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Can a Systems Perspective Help Us Appreciate the Biological Meaning of Small Effects?

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The study of dramatic phenotypes has been pivotal to elucidating biological mechanisms. Effectively approaching low-magnitude quantitative phenotypes, a common outcome of systematic loss-of-function studies, will be critical for understanding how individual components of cells interact to generate functioning systems.

Genetics provides a framework for understanding cells, not only because the genome encodes most of the molecules and interactions present in a cell, but also because the genome is the keyboard for evolution. As a result, a meaningful understanding of the properties and principles of cellular functions requires a rigorous map between the genotype and phenotype. Given this view, it is not surprising that tools to remove, reduce the levels of, or inactivate a gene product (genetic mutants, RNAi, and small molecules) have been pivotal to our current understanding of how cells function and how organisms develop.

Alterations in a single gene are most informative when a gene product has a critical role in a process so that its perturbation results in a large phenotypic change, for example in cellular morphology or population growth. However, one can envision many scenarios in which such a perturbation would not have a significant impact. For instance, the gene product of interest may function only in a particular environment or cell type. In this case, its importance can be unveiled only by a systematic scanning of the genotype-phenotype mapping in different environments or contexts. In other cases, "genetic redundancy" masks the functional repercussions of a perturbation to any one gene. Here, multiple mutations must be combined to observe an impact on phenotype. Occasionally, redundancy is something that is easily understood. For example, redundancy is intuitively obvious when considering a cell that has high-affinity/low-capacity and low-affinity/high-capacity permeases for the same small molecules. However, in most instances the precise biological meaning of redundancy is opaque. Moreover, the fact that the environment can affect the expression or activities of genes blurs the line between these two causes of small phenotypic changes.

Quantitative experimental tools are making subtle phenotypes accessible and are increasingly documenting examples where perturbations of genes and pathways generate quantitative rather than qualitative effects. Although quantitative phenotypes have been appreciated in many fields, there are many disciplines (e.g., control of gene expression or signal transduction) in which an effect that is less than 2-fold is often considered "weak." This stance is often justified by the fact that modest changes might not be statistically distinguishable from experimental noise, given the limited resolution of the assays used. However, an equally common view contends that even when small effects can be accurately assessed, components of a network that have quantitatively limited roles might not be worth studying. When is a 10% change in the output worth considering at the level of a single cell? When are small effects meaningful?

We suggest that there are a number of biologically relevant situations in which a modest quantitative phenotype is important. We discuss a handful of such situations and argue that, at least in these cases, discounting quantitative effects might lead to an incomplete understanding of the elaborate dynamic processes that contribute to the functioning of cells and organisms.

Fidelity

This term is usually used in situations where an output is normally highly reproducible (e.g., the segregation of chromosomes, the replication of DNA, the charging of tRNA, and the translation of mRNA), yet there are rare but biologically important errors. In these instances, there are components (such as the proofreading components of DNA polymerases) whose loss goes unnoticed until a rare error occurs. While this thinking is well appreciated in certain fields where it is obvious that the rare errors are important, the degree to which rare errors are important for other biological processes and the extent to which error suppression/correction mechanisms have evolved to limit rare catastrophes remains less explored. These include the fidelity of epigenetic state inheritance, of organelle inheritance, and of division plane choices. Perturbations to the mechanisms that regulate the fidelity of these important biological processes are likely to generate subtle quantitative rather than large qualitative effects. Nevertheless, delineating these effects is essential for the holistic understanding of the underlying biology of these systems. It will also be invaluable for unraveling the full spectrum of error correction mechanisms accessible to biological processes.

Nongenetic Individuality

Nongenetic individuality, also called "population heterogeneity" or "cell-to-cell variability," is used to describe the degree of variability in a measured parameter of genetically identical cells in an ostensibly uniform environment. The causes of nongenetic individuality can be broadly grouped into two general categories: cellular "noise" and heterogeneity of physiological cellular variables across a population (e.g., cell cycle state,

epigenetic state, or position in a colony or a tissue).

