A Potpourri of Membrane Receptors

Research on receptor structure and function progresses on a broad front while revealing some surprises about the cell's protein-sorting operations

Both the external and internal communication systems of cells depend on the operation of membrane receptors. Receptors on the outer cellular membrane collect information from other cells and the environment in the form of growth factors, hormones, neurotransmitters, and electrical changes and set in motion the biochemical events that produce appropriate responses. Internally, receptors help to direct newly synthesized proteins to their ultimate destinations. This wide diversity of receptors was the focus of the recent Whitehead Institute Symposium,* which was held in honor of Salvador Luria who is retiring as chairman of the Center for Cancer Research at the Massachusetts Institute of Technology.

Presentations at the symposium illustrated the current progress toward a better understanding of receptor structure and function, which is now being aided by gene-cloning successes, but they also revealed a number of surprises, especially regarding the internal protein-sorting operation.

Nobel winners Michael Brown and Joseph Goldstein

LDL Receptor Mutations

By a fortuitous quirk of fate, the symposium opened on the night of Sunday, 13 October, with a preview of the 1985 Nobel Prize award ceremonies when Michael Brown of the University of Texas Southwestern Medical School at Dallas described the operation of the LDL (low-density lipoprotein) receptor and its importance in regulating blood cholesterol concentrations. Scarcely 12 hours later, Whitehead Institute director and Nobel Laureate David Baltimore broke out a bottle of champagne as he told the assembled participants that Brown and his longtime collaborator Joseph Goldstein, also from Texas and also attending the meeting, had just been awarded the 1985 Nobel Prize for Physiology or Medicine for their work in solving the mysteries of the LDL receptor.

One of the more recent accomplishments of the Texas group is the cloning of the LDL receptor gene. Analysis of the gene has shown, among other things, that it was assembled by combining coding segments from genes for several other functionally unrelated proteins. Cloning of the gene is also providing a means of dissecting the contributions of the different regions of the receptor protein to its function. This can be done by analyzing the effects of various mutations, whether these be naturally occurring or experimentally generated.

Natural mutations are common. Approximately one person in every 500 carries a single defective copy of the LDL receptor gene. As a result their cells are deficient in removing the cholesterol-bearing LDL's from the bloodstream, and the individuals have a higher risk of having a heart attack. Individuals who inherit two defective copies of the gene have extremely high blood cholesterol concentrations and often die of heart attacks before their 20th birthdays. Brown, Goldstein, and David Russell, who is also in Texas, have found that a variety of different mutations occur naturally and can be located anywhere in the gene. "The multiple mutations may be the reason for the high frequency of defective genes in the population," Brown suggests.

About 50 percent of the mutations result in failure to synthesize the LDL receptor protein and are uninteresting in the sense that they cannot yield useful information about how the receptor works. However, other mutant genes produce defective proteins that can provide such clues. For example, in one subclass of mutants, the proteins are located on the membrane where they bind LDL as expected. Normally, the receptors with their bound LDL would form clusters and the cell membrane bearing the clusters would pouch inward, eventually forming vesicles that transport the receptor and the LDL's to the cell interior. However, the mutant receptors do not form clusters and thus fail to bring the bound LDL's into the cell.

A large portion of the LDL receptor projects to the outside of the cell where it can bind LDL. There is a single short segment spanning the membrane and another segment of about 50 amino acids projecting into the cytoplasm. Brown, Goldstein, and Richard Anderson of the Texas group predicted years ago that mutations in this cytoplasmic region would result in the failure of the receptor to cluster and they have now confirmed this prediction. Each of three patients with the clustering defect had mutations in the region of the LDL receptor that codes for the intracytoplasmic segment. In one case, a single amino acid was changed. When the Texas workers introduced that mutation into a cloned LDL receptor gene and transferred the gene into cultured cells that lack LDL receptors, they reproduced the defect seen in the patient. "This provides very strong evidence that the intracytoplasmic domain is important for moving the receptor crossways and getting clustering," Brown reported.

Mutations in other areas of the gene produce different defects. The Texas workers identified one patient with a

*Whitehead Institute Symposium, at the Massachusetts Institute of Technology, 13 to 15 October.

deletion of the transmembrane-coding region. The protein was transported out of the cells instead of being inserted into the membrane.

The Watanabe strain of rabbits, which develops a form of coronary-artery disease much like that of human patients, makes a defective LDL receptor in which sugar addition and the reactions needed to make a mature receptor are delayed. In addition, the final receptor product does not bind normal amounts of LDL. Here the mutation turned out to be in the LDL-binding region.

As one example of how the LDL receptor research might guide therapy, Brown concluded his talk by describing a young patient who did not make functional LDL receptors and who had had several heart attacks and two coronary-bypass operations by the age of six. Normally the liver bears about 70 percent of the load of removing LDL's from the bloodstream. About 2 years ago, the patient was given both a heart transplant to replace that diseased organ and a liver transplant in the hope that this would allow her to maintain a more normal blood cholesterol concentration. That proved to be the case and the patient has survived, although Brown cautions that her condition is still precarious because of the immune suppression required for the transplants.

