CELL COMMUNICATION EQUIPMENT: DO-IT-YOURSELF KIT

Molecular biologists have built the four components of a cell's chemical signal receptor and have assembled them into an operational system

By JULIE ANN MILLER

Conversation between nerve cells or between nerves and muscle is mainly a matter of chemistry. But it relies on sophisticated molecular hardware. One cell deposits a dollop of signal chemical into a cleft, and it is up to the cell opposite to recognize the chemical and react to it. A single type of molecule that sits in the receiving cell's membrane performs both the recognition of a given signal chemical and the appropriate response. Scientists are now using recombinant DNA techniques to analyze one such molecule and learn how it performs its precise and dramatic functions.

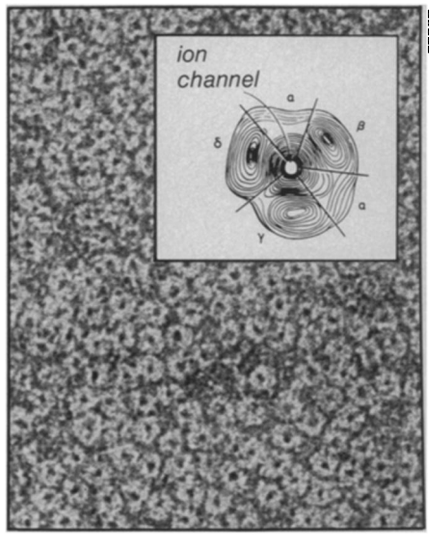
For almost 80 years scientists have been studying this molecule, the nervous system's receptor that responds to the small chemical called acetylcholine. When acetylcholine binds to its receptor, a water-filled channel opens for about a millisecond and lets half a million ions pour across the membrane. Such brief floods of ions across membranes are a basic nervous system response.

The tools and preparations being used by biologists to study the acetylcholine receptor resemble the ingredients of a witch's brew: electric organs of eels and rays, poisons from snakes and plants, as well as antibodies, drugs, detergents and alcohols. Work with these fortuitous natural materials has revealed a great deal about the structure and position of the molecule and has given some clues to how it carries out its tasks.

Recent studies, for example, indicate the approximate locations of important regions on the acetylcholine receptor molecule. These include the site where acetylcholine binds, the segments that extend across the membrane and form the walls of the pore and the region to which antibodies bind in causing myasthenia gravis, an autoimmune disease.

"The three-dimensional structure of the acetylcholine receptor holds one of the keys to its function," says Robert M. Stroud of the University of California at San Francisco. An extraordinarily detailed image of the receptor has been provided by Stroud and numerous other scientists using techniques of electron microscopy and X-ray diffraction analysis.

The acetylcholine receptor is shaped like a "lopsided dumbbell," says Arthur Karlin of Columbia University College of Physicians and Surgeons in New York. It spans the cell membrane, the larger sphere of the dumbbell extending about 50 angstroms (1 angstrom is 10^{-10} meter) above the extracellular surface and the other sphere extending about 20 angstroms on the intracellular side. The bar of the dumbbell is actually funnel-shaped, widest at the extracellular surface.



Stroud

The cores of acetylcholine receptors, densely packed in the membrane, fill with a dark-staining heavy metal. Each receptor is about 85 angstroms in diameter. (Inset) A tentative arrangement of subunits (identified by Greek letters) is superimposed on the reconstructed image of the projection of a receptor.

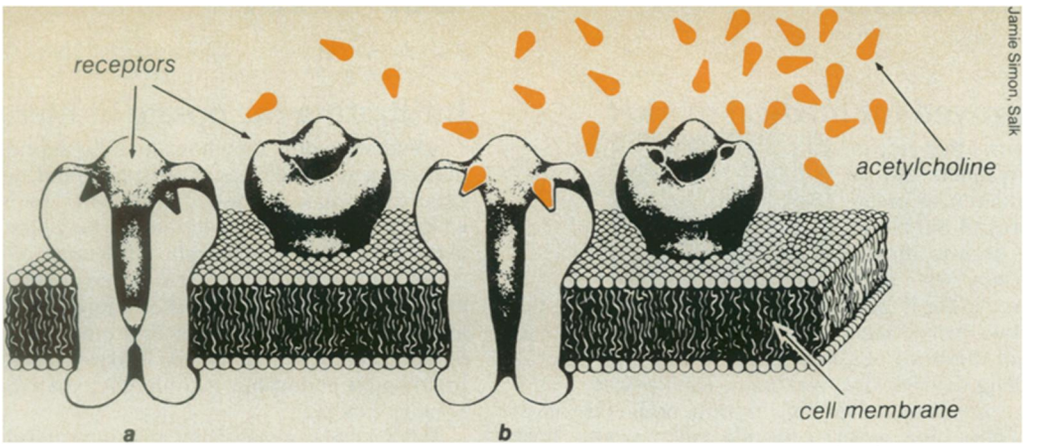
There is strong evidence that the receptor's central region, the funnel, is a channel through which ions flow. For example, when scientists fill it from the outer surface of the membrane with uranyl ions, which are visible in the electron microscope, the ions occupy nearly all the depth of the receptor molecule. The micrographs demonstrate that the receptor makes a cylindrical pore, about 7.2 angstroms in diameter, perpendicular to the membrane. Experiments measuring the ability of ions of various sizes to cross the channel also indicate that the pore is about 7.2 angstroms wide.

