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Research review paper

In vitro regulatory models for systems biology

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ABSTRACT

The reductionist approach has revolutionized biology in the past 50 years. Yet its limits are being felt as the complexity of cellular interactions is gradually revealed by high-throughput technology. In order to make sense of the deluge of "omic data", a hypothesis-driven view is needed to understand how biomolecular interactions shape cellular networks. We review recent efforts aimed at building *in vitro* biochemical networks that reproduce the flow of genetic regulation. We highlight how those efforts have culminated in the rational construction of biochemical oscillators and bistable memories in test tubes. We also recapitulate the lessons learned about *in vivo* biochemical circuits such as the importance of delays and competition, the links between topology and kinetics, as well as the intriguing resemblance between cellular reaction networks and ecosystems.

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1. The molecular revolution of biology

The publication of the double-helix structure of DNA ushered the molecular revolution of biology. In the last 50 years, biologists have broken cells into finer and finer components in an attempt to unravel their inner mechanisms. This reductionist approach has relied on three complementary paradigms: *in vivo*, *in vitro* and *in silico*. *In vivo*, for example, genetic studies have uncovered genes and mutations underlying numerous physiological and pathological pathways—such as cystic fibrosis (Riordan et al., 1989) or oncogenesis (Hanahan and Weinberg, 2011). Genetic studies often discover the function of an unknown gene by mutating or knocking out its protein. On the one hand, *in vivo* studies offer the advantage of studying proteins in their natural environment. On the other hand, the complexity of cells often obfuscates the role of a given protein.

The second approach, based on *in vitro* protocols, aims to isolate a protein from its environment to observe its action in detail. For example, enzymology uses *in vitro* assays, sometimes very elaborate (Rondelez et al., 2005), to discover the mechanism and measure the kinetic and thermodynamic parameters of various key biochemical transformations. Crystallography is another technique that epitomizes reductionism, seeking to explain the role of proteins based on their atomic arrangements. Hypotheses about cellular mechanisms are often not completely accepted until their molecular basis has been validated by crystallographic

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studies. However, *in vitro* studies offer a controlled albeit artificial environment, which may lead to artifacts or omissions of crucial mechanisms.

Lastly, *in silico* techniques or the building of theoretical frameworks have seconded experimental approaches. For example, protein folding algorithms link the 3D structure of a protein—and potentially its function—to the sequence of its amino acids (Dill et al., 2008). In drug discovery, docking simulations are a valuable tool to assess the binding of drugs to their target (Kitchen et al., 2004).

Yet, numerous lines of evidence point to the limits of what can be understood by breaking cells into finer and finer components and looking individually at each of these elements (Hartwell et al., 1999; Kitano, 2002). Challenges to reductionism were for example raised by bioengineers who sought to alter metabolic pathways to boost the production of useful molecules such as ethanol. They realized that in order to tailor metabolism locally, they first needed to understand it globally (Nakatsui et al., 2010; Westerhoff and Palsson, 2004). Similarly, drug discovery increasingly requires a systems approach to predict far-reaching and off-target effects (Hood and Perlmutter, 2004).

The limitation of reductionism is not surprising given that cells are defined not only by their components (proteins, genes, factors…) but also—and mostly—by the interactions between these components (repression, activation, allostery…). In other words, most cellular processes are not performed by a dedicated molecular compound, but orchestrated by networks of interdependent chemical events. Gene regulatory networks provide a prominent example. They can be seen as directed networks of transcriptions and translations. Their nodes are proteins and genes, and their edges are chemical transformations or interactions between them. Transcription networks participate in the regulation of virtually all biological processes, ranging from cellular differentiation (Herskowitz, 1989) to apoptosis (Haupt et al., 2003) or immune response (Calvano et al., 2005; Eulgem and Somssich, 2007).

Reductionism typically apprehends genetic regulation with knockout assays, in which a studied protein is temporarily or permanently repressed. By observing the effect of the knockout on the phenotype, assumptions are drawn on the role of the missing element. But knockout assays are crude because they focus more on proteins than on their interactions. In fact, knockout assays not only remove a node from a collection of proteins, but also prune all the edges of the regulatory network that lead to or originate from this node (the regulations). The limitations of the reductionist approach are creatively illustrated by Yuri Lazebnik (Lazebnik, 2002). The author wonders whether reductionism would help a biologist to fix a broken radio and concludes that an integrated and functional language—similar to that used by engineers—is required to capture the complexity of cellular behaviors.

