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CELL BIOLOGY

The Complexity of Cell Signaling and the Need for a New Mechanics

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Cell signaling systems respond to multiple inputs, such as ligands of cell-surface receptors; and produce multiple outputs, such as changes in gene expression and cellular activities, including motility, proliferation, and death. This "macroscopic" input-output behavior is generated by a web of molecular interactions that can be viewed as taking place at a lower, "microscopic" level. These interactions prominently involve posttranslational modification of proteins and the nucleation of protein complexes. Behaviors at both the micro- and macroscopic levels are complex and must be probed systematically and characterized quantitatively as a prelude to the development of a predictive understanding of a cell signaling system. We must also have a theoretical framework or a mechanics within which we can determine how macroscopic behaviors emerge from known microscopic behaviors or change with manipulations of microscopic behaviors. To connect behaviors at both levels, we suggest that a new mechanics is now required. Newly available data support the idea that this mechanics should enable one to track the site-specific details of molecular interactions in a model, such as the phosphorylation status of individual amino acid residues within a protein.

As exemplified by our understanding of the role of tyrosine phosphorylation in cell signaling (*1*), we now have a fairly clear picture of the types of molecular mechanisms that are responsible for cellular responses to environmental changes, even as the molecular-level details of particular signaling systems continue to emerge. In cell signaling systems, information processing is mediated by posttranslational modifications of proteins, which turn binding and catalytic activities on and off; and the nucleation of protein complexes, in which enzymes and substrates are colocalized to control enzyme specificity and activity. This picture is powerful enough to have contributed to the development of drugs that target specific signaling molecules that drive disease, such as inhibitors of tyrosine kinases (*2*). Current understanding of cell signaling, however, is often insufficient to predict how mutations or potential therapeutic interventions at the molecular level will affect cellular behavior.

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To make further progress in the treatment of diseases caused by dysfunctional cell signaling, we are faced with the challenge of obtaining a predictive understanding of cellular decisions in terms of the underlying molecular mechanisms of cell signaling, which are exceedingly complex. This challenge is similar to the one faced by those in the 19th century who embraced the atomic theory, such as Maxwell, Boltzmann, and Gibbs, and developed statistical mechanics to explain the macroscopic properties of matter in terms of the microscopic properties of atoms and molecules. As at that time, a new mechanics is needed to bridge two levels of behavior (Fig. 1): "macroscopic" behavior at the level of the inputs and outputs of cell signaling systems, and "microscopic" behavior at the level of the molecular interactions that process the inputs and generate the outputs. Just as importantly, experimental studies are required to systematically probe and quantitatively characterize the behaviors of cell signaling systems at both the macro- and microscopic levels. Here, we comment on two such studies (*3*, *4*), which encourage us to believe that the complexity of cell signaling will eventually be understood and which point to the new mechanics that will be needed to achieve this goal.

At the macroscopic level of cell signaling (Fig. 1), the number of potential inputs to which a cell can respond is very large—the typical eukaryotic cell possesses many different types of receptors that respond to an even larger repertoire of chemical ligands and other physiochemical stimuli. The array of potential responses is similarly vast, ranging from changes in the abundance of gene products to changes in shape and locomotion, and even cell death. At this level, the signal-processing machinery of the cell may be thought of as a black box, and a major goal of experimentation is to determine the responses of a cell to the full range of possible inputs. Most current experimental studies, however, focus on the effects of one input at a time, and the derived information is sufficient to predict cellular responses to a combination of inputs only if each input acts independently, in which case the response to a combination of two signals can be written as the sum of the individual responses, a property known as additivity (Fig. 1A). Additivity should not be confused with another mathematical property of many human-engineered control systems; namely, linearity, which requires that the contribution to the output from any single input be proportional to the level of input and is more restrictive in this context. Responses that are highly nonlinear with respect to input levels may still be additive if they can be decoupled into a set of responses to individual stimuli. Nonadditive responses arise when one input modulates the output induced by another. The extent of additivity is a useful measure of the complexity of any signal processing system: The more nonadditive terms that must be used to describe the response, the greater the degree of complexity. Probes of additivity test the current prevailing paradigm of studying one input at a time when characterizing biological responses.

