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MOLECULAR MECHANISMS OF INTERCELLULAR COMMUNICATION: TRANSMEMBRANE SIGNALING

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Introduction

Active immunocytes are among the most dedicated and apt practitioners of information exchange. Often, immunocyte conversations depend upon molecular terms; the molecular message can be found in the medium (1). In many instances the rationale of these interactions is well understood. Lymphocytes and macrophages working in close collaboration recall, initiate, amplify, or suppress particular immunological responses to a wide array of antigens (2). It is within the context of these purposeful immunocyte interactions that we introduce this discussion of transmembrane signaling.

The compelling importance of cell-cell interactions is clearly not limited to the rather hectic exchanges between lymphocytes and macrophages. The essential nature of such interactions becomes increasingly apparent in informationally complex endocrine and neuronal functions as well. We wish here to emphasize the molecular anatomy of transmembrane signaling, the principal focal point in cellular dialogues.

The Need

The fossil record reveals that over 3.5 billion years ago, living things had already provided a unique leavening to the primordial dough. These earliest preserved life forms were single-celled organisms with the appearance of rod-shaped bacteria. The habit of solitary living was not one which cells readily abandoned. Another 2.5 billion years elapsed before living creatures began to experiment with multicellular organization(3). What had these primitive collections of cells gained by abandoning their splendid isolation? Clearly, size alone could prove to be an advantage in food gathering and predation. The emergence of multicellularity also allowed cellular specialization. This capability was of greater ultimate significance for the future of the emergence of more complex living forms. Different parts of the organism could perform specialized tasks for all of the other cells. This gradually permitted enormous advances (at the whole organism level) in locomotion, environmental surveillance, object manipulation, the development of cellular and humoral defenses, and eventually, to neuronal specialization and problem-solving behavior.

Many, if not all, of these specializations and improvements were absolutely dependent upon intercellular communication. Accurate, rapid, sensitive, and dynamic communication was indispensible to allow the degree of coordination required by an emerging multitude of new and remarkably specialized cells.

Transmembrane Signals: A Mechanism for Transduction

A central process in the molecular anatomy of intercellular communication is signal transduction. By this is meant the process by which an external molecular signal can be communicated across the cell membrane. This process involves, in some instances, binding of the signal molecule to a cell surface receptor. Subsequent events produce the elaboration of a

second,intracellular signal which can implement the intent of the primary, external, receptor-bound molecule (4).

There are variations on this theme. The binding of signal molecules to cell surface receptors can modulate the gating of an ion channel. Extracellular ions, such as calcium, are then permitted to enter an otherwise forbidden compartment such as the cytoplasm where they can serve as evanescent intracellular signals (5).

Yet another widely exploited solution to the problem of transduction is exemplified by the steroid hormones. The cell membrane does not constitute a barrier to the lipid soluble steroids. These molecules can readily enter the cytoplasm and bind with high affinity and specificity to soluble binding proteins. These (now occupied) steroid binding proteins can enter the nucleus where they modulate transcriptional events. This steroid-induced perturbation results in the increased production of specific classes of messenger RNA, and ultimately the proteins which they encode (6).

Two Signal, Multicomponent Membrane Switches: Hormone Activated Cyclase and Light Activated Phosphodiesterase

We will focus upon two remarkably effective, widely utilized, and closely related membrane switching mechanisms. The hormone-activated adenylate cyclase system (7) and the light-activated phosphodiesterase system of the photoreceptor (8,9) share a remarkable number of regulatory features and homologous components (10). Unlike the photoreceptor phosphodiesterase system which has evolved the specialized feature of activation by light, the hormone-activated cyclase system responds to a great variety of hormonal agonists (7). Each of these agonists binds with resolute specificity and high affinity to an appropriate, cyclase-affiliated, integral membrane receptor protein. This ligand/receptor interaction reliably causes the production of the intracellular second messenger, cyclic-AMP, which modifies enzyme function by orchestrating the phosphorylation of specific target proteins (11).

