and other parts of Europe, were invented independently or were the result of cultural diffusion—borrowing, in short.

During the discussions, however, it became clear that the evolution of cultural traits is too complex to be explained by the simple framework of Neo-Darwinian theory. "Darwinian evolution, with its emphasis upon common ancestry, offers an ineffective framework for the analysis of cultural diversity," says Colin Renfrew, a professor of archeology at the University of Cambridge. Renfrew argued that so far it has been too difficult to measure cultural similarities precisely and objectively enough to know the value of the comparison. Just because two structures happen to look alike, for example, it doesn't mean that they were built according to similar principles of construction learned from a common ancestor.

What anthropologists need, says Renfrew, is greater intellectual sophistication. Instead of merely borrowing Darwinian mechanisms, they need to elaborate their own theory of how changes arise in different societies. That theoretical backbone would then serve as a reference point for analyzing complicating effects such as population migration and diffusion, ethnicity, the development of production, wealth and technology, and the emergence of power hierarchies. "I'm sure we do have a lot to learn from the analogies between different fields, but I believe we're straining when we try to explain culture in Neo-Darwinian terms."

Others agreed. "There really is no need for some of us to be here: I know all of the archeology and linguistics I need to know," remarked Maynard Smith, after listening to the sessions on the social sciences. He warned that drawing analogies between such disparate fields can be misleading, if not dangerous—particularly when the conclusions from one field don't apply to another. "The question of the conference is whether any of the similarities between the human sciences and the biological sciences are sufficiently close to be useful in a formal way," he said.

But the answer for some was clearly yes. Indeed, the evolutionary biologists and linguists found parallels between their disciplines so compelling that they are planning a follow-up meeting in a year or so. Other participants said the hallway talk suggested new ideas or provided new tools. And a few biologists were pleased to see social scientists be so open-minded about applying evolutionary theory to their work—after years of skepticism. For most, however, the symposium's ambitions remain plausible, but not yet realized. "One can now say there is a general science of evolution," concluded Diamond. "Is it going to be useful? That remains to be seen." ■ ANN GIBBONS

Playing Tetherball in the Nervous System

A simple "ball and chain" model turns out to explain a key feature of how some ion channels work in nerve cells

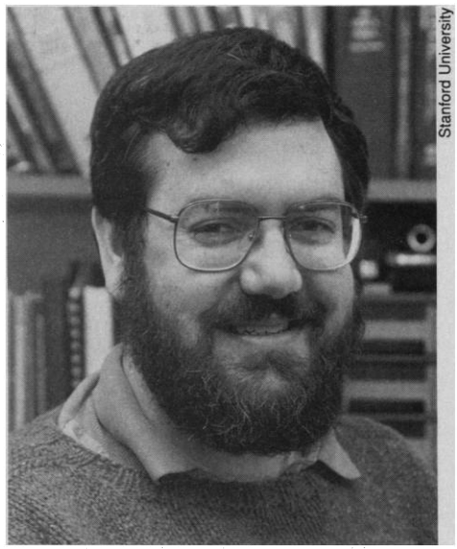
IMAGINE A PROTEIN TETHERBALL, DANGLING by a protein cord on the inside of a nerve cell's outer membrane: while the tetherball floats free, ions pass into or out of the cell; but when the ball pops into the mouth of a pore, the ion flow stops. Sound too cartoon-like to be taken seriously as a scientific model? Two articles in this issue of *Science* provide evidence that this "ball and chain" model of channel inactivation—a notion first proposed 13 years ago but for which there was little hard data—does in fact explain how some of the ion channels that control electrical excitability in nerve cells work (see papers on pages 533 and 568).

Not only is this work—by Stanford neurophysiologist Richard Aldrich, with postdocs Toshinori Hoshi and William Zagotta—generating great excitement on its own, it is also being heralded as the most elegant application to date of a molecular genetic technique that may be the realization of a 40-year-old dream in neuroscience. In that technique, called site-specific mutagenesis, researchers make specific mutations in an ion channel and then observe the effects of those mutations. The technique has already begun to make it possible to understand, for the first time, the precise relations between structure and function in these essential nerve-cell proteins.