2. Systems biology

Given this necessity to understand biological functions as emerging from fully integrated systems, a purely descriptive approach is no longer efficient. At some point one must make informed guesses about the kind of general architectures that could provide a given function, and then submit this hypothesis to the filter of experimental facts.

This "hypothesis-driven" systems biology emerged concomitantly to the realization of the human genome project (Furusawa and Kaneko, 2012; Huang, 2009) and is now a powerful driving force to our understanding of biological systems (for recent examples see (Dodd et al., 2007; Salmena et al., 2011). It asks whether there exist design principles for cellular networks—which is not obvious in the first place since biological networks are evolved rather than engineered (Alon, 2003; Jacob, 1977). Typical examples of questions it addresses are as follows. What kind of topology ensures concentration–robustness (the property that a species has an identical concentration for all legitimate steady states) (Shinar and Feinberg, 2010)? What is the simplest way of making a biochemical oscillator (Novak and Tyson, 2008)? What is the interplay between the dynamics of a network, its topology and the degree of nonlinearity of its chemical reactions (Novak and Tyson, 2008)? What are the fail-safe mechanisms that cells use to compensate for the failure of some of their components (Kitano, 2004)?

This "hypothesis-driven" systems biology draws many of its foundations from the theory of dynamical systems. Cellular networks are described as biochemical instantiations of these mathematical concepts, forming out-of-equilibrium systems that display dissipative spatiotemporal behaviors (multi-stability, oscillation, spatial patterns…). This approach proposes experimentally testable hypotheses in order to validate putative mechanisms, or verify commonly accepted assumptions. Like its reductionist counterpart, it relies on *in vivo*, *in silico* and *in vitro* methods to put to a test the proposed design principles about biochemical circuits.

2.1. In vivo systems biology

In vivo, "hypothesis-driven" systems biology is supported by the rise of synthetic biology, whose birth dates back to two papers in 2000. In the first one, Elowitz and Leibler synthetically engineered an oscillator by expressing three mutually repressing proteins into *E. Coli* (Elowitz and Leibler, 2000). In the other paper, Gardner et al. engineered a bistable switch with two mutually repressing proteins (Gardner et al., 2000). Their work departed from reductionism because it sought to alter edges rather than nodes in a network of cellular components. The success of the approach strongly anchored key concepts of dynamical systems theory (including bifurcations, attractors and so on) to the study of cellular behaviors. Since then, the *in vivo* synthetic approach to systems biology has shed a new light on genetic regulation and provided a wealth of re-wired cellular devices (Qi et al., 2013). For example, synthetic circuits helped to understand the role of noise in gene expression (Eldar and Elowitz, 2010; Elowitz et al., 2002; Suel et al., 2007), or highlight the minimal units required to drive cell cycles (Coudreuse and Nurse, 2010)

2.2. In silico systems biology

Mathematical toy models are often used in physics to capture essential features of a complex system. Similarly, toy models have proved indispensable in biology to sharpen intuition and verify assumptions, because they condensate in a few molecular components and reaction steps the essence of a biological process. Classical toy models include: kinetic proofreading (which drastically reduces error rates in biosynthesis or antigen recognition (Hopfield, 1974; Ninio, 1975)), ultrasensitivity (which bestows a digital response to some circuits (Goldbeter and Koshland, 1981; Buchler and Louis, 2008) or morphogenesis robustness (which ensures stability of morphogen gradients against perturbations (Eldar et al., 2002).

Conversely, fully descriptive simulations are equally needed to rigorously verify and predict the integrated dynamics of cellular networks—provided a corpus of their mechanisms already exists (Tomita, 2001). *Mycoplasma genitalium* proved small enough (~500 genes) to be tackled by a "whole-cell" approach. Karr et al. gathered 1900 parameters from 900 publications in order to simulate in greatest detail the interactions between the metabolome, transcriptome, genome and proteome of *Mycoplasma genitalium* (Karr et al., 2012). In some sense, "whole-cell" simulations are the systems biology's pendants to atomistic simulations.

In silico simulations often make predictions that are experimentally verifiable. Mather et al. (2010) analytically studied competition of substrates for an enzyme using queuing theory. They predicted a striking effect (correlation resonance) in which the levels of competing substrates suddenly correlate around a balancing point. Correlation resonance was subsequently verified *in vivo* with a synthetic circuit that saturated the degradation machinery of *E. Coli* (Cookson et al., 2011).