Receptors Highlighted at NIH Symposium

Research on receptors is flourishing, at least if attendance at a recent symposium on "Receptors and Cell Activation" is any indication. The symposium, held on 17 and 18 September by the National Heart, Lung, and Blood Institute and the American Heart Association as part of NIH's centennial year celebrations, attracted a standing-room-only crowd of approximately 850 participants.

Receptor Gene Family Is Growing

Although researchers have been studying receptors for decades, they did not get their first direct looks at the molecules they have been investigating until just recently, largely as the result of the cloning of the genes in question. "The concept of receptors remained a theory for about 50 years. But the critical evidence for their existence has only come in the past 5 years," says Michael Beaven of the National Heart, Lung, and Blood Institute (NHLBI), who chaired the receptor symposium with Alfred Gilman of the University of Texas Health Sciences Center in Dallas.

Receptors are cell-surface molecules that bind specific hormones, neurotransmitters, or growth factors, and then transmit a signal to the cell interior that causes the cell to respond in an appropriate manner. The NHLBI symposium was devoted to the large number of receptors—50 or more—that interact with the cell interior through intermediaries called "G proteins."

Researchers have now cloned the genes for some seven or eight of these receptors. The information that is being gleaned from the gene sequences is not only leading to a better understanding of how the receptors work, but has also provided a surprise or two. The gene sequences have revealed, for example, that all the receptor proteins are structurally related, even in cases where the agents they bind are chemically diverse.

The prototype G protein–linked receptor is the visual pigment rhodopsin, according to Paul Hargrave of the University of Florida College of Medicine in Gainesville. Like other receptors, rhodopsin is embedded in a membrane, in this case the membranes of disks contained within the light-sensitive cells of the eye. When activated by light, the rhodopsin protein undergoes a change in its three-dimensional structure that allows it to react with the corresponding G protein, thereby triggering the enzymatic and other changes that produce the cell's responses.

Several rhodopsin genes have been cloned in the past 4 to 5 years by Jeremy Nathans, David Hogness, and their colleagues at Stanford University.

The other G protein–linked receptors for which the genes are now in hand include the β_2-adrenergic receptor, which binds catecholamine hormones such as epinephrine. Robert Lefkowitz, Marc Caron, Brian Kobilka, and their colleagues at the Howard Hughes Medical Institute at Duke University Medical Center in Durham and Merck Sharp & Dohme Research Laboratories in West Point, Pennsylvania, cloned this gene about 18 months ago.

Since then, genes for several muscarinic receptors, which bind the neurotransmitter acetylcholine, have been cloned. The groups who did this work include those of Shosaku Numa of the Kyoto University Faculty of Medicine; Michael Schimerlik of Oregon State University in Corvallis and Daniel Capon of Genentech, Inc., in South San Francisco; and Tom Bonner of the National Institute of Mental Health.

In addition, Lefkowitz reported at the meeting the cloning of the gene for the human α_2-adrenergic receptor (see p. 650 of this issue) and of another gene that may encode an adrenergic-type receptor, the function of which is currently unknown. The Lefkowitz group also has a clone for the human β_1-adrenergic receptor.

Sequence analysis of the various genes shows that the adrenergic and muscarinic receptor proteins all resemble one another and the rhodopsin protein both structurally and functionally. "The insights that have been gained have tied the receptors to the rhodopsin system more closely than we could have imagined a few years ago," Lefkowitz says.

Comparisons of the amino acid sequences of the receptors can provide information about the functions performed by the different regions of the molecules. The proteins are similar in size, ranging from about 415 to 480 amino acids long. Each protein contains seven stretches of predominantly hydrophobic amino acids that are separated by segments of hydrophilic amino acids. The receptors apparently weave through cell membranes with the hydrophobic regions becoming embedded in the membrane and the hydrophilic segments forming loops that project either to the cell interior or exterior.

