Controlling Life's Gateway

Opening and closing cell membranes on demand

By RICHARD LIPKIN

It is a spongy, porous sheet that marks life's boundary. A gatekeeper, it regulates the two-way molecular traffic of every living cell. Up close, this web of proteins and fatty acids, a lipid bilayer, resembles a molecular thicket.

A cell membrane: It serves as a barrier between a cell's internal machinery and the external fluids on which it depends.

The membrane chooses what will enter and what will exit. Selectively, it decides whether a cell will admit a molecular messenger, send out a signal, or drink heartily from its enveloping saline sea.

What if one could control a cell's gateway? What if, by tampering with its machinery of selection, one could open and close a cell's floodgates? What if one could choose which molecular visitors may pass through a cell's portals and enter its innards?

Such an ability might well deepen our understanding of a cell membrane's function and make possible new approaches to cancer therapy, drug delivery, biosensors — even metal-ion detectors.

In a laboratory at the Worcester Foundation for Experimental Biology, cloistered in the green hills of Shrewsbury, Mass., molecular biologist Hagan Bayley puzzles over this very problem of membrane control.

"We began by asking a simple question in basic science: How does a water-soluble protein secreted by the bacterium *Staphylococcus aureus* penetrate and assemble itself in the lipid bilayer of a cell?" he says. "That process, which happens spontaneously through self-assembly, is interesting in itself. But it also has great relevance to other biological processes, such as how membrane proteins are synthesized, how viruses fuse with a cell, and how enzymes are secreted."

What Bayley and his colleagues found was a host of interesting questions stemming from the basic science. They began to wonder whether this bacterial protein could be re-engineered to form pores in cell membranes. And might these pores be useful for making a sensor or drug-delivery system?

This pursuit has taken Bayley and his colleagues on a roundabout journey into the molecular machinery of cell membranes. They have worked out a way to open and close a membrane's pores on demand, using what he calls "molecular triggers and switches."

The researchers have isolated a protein, called alpha-hemolysin, that can form pores in a wide variety of cell membranes. Composed of 293 amino acids, the protein digs a hole into a membrane bilayer and opens up a stable, hexagonal portal measuring 1 to 2 nanometers across. They chose this protein because, as a toxin, it is programmed to seek out and penetrate the membranes of other cells. In fact, that's how it does its damage.

The protein's main active site is a large loop, rich in the amino acid glycine, in the molecule's center. The loop connects two large portions — the N and C terminal halves — of the polypeptide chain. Once the protein has bound itself to a target membrane, the loop becomes submerged in the lipid bilayer.

Ordinarily, it will burrow in and open up a hole. And yet the protein can be made inactive, spawning a pore only when triggered.

Bayley believes that the loop actually causes a membrane channel to emerge. Most likely, the loop goes on to become part of the pore's permanent lining. Consequently, the researchers' primary discoveries have come by way of tinkering with the protein's main loop. Using genetic engineering, the scientists have snipped out portions of that loop and replaced them with other amino acid sequences, some of which have made the pores sensitive to controlled openings and closings.

For instance, through a technique called site-directed mutagenesis, Bayley has been able to cut "nicks" into the loop and stitch in strings of the amino acids cysteine and histidine. "We've literally made hundreds of mutants of this protein, altering more than 80 of the 293 amino acid sites," he says. "About 10 percent of the mutations, particularly in the central loop, affect the activity of the protein and the way it forms a pore."

Indeed, the researchers found they could create nicks, gaps, and overlaps in the central loop that affect the way the molecule binds to other molecules. Mutant proteins with drastically altered loop regions would bind to a membrane, yet only those nicked near the midpoint of the loop could form pores efficiently. Thus, the team realized that by manipulating the loop structure, it could control the protein's ability to form, open, and close a pore.

If the scientists hooked into the loop a dangling, "overlapping" amino acid string, for example, the protein would become inactive and form no pores. When subjected to a specific enzyme — a protease that snips off the overlapping amino acid tab — the protein would reactivate and open up a channel in the membrane.

