogenic bacteriophage that, in the prophage state, is induced by DNA damage. The rationale usually given that if a bacterium's DNA is damaged so badly that it may not survive, it is in the interest of lambda to enter the lytic cycle and escape. According to this idea, the penalty for a failure to induce would be death to the prophage.

However, if a lambda prophage is sitting in an E. coli amidst a sea of other E. coli that also contain a lambda prophage, induction also means death – the daughter phage particles from the induced bacterium will infect other lysogenic bacteria and be repressed without integrating. Thus, the penalty for spontaneous induction is high, and the spontaneous induction rate is in fact quite low. Thus, the requirements for levels of lambda repressor appear to be stringent: too little repressor or too much may be lethal.

In the early days of molecular biology, such explanations were often derided as 'molecular psychology' – ideas that could never be proven. Systems biology asserts that design questions should be answerable and are worth studying.

Hypotheses about biological systems design should make testable, falsifiable predictions. In the Lac and lambda cases, this is possible. For example, the hypothesis about the design of the Lac system makes the following predictions. First, if we examine other systems involving repressors for catabolic pathways, the repression should generally be more efficient when the repressor uses the substrate itself instead of a reaction side-product. Second, we could redesign Lac repressor to bind to lactose instead of allolactose, and also redesign the gene for Lac repressor to resemble that of lambda repressor: increase the strength of the promoter and decrease its translation efficiency. We might also insert a weak constitutive promoter in front of *lacY*, so that a bit of lactose

could initially enter the cell. Such a system should work as well as the natural Lac repression system and may be slightly more fit, working without the occasional wasteful burst of expression of the lactose operon in the absence of lactose and allowing all cells to immediately respond to lactose instead of depending on stochastic induction.

A final point about the design of the Lac system. Why is allolactose the inducer, rather than lactose? Systems biology would insist that this is a legitimate question. A human designer would choose lactose as the inducer. The answer may be that a repressor that recognized allolactose by chance evolved before a repressor that recognized lactose. Once the allolactose-recognizing repressor came into existence, it provided such an advantage that it swept through the bacterial population. Subsequent evolutionary tweaking could minimize some of the inefficiency that results from choosing a suboptimal inducer and add other value, such as genetic linkage between this repressor gene and the lactose operon. As a result, if a lactose-binding Lac repressor later came into existence, it would be at a competitive disadvantage. Using the language of economists, the allolactose-binding repressor would have a 'first-to-market' advantage.

One of the challenges for systems biology will be to understand how evolution-based design strategies differ from those that humans would use. Biological design may be seen as a long series of kludges, in which each intermediate has the constraint that it must work well enough to survive, while human design can proceed through intermediates that don't work at all, but which inform the designer about new possibilities.

(5) Systems biology is needed to address the quiet revolution in how we understand

organisms: "Poorly oiled machines"

Because of the remarkable properties of organisms, and perhaps because scientists are organisms, we have tended to wax poetic about how well organisms function as machines. Recently, this view has been re-examined, and evolutionary biologists have pointed out that organisms are, in some ways, poorly designed and don't function as well as they could. The newer conception is that the organism is a contraption that works just well enough to get by.

Creationists have argued that if organisms are really as amazing as we say they are, they cannot possibly have arisen by a random process that depends on an occasional mistake being useful. This has forced evolutionary biologists to scrutinize organisms for examples of poor design, which are plentiful. The panda's thumb (Gould, 1980), the placement of the windpipe in front of the esophagus (so that food can go down the wrong tube), traversal of the urethra through the prostate gland (so that if the prostate becomes inflamed and swells, it becomes difficult to urinate) are just some of the examples.

