Minireview

Systems biology approaches in cell signaling research

Raymond E Chen and Jeremy Thorner

Address: Division of Biochemistry and Molecular Biology, Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720-3202, USA.

Correspondence: Jeremy Thorner. E-mail: jthorner@berkeley.edu

Published: 29 September 2005

Genome ***Biology*** 2005, **6:**235 (doi:10.1186/gb-2005-6-10-235)

The electronic version of this article is the complete one and can be found online at http://genomebiology.com/2005/6/10/235

Abstract

The use of methods for global and quantitative analysis of cells is providing new systems-level insights into signal transduction processes. Recent studies reveal important information about the rates of signal transmission and propagation, help establish some general regulatory characteristics of multi-tiered signaling cascades, and illuminate the combinatorial nature of signaling specificity in cell differentiation.

The most useful road maps are those that provide an overview of the major highways, as well as displaying street-by-street detail for specific locations to reveal the connections at points of interest. In the same sense, a major goal of current research is to collect information and devise tools to help us understand biological phenomena at multiple levels of abstraction. Traditional biochemistry and molecular biology focus on the properties of individual molecules, including, for proteins and enzymes, their immediate subparts (domains), their substrates and ligands, and the company they keep (interacting partners and complexes). This approach has been remarkably successful at elucidating the structures and functions of many cellular constituents and will continue to be so for years to come. In contrast, our understanding of biological processes at larger scales of resolution, such as entire intracellular signal transduction networks, is much less developed.

Over the past few years, powerful methodological advances have enabled high-throughput data acquisition in biology, including sequencing of entire genomes, microarray analysis of global patterns of gene expression, evaluation by mass spectrometry of the nature and modification state of cellular proteomes, and genetic and biochemical methods for identifying protein-protein complexes and entire gene and protein interaction networks. Fortunately, this progress has occurred contemporaneously with other technological advances that have increased the power, versatility, and accessibility of computers. Hence, we now have the capacity to extract a plethora of new insights from what would otherwise be an overwhelming amount of primary information. Of course, deducing the biological relevance of the observations made on such a large scale depends crucially on the understanding and annotation of cellular molecules and processes gleaned from the knowledge base accumulated from decades of small-scale studies. But teasing the meaning out of genome-wide data also depends on conceptual and quantitative frameworks imported from other scientific disciplines, such as electrical and chemical engineering, mathematics, statistics, and computer science. As a result, large-scale approaches combined with computational methods are now facilitating the expansion of biochemistry and molecular biology to the whole-systems level. The new perspectives that such approaches provide are illustrated by three recent studies focused on cell signaling - two investigations of the properties of complex multi-step pathways that include *in silico* simulations [1,2], and a large-scale proteomic analysis of the difference in cellular responses to epidermal growth factor (EGF) and platelet-derived growth factor (PDGF) [3].

Properties of signal transduction cascades

Quantitative analysis is increasingly being used to discover the general principles relating the functional properties of a

signaling pathway to its basic topological characteristics. Among the various signaling modules employed by eukaryotic cells, some involve the activation of only one component downstream of the receptor. One example is the transforming growth factor-beta (TGFβ) receptor-catalyzed phosphorylation of Smad transcription factors, which permits their nuclear entry (which is crucial for pattern formation and cell-fate determination in metazoan embryonic development) [4]. Similarly, after binding of cyclic 3′,5′-AMP to the regulatory subunit of protein kinase A (PKA), the dissociated catalytic subunit can enter the nucleus and phosphorylate the CREB transcription factor [5]. We refer to such pathways as 'single-step'. Other signaling systems, including mitogen-activated protein (MAP) kinase cascades, involve the sequential activation of multiple intermediaries and we refer to these as 'multi-step' pathways [6].