Recently, researchers from the Alliance for Cellular Signaling (AfCS) carried out a systematic investigation of additivity in the responses of macrophages to a wide range of stimuli. Natarajan *et al*. (*5*) investigated the intermediate and final responses to stimulation with 22 individual ligands and all 231 possible pairwise combinations of these ligands to assess the degree of nonadditivity arising from combining two inputs. They found that approximately 90% of the responses to the combined inputs were additive, whereas the remaining 10% displayed nonadditivity that reflected varying levels of interaction between the biochemical pathways leading to the response. In

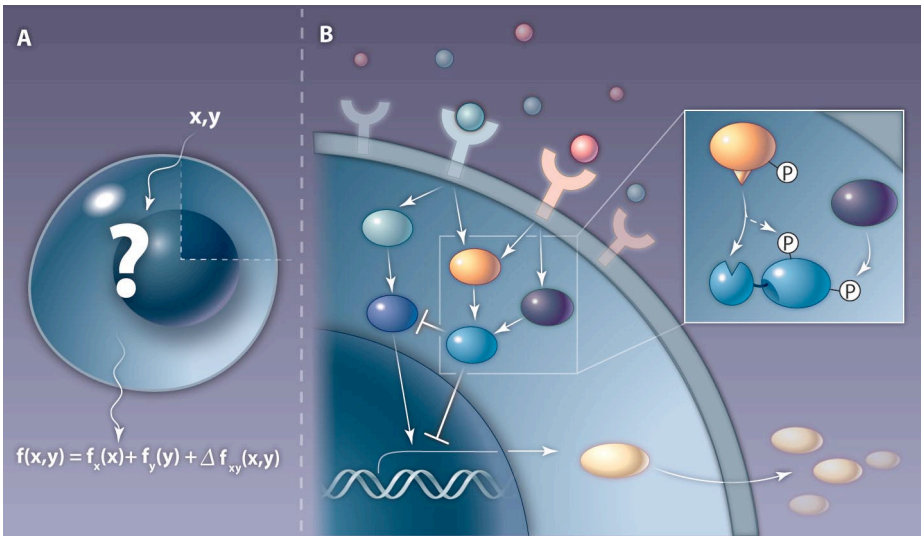

Fig. 1. Multiple layers of complexity in cellular information processing. (**A**) At the highest level, the cell can be considered a black box that generates a range of possible responses, $f(x,y)$, to many different inputs, x and y. The signal can be resolved into additive and nonadditive terms, of which the former are the individual contributions arising from each input, $f_x(x)$ and $f_y(y)$, and the latter arise from interactions between inputs, $\Delta f_{xy}(x,y)$. (**B**) Opening the black box reveals a complex network of signaling molecules that couple ligand-receptor binding to transcriptional regulation. (Inset) The basic building blocks of the network are protein-protein interactions and enzyme-catalyzed posttranslational modifications, such as phosphorylation (P).

some cases, measurements of the intermediate responses suggested novel hypotheses about the mechanisms coupling the pathways, which were subsequently characterized (*6*, *7*).

A natural question that arises from these results is whether additivity, and thus predictability, extends to higher-order combinations of inputs. If not, then our ability to predict signaling outcomes would be seriously limited, because the number of possible nonadditive interactions grows exponentially with the number of combined inputs. For example, there are more than 1500 possible three-way combinations of the 22 ligands used in the study of Natarajan *et al*. (*5*). A three-way combination is said to be additive when the response can be predicted as the sum of the single-ligand and pairwise responses. To characterize the complexity of higher-order responses, Hsueh *et al*. (*3*) measured macrophage cytokine responses to selected three-, four-, and five-way combinations of ligands that exhibited substantial pairwise interaction in the earlier study. Although novel interactions were found, only a minority of the input-output combinations tested (33%) exhibited nonadditivity beyond the pairwise level. In addition, the interactions that were found are consistent with known regulatory mechanisms, suggesting that current paradigms for studying signaling biochemistry are likely to be sufficient for providing a mechanistic basis for biological signal processing. In other words, the network wiring diagrams or canonical signaling pathways that have emerged from several decades of molecular biology and biochemistry (Fig. 1B) are surprisingly reliable. If the macrophage is representative of other eukaryotic cell types, these results suggest, as others have argued (*8*, *9*), that the complexity of interactions between cellular networks is limited, although the basis for this robustness is not entirely clear.

