

In their new study, Bichot *et al.* (1) demonstrate that both sorts of processing occur in area V4 of the visual cortex during a single search task. They recorded neuronal responses from area V4 in the brain of alert macaque monkeys who were searching through displays of colored shapes similar to those shown in the figure. In the first experiment, at the start of a trial, the monkey would be shown a stimulus that cued him to the target color (for example, red) or shape (for example, star). To find this target, the monkey typically made a succession of eye movements that changed the position of the visual stimuli on the retina. During a trial, Bichot *et al.* recorded from a V4 neuron that was sensitive to stimulation in one specific region of the retina (its "receptive field"). Beyond being sensitive to one location in space, a V4 neuron might also have a preferred color and/or shape. Thus, as the monkey moved his eyes around, he presented different stimuli to the receptive field of the neuron under study. If the neuron preferred red stimuli, then, by definition, that neuron responded more vigorously whenever the monkey saw red. Of more interest, on trials when the target color was red, the neuron produced a still larger response. This happened even when the monkey was not about to make a quick eye movement to the target. The red item had not yet become the specific object of attention, but the response of the neuron still received a boost because red was the desired color. Moreover, neurons that preferred the target feature synchronized their activity, perhaps giving them a better chance of activating subsequent postsynaptic neurons.

So much for the parallel enhancement of all items on the basis of a feature like color. What about the selection of specific items? Imagine the situation in which the monkey is searching for a red item, and a red item lies in the receptive field of the studied neuron. We know from previous work (8) that covert attention shifts to an object before it is fixated by the eyes. So Bichot *et al.* went back over their data and sorted the responses into two categories: responses from just before the monkey made an eye movement toward the red item and responses from just before he made an eye movement somewhere else. They found that the neuron responded more strongly just before the eyes fixated on the red item. Thus, it seems that the act of attentional selection that precedes serial fixation also enhanced the response of the neuron. What we have here is attractive evidence at the level of single cells indicating that parallel feature processes are guiding serial selection of plausible targets for further scrutiny.

Understanding how monkeys (and presumably other primates, such as ourselves)

perform search tasks is of more than academic interest. Visual search is a task each of us performs a thousand times a day, from searching for a coffee cup to looking for a face in a crowd. However, some searches are more important than others. As a society, we have created many artificial but critically important search tasks, such as airport baggage screening and routine mammography. Many of these tasks are complicated and currently performed imperfectly. We eagerly await development of new ways to improve human performance on such tasks or the invention of machines that could take over or assist with them. Understanding how biological systems do so well at performing a range of search tasks

should help us to improve the outcome of those artificial search tasks on which we, quite literally, stake our lives.

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CELL BIOLOGY

A Fishing Buddy for Hypothesis Generators

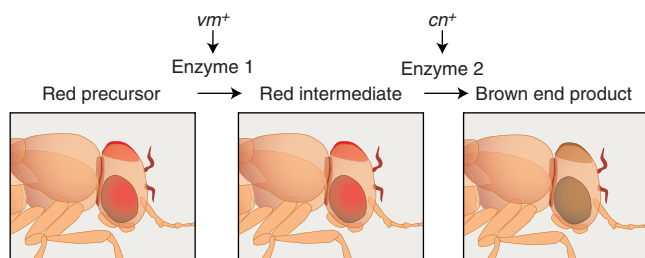
Roger Brent and Larry Lok

In the last half of the 20th century, the dominant experimental modality in biology was hypothesis-directed research. Of course, biology has a proud tradition of important insights arising from undirected poking around and following hunches. However, until genomic biology made undirected fishing for information more respectable (1), the most common response to requests for money for such projects was dismissal with the term "fishing expedition." The study by Sachs *et al.* (2) on page 523 of this issue suggests it may be time to reexamine this prejudice.

In their study, Sachs, Nolan, Lauffenburger, and their co-workers outline what may be a powerful new way to fish. They combine measurements of different signal transduction proteins in large numbers of individual human CD4⁺ T lymphocytes and computational frameworks called Bayesian networks with experimental perturbations that are close to hypothesis-free. These investigators not only regenerated known causal relationships among the signaling proteins but also predicted new connections that they verified by targeted testing. For example, they predicted interpathway cross-talk between the Erk1 and Akt kinases. Their approach suggests a way that "fishing expeditions" and investigator reasoning might supplement one another, generating testable assertions about chains of causation and action in biological systems.