What are the biological consequences, if any, arising from the structural designs of single and multi-step signal transduction pathways? Depending on the concentrations and inherent kinetic characteristics of the components of signal-transduction systems, the output observed in response to a stimulus of increasing intensity can display a graded response (akin to an enzyme that possesses standard Michaelis-Menten characteristics), an ultrasensitive response (akin to the behavior of allosteric enzymes that display a high degree of cooperativity), or even a bistable response (that is, having the character of an all-or-none shift, like an 'off-on' switch) [7]. Previous work has shown that in addition to amplifying small signals into large responses, MAP kinase cascades also combine the inherent cooperative behavior of the constituent enzymes and the nature of the chemical reactions they catalyze into an enhanced systems-level ultrasensitivity [8-11]. This feature has the effect of filtering out stochastic noise and converting graded stimuli into more switch-like behaviors when input exceeds a preset threshold. Thus, even in the presence of a cue of intermediate strength, an individual cell can make a biologically appropriate all-or-nothing decision, such as whether to divide or differentiate. Some of the differences in the signal-response characteristics of single- and multi-step pathways are summarized in Table 1.

Table 1

Properties of single- and multi-step signaling pathways

	Single-step	Multi-step
Noise filtering	No	Yes
Output characteristic	Graded	Switch-like
Potential amplification	Low	High
Transmission speed	Optimized for high input strengths	Optimized for low input strengths

A recent paper by Nakabayashi and Sasaki [1] suggests that signaling cascades exhibit another important emergent property: optimization of the speed at which information is transmitted through the system. The authors analyzed *in silico* a simplified linear kinase-phosphatase cascade that is frequently employed as a model of the core MAP kinase module [2,8,12]. For any particular input signal strength, the time required for the pathway output to reach a desired level depends on the number of steps in the cascade. Nakabayashi and Sasaki [1] sought to determine the number of steps that would minimize this signal transmission time. Interestingly, they observed that the shortest (single-step) pathways were not always the fastest. Specifically, for a given output level, the optimal number of steps increased as the input strength decreased, consistent with earlier analyses performed on models with decaying inputs and weakly activated kinases [12]. Furthermore, for pathways of sufficient length, the optimal step number is proportional to the order of magnitude of the response amplification [1].

Another property of a cascade is that it also provides multiple nodes for potential regulation. This feature is particularly notable in the light of studies indicating that different reactions within a cascade influence qualitatively distinct characteristics of the signal response. Again utilizing *in silico* simulations of a kinase cascade, Hornberg *et al.* [2], in a confirmation of work by Heinrich *et al.* [12], showed that activating processes (in a MAP kinase cascade these are phosphorylation reactions) tend to exert their influence on the characteristics of signal strength, including both output amplitude and basal pathway activity. In contrast, inactivating processes (primarily dephosphorylation reactions) control not only output strength, but also its temporal properties, such as the time to peak intensity (which is inversely related to signaling rate) and the overall duration of pathway stimulation. These findings were formalized mathematically [2,12] and have now also been validated experimentally by Hornberg *et al.* [2], by measuring the time-course of extracellular signal-related kinase (ERK) phosphorylation in fibroblast (NRK) cells treated with EGF in the presence or absence of inhibitors of the upstream MAP kinase kinase (MEK) or an inactivating MAP kinase phosphatase (PTP). These results imply that the effects of simultaneously reducing (or increasing) the activity of kinases and phosphatases will not cancel each other out - the activating and inactivating processes are not purely antisymmetrical. How cascades (as opposed to other mechanisms for signal dissemination) are well designed for speed, ultrasensitivity and complex regulation is illustrated in Figure 1.

Hornberg *et al.* [2] further found that although the activating and inactivating processes together are indeed balanced with regard to response amplitude, individual kinase-phosphatase pairs generally are not: equivalent increases in the activities of a kinase and a phosphatase that act on the same target will lead to a net increase in signal strength. This