Electron microscopy has revealed that the channel is lined by the subunits of the receptor molecule arranged like the staves of a barrel. There are five subunits, each a chain of about 500 amino acids. Two of these subunits (called alpha) are identical; the other three subunits are each represented once per molecule. Each subunit extends from one side of the cell membrane to the other; the subunit's helices, with axes perpendicular to the membrane, forming the channel walls.

To investigate the role of the five subunits in the function of the receptor, researchers have used a variety of chemicals that interfere with receptor function. Work with quinacrine mustard, a derivative of a potent local anesthetic, for example, indicates that sites on the alpha and beta chains are involved in the channel activity, but does not exclude sites on the other chains.

What the scientists would like to do is to vary sites systematically on the chains to see what changes interfere with acetylcholine binding or channel opening. The important first steps toward applying this more direct analytical approach using modern methods of molecular biology were reported in the Feb. 16 NATURE by Masayoshi Mishina, Shosaku Numa and eight colleagues at the Kyoto University Faculty of Medicine in Japan and Jon Lindstrom of the Salk Institute in San Diego, Calif.

In cells growing in laboratory culture, the scientists have produced from genes the four types of subunits of the acetylcholine receptor. The group combined three approaches that had previously been used independently in different contexts. They used as a carrier for each of the receptor subunit genes a plasmid — a ring of bacterial DNA — containing genetic pieces from a virus that normally infects



In the absence of acetylcholine, receptor channels are closed (a). When receptors bind acetylcholine (b), the channel into the cell opens.

monkey cells and pieces from the rabbit gene for the protein beta-globin. This gene carrier had previously been used for carrying a bacterial gene for drug resistance into mouse cells.

To make messenger RNA from the receptor subunit genes, the Kyoto scientists used a line of monkey cells, called COS, that had been established to allow propagation of viruses carrying recombinant DNA.

Finally, to synthesize and assemble the receptors and insert them into a surface membrane, the investigators isolated from the COS cells the messenger RNA carrying the information that directs production of receptor subunits. They transferred this messenger RNA into frog immature egg cells, called oocytes. Frog oocytes had previously been used to make acetylcholine receptors from messenger RNA isolated from the electric organs of rays. These organs are a rich source of the RNA, since the organ contains an abundance of receptors, about 10,000 per millimeter of membrane.

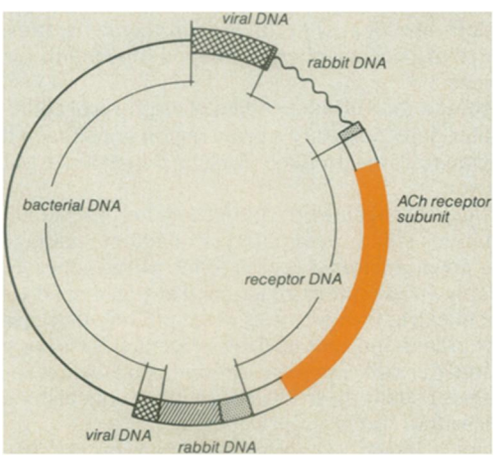
Commenting on the work of the Kyoto group, Charles F. Stevens of Yale University says, "The advantages of having separate systems for making message [messenger RNA (mRNA)] and for using it are that proportions of the message for various subunits can be controlled — for example, mRNA for a given subunit can be completely omitted—and quantity of message can be varied at will."

In the first experiments using this system, Numa and colleagues find that in general all four different subunits are required for normal receptor function. When they injected an oocyte with messenger RNA instructing the cell to make only three of the four subunits, in most cases no operational receptors were formed. However, there is a possibility that two subunits, those called gamma and delta, can replace each other to some extent. In 2 to 3 percent of the oocytes injected with messenger RNA coding for all the subunits except either gamma or delta, the cell responded slightly to acetylcholine.

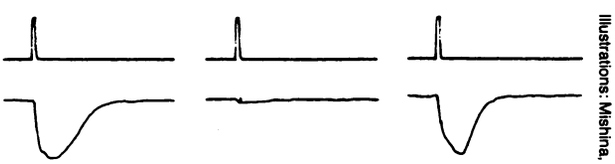
The new work confirmed earlier observations on the binding site of a Formosan snake toxin, called alpha-bungarotoxin, that has been extensively used in experiments with the acetylcholine receptor. Other scientists had reported that the toxin, which interferes with acetylcholine binding, attaches to alpha subunit isolated from the rest of the receptor. Similarly, the Kyoto group finds no alpha-bungarotoxin binding to cell extracts containing no alpha subunit. In extracts lacking one of the other types of subunit, the amount of alpha-bungarotoxin binding correlates with the quantity of alpha subunit present. (The amount of alpha subunit present differs from experiment to experiment because individual subunits appear to be degraded at an increased rate when any subunit is lacking, and that rate is different according to which subunit is missing.)

The scientists next plan to make changes in the receptor by introducing mutations into the isolated genes in order to determine how structural changes affect the molecule's functioning.

Stevens concludes his comments in NATURE, "Before this new work from the Kyoto group, the extent to which expression of this rather complicated and delicate membrane protein could be achieved was uncertain. We now know it works; next will come an exploration of altered regulation and function due to systematic and rational changes in amino acid sequence." □



Carrier for the receptor genes was constructed by inserting stretches of viral and rabbit DNA into a ring of bacterial DNA.



Illustrations: Mishina, Nature

When a frog egg was injected with messenger RNA produced from four such plasmids, each carrying the gene for one of the four receptor subunits, the egg showed an acetylcholine response. A pulse of the chemical (top trace) produced an ionic current (bottom trace) across the egg cell membrane. This activity was abolished by curare (center), a poison that binds to acetylcholine receptors, but was restored when the curare was washed out (right).