Exploring the Systems of Life

No longer content to inventory cells' molecular parts, biologists are teaming up with physicists and engineers to study how these parts work together

A rule of thumb among drugmakers is that the more tightly a compound binds to its molecular target, the more potent it will be. But not always, it turns out. Take cytokines, natural protein messengers that bind to receptors on cells and cause them to proliferate during wound healing or an immune response. A cytokine molecule follows a complex life history before and after it binds to its receptor. It shuttles in and out of cells, risking destruction by proteases, and eventually finds its way into a recycling bin once its work is done. These steps interact, adding to the complexity. When proteases destroy a cytokine molecule, for example, they can also wipe out its receptor, in a feedback that further reduces the compound's effectiveness.

By modeling these and other interactions on a computer, Douglas Lauffenburger and his colleagues at the Massachusetts Institute of Technology have found that in many cases the best way for genetic engineers to boost the potency of a cytokine drug is not by remodeling it to bind more tightly to its receptor but by altering other steps in the chain. Tweaking the structure to help it avoid destruction within the cell, for example, increases its chances of being recycled. "You would think that the stronger the binding, the more potent it would be," says Lauffenburger. "But that's often not the case."

> **"The convergence of chemistry, physics, biology, and engineering is upon us."**
>
> **—Lucy Shapiro**

As he and his colleagues have realized, understanding how parts of a biological system—genes or molecules—interact is just as important as understanding the parts themselves. It's a realization that's beginning to spread. Leading research universities around the United States have begun shelling out tens of millions of dollars to set up new interdisciplinary institutes and departments that will bring together specialists from physics, chemistry, engineering, computer science, mathematics, and biology to document how all the different cellular players work together in complex tasks such as determining when a cell divides and how gene expression is regulated. Says Lucy Shapiro, a developmental biologist at Stanford University: "The convergence of chemistry, physics, biology, and engineering is upon us."

The new centers will take a variety of approaches to exploring the complex systems of life. A proposed center at Stanford, for example, is likely to focus on biophysics, while one at Princeton will lean toward probing networks of genes and proteins. Drug companies, too, such as the Palo Alto, California–based startup Entelos, are turning to computers in the hope that "in silico" biology will lead to improved therapeutics. All these efforts are a response to the growing sense that gene sequencing and other techniques will soon have isolated all the cell's individual parts and

Building Working Cells 'in Silico'

Cells provide living proof of that old saw about the whole being greater than the sum of its parts. "Even if you construct a complete list of all the processes known to occur within a cell, that won't tell you how it works," says Masaru Tomita, a professor of bioinformatics at Keio University in Fujisawa, near Tokyo. But Tomita, who is a computer scientist as well as a biologist, has a scheme for exploring the effects that only emerge when those many processes interact: a simulation program that can reproduce, in simplified form, a cell's biochemical symphony.

His group's E-CELL simulation software will go on the Web for public "beta" testing this June (www.e-cell.org). Other computer models of the cell are being developed, but they often try to reproduce individual cellular processes in detail. E-CELL, in contrast, is designed to paint a broad-brush picture of the cell as a whole. Such efforts "are a next logical step" now that genome sequencing is giving biologists the complete parts lists for living things, says Peter D. Karp, a bioinformaticist at Pangea Systems, a bioinformatics software company in Menlo Park, California.

E-CELL is actually a model-building kit: a set of software tools that allows a user to specify a cell's genes, proteins, and other molecules, describe their individual interactions, and then compute how they work together as a system. It should ultimately allow investigators to conduct experiments "in silico," offering a cheap, fast way to screen drug candidates, study the effects of mutations or toxins, or simply probe the networks that govern cell behavior.

Written to run under the UNIX or Linux operating systems, the software relies on the user to input a cell's molecules, their locations and estimated concentrations within the cell, and the reaction rules that govern them. E-CELL then computes how the abundance of each substance at a particular location changes at each time increment. With a single mouse click, the user can knock out particular genes or groups of related genes, expose the cell to a foreign substance or deprive it of a nutrient, and then run the simulation again. Graphical interfaces allow the user to monitor the cell's changing chemistry.

