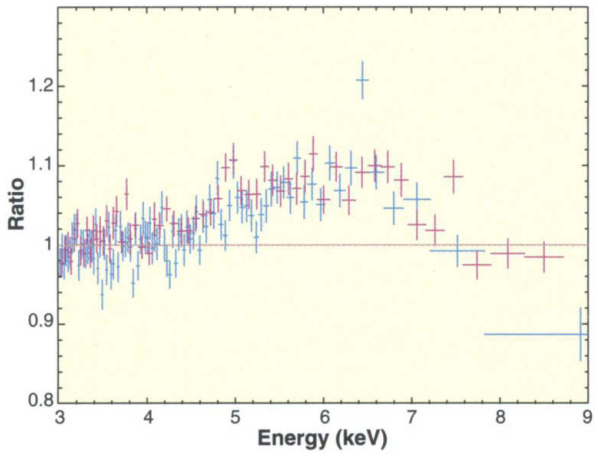

Iron lines in stellar mass black holes. (Red) Ratio of the spectrum of XTE J1650-500 (observed with XMM-Newton) to a simple disk blackbody and power law. (Blue) Same ratio for Cygnus X-1 (from Chandra). Plotting the spectrum in this manner reveals the shape of the iron line, which can be seen as a broad bump between 4 and 7 keV. [Data from (*7*)]

power source; for example, rotational energy may be extracted from a spinning black hole via magnetic connections to the inner accretion disk (*14*).

Spinning (Kerr) black holes have been postulated, but evidence for the spin itself is only now emerging. For several black holes, the inner radius of the disk deduced from the iron line indicates that matter is orbiting much closer than is possible for a nonspinning black hole. The orbit is closer to the stable orbit of a spinning black hole. The hypothesis that the fastest spinning black holes are those with the strongest radio emission remains untested.

A further quantity determined by the profile of the broad iron line is the disk inclination. The Doppler shifts are larger when the disk is seen more edge on, affecting mostly the "blue" (high energy) wing of the line. It is reasonable to suppose that the disk inclination is the same as that of the orbit of the binary companion, in the case of a stellar mass black hole. This has been demonstrated for some systems with optical measurements.

The iron line is a powerful diagnostic of the immediate environment of stellar mass black holes. It will enable us to test strong gravity, catalog black hole spin, and test models for the accretion inflow and jetted outflows from these objects. Further observations with Chandra and XMM-Newton, combined with the Rossi X-ray Timing Explorer (RXTE) (*15*), promise more revelations about stellar mass black holes.

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10. A gravitational radius is G^*m_{BH}/c^2, where G is Newton's gravitational constant, m_{BH} is the mass of the black hole, and c is the speed of light.
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15. RXTE can measure fast timings and covers a broad spectrum, thereby complementing the high resolution over a narrow spectrum of Chandra and XMM-Newton.

PERSPECTIVES: SIGNAL TRANSDUCTION

History Matters

Nicholas T. Ingolia and Andrew W. Murray

From the swimming of bacteria to communications among scientists, organisms depend on the collection and processing of information from the environment, a process called signal transduction. The simplest signaling pathway is a linear cascade, a collection of unidirectional arrows that connect a stimulus to a response via multiple intermediates. If you lived in this oversimplified world, you would still be staring at the blinding light that illuminated the obstetrician who delivered you from the darkness of the womb, because your visual system would neither have adjusted its threshold to maximize the sensitivity of its response (adaptation), nor returned to its ground state (recovery) once your gaze turned to something else. Properties like adaptation and recovery result from interactions among components of signaling networks. How do these properties reflect the topology of connections within a network, in addition to the detailed properties of the network's biochemical components? On page 1018 of this issue, Bhalla *et al.* (*1*) tackle this question with a combination of theory and experiment. They reveal that the behavior of a common cellular signaling network is history dependent, that is, the network output depends on the recent history of a cell's exposure to the network's activating stimulus.