The light-activated phosphodiesterase and hormone-sensitive cyclase systems share a complex regulatory algorithm which exhibits some prototypic regulatory characteristics. These include rapid response, signal amplification, and exquisite sensitivity which can be altered by prolonged exposure to the signal (12). This shared algorithm has been extensively studied and is well characterized (13). It is an algorithm of control which appears to be utilized by many different types of specialized cells, including immunocytes (14).

The Generalization of an Effective Membrane Switching Device

How can one explain the fact that such specialized and complex systems have been exploited by many different cell types, all displaying unique responses to different cell-specific signals? The macromolecules which cooperate to convey the signal across the membrane depend upon a guanine nucleotide binding protein which is remarkably well conserved. Although the core of the cyclase system is strongly conserved, it has been adapted to

control an extraordinary range of cellular functions; many classes of agonists function via the cell-specific cyclase system.

There is presently no decipherable molecular fossil record. While it is possible to visualize fossilized cellular and whole organism morphology, we can only make inferences about the molecular features which characterized and vivified these preserved forms. However, contemporary primitive life forms suggest that transmembrane molecular signaling devices, such as adenylate cyclase, appeared very early in evolutionary history. Moreover, these systems appear to exploit "plug compatible" molecular connectors which permitted substitution at the specialized receptor domain. The GTP binding and hydrolyzing components and their contiguous, interacting domains within the signal receptor and enzymatic "read out" components, were retained in each of the systems. The earliest signals were probably concrete, physical or molecular aspects of the cellular environment, such as photons, sugars, or amino acids. It may have taken evolutionary millennia for the development of abstract signals such as glucagon (15), vasopressin (16), or lymphokines(1), which present systemically relevant functional requests to their target cells.

The currently recognized catalogue of signaling elements include photons, peptide hormones, catecholamines and other neurotransmitter molecules and the prostaglandins. Within each of these large classes of agonists or signal molecules, there are many individual components. Moreover, a number of membrane switching devices have evolved to respond exclusively to a single or a few members of each class. E.g., different adenylate cyclase systems exhibit marked differences in specificity and susceptibility to the more than thirty known prostanoids (17). The same may be said of the many peptide and neurotransmitter signal molecules which influence a host of cell-specific adenylate cyclase systems (4).

The Molecular Anatomy of the Membrane Switch

In Figure I the components of the retinal rod-cell version of the transmembrane signaling apparatus are schematically depicted. When we began to study this system about twelve years ago (18), the identified components were few indeed. In the course of studying such transmembrane signaling systems, the components are uncovered sequentially and the initial working model is inevitably too simple. The naive simplicity of initial working models emerges when one attempts to reconstitute the functional system from its purified components. This effort will often demonstrate that unknown or unidentified components have been omitted from the reconstitution protocol, and that the partially reconstituted system either does not work at all or exhibits only rudimentary function, lacking many features of the fully integrated system as it exists *in vivo* (19).

The astonishing complexity of the actual mechanism is neither capricious nor inadvertant. It would appear that each of the components endows the system with added levels of functional efficacy, i.e., each of the components provides either opportunities for regulation, amplification, or enhanced function, which could not be accomplished with a simpler molecular anatomy.

It is possible that the components which are depicted in Figure I are an incomplete set. While these components appear to largely support the transduction mechanism of light-activated rod phosphodiesterase, it is entirely possible that additional, as yet unidentified components, will be revealed by future studies.

Compositional Analysis of the Membrane Switch: The Three Segment Analysis

Analysis of Figure I reveals three major segments of the regulatory algorithm. The receptor segment includes rhodopsin [the photon capturing molecule, whose entire amino acid sequence and membrane topology have been described (20)] , opsin kinase [an ATP utilizing protein which can phosphorylate rhodopsin (21)], and a phospho-opsin phosphatase which can remove enzymatically attached phosphate moieties. Evidence for the latter component is indirect.

A second major segment of the photoreceptor transmembrane signaling device is assembled about the GTP binding protein (22,23). The segment also includes a "helper" protein and a low molecular weight component, now called 6K. This middle section of the regulatory algorithm is best described as a communicating segment. It communicates formation of the activated receptor to the catalytic readout apparatus.

A third segment embodies the catalytic function, in this case carried out by cyclic GMP phosphodiesterase (24). An inhibitor component is directly involved in the regulatory algorithm, i.e., light activation of the system requires relaxation of the inhibitor's interaction with the catalytic moiety (25).