Cellular noise is thought to ultimately take root in the inherently stochastic and discrete nature of biomolecular reactions (Maheshri and O'Shea, 2007). Recently, genome-wide studies of cell-cell variability in mRNA or protein levels in *E. coli* and *S. cerevisiae* have indeed revealed that a tremendous amount of variability exists in the molecular make-up of genetically identical cells and that different molecular constituents of a cell can exhibit widely different patterns of variability (Newman et al., 2006; Taniguchi et al., 2010).

Has variability been regulated during the functional evolution of cellular pathways? There are many indications that this might be the case. For example, it has been shown that single-photon responses of retinal rod cells are remarkably uniform despite the fundamental stochastic nature of the underlying biochemical reactions (Doan et al., 2006). In human tissue culture cells, at least for virus infection efficiency and endocytosis, variability seems to be strongly regulated in response to factors that shape a population of cells, such as cellular crowding and cell-cell contacts (Snijder et al., 2009). Bacterial populations are also thought to regulate noise levels in their stress responses to "hedge their bets" against variable environments (Avery, 2006).

If variability were indeed regulated, then removing components involved in its regulation should lead to quantitative yet biologically significant effects. Such a quantitative role of variability has long been appreciated in systems where a stochastic component is needed to initiate a probabilistic differentiation of otherwise identical cells (Kalmar et al., 2009; Lidstrom and Konopka, 2010; Suda et al., 1983). Examples range from phenomena such as persistence and competence in bacteria to fate determination in stem cells.

However, even in the absence of such dramatic changes in the frequency, rate, or duration of some binary cellular outcome, changes in variability could still be critical to the underlying cellular physiology. In the pheromone response signal transduction pathway of the yeast *S. cerevisiae*, deletion of the gene encoding Dig1, a redundant negative regulator of the main transcription factor Ste12, induces little change in the mean output of the pathway. The mutant, however, exhibits increased cell-to-cell variability, which correlates with an important fitness cost to the organism in terms of both growth and mating efficiency (McCullagh et al., 2010). In the context of human physiology, a striking observation has been made that variability in red blood cell size can predict mortality from all causes (van Kimmenade et al., 2010). While the biological underpinnings of this remarkable correlation remain under study, it strongly implicates cell-to-cell variability as an important quantitative phenotype whose elucidation is essential for understanding the full physiological spectrum of cells and organisms. As a result, we suspect that using "noise" and its quantitative changes upon cellular perturbations as a phenotype will have substantial explanatory power in the future.

Selective Advantage of Fine Control

"Fine control" can refer to either strict regulation around a steady-state value or prompt reestablishment of a steady-state quickly after a perturbation. The importance of both types of control has long been recognized in relation to the physiological states of a human being. Consider virtually anything one can measure: blood pressure, temperature, tissue oxygen levels, the concentration of any ion in the bloodstream, brain function, etc. Functional consequences can be attributed even to small fluctuations in these quantities. As a result, most medically relevant measurements occur in this quantitative realm. One imagines that the larger the assemblage of cells (tissue, organ, whole organism), the more critical it might be to maintain the average behavior of the system within certain operating limits.

But what about single cells, be they microorganisms or cells in the culture dish? One approach to this question is to ask whether reducing the dose of a gene by 2-fold has an effect on growth. This is feasible to do in yeast, as there appears to be little dosage compensation (Springer et al., 2010). Indeed, merely halving the dose of over 100 genes results in measurable defects in growth in rich media (Deutschbauer et al., 2005), suggesting that for many cellular processes a 2-fold difference in expression of a single gene matters. As these measurements were done in unstressed cells and required an obvious fitness defect in a relatively short-term experiment, one anticipates that the precise dose of many additional gene products will have an impact on fitness. For microorganisms, modest changes can have large effects on fitness over time, so one suspects that any measurable effect on growth is likely to be biologically relevant.