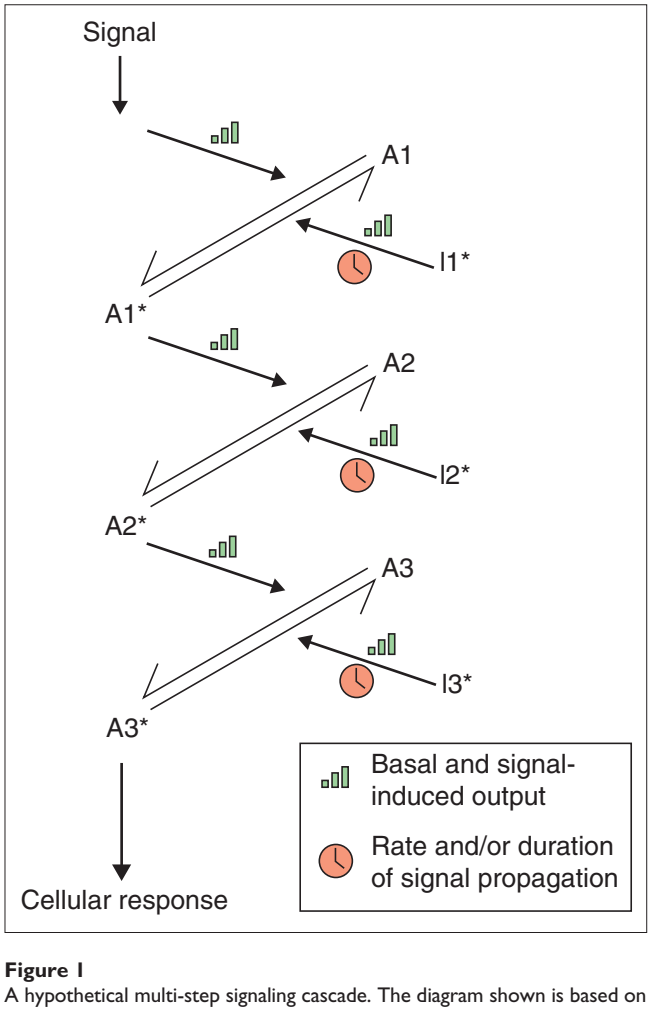

Figure 1
A hypothetical multi-step signaling cascade. The diagram shown is based on the classical MAP kinase activation pathway. The core of such signaling cascades comprises a series of enzymes (protein kinases) that sequentially activate each other (shown as A1, A2 and A3 in the unphosphorylated and inactive state, and as A1*, A2* and A3* in the phosphorylated and active state) so as to propagate a cellular response to a signal, as well as the opposing enzymes (for example, phosphatases) and other factors (such as ubiquitin-mediated degradation) that inactivate them (shown as I1*, I2* and I3*). Upstream and downstream factors in this schematic multi-tiered signal transduction cascade are not shown. The *in silico* analyses discussed in this article indicate that activating processes primarily control the strength of both the basal and signal-induced output (indicated by bars), whereas inhibitory processes control both output strength and the rate and/or duration of signal propagation (indicated by clocks). These studies conclude that, compared with single-step pathways (like the TGFβ- and PKA-mediated transcription factor activation described in the text), a cascade exhibits ultrasensitivity (resistance to stochastic noise and switch-like responsiveness), signal amplification and optimized signal transmission speed (see also Table 1). In addition, in a cascade, there is the opportunity potentially to exert very fine-tuned regulation of pathway output because there are multiple points at which different factors can be used to control the amount and/or level of activity of the pathway constituents and their temporal response characteristics.

asymmetry is counterbalanced at the level of the upstream receptor, where inactivation exerts stronger control than activation. Thus, even within a single level of a cascade, counteracting signaling components cannot be treated as mere opposites, and they can be differentially controlled to regulate distinct response characteristics.

A few years ago, Bhalla *et al.* [13] showed that the concentration of MAP kinase phosphatase is crucial for determining whether MAP kinase signaling in NIH-3T3 fibroblasts displayed bistability or not. What about other cell types? It is well known that treatment of cultured neuroendocrine (PC12) cells with EGF induces only transient ERK activation and results in cell proliferation, whereas treatment of the same cells with nerve growth factor (NGF) causes sustained ERK activation and results in cell differentiation, including extension of dendritic and axonal projections [14]. A recent analysis by Sasagawa *et al.* [15] found that this difference depends on differences in the regulation of the GTPase-activating proteins (GAPs) that inactivate the small GTPases, Ras and Rap1 - the activators of the respective MAP kinase kinase kinases in the proliferation and differentiation pathways. In these systems, therefore, inactivating enzymes in MAP kinase cascades have a key role not only in suppressing the level of pathway activity in unstimulated cells and during recovery from stimuli, but also in regulating the specific dynamics of signaling in ways that are biologically meaningful. The studies by Hornberg *et al.* [2] and Heinrich *et al.* [12] suggest that this may be a general feature of MAP kinase signaling systems.