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Consider how biologists identify candidate gene products and suggest how these might act in chains of cause and effect. With "genomic" methods, two grounds for identification correspond to logical fallacies: "guilt by association" (for example, these two proteins touch one another, and might cooperate in the same cellular process) and "post hoc ergo propter hoc" (for example, this gene regulator is expressed before this transcript appears and therefore might regulate production of that transcript) (3). In contemporary biology, such observations are supplemented by additional information including DNA sequences, which can suggest direction of action from the biochemical function of the encoded proteins and similarities to known pathways in other organisms. Such inferences are complemented by experimental methods that more rigorously establish flows of action and consequence. These approaches go back to the work of Ephrussi and Beadle in the 1930s. These experimenters isolated fruit flies with mutations in genes for eye color. They then showed that an eye disk containing the *cinnabar* (*cn*) gene product but lacking the *vermillion* (*vm*) gene product produced a wild-type eye when transplanted into flies that lacked *cinnabar* but contained *vermillion*; they also showed that the converse was not true (4, 5) (see the top figure). This phenomenon, called "epistasis," established both that *cn* and *vm* act in an eye-color "pathway" and that the wild-type *vm* gene product must act first in order for the wild-type *cn* gene product to exert its effect.



Order of action in eye color. Cause and effect relationships established by epistasis (4, 5). The vm^+ gene product from the recipient acts on red precursor pigment to produce a red intermediate, and the cn^+ gene product from the eye disk acts on the intermediate to produce wild-type brown pigment. Flies with mutations in the *vermillion* (vm^-) and *cinnabar* (cn^-) genes have red eyes. An eye disk from a $vm^- cn^+$ larva transplanted into a $vm^+ cn^-$ host results in wild-type brown eyes, but the converse transplantation results in red eyes, due to the enzyme pathway depicted. An eye disk from a $vm^- cn^+$ donor receives the vm^+ product from a $vm^+ cn^-$ recipient. The vm^+ product from the recipient acts on red precursor pigment to produce substance A, and the cn^+ product from the disk acts on A to produce B, the wild-type pigment.

Sachs *et al.* (2) provide real progress in developing descendant methods to assign gene products to pathways and to learn the order of their actions. This progress rests on their ability to measure the amounts of various proteins (in this case signaling proteins) in large numbers of individual cells. Nolan and co-workers have spent years perfecting techniques for targeting proteins in (fixed) lymphocytes with fluorescent antibodies and for measuring the amounts of proteins by flow cytometry (6). Their work culminated last year in a beautiful paper (7) that quantified different protein species involved in signal transmission in lymphocytes from patients with acute myeloid leukemia. These investigators stimulated the leukemic cells by various means and then classified the leukemias according to the differences in their complements of signal transduction proteins. They proposed that this method of classification could eventually guide mechanism-based treatment.

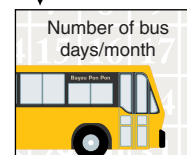
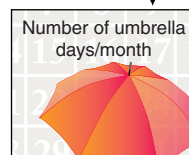
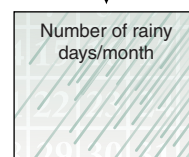
A second element necessary for this progress is the development of Bayesian statistical methods to formalize common concepts of causation, work begun by Judea Pearl (8, 9). Bayesian networks compute and display relationships of statistical dependency between system variables. Variables are represented by nodes in a graph. Arrows between nodes represent some dependencies between the corresponding variables explicitly; other dependencies are left implicit. If arrows point from “cause” nodes to “effect” nodes, the network is called a causal Bayesian network. The form of such graphical models is familiar to geneticists, but the difference is that Bayesian network methods automatically generate numerous alternative models from experimental data and assign probabilities to each different network structure and each different arrow. The methods initiated by Pearl take into account measurements after experimental per-

turbations of the process to obtain causal Bayesian networks. In these networks, the adage “correlation does not imply causation” is still true, but insights from perturbations provide additional information that allows, *inter alia*, determination of which correlations arise from causal relationships, and the direction each arrow of causality points. The third element is the ability to carry out interventions that initiate the process and perturb its function. These three capabilities enabled Sachs *et al.* to perturb human CD4⁺ T lymphocytes in different ways in order to collect data from thousands of cells subjected to each trial, and to infer casual relationships among the measured variables.

