crobial community is extremely sensitive to temperature, oxygen content, pH, and other environmental variables. It thrives in subsurface zones where the hot hydrothermal fluid mixes with entrained seawater (*13*). Changes in crustal fluid temperatures of only a few degrees, or a minor alteration of crustal permeability, can cause the prevailing microbial species to weaken and encourage new species to thrive and become dominant (*14*). In crustal fluids, the temperature elevation due to seismic activity can last for months or even years, implying that the species distribution in the oceanic crustal biosphere can shift substantially with every large earthquake or earthquake swarm.

The impact of earthquakes on biological communities associated with terrestrial hydrothermal systems is largely unexamined. However, most terrestrial microbial populations rely on photosynthetic energy, and the impact of changes in fluid temperature and flow rate may hence be greatly reduced. In contrast, ocean-crust hydrothermal fluid provides the primary thermal and chemical energy that fuels the microbial subsurface biosphere and most of the vent-specific macrofauna. Earthquake-induced changes in this circulation, which can occur over areas much wider than previously expected (*3*), will strongly affect these communities.

The dissimilarity in oceanic and terrestrial responses probably results from differences in crustal architecture. On land, where geological structure and fluid reservoir geometries are diverse, the primary response of hydrothermal systems appears to be immediate as temperature and volume changes coincide with the passage of seismic waves that compress or dilate the pore space of the reservoir (*6*). In contrast, hydrothermal fluid circulation in the porous ocean floor is a thermally driven process within an aquifer that is more uniform and can be continuous over thousands of kilometers (*15, 16*).

The response of marine systems to earthquakes can be complex, even oscillatory (*3*), with delay times of days or weeks before a reaction is observed, and a far wider spatial impact than expected from the amount of seismic energy involved. We do not yet understand the physical processes that link crustal strain, earthquakes, and hydrothermal circulation in the oceans, but we can predict with some certainty that seismic activity has a major impact on the biological communities that inhabit the seafloor.

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PERSPECTIVES: GENETICS

Wild by Nature

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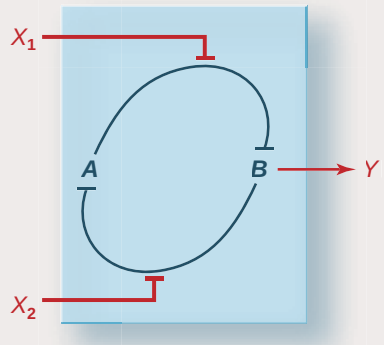
Much of modern biology has centered on dissecting the series of molecular events that we call signal transduction. Typically, the response to a singular event—such as the binding of a hormone, growth factor, neurotransmitter, or some other small chemical moiety to its receptor—is depicted in a wiring diagram of molecules that in temporal order become altered, some acting to propagate the signal, others acting to inhibit it. As these pathways ramify, interconnect, and incorporate components such as feedback inhibition, we begin to speak of regulatory networks. And as the diagrams grow, they reach their apotheosis as office wall decorations, ever-present reminders of the complexity of living things.

But do we really need complex diagrams to generate biological complexity? Can we achieve the imponderable by simpler means? What if we studied cellular responses not to a single signal, but to two signals, which may not necessarily even arrive at the same time? The study by Guet *et al*. (*1*) on page 1466 of this issue describes the creation of very simple pathways that respond to two inputs with a range of unpredictable behaviors.

Guet *et al*., using DNA fragments as cassettes, constructed a library of plasmids. Every plasmid contained three genes encoding transcription regulators (LacI, TetR, and lambda CI) of the bacterium *Escherichia coli*, and each gene was driven by one of five promoters. The promoters were chosen so that each was controlled by one of the three regulators. In this way, a total of 125 possible combinatorial "circuits" are contained within this plasmid library. The LacI and TetR gene products are responsive to the small molecule inducers isopropyl β-D-thiogalactopyranoside (IPTG) and anhydrotetracycline (aTc), respectively. The two inducers act as the two "inputs." The entire circuit reads out through green fluorescent protein (GFP)—the "output"—which is under the control of a promoter inhibited by lambda CI. Each combinatorial network resembles a binary logic circuit with two inputs and one output. Guet *et al*. introduce these plasmids into two different *E. coli* hosts and then monitor the response of the bacteria to different concentrations of IPTG and aTc. Plasmids that induce interesting behaviors in the bacterial hosts are rescued



A simple metastable biological circuit. The combinatorial genetic network depicted consists of two mutually inhibiting repressor genes, *A* and *B*, which are modulated by two small molecule inducers, X_1 and X_2. The gene products encode the state of the system, and the inducers act as inputs to the network. The state of *B* encodes the output (*Y*). This network has two stable states (output is "high" or "low"), but also a metastable state (output assumes an intermediate state between "high" and "low") that is achieved by withdrawing both inputs simultaneously. For these reasons, the network is also extremely sensitive to the relative order in which the inputs arrive and, thus, is unpredictable.

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and their structures solved. In each case tested, the host behaviors are re-evoked by again transforming *E. coli* with the rescued plasmid.