Mitogen-activated protein kinase (MAPK) cascades are well-studied signal transduction systems present in a wide variety of eukaryotes. MAPK signaling pathways transduce signals for processes as diverse as mating, cell proliferation, and organ development. Depending on the cellular context, MAPK cascades may provide a switchlike all-or-none decision between two different responses (*2*), or a graded response over a wide range of stimulus strengths (*3*). Bhalla and colleagues present a systems-level analysis of how the MAPK signaling network of cultured mammalian cells processes signals (*1*). They couple computational simulations with pharmacological inhibition of network components, and discover that the network produces two qualitatively different intracellular responses that depend on the cell's prior history.

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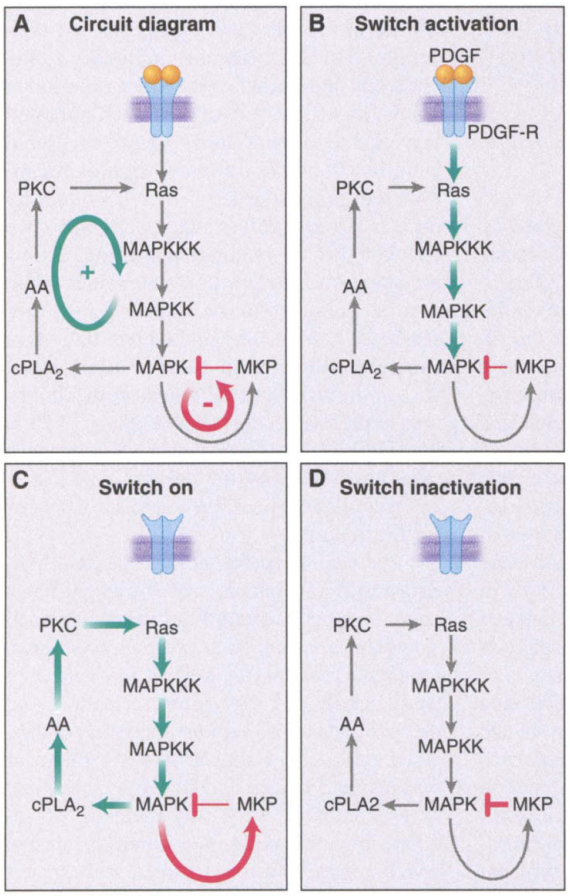
There is a powerful analogy between biological signal transduction networks and the signal processing systems conceived by engineers. The input of a typical signal transduction system is the concentration of some extracellular stimulus or ligand, whereas the output is the activity of an intracellular factor such as a protein kinase. For Bhalla *et al.*, the concentration of platelet-derived growth factor (PDGF) is the input of the MAPK network, and the activity of MAPK is the output. The amount of MAPK activity is related to the extracellular signals to which the cell is exposed, but this may not be a simple relation. One reason is that the signaling network features feedback regulation, which produces many complex behaviors (see the figure).

Bhalla and co-workers produce a quantitative model of the MAPK network and experimentally test its qualitative predictions. MAPK initiates two feedback loops, one positive and one negative. In simulations and experiments, a 5-minute pulse of PDGF induces MAPK activity that persists for about 30 minutes before slowly declining. Positive feedback results in this bistable (switchlike) behavior, where a brief stimulus flips the system into a state in which a positive-feedback loop sustains the active state. Bistability thus provides a cellular memory, allowing a response to outlast the stimulus that elicited it. Inhibiting either of the two proteins involved in posi-

tive feedback has no effect on the initial response, but MAPK is inactivated as soon as PDGF is removed, showing that abolishing positive feedback destroys the switch.

A negative-feedback loop produces recovery and ensures that a pulse of PDGF does not permanently activate the switch. The key component of this loop is the accumulation of MAPK phosphatase (MKP), which switches off MAPK activity by removing a phosphate group. Experimentally elevating MKP levels decreases the initial response to PDGF stimulation and prevents active MAPK from persisting after PDGF has been removed. In many settings negative-feedback loops cause adaptation (*4*), and recovery after PDGF removal can be thought of as adaptation to the high–MAPK activity state of the bistable system. Theory predicts that prior exposure to one pulse of PDGF will make the response to a second, later pulse shorter and more proportional to the PDGF concentration. Experiment confirms this prediction: Experienced cells show a response that increases with pulse strength, in contrast to the switchlike behavior of naïve cells.