Functional Dynamics of the Membrane Switch

In examining the workings of this model, the following features emerge. The first event in signal transduction is, of course, signal capture. This is a stochastic process, a numbers game which pairs signal and receptor in a mating ritual. The process is imperfect. In the photoreceptor adaption of the membrane switch, the signal is a photon which is captured by rhodopsin. The probability of bleaching following photon absorption by rhodopsin is only 50%; only about 70% of incident photons are absorbed in spite of the fact that each retinal rod contains an array of 3×10^9 rhodopsin molecules. In the hormone-receptor variation on this theme, much lower efficiencies can result from hormonal loss as a consequence of the precarious circulatory odyssey which is the obligatory fate of many signal molecules.

Interaction of signal with receptor gives license, via putative conformational changes, for the acquisition of a GTP by the GTP binding protein which constitutes the middle portion of the transmembrane switching apparatus (22). When the GTP-binding protein acquires GTP, it thereby becomes an activator (23). This activator interacts with the bound inhibitor of the third segment, releasing the catalytic activity of

phosphodiesterase from inhibitory constraint (26). Thus, the feed-forward or activation step depends upon signal capture, GTP acquisition (formation of activator), and activation of the catalytic unit by physical interaction of the activator with the inhibitor moiety.

The turnoff mechanisms of the photoreceptor system appear to involve the following:

1. The activation of bleached rhodopsin. Since there is striking amplification at the rhodopsin locus, (i.e., bleached rhodopsin produces many activator-GTP complexes), it is clearly necessary to rapidly remove the bleached rhodopsin. Regeneration of rhodopsin by retinal is far too slow to satisfy the needs of the system for a functional recovery. Inactivation of rhodopsin by phosphorylation is an important possibility, though evidence is indirect (27).

2) The rapid inactivation of the GTP-binding protein is accomplished by the hydrolysis of bound GTP to GDP forming an inactive complex (28). This hydrolysis requires the presence of the "helper" subunit (29) and possibly the 6K protein. The inactivation of phosphodiesterase is complete with the reassociation of the inhibitor moiety to the phosphodiesterase (26).

Conceptual and Functional Homology Between a Light-Activated Retinal Phosphodiesterase and the Hormone-Activated Adenylate Cyclase System

An analysis of the algorithm which modulates the light- and the hormone-activated systems reveals remarkably similar regulatory components (Figure II). There are certainly exceptions to this homology, in the adaptations of receptor specificity (light verses hormones) and different catalytic readouts. Moreover, a number of components in both the cyclase and phosphodiesterase systems are not yet definitively identified. However, the power of the analogy and the reality of the homology are emphasized by recent studies which indicate that it is possible to functionally exchange components between the two systems (10,13). For example, an adenylate cyclase system derived from hypothalamic synaptosomal particles will respond either to the GTP-binding protein and/or illuminated rhodopsin from both amphibian and mammalian photoreceptor systems. Such component exchange gives clear support for the idea that the regulatory algorithm exhibited by these transmembrane signaling mechanisms was developed early in evolutionary time and has been highly conserved. Other component exchanges have used the inhibitor moiety of the photoreceptor system. This heat stable protein inhibits the catalytic moiety of adenylate cyclase and this inhibition can be reversed by the activator (GTP/GTP binding protein) complex from the photoreceptor system.

Membrane Fluidity and the Cytoskeleton

It will be readily seen from the structural and functional analysis of this switching system that many of the components are integral or peripheral membrane proteins which, in the course of active transmembrane signaling, need to physically interact. For this reason membrane "fluidizing"

molecules, such as cis-vaccinic acid, enhance the function of this signal transduction cascade while molecules which diminish fluidity such as cholesterol, have the opposite effect (30). In addition, cytoskeletal interactions with membrane lipids and integral membrane proteins also appear to constrain lateral diffusion of the adenylate cyclase components. Thus, compounds which can disorganize microtubular arrays such as colchicine and vinblastine also facilitate interaction between the cyclase components, thereby producing a striking enhancement of activation by cyclase agonists (31). Axelrod has recently described a series of membrane embedded enzymes whose activation by catecholamine binding also facilitate the adenylate cyclase mechanism as a consequence of membrane fluidization. Hormone activated methylation of membrane phospholipids is postulated to cause their translocation from inner to outer membrane leaflets, thereby causing an increase in membrane fluidity (32).