Coba *et al*. (*4*) used proteomic methods to define the molecular circuitry (Fig. 1, right panel) of the mammalian postsynaptic density (PSD), the region of a neuron that receives electrical signals from other neurons and contains hundreds of different protein types (*10*). In addition to its electrical signal transmission properties, the PSD plays a critical role in the process of synaptic plasticity: the modification of the coupling between two neurons, which may form the basis of learning and memory (*11*). At the microscopic level, however, the biochemistry of the PSD is similar to that of many other signaling systems that have a wide range of functions, and the architecture of the postsynaptic phosphoproteome network revealed by Coba *et al*. (*4*) is likely to share generic features with many other eukaryotic signaling networks. Indeed, as in a number of studies of other systems through similar methodologies (*12*), Coba *et al*. (*4*) found that brief stimulation of a single key receptor led to changes in the phosphorylation status of more than 100 proteins. Interestingly, the majority of the observed changes (77%) were decreases in the level of phosphorylation, suggesting that activation involved a complex pattern of phosphorylated and unphosphorylated residues. The phosphorylation pattern of 10 specific receptor sites in response to stimulation by different ligands was monitored and found to be unique for four of the five ligands studied. Many of the sites exhibited similar responses to two or more ligands, indicating a high degree of potential interactions between inputs, and it would be interesting to know whether these ligands exhibit nonadditive responses in combination, because this would provide a partial answer to the question of whether overlaps at the microscopic level feed up to interactions at the macroscopic level.

To map what they call the phosphoproteome (the network of kinase-substrate interactions at the level of individual sites), Coba *et al*. (*4*) constructed a peptide array to probe more than 300 sites identified in vivo either by their initial screen or from other studies, and measured phosphorylation by 25 postsynaptic kinases. The resulting network contains more than 700 wires connecting all 25 kinases and more than half of the phosphorylation sites assayed. Each kinase phosphorylated 29 different sites on average, and more than half of the sites and 85% of the proteins were phosphorylated by more than one kinase, with some hub sites being phosphorylated by as many as 12 kinases. Although clustering of the network revealed a certain level of organization based on substrate specificity and the temporal sequence of signaling events, it is impossible to predict by inspection or high-level analysis how signals would propagate through such a densely connected network to produce a stable end point. One mechanism for pruning the phosphorylation chaos is cooperativity between sites, a phenomenon the authors call priming, wherein the phosphorylation of one site either enhances or inhibits the phosphorylation of a neighboring site. The priming effect was found in 66% of the cases studied and

was more often inhibitory by a nearly 3-to-1 ratio. Phosphorylation acts as a binary switch to turn on or off the activity of a protein; in a large fraction of the proteins assayed, the apparent role of phosphorylation was to modulate ligand-binding motifs that controlled interactions with other proteins. The net effect then of stimulating the complex phosphoproteome network is to remodel the protein complexes in the network for the purpose of generating a response, as in transcriptional regulation, and possibly also to encode information that a signal has been received for the purpose of modulating subsequent responses; that is, memory. The most obvious form of cellular memory encoding is a change in the abundance of proteins, but the richness of the phosphoproteome suggests the possibility that memory may also be encoded by changes in the pattern of phosphorylation.

The results of Coba *et al*. (*4*), like those of earlier phosphoproteomic studies (*13*), have important implications for those who seek to develop a mechanics to connect the microscopic and macroscopic levels of cell signaling behavior. The salient results can be summarized as follows: (i) Any given phosphorylation site is likely to be the substrate of multiple kinases, (ii) phosphorylation of nearby phosphorylation sites is likely to be cooperative, and (iii) many phosphorylation sites regulate the binding activities of protein interaction domains. Although these results clearly reveal that microscopic behaviors are dominated by the phosphorylation status of individual sites, tracking the site-specific details of molecular interactions is challenging because of their combinatorial potential (*14*), which may be an essential feature of cell signaling systems that facilitates the encoding and transmission of information (*15*, *16*). Current efforts to model cell signaling processes are dominated by assumptions such as that of the "virtual phosphorylation site" of Birtwistle *et al*. (*17*), which states that all phosphorylation sites within a protein can be lumped together into a single effective site. Such assumptions are made to simplify model specification and simulation, because classical modeling approaches are poorly equipped to deal with a system characterized by a large (combinatorial) state space.

Recently, researchers have proposed an array of new model specification and simulation approaches that are better suited for tracking the site-specific details of molecular interactions in cell signaling systems (*18*–*21*). We expect that further development of such methods will eventually provide the mechanics needed to connect the microscopic and macroscopic levels of cellular information processing. Such mechanics will help us to control cellular activities through manipulation of the molecular machinery of cell signaling and to gain greater insights into the design principles of cell signaling from phosphoproteomic data. In particular, the ability to reason about single-site events in signaling systems will be important for deciphering how patterns of posttranslational modifications affect cellular activities (*22*).

