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5

Signal Transduction in Biological Membranes

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1. Information Processing: Some Generalities

Information processing is the key to the biology of learning and adaptation. Receptors are essential components of such cognitive systems. However, it should be emphasized that receptors *per se* have no intrinsic function; i.e., as with facts, they cannot speak for themselves. To be functional, receptors must be incorporated into systems that process or transduce the incoming signals. On the other hand, to the extent that their removal results in loss of information processing, receptors are the key to the phenomenon known as habituation or desensitization, i.e., the waning of information processing observed on repeated signal input.

Two general types of information-processing systems are commonly observed. One is short term in that signal processing is rapid in both onset and offset. The other is long term and expresses memory of the input system long after the initial signal has been withdrawn. For experimental purposes, the former systems are more amenable for detailed study and are the subject of most of the discussion in this volume.

Over the past 20 years, receptors have passed from the near-mythical quality with which they were held to that of the macromolecules they have proven to be. For example, the nicotinic cholinergic receptor has been purified and shown to consist of five separate proteins that are tightly integrated and contain all of the elements necessary for information processing including signal recognition, transduction, and conductance of ions. Hence, information processing with this system involves multiple

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components integrated and incorporated into a membrane as a unit. Thus far, this is the only purified receptor system that displays all of the qualities of an information-processing unit and is clearly responsible for responses of the cell to the incoming neurotransmitter.

As is discussed in detail in this volume, hormone-sensitive adenylate cyclase systems are also multicomponent systems. However, unlike the cholinergic system, the components are not bonded in a manner that allows isolation of the complete system as a unit. Moreover, in the case of certain steroid receptors, the recognition component is compartmentalized from the processes in the nucleus where signal transduction takes place. Thus, information processing can be composed not only of distinct molecules but also of molecules that are spread over different parts of the cell.

Because of our ability to tag receptors with radioactive markers, these elements have proven to be the easiest to isolate and characterize. The molecules responsible for transduction have proven to be most difficult to detect and isolate. A major problem is knowing the nature of the signal processing that takes place subsequent to ligand interaction with the receptor. As is emphasized in this and subsequent chapters, it is essential to understand the nature of the signals arising initially from the stimulus of the external signal before one can begin to undertake the task of unraveling the nature of transduction. The enormity of the problem is underscored by the actions of such hormones as insulin, which induces a variety of different responses in its target cells, including enhancement of growth, stimulation of synthesis of proteins, fat, and carbohydrates, and alterations of ion, amino acid and sugar transport. Are these various responses the result of a unique chemical signal produced by a single information-processing system, or are there multiple information-processing systems intercalated with a common pool of receptors?

2. *Transduction and the Adenylate Cyclase System*

As a biological term, transduction has classically referred to the transfer of information between viruses and bacteriophages and their host cells. Put into a somewhat different context, several years ago transduction was used as a term for describing the transfer of information between the receptors for a variety of hormones and adenylate cyclase, the enzyme responsible for the production of cAMP (Rodbell *et al.*, 1969). At that time, hormone-sensitive adenylate cyclase systems were the only information-processing systems that could be investigated at the level of isolated plasma membranes. As a model for information transfer, it was

suggested that the system can be described in abstract terms as a tripartite system composed of recognition (R) or discriminator units, transducer, and an effector (E) or amplifying component that produces signals at a higher level than the incoming signal. After a decade of research, it became clear that, in fact, recognition, transduction, and amplification are carried out by distinct macromolecular components. Although not fully understood, transduction involves GTP-binding proteins (abbreviated N). Here I discuss briefly the possible role of receptors in the function of the N units, the possible organization of receptors and N units in the membranes, and the growing evidence that N proteins may be responsible for signal transduction in other membrane information transfer systems.

The production of cAMP in animal cells is one of the most highly regulated processes known in biology. A large and ever growing number of hormones and neurotransmitters, each acting through distinctive receptors, regulate the production of cAMP by acting on adenylate cyclase systems in the outer cell membrane. Because of the multiplicity of receptor types, it is clear that receptors are distinct molecules from the enzyme. The discovery that hormone action invariably requires the presence of micromolar concentrations of GTP, whether the hormones act by stimulating or inhibiting adenylate cyclase, led to the concept (schematically represented in Fig. 1) of distinct GTP-binding proteins, designated N_s and N_i, that control, respectively, stimulation and inhibition of adenylate cyclase. This concept has been verified recently by the isolation of two GTP-binding proteins that have the properties of N_s and N_i (Sternweiss *et al.*, 1981; Bokoch *et al.*, 1983).