Sensitivity

The systems here described vary in their degree of sensitivity and their capacity to respond to signals of varying intensity. In the photoreceptor system a true quantum counting mechanism exists. The dark adapted vetebrate retina can reliably detect a single photon (33). Because of the high affinity and specificity of the ligand/receptor interactions at the hormonal receptor locus, small amounts of circulating hormone molecules can elicit striking responses from target tissues. Typical circulating levels of glucagon (a peptide which activates hepatocyte adenylate cyclase) fall in the picomolar range where the peptide can effectively orchestrate hepatocyte glycogenolysis (34).

Many of the signal detection systems exhibit a profound modulation of sensitivity. This is seen both with the photoreceptor system (35) and in the cyclase-related hormone receptor systems (12). Sensitivity loss is transient and follows upon exposure to specific agonists. Such exposure may consequently cause refractory periods during which transmembrane signaling is inhibited. Intriguing models exist suggesting that this desensitization process may reflect receptor modification as well as the binding of guanine nucleotides to an inhibitory moiety (12,36).

Amplification

The systems exhibit extraordinary amplification. The photoreceptor system has been extremely useful in the analysis of this parameter. It has been found that a single photon can program the formation of over 500 activator moities, i.e., complexes between GTP and its binding protein. If each of these activate a phosphodiesterase, which exhibits a turnover number of 800 sec^{-1} (24), the number of molecules of cyclic GMP hydrolyzed per photon captured per second can approach 4×10^5 (37). These displays of signal amplification and system sensitivity are characteristic of transmembrane switches and provide communicating cells with greater plasticity and dynamic range for their signaling transaction.

Derivative Effector Systems:

In the systems described here, elaboration of a second messenger, i.e., cyclic AMP, or the generation of protons by the light-activated hydrolysis of cyclic GMP (37), represent a secondary level of response which still has to be transduced into molecular and cellular consequences. In many cases, protein phosphorylation is the mode of signal propagation, especially where cyclic AMP or Ca^{++} function as second messengers (11). The metabolic changes produced by light-activated rod phosphodiesterase appear to control the uptake and release of calcium (perhaps in exchange for generated protons) which in turn modulates the photoreceptor sodium conductance (39). The resulting control of rod membrane voltage by Ca^{++} is the strategic event which supports visual excitation in vertebrate rods (40).

Summary and Conclusions:

In this short discussion of transmembrane signaling, we have attempted to depict a particular class of signaling devices whose functional characteristics may well be representative of broader classes of membrane switches. These multicomponent aggregates are characterized by tight organization of interacting components which function by conformational interactions to provide sensitive, amplified, rapid, and modulated responses. It is clear that the essential role of such switches in cell-cell interactions necessitated their appearance early in the history of the development of multicellular organisms. It also seems clear that once such devices made their appearance, the conformationally interactive moieties were firmly locked into a regulatory relationship. Since modification of interacting components could perturb or interfere with the functional integrity of the whole switch, genetic drift was only permitted at the input and outflow extremes. I.e., adaptive changes in receptor input or enzymatic readout were permitted. However, the GTP binding moiety and its interacting protein domains on contiguous portions of the receptor and readout components were highly conserved.

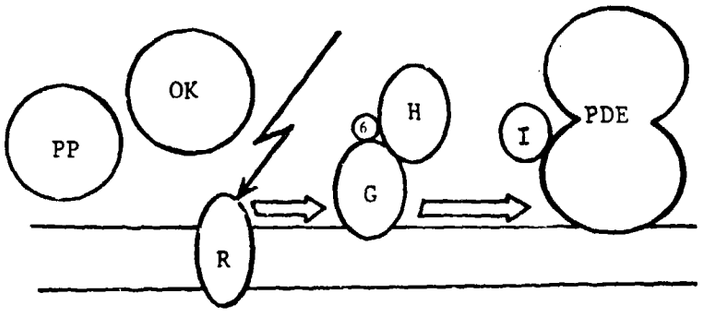
The observed stringent evolutionary conservation of the molecular features of these membrane switches thus applies primarily to the central (GTP binding) elements. An extraordinary degree of variation was permitted within the domains of signal recognition and enzymatic output. Thus, time and evolution have adapted the central logic of the regulatory algorithm to serve a great variety of cellular purposes and to recognize a great variety of chemical and physical signals. This is exemplified by the richness of the hormonal and cellular dialogues found in primates such as man. Here the wealth of intercellular communication can support the composition and performance of symphonies and the study of cellular immunology.