A second group of revisionists are evolutionists who examine biology from the gene's point of view, seeing organisms as transitory vessels for carrying DNA (Burt and Trivers, 2005; Dawkins, 1976). This view predicts that, since genes are ultimately running the show and often battling with each other, organisms will often be caught in the crossfire. For example, if an autosomal mutation arises that increases the reproductive fitness of females by a certain amount and decreases the reproductive fitness of males by less than that amount, such a mutation will spread by natural selection, even though the

males may suffer in the process. (If such a mutation arises on an X chromosome, the benefit to females needs to be only 1/2 as strong as the detriment to males to be favored by natural selection, because an X spends twice as much time in females as in males.) A second example: if a chromosome can insure that its homologue is not transmitted to offspring (for example by killing sperm that contain the homologue), then such a chromosome will spread through a population even if it has other deleterious consequences, and organisms containing it will derive no benefit.

The typical view of an unreconstructed molecular biologist is usually that an organism in a given environment has a particular ideal genotype, and as long as that genotype is present, the organism will fare pretty well. Organisms that deviate from this ideal are regarded as mutants. According to the naïve systems molecular biology view, the genotype is a system that designed to optimize the function of the organism. The newer view is that each gene has its own agenda – to increase the number of copies of itself. Accordingly, some genes play by the usual rules, such as housekeeping genes that encode ribosomal subunits. Other genes, such as transposable elements, selfish chromosomes, and genes that promote altruistic behavior, may reduce the fitness of their host to increase their own rate of replication.

Fontana (1994) has argued for the importance of developing "a theory of the organism." We propose that Trivers, Dawkins and their colleagues (Burt and Trivers, 2005; Dawkins 1976; Dawkins 1982) have in fact articulated a theory of the organism (although they may have not have labeled it as such). In a nutshell, their theory is that an organism is a transient alliance of the genes present in a zygote, along with the phenotypes are produced by the particular alliance.

A few books elaborate these ideas at some length. In *Genes in Conflict*, Burt and Trivers review the biology and molecular mechanisms of various DNA elements that propagate themselves not by contributing to the fitness of their host organism, but by refusing to play by the rules of the Mendelian system. These include clearly selfish elements such as transposons and chromosomes that segregate into more than 50% of progeny.

Other selfish phenomena include genomic imprinting, in which differential DNA methylation on paternal and maternal chromosomes in a fetus represents the differential interest of each parent. In sexual species with little or no pair-bonding, it is in the interest of the father (more precisely, the father's genes) to maximize the fitness of the offspring at the expense of the mother, while it is in the mother's interest to balance the fitness of a given offspring with that of future offspring. In the best-understood case, the insulin-like growth factor 2 (*Igf2*), which promotes fetal growth, is expressed from the paternal chromosome but not from the maternal chromosome. *Igf2R*, whose protein product binds and inhibits Igf2, has the opposite pattern of expression. The Igf2/Igf2R case illustrates how different genes within an individual can work toward conflicting ends (Wilkins and Haig, 2001).

In *The Extended Phenotype*, Richard Dawkins argues for a revised view of organisms. Dawkins starts with the view that organisms are simply vessels for protecting genes and enhancing their replication. We should therefore consider everything that a gene causes in enhancing its replication to be part of its phenotype. For example, the characteristics of birds' nests are known to vary from species to species. In the view of *The Extended Phenotype*, the bird's body is a set of phenotypes and the nest is another set

of phenotypes; it may be somewhat arbitrary to draw a distinction between the two. (In fact, our emphasis on physical bodies may derive from the way that natural selection has shaped our consciousness, rather than because of its inherent scientific rigor.)

The implications of this worldview for systems biology are too numerous to summarize here. One point is that when we think about the behavior of an organism, it is an oversimplification to think of its genome as acting for the single common purpose, and generating quantitative models with a view to explaining that purpose. In some cases, it may be more appropriate to consider the genome as a bag of genes encoding products with certain properties, and the job of a systems biologist is to calculate the behavior of the aggregation, keeping the notion of organismal purpose at an arm's length.

(6) Practical considerations

Diseases and disorders are properties of organismal systems. For purposes of illustration, we return to oscillations, but it should be apparent that all sorts of diseases may be seen as quantitative failures in system function. It is striking that oscillatory phenomena appear to arise in a number of disease states, such as cyclic neutropenia, manic-depressive disorder, and multiple sclerosis.