Tomita's group has used early versions of E-CELL to construct a hypothetical cell with 127 genes, which they figured was a minimal set for a self-sustaining cell in their system. Most of the genes were based on those of *Mycoplasma genitalium*, a microbe that has the smallest known gene set of any self-replicating organism. But the genes for some vital cellular processes still have not been identified in the mycoplasma, so the group added genes from other organisms. The virtual cell "lives," maintaining a simple, stable metabolism: It takes up glucose from the virtual culture medium, generates the enzymes and proteins to sustain internal cell processes, and exports the waste product lactate.

This bare-bones cell has already delivered one surprise. As expected, starving it of glucose causes a drop in levels of adenosine triphosphate (ATP), a key compound that provides the energy for many intracellular processes. But unexpectedly, before ATP levels drop they briefly rise. The reason, Tomita suspects, is that the early part of the ATP-producing pathway itself consumes ATP. Cutting the supply of glucose shuts down the early stages of the pathway, stopping ATP consumption there even while ATP continues to be produced from intermediary metabolites further down the pathway. Tomita thinks the effect may eventually be confirmed in living cells.

More surprises could be forthcoming when E-CELL is eventu-

spelled out their isolated functions. Now, it's time to move beyond reductionism.

"We have generated an enormous mass of information on the molecular events that occur in cells," says Marvin Cassman, director of the National Institute of General Medical Sciences (NIGMS) in Bethesda, Maryland. "Now we need to know how all these things are integrated." John Doyle, an electrical engineer at the California Institute of Technology in Pasadena who is turning his attention toward biology, puts it this way. "Biology has spent decades trying to be like physics," trying to understand complicated systems by understanding each part at its most basic level. "Now they're interested in putting it all back together."

Doing so, says Shapiro, will take "physicists, engineers, and biologists at lab benches next to one another working on the same problem." Foremost among these problems, say Shapiro and others, will be understanding the complex chemical networks that govern cell functioning. Genome analysis, for example, has already isolated hundreds of genes that code for transcription factors, proteins that help regulate the expression of other genes. "The expression of individual genes is not being regulated by one, two, or five proteins but by dozens," says Shirley Tilghman, a molecular biologist at Princeton University. Some regulate specific genes; others work more broadly. Some sit on DNA all the time, while others bind temporarily. "The complexity is becoming mind numbing," says Tilghman.

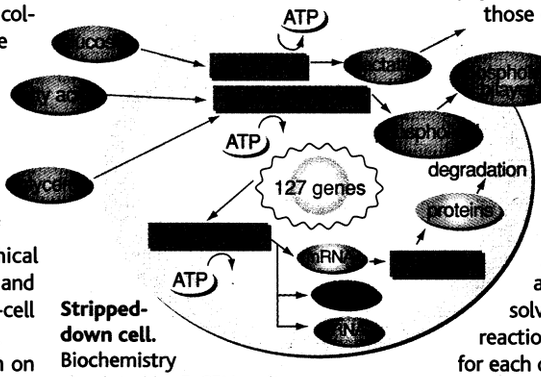
Simply determining the individual role of each protein only gets you so far. "Even if you have all the chemistry, it's hard to understand how the cell functions," says Adam Arkin, a physical chemist at Lawrence Berkeley National Laboratory (LBNL) in California. That's because interactions between different molecules can have a feedback effect that increases or decreases the expression of other compounds. "When we get to a certain network complexity, we completely fail to understand how it works," says Arkin.

BUILDING BOOM IN MULTIDISCIPLINARY CENTERS

	Institution	Plan	Cost (in $ millions)
PROPOSED	Stanford	BioX (biophysics research)	??
PROPOSED	UC Berkeley/LBNL	Physical Biosciences	>100
PROPOSED	Univ. of Washington	Institute for Quantitative Systems Biology	??
PROPOSED	Harvard	Center for Genomics and Proteomics	~50
APPROVED	Princeton	Inst. for Genomic Analysis	70
APPROVED	Caltech	Biological Sciences Initiative	100
APPROVED	Univ. of Chicago	Biophysics Institute	110
APPROVED	Univ. Pennsylvania	Inst. for Medicine & Engineering	20
APPROVED	Stowers Institute for Medical Research	Genomics and Biotech Center	125
APPROVED	Johns Hopkins	Whitaker Biomed. Eng. Institute	34
APPROVED	Georgia Tech/ Emory University	Biotechnology Initiative	~75

Such complexity is well known in fields such as engineering. Take the latest Pentium chip in your desktop computer. The chip contains millions of individual elements, such as transistors, connecting wires, and gate arrays. The behavior of each element is understood to many decimal places. But for the engineers designing the chip, predicting how all the different elements would interact was a trickier proposition. Chip designers have to rely on sophisticated modeling programs to simulate how different collections of the elements interact and predict their collective behavior, so that they can iron out bugs in advance.