In fact, by tweaking the loop with subtle "point mutations," or single changes in the amino acid sequence, they found they could train the protein to respond only to specific proteases, to certain physical and chemical signals, and even to stimulation by light.

In one mutation, Bayley's team spliced in a string of five histidine molecules near the loop's center. The protein remained active, opening pores on command. Yet, when exposed to very small concentrations of certain metal ions, such as cobalt, nickel, copper, and zinc, pore formation stopped. Apparently, the metal ions had the ability to block the tiny pores. When exposed to chelating agents, which pull off the metal ions, the pores reopen.

In effect, this sequence of five histidines serves as a switch that can be turned on and off to open or close membrane channels.

"This is first-class work," says M. Reza Ghadiri, a molecular biologist at the Scripps Research Institute in La Jolla, Calif. "It's an elegant use of the tools of molecular biology to learn how a natural system works and to modify it. These systems are very difficult to design. Anything that goes into a membrane belongs to a different world."

"This is a whole package of interesting research," Ghadiri adds. Bayley is "working at the interface between chemistry, biology, and materials science, taking advantage of the resources of each discipline. This is the future of molecular biology."

Frances H. Arnold, a chemical engineer at the California Institute of Technology in Pasadena, concurs. "When it comes to making complex structures, nature certainly has one up on us," she says. "But for something as complex as hemolysin, I

think Bayley's come up with a fabulous experimental system. Now he can test specific ideas."

To create other types of reversible membrane switches, Bayley's group is currently exploring how to splice photosensitive sequences into the loop. In theory, cells bathed in the pore-forming protein, then exposed to a specific wavelength of light might open themselves up just long enough to admit a drug before shutting back down.

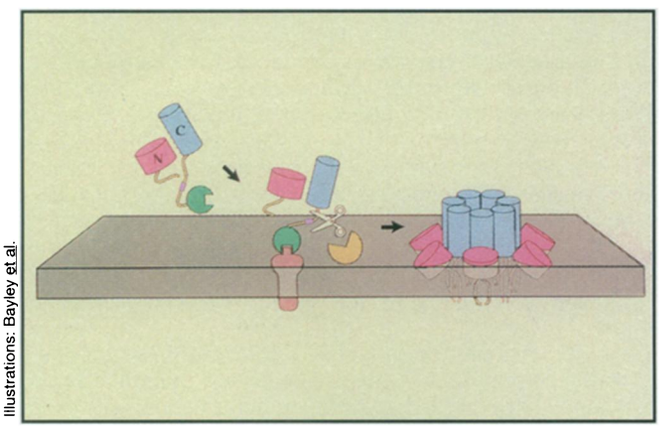
"One idea is to use these proteins to get metastatic cancer cells to commit suicide," Bayley says. "The idea is to genetically engineer the protein so that it's inactive but becomes activated by the proteases in certain tumor cells. That would cause pores to form only in those tumor cells.

"Now that alone won't kill tumor cells, but it will make them more permeable to some cytotoxic agents," Bayley adds. "We could then design agents that normally aren't absorbed by cells. Yet when they are absorbed, they're lethal. Because only tumor cells activate the protein, the toxin will kill only them."

Bayley also envisions a system that delivers drugs to a specific site. For instance, tiny fatty capsules called liposomes could ferry a chemical to a set of target cells in a remote region of the body. The liposomes might themselves have pore-forming proteins imbedded in their membranes. When they came in contact with the sought-after cells, the proteases of those cells could trigger a release of the drug.