Oscillations in blood cell populations. Cyclic neutropenia (reviewed in Berliner et al. 2004) is striking because because the oscillations occur in a regular 21-day cycle, as opposed to the more variable periodicities seen in multiple sclerosis and manic-depressive disorder. Congenital cyclic neutropenia in humans is caused by dominant mutations in ELA2, which encodes neutrophil elastase. In dogs, a cyclic neutropenia syndrome results from recessive mutations in AP3B1, an intracellular transporter protein

that directs neutrophil elastase to intracellular vesicles that remain in the neutrophil until needed. The effects of both the ELA2 mutations and the AP3B1 mutations appears to be that a fraction of neutrophil elastase is constitutively transported to the plasma membrane. Granulocyte-colony stimulating factor (G-CSF) is the major cytokine that promotes production of neutrophiles. When neutrophil elastase is on the plasma membrane, it may degrade both G-CSF and the G-CSF receptor, both of which are known substrates for this elastase.

Clinically, patients with this syndrome are at risk for serious infections at the nadir of the cycle, when neutrophil counts are essentially zero. Patients can be treated with G-CSF, which causes a shortening the cycle period so that the time around the nadir of the cycle is shorter (as well as causing an increase in the average number of neutrophils).

Similar cyclic phenomena are seen in disorders of other blood cell types, such as chronic myelogenous leukemia (Hirayama, *et al*, 2003), hypereosinophilic syndrome (e.g Xiao *et al*, 2003), and in normal T cell responses to persistent antigens (Shanklin and Smalley, 2006). Colijn and Mackey (2005a, b) have proposed models for the oscillations in cyclic neutropenia and CML.

Our conclusion is that oscillations may arise spontaneously in stressed biological systems simply because these systems have many moving parts, built-in time delays, and feed-back loops, and are designed to run in a steady state with a continuing supply of energy instead of naturally going to a low-energy equilibrium. We might imagine that such oscillations occur often enough to be harnessed by evolution.

Conclusions

In this review, we have tried to make a few key points. First, in order to be a meaningful discipline, systems biology needs to be clearly defined in a way that relates it to other disciplines. This will be important in clarifying the distinct questions that this discipline can address.

Second, there are in fact a number of observations that are not explained by existing disciplines. Why are biological systems designed the way they are, and not some other way? Why do oscillations sometimes arise spontaneously in perturbed biological systems, and what controls their characteristics? How do the conflicts between genes play out within an organism? Can a quantitative understanding of system failure inform therapy for diseases and disorders?

Finally, the best justification for creating a new discipline is to give scientists a new 'cognitive framework' to define issues that might not otherwise even be addressed (C. Pabo, pers. comm.). This framework is social as well as intellectual: the existence of new departments, colleagues, meetings, and funding mechanisms should allow us to ask and answer important new questions.

References

Bayliss P, Choquenot D (2002) The numerical response: Rate of increase and food limitation in herbivores and predators. *Phil Trans R Soc London B* **357:**1233-1248.

Berliner N, Horwitz M, Loughran TP Jr (2004) Congenital and acquired neutropenia. *Hematology [Am Soc Hematol Educ Program]* 63-79

Burt A, Trivers R (2005) *Genes in Conflict: The Biology of Selfish Genetic Elements.* Harvard University Press. Cambridge, Massachusetts USA

Calos MP (1978) DNA sequence for a low-level promoter of the lac repressor gene and an 'up' promoter mutation. *Nature* **274:** 762-5

Colijn C, Mackey MC. 2005 A mathematical model of hematopoiesis--I. Periodic chronic myelogenous leukemia. *J Theor Biol* 237: 117-32

Colijn C, Mackey MC. 2005 A mathematical model of hematopoiesis: II. Cyclical neutropenia. *J Theor Biol* **237:** 133-46