But how should we think about the mechanistic role of factors that have modest quantitative effects on the dynamic, rather than the static, performance of a system? Components mediating these small effects might not be necessary for a network to function per se (just as antilock brakes are not necessary for a car to move forward), but they might affect how reliably and how rapidly they do function. Therefore, in examining this question, the notion that the architecture of biological networks might reflect the need to satisfy multiple desired characteristics (known as "performance specifications" in engineering terminology) could be useful. In this framework, components with seemingly "weak" effect on one performance aspect, such as the steady-state behavior of a system, might have considerable effects on the time to converge upon a steady-state (system dynamics) or how robustly this steady-state is maintained in variable environments or in the face of intracellular fluctuations.

Still, which dynamic measures should be considered to be biologically meaningful? Answering this question is far from trivial in complex biological networks. For example, circadian oscillators provide stable oscillations that are coordinated with rotation of the earth, but what are the most important performance variables? Period, amplitude, and entrainment by light all seem likely to be important, but to what degree? Is the precise amplitude or phase of any clock-controlled component important and, if so, what are the optimal parameter ranges? It is even more complicated to pinpoint important variables for cellular signaling and information processing, which need to balance sensitive and robust detection with amplification and decoding of multiple input signals. Perhaps the only defensible way of making these assessments is to examine

the impact of experimental alterations on measurable fitness outcomes.

A plausible view that emerges, then, is one in which biological networks are navigating a complex performance landscape. A low-dimensional projection of this landscape might reveal the components that impact the core functionality of the system (e.g., whose loss abolishes oscillations). However, perturbations to other components, especially those that are modulating fine homeostasis, would generate quantitative rather than qualitative changes. Accounting for these effects will be necessary for developing predictive models of the non-steady-state behavior of cells during their dynamic responses to internal and external perturbations. It will also be essential for generating a holistic understanding of cellular homeostasis in health and of its multifaceted breakdown in disease.

Evolutionary Neutral Changes May Still Be Worth Studying

The traditional theory of neutral evolution prescribes that the vast majority of molecular differences are selectively "neutral." That is, the molecular changes represented by these differences do not influence the fitness of the individual organism. Viewed in this light, most small differences in cellular regulation strategies will not induce any fitness repercussions and therefore should be dismissed.

Are small changes that don't induce any detectable fitness effect worthy of study? Elaborations of neutral evolution theory introduced the concept of isoneutrality and argued that it is a ubiquitous evolutionary strategy (Proulx and Adler, 2010). In this framework, two types are equivalent in some population or ecological context, but not in others. Also, two genotypes could differ along multiple phenotypic axes and yet still be exactly equal in terms of their mean fitness. Such alternative ways of producing the same mean fitness will differ in their variance or in other properties (e.g., skew) of observed phenotypic distributions. Obviously, and as argued above, this calls for the interrogation of small phenotypic effects under different conditions and in a population context.

However, more intriguingly, it brings forward a daunting proposition: could cellular pathways be wired in such a way that a small change along one phenotypic axis (e.g., in one cellular pathway) induces many small changes along other phenotypic axes (e.g., in other cellular pathways) in order to uphold a given fitness value? If this were the case, then a cell (or some subset of its pathways) should be viewed as implementing a massive buffering strategy, unfolding in real time, all the time. Viewed in this context, small changes that don't result in dramatic fitness effects could still be valuable as our window into the extent and purpose of intracellular regulatory connectivity. Increasingly, high-throughput technologies are revealing the extent of this connectivity. Coupled with quantitative measures of regulation and accurate snapshots of cellular physiology, this framework might be an invaluable tool for understanding the inherently dynamic underpinnings of life.

Concluding Remarks

Order-of-magnitude effects have allowed us to identify many regulators of biological pathways whose perturbations induce severe fitness defects by causing massive network failures. We have argued that understanding how cells work requires us to identify and investigate in-depth factors whose contribution to a process of interest is more quantitative than qualitative. While there are realms of biological investigation in which this sort of thinking is de rigueur, it does not seem to be a universal view, especially among molecular, cell, and developmental biologists. Given that the technology for making automated, highly quantitative single-cell observations is rapidly maturing, the time is ripe for a reevaluation of perspectives on the biological meaning of quantitative effects.

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