To illustrate how the three elements work together, imagine a city dominated by a secret police agency, “the Instrumentality,” which is distinguished by a combination of power and myopia. The Instrumentality conducts grand operations, and also targeted interventions that change the value of almost any variable. It can perturb many more variables than it can measure, and its measurements are often imprecise.

The Instrumentality has become interested in the process by which an inhabitant, Joe, goes to work (see the bottom figure). Joe is clinically depressed, but, after sunrise each morning, if he is not too depressed, he gets out of bed. When the weather is good he walks to work. When it is raining, he uses his umbrella as he walks to the bus stop, and then rides the bus. By the end of the month, riding the bus depletes his bank balance. The Instrumentality can measure values for all of these variable aspects of Joe’s life, and, because it can make the sun come up in the morning, it can perform

turbations of the process to obtain causal Bayesian networks. In these networks, the adage “correlation does not imply causation” is still true, but insights from perturbations provide additional information that allows, *inter alia*, determination of which correlations arise from causal relationships, and the direction each arrow of causality points. The third element is the ability to carry out interventions that initiate the process and perturb its function. These three capabilities enabled Sachs *et al.* to perturb human CD4⁺ T lymphocytes in different ways in order to collect data from thousands of cells subjected to each trial, and to infer casual relationships among the measured variables.



How Joe gets to work. Causal connections among variables in the process. By measuring the values of these variables, even imperfectly, for most months of the year, an outside observer can establish correlations among them. By using Bayesian network methods together with “non-hypothesis-directed” perturbations to the values of these variables, the observer can identify causal connections and assign directionality to them. Given enough trials, the methods can operate efficiently on “noisy” data. In most biological systems, application of these methods is limited by the kinds of variables researchers can quantify and the number of times the researchers can repeat each set of measurements.

as many trials as it wants. Note that the values of some variables are conventionally correlated even though they are not causally connected. For example, the number of days Joe carries an umbrella to work each month correlates positively with the number of days he rides the bus, and negatively with his bank account at the end of the month, although taking the umbrella doesn’t cause him to ride the bus. Even without perturbing the system, the Instrumentality could posit from these observations that rain days caused umbrella days and bus days, or that rain days, umbrella days, and bus days all had a common cause.

The Instrumentality learns more by intervening in downstream steps of the process. For example, it can decrease the number of umbrella days to zero by hiding Joe’s umbrella, or can increase the number to 30 by gluing the umbrella to his hand. Whatever the number of umbrella days, Joe will continue to take the bus on rainy days and walk on sunny ones; so the number of bus days will be unaffected by the number of umbrella days. The Instrumentality can let the air out of the tires of the city bus fleet, and deposit and withdraw money from Joe’s bank account. These interventions have no effect on the number of umbrella days.

But when the Instrumentality increases the number of rain days, it observes an increase in the number of umbrella days and bus days, and a decrease in his end-of-month bank balance. By contrast, no manipulation of umbrella days or

bus days affects the number of rain days. Thus, by taking measurements after interventions, the Instrumentality reveals relationships between the observables of Joe’s life that correspond to the chain of causation in our natural-language narrative, and, for some variables, which way the arrows point: in this case, from rain days to umbrella days and not the other way around.

Four more points about this example merit mention. First, in many cases, causal independence can be established by observation alone. For example, although the Instrumentality vigilantly tracks variables related to the purchase

of water skis, these show independence from Joe-related variables under all conditions, and drop out of consideration. Second, the Bayesian network approach allows the observer to start with a hypothesis about the structure of a network, and to perform targeted (as opposed to blundering) experiments to test particular network structures. Third, the particular approach used by Sachs *et al.* fails to identify cases in which downstream events feed back into upstream events (for example, when Joe's bank balance hits zero, he cannot take the bus). However, coupling measurements of variables at different time intervals with "dynamic Bayesian networks" may allow identification of feedback relationships. Finally, existing methods cannot identify causal connections between variables the Instrumentality does not know exist. In this example, the probability that Joe gets out of bed is influenced by whether he has filled a prescription for an antidepressant drug at the nearby drugstore the month before. Thus, Joe's antidepressant purchases seem to be relevant upstream "causal" input for the number of bus days. But if the Instrumentality has not yet learned about

antidepressants and drugstores, it will not be able to discover the additional causal link.