Dawkins R. (1976). *The Selfish Gene.* Oxford University Press, Oxford UK

Dawkins R. (1982). *The Extended Phenotype: The Long Reach of the Gene.* Oxford University Press, Oxford UK

Gould, SJ. (1980) *The Panda's Thumb: More Reflections in Natural History.* W. W. Norton & Company, Inc. New York, New York USA

Hirayama Y, Sakamaki S, Tsuji Y, Matsunaga T, Niitsu Y (2003) Cyclic platelet and leukocyte count oscillation in chronic myelocytic leukemia regulated by the negative feedback of transforming growth factor beta.
Int J Hematol **77:** 71-4

Kleppner and Kolenkow, *An Introduction to Mechanics.*

Kuhn, T. (1962) *The Structure of Scientific Revolutions.* Chicago University Press. Chicago, Illinois USA

Luria S, Delbruck M (1943) Mutations of bacteria from virus sensitivity to virus resistance. *Genetics* **28:** 491

Meyer BJ, Maurer R, Ptashne M. (1980) Gene regulation at the right operator (OR) of bacteriophage lambda. II. OR1, OR2, and OR3: their roles in mediating the effects of repressor and cro. *J Mol Biol* **139:** 163-94

Migliaccio AR, Migliaccio G, Dale DC, Hammond WP (1990) Hematopoietic progenitors in cyclic neutropenia: effect of granulocyte colony-stimulating factor in vivo. *Blood* **75:** 1951-9

Pardee AB, Jacob F, Monod J. (1959) The genetic control and cytoplasmic expression of "inducibility" in the synthesis of beta-galactosidase by E. coli. *J Mol Biol* **1:** 78.

Ptashne M, Jeffrey A, Johnson AD, Maurer R, Meyer BJ, Pabo CO, Roberts TM, Sauer RT. (1980) How the lambda repressor and cro work. *Cell* **19:** 1-11.

Richard P, Bakker BM, Teusink B, Van Dam K, Westerhoff HV (1996) Acetaldehyde mediates the synchronization of sustained glycolytic oscillations in populations of yeast cells. *Eur J Biochem* **235:** 238-41.

Shanklin DR, Smalley DL (2006) Kinetics of T lymphocyte responses to persistent antigens *Exp Mol Pathol* **80:** 26-37.

Wilkins JF, Haig D. (2001) Genomic imprinting of two antagonistic loci. *Proc Biol Sci* **268:** 1861-7.

Wolf J, Passarge J, Somsen OJ, Snoep JL, Heinrich R, Westerhoff HV (2000) Transduction of intracellular and intercellular dynamics in yeast glycolytic oscillations. *Biophys J* **78:** 1145-53.

Xiao Z, Hao Y, Qin T, Han Z (2003) Periodic oscillation of blood leukocytes, platelets, and hemoglobin in a patient with chronic eosinophilic leukemia. *Leuk Res* **27:** 89-91

Yildirim N, Santillan M, Horike D, Mackey MC (2004) Dynamics and bistability in a reduced model of the lac operon. *Chaos*. **14:** 279-92.

Figure 1 Legend. Stochastic features of expression of the lac and lambda repressors. (A) Lac repressor is driven by an unregulated, weak promoter that is transcribed once every few cell divisions, so that the lac operon is spontaneously induced in a fraction of cells. When lactose appears in the environment of such a population, cells that have recently expressed the lac operon transport lactose into the cell and convert it into allolactose, so that the operon remains on in those cells (see cell in lower right). Complete induction of a culture of E. coli transferred into lactose takes some time, because it depends on stochastic induction in most of the cells, followed by outgrowth of the lac operon-expressing cells. (B) The gene encoding lambda repressor (cI) is transcribed at a high rate, but translated poorly. As a result, there is little cell-to-cell variation in levels of lambda repressor. If repressor levels become too high, occupancy of O_R3 by repressor shuts off its own synthesis, further limiting cell-to-cell variation (see cell in lower left).