References and Notes

1. T. Hunter, Signaling—2000 and beyond. *Cell* **100**, 113–127 (2000).
2. T. Hunter, Treatment for chronic myelogenous leukemia: The long road to imatinib. *J. Clin. Invest.* **117**, 2036–2043 (2007).
3. R. C. Hsueh, M. Natarajan, I. Fraser, B. Pond, J. Liu, S. Mumby, H. Han, L. I. Jiang, M. I. Simon, R. Taussig, P. C. Sternweis, Deciphering signaling outcomes from a system of complex networks. *Sci. Signal.* **2**, ra22 (2009).
4. M. P. Coba, A. J. Pocklington, M. O. Collins, M. V. Kopanitsa, R. T. Uren, S. Swamy, M. D. R. Croning, J. S. Choudhary, S. G. N. Grant, Neurotransmitters drive combinatorial multistate postsynaptic density networks. *Sci. Signal.* **2**, ra19 (2009).
5. M. Natarajan, K. M. Lin, R. C. Hsueh, P. C. Sternweis, R. Ranganathan, A global analysis of cross-talk in mammalian cellular signalling network. *Nat. Cell Biol.* **8**, 571–580 (2006).
6. L. I. Jiang, J. Collins, R. Davis, K. M. Lin, D. DeCamp, T. Roach, R. Hsueh, R. A. Rebres, E. M. Ross, R. Taussig, I. Fraser, P. C. Sternweis, Use of a cAMP BRET sensor to characterize a novel regulation of cAMP by the sphingosine 1-phosphate/G13 pathway. *J. Biol. Chem.* **282**, 10576–10584 (2007).
7. L. I. Jiang, J. Collins, R. Davis, I. D. Fraser, P. C. Sternweis, Regulation of cAMP responses by the G12/13 pathway converges on adenylyl cyclase VII. *J. Biol. Chem.* **283**, 23429–23439 (2008).
8. S. Noselli, N. Perrimon, Are there close encounters between signaling pathways? *Science* **290**, 68–69 (2000).
9. S. Maslov, K. Sneppen, Specificity and stability in topology of protein networks. *Science* **296**, 910–913 (2002).
10. M. Sheng, C. C. Hoogenraad, The postsynaptic architecture of excitatory synapses: A more quantitative view. *Annu. Rev. Biochem.* **76**, 823–847 (2007).
11. M. Sheng, M. J. Kim, Postsynaptic signaling and plasticity mechanisms. *Science* **298**, 776–780 (2002).
12. B. Macek, M. Mann, J. V. Olsen, Global and site-specific quantitative phosphoproteomics: Principles and applications. *Annu. Rev. Pharmacol. Toxicol.* **49**, 199–221 (2009).
13. J. V. Olsen, B. Blagoev, F. Gnad, B. Macek, C. Kumar, P. Mortensen, M. Mann, Global, in vivo, and site-specific phosphorylation dynamics in signaling networks. *Cell* **127**, 635–648 (2006).
14. W. S. Hlavacek, J. R. Faeder, M. L. Blinov, R. G. Posner, M. Hucka, W. Fontana, Rules for modeling signal-transduction systems. *Sci. STKE* **2006**, re6 (2006).
15. J. R. Faeder, M. L. Blinov, B. Goldstein, W. S. Hlavacek, Combinatorial complexity and dynamical restriction of network flows in signal transduction. *Syst. Biol.* **2**, 5–15 (2005).
16. M. Thomson, J. Gunawardena, Unlimited multistability in multistate phosphorylation systems. *Nature*, 10.1038/nature08102 (2009).
17. M. R. Birtwistle, M. Hatakeyama, N. Yumoto, B. A. Ogunnaike, J. B. Hoek, B. N. Kholodenko, Ligand-dependent responses of the ErbB signaling network: Experimental and modeling analyses. *Mol. Syst. Biol.* **3**, 144 (2007).
18. J. Colvin, M. I. Monine, J. R. Faeder, W. S. Hlavacek, D. D. Von Hoff, R. G. Posner, Simulation of large-scale rule-based models. *Bioinformatics* **25**, 910–917 (2009).
19. J. Feret, V. Danos, J. Krivine, R. Harmer, W. Fontana, Internal coarse-graining of molecular systems. *Proc. Natl. Acad. Sci. U.S.A.* **106**, 6453–6458 (2009).
20. M. Lis, M. N. Artyomov, S. Devadas, A. K. Chakraborty, Efficient stochastic simulation of reaction-diffusion processes via direct compilation. *Bioinformatics*, in press.
21. N. A. Sinitsyn, N. Hengartner, I. Nemenman, Adiabatic coarse-graining and simulations of stochastic biochemical networks. *Proc. Natl. Acad. Sci. U.S.A.* **106**, 10546–10551 (2009).
22. B. A. Benayoun, R. A. Veitia, A post-translational modification code for transcription factors: Sorting through a sea of signals. *Trends Cell Biol.* **19**, 189–197 (2009).

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