Still controversial is how the receptors are linked to the N units. One hypothesis suggests that R units only become coupled to N units on liganding of hormones to their receptors (Stadel *et al.*, 1982). In this theory, the act of coupling induced by hormones is the mechanism by which the N units become "activated." An alternative theory (Rodbell, 1980) suggests that receptors and N units may exist in free and coupled forms but that only the latter are responsible, when occupied by hormone and GTP, for activation of N. Indirect evidence that R and N may be complexed as large aggregates or complexes was obtained from target analysis of two systems (Schlegel *et al.*, 1979, 1980). In these studies it was found that the combined actions of GTP and hormones converted the large targets to smaller targets. This finding led to the theory that the concerted actions of hormone and GTP cause disaggregation of oligomers of RN complexes into forms ("monomers") that were capable of interacting with adenylate cyclase. In contrast to the coupling theory of hormone action, this theory suggests that receptors complexed to the N units prevent the latter from interacting with the enzyme; the small ligands (hormones and

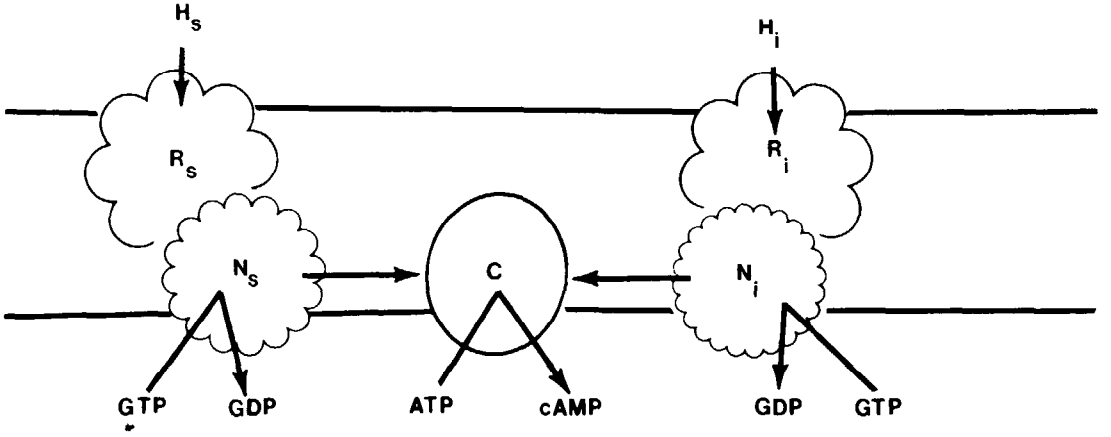

Figure 1. A schematic representation of GTP binding proteins that stimulate (N_s) or inhibit (N_i) adenylate cyclase and also have GTPase activity.

GTP) serve to release these structural constraints, bringing about "activation" of the N units.

Consistent with this theory are recent studies of the reconstitution of purified N units and β-adrenergic receptors in lipid vesicles (Brandt *et al.*, 1983). Hormone action was followed by measuring the production of inorganic phosphate and GDP by a GTPase activity associated with the N_s unit. In the absence of receptor (and detergents that inhibit GTPase activity), the N_s unit displayed appreciable GTPase activity. However, when N_s units were reconstituted in lipid vesicles with receptor, no GTPase activity was detected. Following the addition of hormone, GTPase activity was restored to levels observed with the uncoupled N_s units.

Based on mathematical modeling of the action of a nonhydrolyzable analogue of GTP (guanylylimidodiphosphate), it has been suggested that the enzyme system can take distinct transition states and that there is a slow transition to the "activated" state in the absence of hormone. The role of the hormone is to accelerate the rate of this transition, leading to activation of both adenylate cyclase and the GTPase associated with the N units (Rendell *et al.*, 1975, 1977). According to the "disaggregation" theory of hormone action, the hormones accelerate the rate of release of the N unit from its oligomeric association with the RN complex; in the absence of hormone, nonhydrolyzable analogues induce slow release of the N units. Presumably GTP is relatively inactive because the rate of hydrolysis by the "activated" GTPase on the released N unit is faster than the rate of activation (or coupling) with adenylate cyclase.

Although the disaggregation theory can explain the kinetic behavior of adenylate cyclase systems and has the merit of relating structure with function, the actual process of hormone transduction is still poorly understood. The recent findings that N_s and N_i are heterodimers and that guanine nucleotides or fluoride ions induce dissociation of the heterodimers into "active" forms of the GTP-binding protein (see Gilman *et al.*, Chapter 10) raise the possibility that hormones and GTP act by inducing dissociation of the GTP-binding subunit from the complexes between R and N units.

3. *GTP Binding Proteins: A Family of Membrane Regulatory Proteins*

In addition to N_s and N_i, it was also postulated a few years ago (Rodbell, 1980) that there may be types of N units (N_x) that regulate membrane processes other than adenylate cyclase. The basis for this sug-

gestion was the finding that, as with receptors coupled to N_s or N_i, agonist binding to receptors that were not involved in the regulation of adenylate cyclase activity produced a marked decrease in binding affinities in the presence of guanine nucleotides. Since such changes in receptor affinity states are associated with the transduction process in the case of adenylate cyclase regulation, it seemed reasonable to suggest that other receptor types were similarly linked to GTP-dependent processes involving N units. Since that suggestion, there have been several reports that guanine nucleotides are involved in the regulation of membrane-bound protein kinases (Walaas *et al.*, 1981), cAMP phosphodiesterase (Heyworth *et al.*, 1983), and calcium gating in mast cells (Gomperts, 1983). However, the best evidence thus far that N units regulate processes other than adenylate cyclase is the isolation of an N unit, termed transducin, that regulates a cGMP phosphodiesterase in rod outer segments. Transducin is activated by light activation of rhodopsin. Recent papers have shown that transducin has a remarkably similar structure to that of both N_s and N_i in that not only do they have identical β subunits but their α subunits (the GTP-binding proteins) share structural homologies (Manning and Gilman, 1983; Abood *et al.*, 1982).