Cellular circuits. (**A**) The logic of the cellular circuit analyzed by Bhalla *et al.* (*1*). The circuit diagram depicts positive-feedback (green) and negative-feedback (red) loops and omits several components that lie outside the central switch. (**B**) During switch activation, the binding of PDGF to its receptor (PDGF-R) leads to the activation of Ras. Activating Ras in turn stimulates the MAPK cascade, in which a MAP kinase kinase kinase (MAPKKK) activates a MAP kinase kinase (MAPKK), which activates MAP kinase (MAPK). This cascade activates both a positive-feedback loop through phospholipase A_2 ($cPLA_2$), arachidonic acid (AA), and protein kinase C (PKC), and a negative-feedback loop in which MAPK stabilizes MAPK phosphatase (MKP) and induces transcription of its mRNA. Because the positive-feedback loop is fast, whereas the negative-feedback loop is slow, MAPK activity is initially maintained after the removal of PDGF (**C**). However, as the level of MKP increases, the negative-feedback loop dominates and the switch is turned off (**D**). The level of MKP declines slowly after the switch is turned off, and so the behavior of the switch depends on the recent history of the cell's exposure to PDGF.

Many signaling networks have both positive- and negative-feedback loops. They can give rise to a variety of behaviors, including switches that remain in their active state after the stimulus has been removed, oscillators, and many different logical devices (*5*). Are there features of a network that allow us to predict its behavior? A recent *Science* paper by Guet and colleagues (*6*) reported synthetic networks made out of a variety of promoters and DNA binding proteins. These authors showed that networks with the same topology of connections between activating and inhibitory elements can have quite different behaviors, dashing the hope that such topologies will be sufficient to define the function of signaling networks (*6*). In contrast, general conclusions can be drawn from the quantitative biochemical details of the different steps in a network. For example, the combination of a fast positive-feedback and slow negative-feedback loop can produce either a switch or an oscillator (a relaxation oscillator or a one-shot switch, in engineering terms), depending on the strength of the negative feedback.

Feedback loops make the response of the MAPK system history dependent. Apparently identical cells will respond in different ways to the same stimulus depending on the magnitude, duration, and timing of the last pulses of PDGF they experienced. A linear cascade generally reflects a moving average of its input, but a network can have a more complicated response. For instance, it may have a very different response to pulsed signaling than to continuous signaling at any level. Phenomena of this sort are well known in neurobiology and physiology. For example, when animals respond to gondatropin-releasing hormone, the pulse frequency of stimulation is more important than the average level of the hormone (*7*). The dynamics of PDGF signaling in intact animals are not known, so it is not clear what aspects of the MAPK response are important in vivo. The biology of PDGF suggests that responses on different time scales are likely to be important. This growth factor, released by platelets at wound sites, induces expression of two sets of genes important for wound healing: One set is required for the slow process of cell proliferation, and the other for faster processes, such as recruitment of white blood cells and blood clotting (*8*).

A cell's history dependence raises important questions: How common is this dependence, how important is it in determining the properties of cells, and how long can it persist? Usually, history dependence is discovered by accident rather than by systematic searches, suggesting that we have underestimated its importance in biology. At a practical level, history dependence is an important and frightening reminder of how difficult it is to control biological experiments.

Molecular biology succeeded in reducing the functions of individual proteins to chemistry, and in discovering the general principles that govern the encoding, transmission, and expression of genetic information. But how do collections of different molecules form a signaling network, and how do these networks interact to allow cells to mount appropriate responses to an enormous number of different combinations of stimuli? How do networks arise and evolve? Evolution seems to have maintained only a tiny fraction of the networks that could exist. At what level do existing networks reflect the principles of evolutionary engineering, and how similar are these principles to those conceived by human engineers? How often will we depend on the details of a particular network to understand even its qualitative function? A long-term dialogue between theoretical and experimental analyses is our best hope for answering these questions.

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