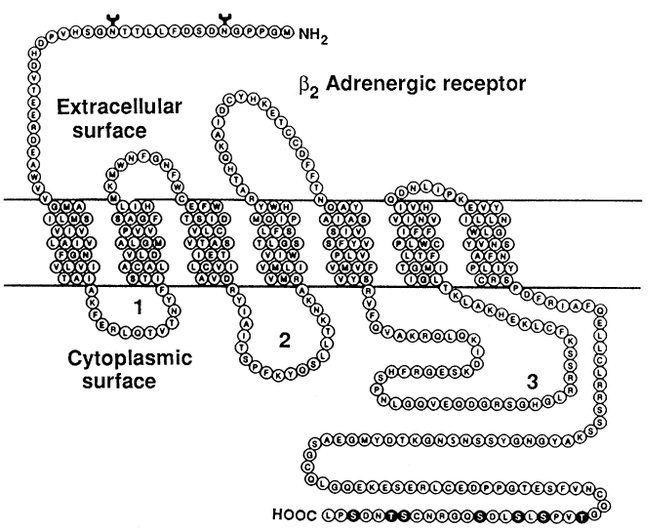
In addition to this overall structural resemblance, the proteins show similarities in their amino acid sequences. The membrane-spanning segments are usually the most similar. Depending on which proteins are being compared, 20 to 50% of the amino acids in those regions may be identical.

The light-adsorbing portion of the rhodopsin molecule, 11-*cis*-retinal, binds in a cavity formed by the transmembrane segments of the protein. Comparisons of the amino acid sequences of the other receptor proteins suggest that hormones and neurotransmitters bind in a similar fashion. The sequence comparisons are also consistent with the idea that the cytoplasmic loops of the receptors interact with the appropriate G proteins in the cell interior.

One of the most surprising aspects of the

Receptor structure

The β_2-adrenergic receptor shows the typical structure of a G protein–linked receptor. It has seven hydrophobic, helical segments that are embedded in the membrane. The three extracellular and three cytoplasmic loops are more hydrophilic.



genes for the G protein–linked receptors is that the protein-coding regions of all except the rhodopsin genes lack the noncoding DNA segments called introns. In the genes of higher organisms, introns ordinarily separate DNA sequences that encode protein segments with differing functional capabilities. As mentioned previously, for example, some segments of the receptor proteins may be involved in hormone or neurotransmitter binding, whereas others react with G proteins. The current view is that new genes evolve by assembling DNA segments with particular functions from existing genes and that these segments end up separated by noncoding introns.

But concerning the intron-lacking genes for the adrenergic and muscarinic receptors, Caron says, "It's difficult to tell exactly how they evolved. The fact that they are intronless really poses a problem."

The assumption is that these genes and those encoding the rhodopsin proteins evolved from the same ancestral gene. If that is the case, then either the genes for the adrenergic and muscarinic receptors had to have lost their introns at some point, or the rhodopsin protein genes had to have acquired theirs in some fashion.

Intron loss may be the more likely possibility. The intron locations in the rhodopsin genes are consistent with the idea that the genes were formed by combining DNA segments with different functions.

Moreover, Kobilka and his colleagues found that the gene for the β_2-adrenergic receptor is flanked by a repeated DNA sequence. This finding suggests that it may have originated as a DNA copy of a messenger RNA that was then inserted back into the genome. Introns are spliced out of messenger RNAs, and genes produced as DNA copies of the messengers end up without introns. Since it is unlikely that each of the intronless receptor genes was formed individually as a DNA copy of a messenger RNA, Caron speculates that they are all descended from the same ancestral gene.

The evidence thus far suggests that the genes for all the G protein–linked receptors belong to the same family. If the trend continues as more of the genes, including those encoding receptors that respond to peptides, are cloned, the family will be a large one indeed.

Calcium Ions May Have Their Ups and Downs

Although the effects of many G protein–linked receptors, including the α_2- and β_2-adrenergic receptors, are mediated by changes in the concentrations of cyclic nucleotides, many others transmit their signals to the cell interior by stimulating the breakdown of a membrane phospholipid called phosphatidylinositol-4,5-bisphosphate. One of the products thus produced causes the release of calcium ions from storage sites within the cell, and these calcium ions in turn bring about the changes in the cell's activities that constitute its response to the original receptor activation.