Signal specificity

Quantitative and large-scale approaches are also proving useful in elucidating the underlying molecular basis of differential cellular responses to similar extracellular cues. Given that many growth-factor receptors are ligand-activated protein-tyrosine kinases, a prominent feature of the behavior induced by such growth factors is the phosphorylation of numerous downstream effector proteins on tyrosine, including autophosphorylation of the growth-factor receptors themselves [16]. Many phosphorylation targets appear to be regulated similarly upon exposure to different growth factors, even when the growth factors induce different biological behavior. For example, while exposure of human mesenchymal stem cells (hMSCs) to either EGF or PDGF leads to equivalent levels of MAP kinase enrichment in phosphotyrosine-containing complexes, EGF induces MAP kinase-dependent differentiation to bone cells, whereas PDGF does not [3,17].

In order to identify differences in the signaling networks activated by EGF and PDGF in hMSCs, Kratchmarova *et al.* [3] compared the entire set of tyrosine-phosphorylated proteins and their interacting partners in EGF- versus PDGF-stimulated cells. Equivalent populations of hMSCs were metabolically labeled with isotopically different (but biochemically identical) variants of arginine and exposed to EGF, PDGF, or no growth factor. Equal amounts of cell

lysates from these populations were pooled and subjected to anti-phosphotyrosine immunopurification, tryptic digestion, and mass spectrometry. For each identified protein, the isotopically distinguished mass spectrum of the arginine-containing peptides indicated the relative cellular level of that species in tyrosine-phosphorylated complexes across the growth-factor treatment conditions.

Using this method, the researchers discovered that among the few proteins (less than 10%) that were uniquely regulated by the two different stimuli, phosphatidylinositol (PI) 3-kinase was preferentially enriched in phosphotyrosine-containing complexes in cells exposed to PDGF relative to those exposed to EGF (or no growth factor) [3]. The SH2 domain-containing subunit (p85) of PI 3-kinase binds to a specific phosphotyrosine-containing motif on the PDGF receptor, thereby recruiting the enzyme to the plasma membrane (owing to the resulting proximity, the receptor also phosphorylates the enzyme at tyrosine) [18]. Tethering PI 3-kinase at the plasma membrane permits generation of PI 3,4,5-P_3, which stimulates activation of additional downstream signaling components, such as the protein kinases PDK1 [19] and c-Akt [20], that promote cell survival and cell migration [21,22]. Hence, the fact that PDGF, but not EGF, leads to PI 3-kinase recruitment suggested a possible and novel negative regulatory role for this lipid kinase in blocking differentiation. Indeed, hMSCs treated with PDGF in the presence of wortmannin, a specific inhibitor of PI 3-kinase, exhibited osteoblast differentiation comparable to that of EGF-stimulated cells both in culture, as assayed by acquisition of a cell-type-specific enzymatic activity and mineralization, and *in vivo*, as assayed by bone formation following implantation in mice [3]. The ability to pinpoint PI 3-kinase as one of the very few major molecular differences between the EGF- and PDGF-stimulated signaling networks, and subsequently to demonstrate that PI 3-kinase is a critical regulatory node for hMSC differentiation, is remarkable and clearly validates the authors' global proteomic approach [3]. This study also highlights the importance of a feature inherent in large-scale analyses in which many components are directly assessed in parallel, namely the ability to rule out the 'uninteresting' players (in this work [3] proteins that were equivalently affected by both ligands).