"These are really a beautiful set of very trenchant experiments," says Chris Miller, a physiologist at Brandeis University who studies ion channels, of Aldrich's work. "They make the case that there is actually a ball and there is a chain; and the ball actually flops in and [blocks] the channel."

The application of site-specific mutagenesis to ion channels has been made possible largely by the cloning in the last few years of the genes for the ion channels. The channels are membrane-spanning proteins that contain pores through which specific ions can pass. But the pores are not open all the time. Instead, they are "gated"—primed to open and close under certain conditions. Some channels respond to changes in voltage across the membrane, others to the binding of neurotransmitters.

The opening and closing of these channels have potent consequences. For example, the action potential—the electrical impulse that travels the length of neurons—depends on the precisely timed opening and closing of voltage-sensitive channels for sodium and potassium ions. Other voltage-dependent ion channels, including calcium and chloride channels and a variety of more specialized potassium channels, play modifying roles in nerve cell function, altering the nature of a nerve cell's action potentials or the timing of their firing.



Molecular confirmation. *Rick Aldrich applied molecular genetic methods to confirm a 13-year-old model for how some ion channels are inactivated.*

But how does the molecular structure of a channel enable it to carry out these functions? It is assumed that the proteins making up the channel snake back and forth many times across the cell membrane to form the channel's functional parts. The sequence of amino acids in the channel proteins supports that notion: stretches of amino acids that would be at home in membranes alternate with stretches of residues that would be more stable surrounded by water.

Beyond these vague assumptions, however, no one knows what precise shape the channel proteins take in the membrane, how they form pores that select one type of ion over another, how voltage changes cause the pores to open and close, or how inactivation—which seems independent of voltage—occurs. One problem is that no one

has yet been able to crystallize an ion channel protein to determine its three-dimensional structure, and so researchers have had to rely on inferences about channel structure and about the position of specific amino acids in that structure.

Site-directed mutagenesis is helping to fill this gap. By systematically changing amino acids in the proteins and studying the consequences of those changes, researchers are zeroing in on regions responsible for some channel functions. "We had 2000 amino acids [in a channel protein] and we had no idea what they were for," says Bertil Hille, of the University of Washington, who has studied ion channels for 25 years. "Now you can modify a specific amino acid and get very definite information."

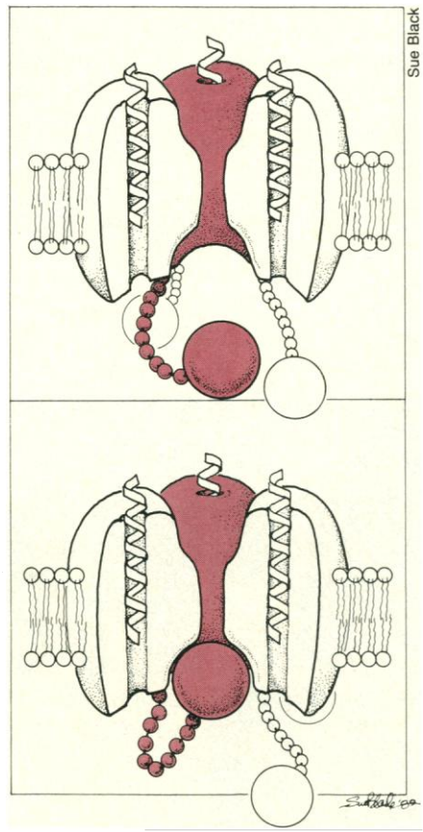
Aldrich and his co-workers chose inactivation for their first mutagenesis studies because it is the easiest function to assay with electrophysiological techniques. In studying inactivation, they were building on experiments done in the 1970s by Clay Armstrong and Francisco Bezanilla at the University of Pennsylvania. Working with sodium channels, Armstrong and Bezanilla found that the channels are inactivated by a voltage-independent mechanism that can be blocked by the introduction of protein-digesting enzymes. That finding suggested that a part of the channel protein inside the membrane—where it was vulnerable to the enzymes—formed a tethered ball that could swing into place and block the pore.