When we return from the Instrumentality to our own world, we find that biologists are very good at making targeted perturbations. In genetically tractable organisms, performing these perturbations often depends on making the right mutant. In cell lines and in less tractable organisms, perturbations might be better effected by RNA interference, "protein genetic," or (for people) pharmacological approaches. We also see that for Bayesian network methods to realize their promise, researchers will need to get much better at measuring relevant variables. For intracellular events, variables include but are not limited to, numbers of regulatory molecules, modified molecules, and specific molecular complexes, and the percent occupation of regulatory sites upstream of genes. To be useful, measurement methods will need to operate on individual cells, or, at the very least, to allow large enough numbers of trials to yield causal assertions reliable enough to merit further experimental testing.

The Sachs *et al.* work is important because it suggests how researchers might develop a

package of capabilities to enable systematic fishing expeditions. Such a package would generate testable ideas based on (relatively) error-prone high-throughput measurements made after (relatively) uninformed experimental treatments and could help experimenters refine those ideas after quick tests. Such capabilities seem well suited to one of the grand challenges of 21st-century biology: the grouping, ordering into pathways, and description of function for the numerous weakly acting and incompletely penetrant genes that quantitatively modify important phenotypes in humans and other organisms.

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PLANT SCIENCES

Recognition at a Distance

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A key step in the evolution of eukaryotic immune systems was the ability to discriminate between self and nonself. Evidence suggests that animals and plants independently evolved dedicated and highly variable receptor families for recognition of nonself structures. The outcome of interactions between plants and the pathogenic microbes that invade them largely relies on a repertoire of receptors that serve as a radar system for detecting pathogen-derived nonself molecules. The function and specificity of these receptors were originally defined by genetic studies. Such studies revealed that for plants to recognize their intruders and to mount an effective resistance response, there needed to be a match between a strain-specific pathogen effector and its corresponding plant host resistance (*R*) gene product (*I*). Detection of a pathogen effector by a plant *R* receptor frequently leads to rapid death of plant host cells at sites of attempted invasion as part of the immune response. Most known *R* genes

encode intracellular receptors containing a nucleotide binding site and leucine-rich repeats (LRRs) or membrane-bound surface receptors containing extracellular LRRs (2). Two new studies—by Coaker *et al.* (3) on page 548 of this issue and by Rooney *et al.* (4) in this week's *Science Express*—describe encounters between pathogen-secreted effector molecules and their host targets in *Arabidopsis* and the tomato (*Lycopersicon*), respectively. Although this interorganismal molecular liaison has entirely different consequences for the effector target proteins, in both cases, their manipulation holds the key to a better understanding of how plant immune receptors recognize nonself.

Many *Arabidopsis* ecotypes contain the plasma membrane-associated intracellular *R* protein RPS2 (see the figure). This protein specifically detects and mounts an immune response to strains of the bacterial pathogen *Pseudomonas syringae*, which produce the AvrRpt2 effector protein. AvrRpt2 is delivered into the plant cytosol by a specialized bacterial secretion system and is cleaved near its amino terminus. The carboxyl-terminal cleavage product is sufficient to trigger the RPS2-dependent immune response and is predicted to adopt

a secondary structure typical of a cysteine protease (5). Although attempts to detect direct interactions between RPS2 and AvrRpt2 have been unsuccessful, both proteins physically associate with the *Arabidopsis* protein RIN4. A complex between RPS2 and RIN4 is constitutively present in healthy (unchallenged) plants, but RIN4 disappears when AvrRpt2 is delivered into plant cells. Importantly, mutations in any of three amino acid residues in the carboxyl terminus of AvrRpt2 (predicted to be essential for catalytic activity of the putative *Pseudomonas* protease) disrupts the processing of AvrRpt2, the RPS2-dependent immune response, as well as elimination of RIN4 (5–7). This finding prompted the proposal that RPS2 might recognize the result of AvrRpt2's proteolytic activity, that is, the removal of RIN4.

Coaker *et al.* started from the puzzling observation that processing of AvrRpt2 could be detected in all eukaryotic but not prokaryotic extracts tested, including those from *P. syringae*. This observation implies the existence of a eukaryotic cofactor required for AvrRpt2 processing. Using a combined biochemical and genetic approach, the authors identified this cofactor as a single-domain cyclophilin, a folding catalyst that facilitates cis/trans isomerization of prolyl bonds. Cyclophilin activity is required for proper AvrRpt2 self-cleavage, and this in turn may be a critical step for the correct subcellular localization of

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