Now researchers are hoping to bring similar types of analyses to bear on understanding whole cells of real organisms. Tomita admits that because building model cells with E-CELL depends on understanding the functions of large numbers of genes, the software is not likely to prove really useful for molecular biologists for some time. But he and his colleagues designed the program so that it should easily scale up to simulating the thousands of genes in a real cell. "Tomita and his group have done a fantastic job of engineering a 'graphical cockpit' for initializing and monitoring a whole-cell simulation," says Karp.



Stripped-down cell. Biochemistry simulated by E-CELL software.

For greater realism on a smaller scale, users can turn to a different model-building kit: the Virtual Cell developed by physiologist Leslie Loew and computer scientist James Schaff of the University of Connecticut Health Center in Farmington. Rather than downloading software to run on their own computer, Virtual Cell users will simply run their simulation on Loew's host computer via the Internet. More important, rather than simulating an entire cell at once, as a biochemical system, Virtual Cell will eventually enable cell biologists to study how a cell's shape, volume, and other physical features affect individual biochemical processes.

Loew's team builds its Virtual Cell models using precise measurements of how molecules diffuse and react within living cells, which they make by labeling key molecules and observing them with a video microscope. The result is a computerized cell with physical properties resembling those of real cells—a framework in which users can unleash specific biochemical reactions. For example, a researcher can add a certain amount of calcium—a key intracellular messenger—and then sit back and let the Virtual Cell solve equations describing reaction and diffusion rates for each of the molecular participants affected by calcium. Then the program generates a movie of the process. "The simulations are comfortable for the biologists to use because they are based on real image data," Loew explains.

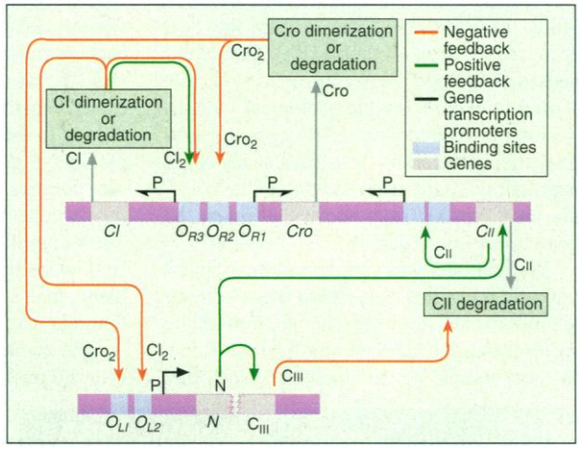
In the case of calcium, the simulation not only looked much like the calcium waves measured in actual cells—indicating that the simulation was realistic—but it also predicted the dynamics of an intermediary molecule called IP3, which cannot be monitored inside the cell itself. (Demonstrations of Virtual Cell can be accessed at www.nrcam.uchc.edu)

"These two approaches can complement each other very well," Tomita says. And both are attracting growing interest from other biologists. Tomita says that when he first starting describing his plans for E-CELL, "I was dismissed as a naïve computer scientist." Now he gets e-mail requests for information on his simulation software nearly every day. Loew, too, has found that "interest has begun to mushroom." He adds, "[Cell biologists] are getting to the point that they are realizing that without computers we are never going to be able to organize all this information."

–DENNIS NORMILE

With reporting by Elizabeth Pennisi.

ing biological networks. At LBNL, for example, Arkin and his colleagues have begun using computer models together with experiments to track how viruses that infect bacteria "decide" whether to replicate inside their host or lie dormant, waiting for a better opportunity. Years of painstaking experimental measurements by numerous teams have shown that the five genes that push the virus either to replicate or lie dormant are controlled by six other genes: four promoters that turn on gene transcription, and two terminators that either partly or entirely shut it off. Embedded in this gene play are numerous positive and negative feedback loops: When one gene called *CI* that promotes the dormancy path is expressed, for example, it feeds back to amplify its own expression while diminishing the output of *Cro*, a gene that pushes immediate viral replication and release. Outside factors, such as the availability of nutrients and the presence of competing viruses, also act as inputs controlling which promoters are turned on and off.



Complex system. A web of interactions among a virus's genes and promoters determines whether it will lie dormant or replicate.