Protein-Sorting Surprises

Cloning can provide new insights into molecular functions, as it is doing for the LDL receptor. It can also provide surprises, as Randy Schekman of the University of California at Berkeley described for the protein clathrin. Clathrin forms a cagelike structure around vesicles, including those that transport materials such as LDL's into cells. The clathrin cage has been considered essential for normal vesicle transport. Vesicles that transport proteins from where they are synthesized on the ribosomes of the endoplasmic reticulum to the proteins' final destinations either inside or outside the cell are also supposed to have clathrin cages—but that now appears not to be true.

An individual clathrin molecule contains two protein chains of unequal molecular weight. Schekman and his colleagues have recently cloned the yeast gene for the heavier of the two. They then created a mutant yeast strain by replacing a normal clathrin heavy chain gene with one that was inactivated by insertion of a piece of foreign DNA. Expecting the mutation to be lethal for yeast cells that inherited only the defective gene because of the resulting disruption of protein transport, they were therefore surprised to find that such cells survive, although they grow more slowly than cells with a normal clathrin gene. The researchers confirmed that no clathrin was made in the mutants. "There is one [clathrin heavy chain] gene per cell, and its expression can be eliminated and the cells can still grow," Schekman concludes.

Success in gene cloning yields insights into communication among cells.

Moreover, Schekman and his colleagues found that transport of one of the proteins secreted by yeast cells is nearly normal in mutant cells without clathrin. What the protein might actually be doing if it is not needed for transporting newly synthesized proteins is unclear. It still might play its postulated role in vesicle transport from the cell surface to the interior; it might also help to prevent vesicles from fusing with one another if they come into contact.

Additional support for the view that clathrin is not needed for transporting proteins from the endoplasmic reticulum to their ultimate destinations came from James Rothman of Stanford University School of Medicine and Kai Simons of the European Molecular Biology Laboratory in Heidelberg, Germany. Their results have also helped to clarify the role of the Golgi complex, which appears in electron micrographs to be a stack of flattened membranous disks.

Newly synthesized proteins travel in vesicles from the endoplasmic reticulum to the Golgi complex where they undergo a series of reactions needed for protein maturation. The Golgi complex is also the site where proteins are sorted for targeting to their ultimate destinations.

Over the years investigators, including Rothman, have found the Golgi complex is subdivided into three compartments, each with its own distinct complement of enzymes for protein maturation. Although the separate compartments might have been used to effect protein sorting, Rothman says that this does not happen. The proteins stay together until the last compartment.

The proteins are transported between Golgi compartments in vesicles. The Stanford workers find that the vesicles can form even when they use yeast cytosolic enzymes with mammalian Golgi complexes. "The Golgi pathway is highly conserved in nature," Rothman says. "The process of sorting is really an extension of the process of protein synthesis."

If the vesicles move from one Golgi stack to another, they can still find the right location, Rothman says. This indicates that there is some targeting mechanism, presumably including receptors for the vesicles on the various Golgi compartments. However, Lelio Orci of the University of Geneva and Rothman were unable to detect clathrin on the transporting vesicles.

Simons has been using viral proteins to study sorting. The viruses in question normally bud from two different surfaces of kidney cells; the Simons group has shown that viral membrane proteins are transported directly to the correct surfaces for the particular virus. They are not transported together to the same surface and then somehow separated. "These studies suggest that the proteins are sorted intracellularly," Simons says.

The most likely place for the sorting to occur, Simons suggests, is a tubular network at the end of the Golgi complex that has been noted by a number of investigators over the years. "It is the only site where proteins are leaving for more than one location in the cell," he points out.

Although the Heidelberg workers find clathrin primarily on the surface of the final compartment of the Golgi complex, its location is not the same as that of the viral protein to be transported to the cell membrane, a finding which indicates that clathrin is not involved in protein export to the surface.

Similarities in Ion Channels

Cloning is also contributing new insights into the design and operation of ion channels—membrane pores that open in response to a stimulus and allow ions to pass through an otherwise impermeable membrane. Shosaku Numa of the Kyoto University Faculty of Medicine described his group's work on the acetylcholine receptor and the sodium channel. Both of these entities allow sodium ions to move into the cell. The receptor does this when it binds the neurotransmitter acetylcholine, thus producing a small voltage change which is the signal for opening the sodium channels. The resulting influx of sodium ions then triggers the changes leading to the cells' responses, such as the firing of

neurons or the contraction of muscle cells. According to Numa, the overall designs of the acetylcholine receptor and the sodium channel appear similar even though the proteins of which they are composed are quite different.

The acetylcholine receptor, which has a total molecular weight of about 250,000, consists of five protein chains, two of which are identical. Genes for all of these have now been cloned in a number of laboratories, including Numa's. More recently, Numa and his colleagues have cloned the gene for the single protein that forms the sodium channel. The molecular weight of this protein is also in the range of 250,000.