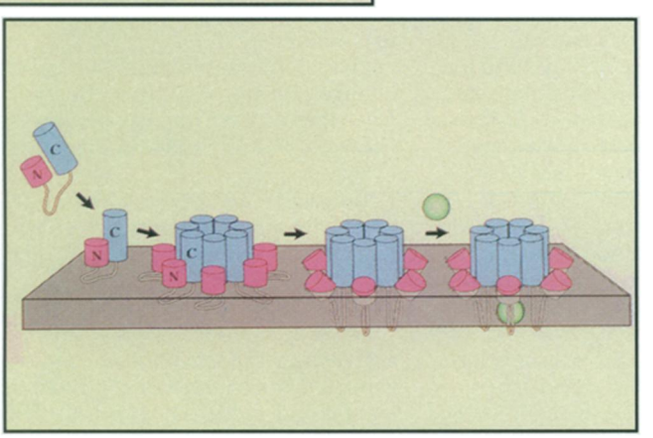
If one rigged alpha-hemolysin to react to light instead of a protease, other intriguing options might arise. The pore formers could be introduced into tumor cells or into a liposome carrying a drug to a tumor site. Shining a light on a particular area with a laser or fiber-optic probe might then permit the tumor cells to absorb toxic drugs. Of course, the cells need not be cancerous. In theory, any organ or cell cluster could receive a directed dose of any agent if the pore-forming proteins and drug delivery system were properly designed.

"This technique could apply to the skin, the lungs, the colon. Someone could inhale or ingest a substance that is activated only at the site where a fiber-optic instrument shining a particular wavelength causes those pore-forming proteins to become active. Liposomes could release a drug only into a small area," Bayley says.



Illustrations: Bayley et al.

***F**orming a pore in a cancer cell: An alpha-hemolysin protein — polypeptides N and C (pink and blue) — is led to a tumor cell membrane (gray slab) by an antibody (green). A tumor protease (yellow) then cuts the protein, triggering it to construct a membrane pore.*



***M**aking a switched pore: An alpha-hemolysin protein — N and C polypeptides (pink and blue) joined by a genetically engineered amino acid loop — forms a membrane pore. A metal ion (green) can open and close the pore, yielding a switch.*

Yet why stop at strictly biological applications? "These proteins can make very sensitive and specific sensors for metals or, ultimately, almost any molecule that will turn on the switch," Bayley says.

He would like to choose the best 100 mutant proteins from a pool of 100,000 and from these create a "sensor library." Each mutant could bind a particular metal ion. By making a protein with an all-purpose loop, the researchers might be able to plug in "cassettes" with specific nucleotide sequences.

"With these cassettes, we could encode thousands of variant proteins," Bayley says.

From this library, Bayley envisions simple sensors that could quickly and sensitively detect low levels of metals in remote locations. "Someone could use it to find toxic metals at dump sites," he says. "Just dip it into a pool of muddy water and see how much zinc, or cadmium, or mercury is there."

In theory, anyway, the same principle could apply to bodily fluids. At some point, a spectrum of mutant proteins might be fashioned in a single instrument that could quickly reveal the makeup of a person's blood chemistry — or at the very least give a fast readout of toxins or metals.

"There's a very small set of people who do protein membrane work, and they wear two hats," says Kenneth J. Rothschild, a molecular biophysicist at Boston University. "One goal is to understand how these membrane proteins function, which is very important since they are among the most crucial components of a living system. But another goal is to find ways to preserve the function of these proteins while incorporating them into synthetic systems. This could have a tremendous technological impact. It's much harder to design an organic component from scratch than it is to take one from a living system and modify it for another use.

"Imagine a whole series of future devices that could incorporate membrane proteins as if they were computer circuits, putting millions of them into a very small space."

Rothschild calls this biomolecular electronics.

"Bayley's project is among the best examples of work at the forefront of this area. He's modifying a protein to do something useful. Imagine a detector that picks up toxins or certain wavelengths of light. One could even make an artificial nose to sniff out pollutants, allergens, [or] pathogens in the air to tell if the air quality around you is good or bad," Rothschild speculates.

"What I'm really interested in, though, is the basic biology of how these proteins assemble," Bayley says.

"Through this project I've also become convinced that basic science and biotechnology can really feed each other," he adds. "But you have to be careful. To do applied science well, you need an underpinning of basic research. Otherwise you can end up with shoddy science that doesn't go as far as it would if you did some basic research first."

"A primitive person might spend a few months building a sled," Bayley muses. "But if he spent a few years working on a wheel, he would go much farther in the long run." □