Although there is still only indirect evidence for the participation of N units in other membrane functions, the reasons are now even more compelling to invoke the general thesis that GTP acts, through a family of N units, on a variety of membrane-associated processes, each of which has specific types of receptors associated with the N units.

Clearly needed for testing this hypothesis are other types of signal-generating systems similar to adenylate cyclase and cGMP phosphodiesterases that can be examined with cell-free membrane preparations. Since many hormones appear to affect the metabolism of phosphatidylinositol, we may learn that there are, indeed, other signal-generating systems with which the thesis can be examined. These may involve both calcium gating and the production of inositol triphosphate.

4. *Organization of Receptors and Transduction Elements in Membranes*

A popular theory of hormone action is that receptors are distributed uniformly over the surface of the membrane and that the action of the hormone is to induce the receptor to react with an effector system. Consistent with this idea are numerous studies showing that some hormones induce receptor aggregation and that aggregation correlates with transduction. A notable example of ligand-induced aggregation of receptors

that demonstrates this point is the immunoglobulin E(IgE) receptors on mast cells, which, when liganded by IgE, initiate exocytosis (Perez-Montfort *et al.*, 1983). The receptor in this case is a composite of at least three subunits. However, it is not known whether this complex contains all of the elements necessary for signal transduction or what the nature of the molecular consequences of receptor aggregation is.

Another model system for receptor organization and transduction is the rhodopsin-stimulated phosphodiesterase system in rod outer sgements discussed briefly in Section 3. In the absence of light, the N units (transducin) are associated with the inner or cytoplasmic face of the rhodopsin membrane in the form of large (9- to 12-nm) particles (Roof and Heuser, 1982). These particles are one-tenth the concentration of rhodopsin, in accord with the ratio of rhodopsin to transducin in these membranes. When exposed to light and GTP, the particles dissociate from the membrane coincident with activation of phosphodiesterase (Roof *et al.*, 1982). Only a quantum of light is necessary to discharge hundreds of transducin molecules (Pober and Bitensky, 1979). The structural basis of such amplification is not understood. As a possibility, one might consider that rhodopsin molecules in the membrane interact in a concerted fashion such that one light-activated molecule is sufficient to "energize" the release of all associated transducin molecules. In this case, the efficiency of transducin is dependent on the organization of a large number of receptor molecules to which relatively few transducer molecules are attached.

As discussed in Section 2, the receptors and N units involved in adenylate cyclase regulation may be complexed in the form of large oligomeric structures. In view of the concentrations of receptors and N units in most membranes, however, they can only cover small patches of the membrane. Less certain than the rhodopsin–transducin relationship is the relative concentration of R and N units involved in the regulation of adenylate cyclase. In many cyclase systems, occupation of only a few percent of the total population of receptors leads to essentially maximal activation of adenylate cyclase. The simplest explanation is that the concentration of the enzyme is far less than that of the receptors and the N units. However, the situation is more complex, since the relative concentrations of N_s and N_i have to be considered in the equation.

5. *Summary*

To summarize this brief introduction to the subject of transduction in biological membranes, two systems—the light-activated phosphodiesterase system in rod outer segments and hormone-sensitive adenylate

cyclase systems in the cell membrane—have proven to be excellent model systems for investigating signal transduction. Perhaps the most revealing aspect of the transduction process thus far ascertained is that they are extraordinarily complex systems. Each system contains a minimum of five units if only the receptor, N units, and enzyme are counted. More, certainly, are required for the overall transduction processes. The second notable point is that transduction in these two systems involves the participation of not only the initial signal input at the receptor level but also regulatory ligands (GTP and metal ions) that act in concert to bring about the large changes in structure accompanying the transduction processes. The third point is that the transduction processes involve changes in the distribution of the components within the membrane or the association of the components with the membrane. Finally, and perhaps most importantly, it appears that the receptors and their associated N units are organized in fashions poised for amplification of the incoming signals. The precise nature of the organization and the events taking place immediately following alterations in the receptor are still largley unresolved issues. One point that is clear from the studies reported thus far with these model systems is that the cascade of events among signal receptor, transduction, and amplification involves dissociations and associations between the various macromolcules comprising these systems. Thus, the actual messengers are the proteins that shuttle back and forth both within and out of the framework of the membranes.

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