Comparison of amino acid sequences of the acetylcholine receptor proteins shows similarities. They all have four regions consisting of hydrophobic amino acids plus a fifth "amphipathic" segment that can form an alpha-helix with charged amino acids on one side and hydrophobic amino acids on the other. Current models indicate that the five protein chains are arranged circularly in the membrane, thus forming a pore. Unlike the LDL receptor, which is anchored by a single membrane-spanning region, the acetylcholine receptor proteins weave back and forth so that the four hydrophobic regions and the amphipathic segment are all embedded in the membrane. The charged amino acids of the amphipathic segments may form the pore lining.

Although the sodium channel is formed by a single protein, the work of the Numa group shows that it can be subdivided into four internal units that have similar amino acid sequences. Numa postulates that the four units are oriented in pseudosymmetrical fashion to form a pore in the membrane. Each of the internal units shows a similar pattern with six distinguishable subregions. Although Numa originally proposed that just four of these are embedded in the membrane while two, including one that is positively charged, would project into the cytoplasm, he now suggests that all six span the membrane for a total of 24 membrane-spanning regions compared to the 25 in the acetylcholine receptor. The four related internal units of the sodium channel protein may thus achieve a pore structure similar to that produced by the separate but related protein chains of the receptor.

The positively charged subregions, which would presumably form a block to the transport of sodium ions, are probably shielded by the other membrane-spanning units. However, the positive segments may serve as the detector of the voltage changes across the membrane. "It seems likely," Numa proposes, "that the positive charges in the segments detect the membrane depolarization and move outward, causing conformation changes and opening the channel."

Receptors on Immune Cells

The operation of ion channels is not limited to nerve and muscle cells but also contributes to immune cell function. John Ding-E Young of Rockefeller University presented evidence indicating that the macrophage receptor for the constant region of antibody molecules is an ion channel, too, in this case permitting the flow of monovalent cations such as sodium and potassium ions. When the receptor is activated, it stimulates the activities of macrophages in removing complexes of antibody and foreign antigen from the blood.

At the end of his talk, Young described a tantalizing observation that may help to explain how some cells can kill other cells. He and his colleagues have isolated a protein from one species of amoeba and from a variety of immune killer cells that may help to do the job. This protein turns out to resemble one of the proteins of the complement system, which also contributes to immune responses by helping to kill foreign bacteria. Molecules of the complement protein associate with one another to form channels that punch holes in the membranes of target cells, and the killer cell protein appears to have a similar capability. "It suggests that there is a family of proteins in the immune system that kill by causing these holes," Young says.

The normal development of functional lymphocytes requires their interaction with the various organs of the lymphoid system, including the thymus gland, spleen, and peripheral lymph nodes. Irving Weissman and his colleagues at Stanford University School of Medicine have recently identified a receptor that allows a particular subclass of lymphocytes to home in specifically on the peripheral lymph nodes. Somewhat surprisingly, the receptor consists of an unusual branched protein in which the peptide ubiquitin forms one of the branches. The ubiquitin structure has been highly conserved throughout evolutionary history and, as its name suggests, is found in all cells. The Stanford results are the first to indicate that the peptide might be an integral part of membrane receptors and thus have a role in mediating intercellular interactions.

Although most of the speakers at the Whitehead Institute symposium concentrated on receptor structure and the more immediate biochemical pathways through which activated receptors induce cellular responses, Eric Kandel of Columbia University's College of Physicians and Surgeons reminded the participants that receptors can also mediate long-lasting effects. Kandel, James Schwartz of the Columbia group, and their colleagues have for many years been studying learning and memory in the marine snail *Aplysia,* which has a simple enough nervous system to permit them to trace the neuronal pathways needed for specific behaviors. In particular they have concentrated on the gill withdrawal reflex and have found that only about 50 neurons are needed for this response.

Stimulation of the *Aplysia* tail causes a form of learning in which the gill withdrawal reflex is strengthened. The Columbia group has found that the short-term memory of the learned response is mediated by neurotransmitters that act by increasing cyclic adenosine monophosphate concentrations in the affected neurons and thus stimulating the activity of an enzyme that causes protein phosphorylation. The short-term memory, which lasts about an hour, does not require the synthesis of new proteins but only the modification of proteins that already exist.

With repeated stimulation of the tail, the memory of the response becomes more or less permanent. Craig Bailey of the Columbia group has found that the affected neurons show structural changes, particularly in the terminals where they contact the next nerve cells in the pathway. Samuel Schacher, who is also at Columbia, has recently developed a new technique for growing *Aplysia* neurons in culture that allows the neurons to make functional connections with one another. Using this system, Piergiorgio Montarolo of Kandel's group has obtained preliminary results indicating that the long-term neuronal changes require the synthesis of new proteins. "The result suggests that you can readily dissociate long-term from short-term memory," Kandel says.

Although a variety of evidence over the years has suggested that long-term memory requires protein synthesis, the new culture system should facilitate experiments aimed at identifying the activating signals and the genes that are turned on. All in all, the Whitehead Institute symposium proved to be a good learning experience for researchers interested in receptor structure and function.—**JEAN L. MARX**