Combinatorial control

The fact that an individual protein can elicit distinctly different biological effects depending on the nature of the interacting partners that are present in the same cell or compartment is a frequently encountered paradigm in transcription factor function and the regulation of gene expression [23,24]. The conclusion of Kratchmarova *et al*. [3] - that EGF induces hMSC osteoblast differentiation by activating the MAP kinase pathway, whereas PDGF stimulation avoids the differentiation response by stimulating PI 3-kinase in addition to MAP kinase - suggests that cells also achieve appropriate signaling outputs through simple combinations of entire signaling pathways. In other words, even among extracellular stimuli that evoke responses leading to essentially identical modification of a substantially similar set of proximal targets, the additional input of differential regulation at one or a few selected upstream nodes can yield dramatically different biological consequences by uniquely triggering the activity of an entire downstream module.

Simulation of the kinetics and behavioral characteristics of various arrangements of signal transduction circuitry, and the ability to interrogate simultaneously all cellular components, provides an unprecedented view of the cell that is unavailable at smaller scales of analysis. When informed by and combined with traditional methods, these system-wide approaches enhance our understanding of complex biological phenomena. Although the application of quantitative systems-level techniques to signal transduction research is still relatively new, compared with the established use of such techniques in investigations of metabolic and neuronal networks [25,26], signs are promising that, in this area too, such methods can help delineate testable hypotheses and generate useful conceptualizations about the biological processes involved [27-30]. The studies by Nakabayashi and Sasaki [1], Hornberg *et al*. [2], and Kratchmarova *et al*. [3] illuminate the functions and relationships among components and pathways in MAP kinase and growth factor signaling and provide insights into properties that may be generalizable to other signal transduction mechanisms and networks. As proteomic and other large-scale methods are continually and rapidly improving [31], in lock-step with new computational methods [32], we are likely to see rapid progress on this front.

References

1. Nakabayashi J, Sasaki A: **Optimal phosphorylation step number of intracellular signal-transduction pathway.** *J Theor Biol* 2005, **233:**413-421.
2. Hornberg JJ, Bruggeman FJ, Binder B, Geest CR, de Vaate AJ, Lankelma J, Heinrich R, Westerhoff HV: **Principles behind the multifarious control of signal transduction. ERK phosphorylation and kinase/phosphatase control.** *FEBS J* 2005, **272:**244-258.
3. Kratchmarova I, Blagoev B, Haack-Sorensen M, Kassem M, Mann M: **Mechanism of divergent growth factor effects in mesenchymal stem cell differentiation.** *Science* 2005, **308:**1472-1477.
4. Attisano L, Wrana JL: **Signal transduction by the TGF-beta superfamily.** *Science* 2002, **296:**1646-1647.
5. Mayr B, Montminy M: **Transcriptional regulation by the phosphorylation-dependent factor CREB.** *Nat Rev Mol Cell Biol* 2001, **2:**599-609.
6. Widmann C, Gibson S, Jarpe MB, Johnson GL: **Mitogen-activated protein kinase: conservation of a three-kinase module from yeast to human.** *Physiol Rev* 1999, **79:**143-180.
7. Ferrell JE Jr: **Self-perpetuating states in signal transduction: positive feedback, double-negative feedback and bistability.** *Curr Opin Cell Biol* 2002, **14:**140-148.
8. Huang CY, Ferrell JE Jr: **Ultrasensitivity in the mitogen-activated protein kinase cascade.** *Proc Natl Acad Sci USA* 1996, **93:**10078-10083.
9. Ferrell JE Jr: **Tripping the switch fantastic: how a protein kinase cascade can convert graded inputs into switch-like outputs.** *Trends Biochem Sci* 1996, **21:**460-466.