The general view is that the concentration attained by the calcium ions determines the extent of the cellular responses to activation of the receptors. At the symposium, however, Michael Berridge of Cambridge University proposed another way in which the ions might be working, a hypothesis that many observers found to be intriguing.

Several investigators, including Berridge, have noted that the membranes of cells that are responding to hormones or neurotransmitters undergo a rapid series of depolarizations and repolarizations. Since the depolarizations result from the opening of a calcium-controlled ion channel, the supposition is that the oscillations in membrane potential are brought about by periodic fluctuations in the calcium ion concentrations within the cells.

The results of Berridge and his colleagues suggest that the oscillating concentrations of calcium ions are themselves a direct result of the way in which the ions are released from the endoplasmic reticulum, the cellular structure that stores the calcium ions, in response to polyphosphoinositide breakdown. The investigators can, for example, duplicate the membrane effects by injecting frog oocytes with inositol-1,4,5-trisphosphate (IP_3), the breakdown product that causes the calcium ion release.

The frequency of the oscillations in membrane potential increases as the concentration of the injected IP_3 increases. This, Berridge suggests, may be how the cell determines how strong a response to make. "The normal view of how the second messenger works is that the cell reads the [calcium ion] concentration and makes a proportional response," he explains. "But the cell may read the frequency of oscillations to get the intensity of response."

How this might work is among the many questions remaining to be answered about the calcium ion oscillations. One possibility is that there would be less time to reverse some enzymatic consequence of the calcium ion releases—phosphate addition to a critical protein, for example—as the frequency of the releases increases. The critical protein might then be in the "on" position for a greater proportion of time, thereby producing a more intense response.

New Role Proposed for α-Adrenergic Receptor

Evidence presented at the receptor symposium by Lee Limbird of Vanderbilt University in Nashville suggests a possible new role for the α_2-adrenergic receptor, and perhaps for other, related receptors as well. Binding of appropriate agents to the α_2-adrenergic receptor results in a decrease in the concentration of cyclic AMP (cyclic adenosine monophosphate) within the cell, and this decrease has been thought to mediate cellular responses to activation of the receptor. "But," Limbird says, "the decline in cyclic AMP does not completely account for the physiological effects."

Her results indicate that increased *p*H within cells, caused by an exchange of internal hydrogen ions for external sodium ions, can account for most of the physiological consequences of α_2-adrenergic receptor activation, at least in platelets. The receptor itself may even be the entity—the "Na^+/H^+-antiporter as it is called—that exchanges the ions across the cell membrane.

Limbird's original results that implicated Na^+/H^+ exchange in α_2-adrenergic receptor activity came from studies of serotonin secretion by activated blood platelets. Platelets are not exactly typical cells, however, and Limbird turned to a line of cultured cells that has many properties characteristic of nerve cells to confirm and extend the platelet work.

She and her colleagues found that agents that bind to the α_2-adrenergic receptor increase the *p*H in the cultured cells, as do substances that activate opiate and muscarinic receptors, which also act by decreasing cyclic AMP concentrations.

If the Na^+/H^+-antiporter is inhibited, the *p*H increases are prevented, a result indicating that they are caused by the exchange of intracellular hydrogen ions for extracellular sodium ions. Moreover, the *p*H increases occur even when decreases in cyclic AMP concentrations in the cells are blocked. Activation of Na^+/H^+ exchange therefore appears to be an independent effect of the receptor activation.

According to Limbird, all the agents that modulate the Na^+/H^+-antiporter also modulate the binding of stimulatory agents by the purified α_2-adrenergic receptor. "It is a very reasonable speculation that the receptor is the antiporter," she says. "But do we have the proof? No." In future experiments, Limbird plans to introduce the purified receptor into an artificial membrane system to determine whether it can behave like an antiporter and exchange hydrogen ions for sodium ions, as she has postulated. ■

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