Aldrich's group repeated the protease experiment with a type of potassium channel that shows inactivation and found that its inactivation could also be eliminated by proteolytic enzymes applied to the inside of the membrane, suggesting that was where inactivation occurs. Both ends of the potassium channel protein are thought to protrude from the inside of the nerve cell membrane, but Aldrich focused on the amino terminal end (named because of the free amino group there). That end is known to vary among potassium channels with different inactivation properties suggesting that the amino terminus is essential to inactivation.

Aldrich says he didn't set out to confirm the ball and chain model. But as the mutation data rolled in, it was the image of a ball and chain that came into focus. The 19 amino acids at the end of the protein seem to make up the ball: deletions in that region destroy the channel's ability to inactivate. Furthermore, changes of amino acids in that region slow inactivation, as if they were reducing the ball's affinity for its receptor site. Beyond the first 19 amino acids is a stretch of protein that serves as the chain. Deletions there speed up inactivation—as if with a shortened tether the ball finds its target more easily.

These are elegant results. But site-directed mutagenesis studies do raise the concern that the mutations change the protein's shape in a global way, and that the effects of the experiment are due to those global changes rather than to the local effects of substituting one amino acid for another. To rule that possibility out, Aldrich's group showed that inactivation of mutant channels could be restored by adding back the "ball" alone, in the form of free synthetic peptides with the same amino acid sequence as the 19–amino acid end of the normal channel protein. The experiments, Aldrich says, "have confirmed the ball and chain model and put it on real molecular grounds."

The power of site-directed mutagenesis, so clearly demonstrated in Aldrich's work, is now being applied to other key ion channel problems as well. Several groups, including those of Walter Stühmer of the Max Planck Institute in Göttingen, Germany, and Lily Jan of the University of California at San Francisco, are using mutagenesis to analyze the voltage-sensing mechanism through which changes in membrane potential cause channels to open and close their pores.



Jack-in-the-box. *A protein ball pops into a pore formed by the bases of four membrane-spanning proteins (one not shown) thereby stopping the flow of potassium ions out of a nerve cell.*

Physiological experiments suggest that the voltage change across the neuronal membrane must cause a shift in a charged part of the protein that spans the membrane. Candidate stretches have been found in both the sodium and potassium channels, and changing their charged amino acids does indeed alter the response of the channels to voltage shifts, suggesting the researchers are on the right track.

Other groups are on the trail of the protein regions that make up the ion pores of several channels. Bert Sakmann, at the Max Planck Institute in Gottingen, working with Shosaku Numa of Kyoto University, as well as a Caltech team from the labs of Norman Davidson and Henry Lester, have identified parts of the channel that might line the ion pore of the acetylcholine receptor, a neurotransmitter-activated ion channel. Mutations in those regions change either ion flow or the binding, suggesting that the altered amino acids are part of the ion pore.

But such questions about pore structure and voltage sensing may not yield to mutational analysis quite as neatly as ball and chain inactivation. These functions depend on segments in the middle of the protein that have unknown shapes. The lack of information on three-dimensional structure leaves researchers working on these problems more profoundly in the dark than Aldrich's team, which was working with an end of the protein that almost certainly dangles into the cell's cytoplasm.

Those working on these questions readily acknowledge the problem. "You form a hypothesis," says Caltech's Davidson, "and say if I change this amino acid I should see an effect. You change the amino acid and you see the effect, and you say this evidence supports the hypothesis. But what you draw are probable inferences, not certainties. In the absence of atomic resolution structure, you don't get a rigorous proof."

For the answers to some questions, such as how an ion pore selects one type of ion while excluding others, site-directed mutagenesis will never provide the details, which may involve differences in spatial clearance on the order of angstroms. "We'd all like to know how ion selectivity works, but until we know something really precise about the structure in there, we're not going to understand it," says Brandeis' Miller who, with Roderick MacKinnon of Harvard, has used site-directed mutagenesis to probe the ion pore of the potassium channel.

But that doesn't take away from the power the method has already shown. Says Miller: "It's very exhilarating to think we can finally begin to attack these questions that have been hanging around for 30 years."

■ MARCIA BARINAGA