10. Brown GC, Hoek JB, Kholodenko BN: **Why do protein kinase cascades have more than one level?** *Trends Biochem Sci* 1997, **22:**288.
11. Ferrell JE Jr: **How responses get more switch-like as you move down a protein kinase cascade.** *Trends Biochem Sci* 1997, **22:**288-289.
12. Heinrich R, Neel BG, Rapoport TA: **Mathematical models of protein kinase signal transduction.** *Mol Cell* 2002, **9:**957-970.
13. Bhalla US, Ram PT, Iyengar R: **MAP kinase phosphatase as a locus of flexibility in a mitogen-activated protein kinase signaling network.** *Science* 2002, **297:**1018-1023.
14. Traverse S, Gomez N, Paterson H, Marshall C, Cohen P: **Sustained activation of the mitogen-activated protein (MAP) kinase cascade may be required for differentiation of PC12 cells. Comparison of the effects of nerve growth factor and epidermal growth factor.** *Biochem J* 1992, **288:**351-355.
15. Sasagawa S, Ozaki Y, Fujita K, Kuroda S: **Prediction and validation of the distinct dynamics of transient and sustained ERK activation.** *Nat Cell Biol* 2005, **7:**365-373.
16. Schlessinger J: **Cell signaling by receptor tyrosine kinases.** *Cell* 2000, **103:**211-225.
17. Jaiswal RK, Jaiswal N, Bruder SP, Mbalaviele G, Marshak DR, Pittenger MF: **Adult human mesenchymal stem cell differentiation to the osteogenic or adipogenic lineage is regulated by mitogen-activated protein kinase.** *J Biol Chem* 2000, **275:**9645-9652.
18. Tallquist M, Kazlauskas A: **PDGF signaling in cells and mice.** *Cytokine Growth Factor Rev* 2004, **15:**205-213.
19. Mora A, Komander D, van Aalten DM, Alessi DR: **PDK1, the master regulator of AGC kinase signal transduction.** *Semin Cell Dev Biol* 2004, **15:**161-170.
20. Brazil DP, Yang ZZ, Hemmings BA: **Advances in protein kinase B signalling: AKTion on multiple fronts.** *Trends Biochem Sci* 2004, **29:**233-242.
21. Debiais F, Lefevre G, Lemonnier J, Le Mee S, Lasmoles F, Mascarelli F, Marie PJ: **Fibroblast growth factor-2 induces osteoblast survival through a phosphatidylinositol 3-kinase-dependent, beta-catenin-independent signaling pathway.** *Exp Cell Res* 2004, **297:**235-246.
22. Fukuyama R, Fujita T, Azuma Y, Hirano A, Nakamuta H, Koida M, Komori T: **Statins inhibit osteoblast migration by inhibiting Rac-Akt signaling.** *Biochem Biophys Res Commun* 2004, **315:**636-642.
23. Remenyi A, Scholer HR, Wilmanns M: **Combinatorial control of gene expression.** *Nat Struct Mol Biol* 2004, **11:**812-815.
24. Istrail S, Davidson EH: **Logic functions of the genomic *cis*-regulatory code.** *Proc Natl Acad Sci USA* 2005, **102:**4954-4959.
25. Fell D: *Understanding the Control of Metabolism.* London: Portland Press; 1997.
26. Laughlin SB, Sejnowski TJ: **Communication in neuronal networks.** *Science* 2003, **301:**1870-1874.
27. Bhalla US, Iyengar R: **Emergent properties of networks of biological signaling pathways.** *Science* 1999, **283:**381-387.
28. Lee E, Salic A, Kruger R, Heinrich R, Kirschner MW: **The roles of APC and Axin derived from experimental and theoretical analysis of the Wnt pathway.** *PLoS Biol* 2003, **1:**E10.
29. Angeli D, Ferrell JE Jr, Sontag ED: **Detection of multistability, bifurcations, and hysteresis in a large class of biological positive-feedback systems.** *Proc Natl Acad Sci USA* 2004, **101:**1822-1827.
30. Barrios-Rodiles M, Brown KR, Ozdamar B, Bose R, Liu Z, Donovan RS, Shinjo F, Liu Y, Dembowy J, Taylor IW, *et al.*: **High-throughput mapping of a dynamic signaling network in mammalian cells.** *Science* 2005, **307:**1621-1625.
31. Sachs K, Perez O, Pe'er D, Lauffenburger DA, Nolan GP: **Causal protein-signaling networks derived from multiparameter single-cell data [Erratum Science 2005, 309:1187].** *Science* 2005, **308:**523-529.
32. Lok L, Brent R: **Automatic generation of cellular reaction networks with Moleculizer 1.0.** *Nat Biotechnol* 2005, **23:**131-136.