In most cases that feedback leads to predictable results: If food is present and competition is absent, the virus proliferates. But by modeling the entire network of interactions on the computer, the LBNL researchers found that the feedback control is inherently "noisy," so not all the viruses make the same decision under identical conditions—an adaptation that ensures some viruses will survive should the other path prove fatal. Understanding how to control such genetic switches could ultimately lead to new ways to control infections, says Arkin.

Still, even with these and other initial modeling efforts (see sidebars on pp. 80 and 82), many researchers argue that biological models have a long way to go before proving themselves. "[Models] haven't had a lot of respect among biologists," says Marc Kirschner, a cell biologist at Harvard Medical School in Boston. "They don't have enough of the biological character built in," and thus often don't reflect the true complexities of real biological systems. Arkin, Lauffenburger, and others say, however, that the new research in this area will improve the sophistication of the models by identifying common circuit motifs used in biological networks and incorporating more complex and realistic feedback mechanisms. Over time, the models will also benefit from better inputs, such as the amount of each protein present in real cells and their reaction and diffusion rates.

Other challenges loom. Among the biggest concerns, say researchers and administrators, are differences in research cultures. In physics, for example, postdocs are often treated like junior faculty, whereas in biology they typically have far less autonomy. Ironing out such differences is "one of the biggest problems we face," says Shapiro.

Promotions and tenure decisions could also prove to be sticking points. "People who work at the boundaries between disciplines are at a real disadvantage," says Chris Overton, who directs Princeton's bioinformatics center. "Who evaluates you for tenure and the quality of your work?" he asks rhetorically. Often, he says, people in one discipline or another fail to appreciate the work's full scope. What's more, discipline-bound funding departments within agencies such as the National Institutes of Health (NIH) or the National Science Foundation can be reluctant to fund interdisciplinary work seen as lying largely outside their area, and grant review panels made up of researchers in a single discipline may not fully understand an interdisciplinary project. Whether the money will be there to support new interdisciplinary programs "is a question we are all worried about," says Carlos Bustamante, a biophysicist at the University of California, Berkeley.

But NIGMS's Cassman says that his agency and others are creating niches for interdisciplinary science. Last year, for example, NIH announced a new bioengineering initiative to fund multidisciplinary research (*Science*, 5 June 1998, p. 1516). And interdisciplinary review panels, he says, are likely to follow. "When we've been able to promote an area of science, it is because it is ready," says Cassman. "From everything I hear about [the systems approach to biology], I think it is." **–ROBERT F. SERVICE**

SOURCE: *TRENDS IN GENETICS* 5 (2), 67 (1999)

NEWS

Life After Chaos

After years of hunting for chaos in the wild, ecologists have come up mostly empty-handed. But the same equations that failed to find chaos are turning up stunning insights into how environmental forces and internal dynamics make populations rise and fall

The complexity of nature may be a beautiful thing, but it came pretty close to crushing Maria Milicich's spirit. On a typical morning 10 years ago she would take her motorboat out to the Great Barrier Reef, where she was studying the ecology of damselfishes. These brightly colored aquarium fish lay their eggs in nests at the reef's bottom. Each month the full moon triggers the larvae to hatch and emerge; they leave the reef and 19 days later return as mature larvae. Milicich wanted to figure out what determined how many larvae reached maturity, so she set up 2-meter-tall traps floating from buoys, each rigged with a light to attract the fish.

You might expect that Milicich would have found a regular pulse of new adults every month. Instead, she logged a wild gyration. When she checked her traps during some pulses, she found only a few fish, but during other months she would find thousands. On one visit to the reef she discovered that the trap had been dragged to the sea floor by a load of 28,000 fish.

Milicich searched for a cause for the fluctuations, seeking a link between the number of new adults and measurements she had made at the reef—everything from rainfall to the brightness of the moon. She tried hundreds of variables but came up empty-handed. Of course, many marine biologists had failed before her and simply labeled the supply of mature larvae as nothing more than random. That wasn't much consolation to Milicich. "To say that I felt depressed is an understatement," says Milicich, who now works as an ecological consultant to the Hong Kong government and private companies. "Something was clearly wrong."

Then Milicich had an epiphany. In 1990, she stumbled onto a paper in *Nature* that had invoked a strange kind of math to describe the abundance of phytoplankton off the coast of California. To decode her damselfish, Milicich had been trying to use linear equations—which produce results that