A diverse set of host behaviors is observed, many of which are surprising. For example, a full range of logical operations on the "inputs" are obtained, including NAND, NOR, and NOT IF, although simple inferential thinking predicts that only NOT IF would be found. As another example, GFP expression in hosts containing plasmid circuits of type 2 (fig. 4 from the Guet *et al*. report) would not be expected to be responsive to aTc, yet some are. Moreover, circuits of the same design but built of different components sometimes show very different behaviors. Thus, simple rules of logical inference have a limited ability to predict the output of the host in response to the two input signals.

Can biological systems be unpredictable? Consider the simple circuit depicted in the figure on the previous page. The circuit is composed of two repressor genes *A* and *B* that mutually inhibit each other. We will call this a "feedback dyad" (also termed a "bistable latch" in electrical circuit design). The output is denoted by *Y*. In the absence of any perturbation, the system has two stable states: one in which *A* is "high" and *B* is "low," and one in which *A* is "low" and *B* is "high." The system could be perturbed by two inhibitors, one nullifying the inhibitory influence of *A* on *B* and the other nullifying the similar inhibitory effect of *B* on *A*. These inputs are labeled X_1 and X_2. Consider the case when both X_1 and X_2 are initially high and then both inputs are lowered simultaneously, thus effectively initiating the interaction of *A* and *B*. To which of the two possible states will the system go? Either of the two states appears to be equally likely. However, if X_1 is lowered slightly earlier than X_2, then the state of the system is *A* "high" and *B* "low," and the output *Y* is "low." Conversely, if X_2 is lowered slightly earlier than X_1, then the output *Y* is "high."

Because the arrival time of these two "input" events cannot be determined exactly—especially in a system built of only a few molecules—the output *Y* cannot be predicted exactly, even when the values of the input are known. Moreover, if the input events occur simultaneously, then the entire system can enter a "metastable" state where *A*, *B*, and the output *Y* assume some intermediate value between "high" and "low," remaining there until small factors or even stochastic fluctuations resolve the entire system into one stable state or the other. Such a system can hardly be considered "predictable."

Many of the circuits constructed by the combinatorial method of Guet *et al*. incorporate a feedback dyad (see, for example, circuit types 5, 9, and 10 of their fig. 4) or another metastable element (for example, circuit type 13). Are these circuits likely to be found in nature? The answer is certain to be yes, because they are virtually the inevitable consequence of wiring pathways together. Moreover, such circuits are likely to be highly useful in a variety of ways. For example, a feedback dyad can function as a simple memory device, its state recording which of two present signals arrived first. Such "memory" may be extremely useful when, for example, a free-living microbe is sensing a complex chemical environment, or a cell exposed to a variety of soluble factors during embryogenesis is deciding its fate. The unpredictability of a metastable circuit may itself be a useful feature—in predator-prey evasion, for example, or when an organism scans its environment by random searches. Furthermore, such systems may be indeterminate at a single-cell level but deterministic in a population at large. For example, to maintain a healthy tissue, it may be advantageous to respond to signals such that some cells divide and some die, maintaining new cells without a net increase in population. Finally, circuits with metastable components may be readily modified by small genetic or biochemical perturbations that bias resolution into one state or another, and thus can be reprogrammed to perform a variety of logical operations.

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PERSPECTIVES: NEUROSCIENCE

Windows into the Human Brain

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The study of the developing human brain promises to move at an unprecedented rate, thanks largely to developments in magnetic resonance imaging (MRI). A decade ago, Kwong (*1*), Ogawa (*2*), and others showed that magnetic resonance is sensitive to blood oxygenation changes in the brain that may reflect changes in blood flow and neuronal activity. The insight that MRI can assess the activity of the human brain without the need for the exogenous radioactive tracers required by other methods began a new era in the study of human brain development and behavior. One key issue that could be investigated with MRI is how brain development and behavior change with growth and experience. Do children use the same cognitive and neural processes as adult humans, or do radically different neural processes underlie the superficially similar accomplishments of child and adult? This issue has been hotly debated by developmental psychologists (*3*). Schlaggar *et al*. (*4*), reporting on page 1476 of this issue, demonstrate how we may use functional MRI (fMRI) to begin to address this question. Specifically, they dissociate brain activity related to age from that related to behavioral performance in the prefrontal and extrastriate cortex of the adult and child brain during single word processing tasks.

The Schlaggar *et al.* report accompanies a surge in developmental fMRI studies (*5*–*9*). Differences between children and adults are typically reported in terms of the location (cerebral gyri, stereotaxic coordinates, Brodmann's areas), magnitude (percent change in MR signal), or volume (number of voxels) of brain activity (*5*). Schlaggar *et al.* examined brain activity (MR signal change) by including time as an independent variable. In this way, they could test whether the change in signal peaked at the same time and intensity for the adult and child groups. A central question is whether developmental differences in the pattern of brain activity are specific to age or to the accuracy and latency of the behavior. Typically, across tasks, children perform more poorly and variably than adults. These same concerns arise when comparing clinical and normal populations (*10*).

How can one tease apart age differences from behavioral performance differences in brain imaging studies? At least three approaches are described in the literature, including the one used by Schlaggar and colleagues. First, we can design tasks a priori that include parametric manipulations in the degree of difficulty, such that children and adults can be compared on the same or different levels of the task to equate behavioral performance. Memory or visual search tasks particularly lend themselves to this design (*11, 12*); many other tasks are not conducive to such manipulations. Second, with sufficient variability and range, we can correlate age and behavioral performance with magnitude or volume of activity, showing which brain regions are predominantly relat-

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