FIGURE I

Top: The molecular components of the light-activated phosphodiesterase system of the vertebrate photoreceptor. Light is absorbed by rhodopsin (R) which activates a guanyl nucleotide binding complex [composed of G, H, and 6(K dalton) subunits]. The activated complex interacts with an inhibitory subunit I of cGMP phosphodiesterase (PDE) to activate the enzyme. Bleached rhodopsin is phosphorylated by opsin kinase (OK) and is presumably dephosphorylated, possibly after regeneration, by a protein phosphatase (PP).

Bottom: The control algorithm of the photoreceptor phosphodiesterase system. Bleached rhodopsin (R*) serially activates many G complexes by stimulating exchange of GTP for GDP bound to the G subunit of the complex. The hydrolytic, "helper" subunit (H) inactivates the complex by hydrolyzing bound GTP. The terminal phosphate of R* continues to activate G complexes until it is itself inactivated by phosphorylation or regeneration.

FIGURE I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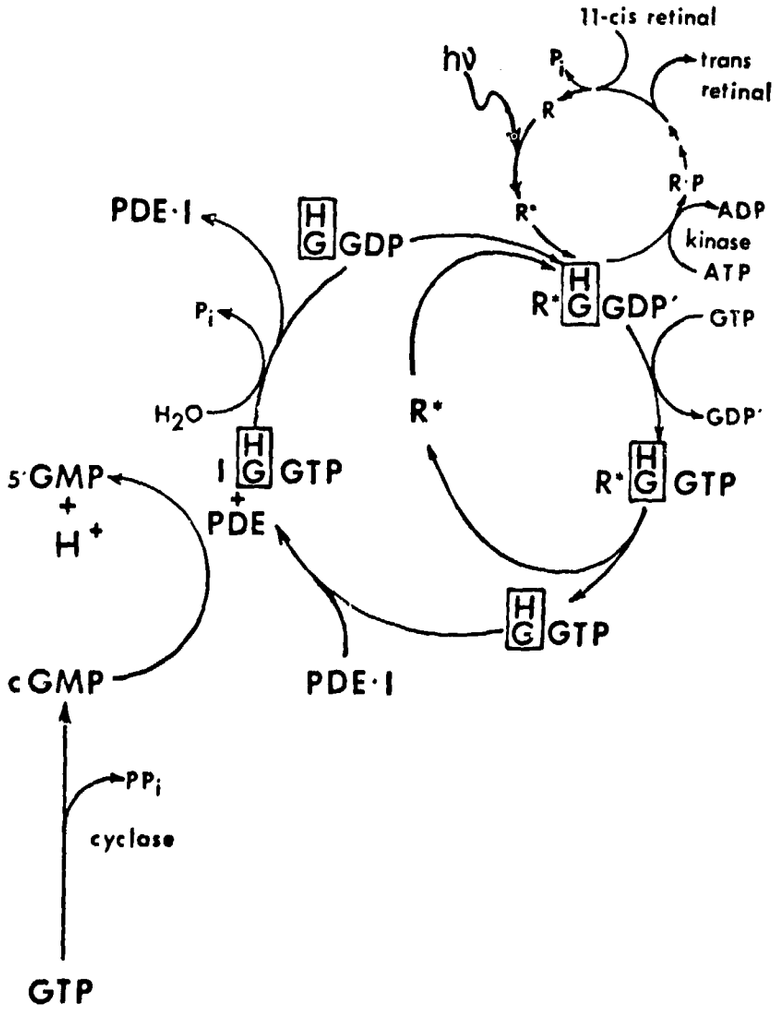
11-cis retinal
hν
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trans retinal
R
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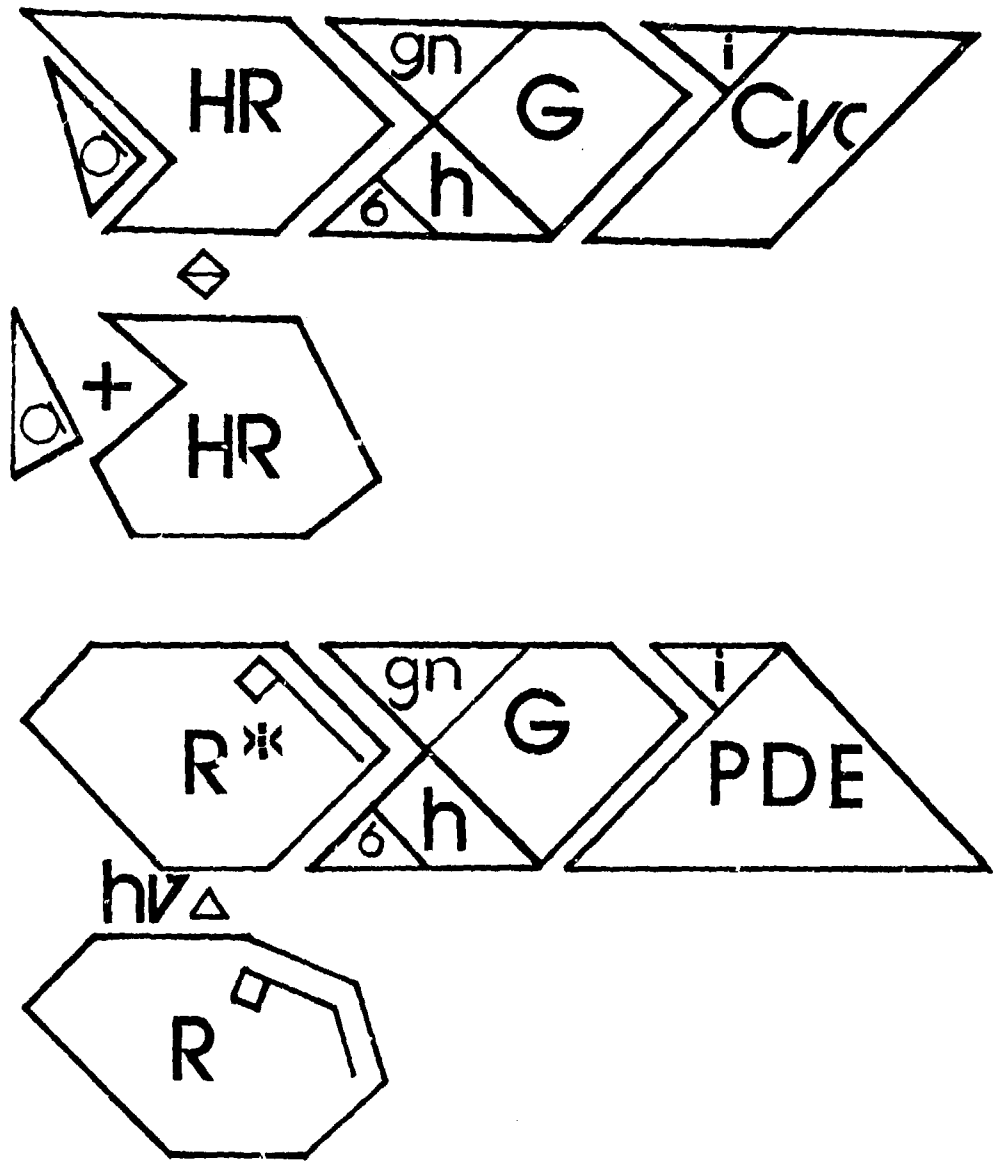
R*
ADP
kinase
ATP
PDE·I
HG GDP
R* HG GDP'
GTP
GDP'
Pi
H2O
R*
I HG GTP
PDE
R* HG GTP
5'GMP
+
H+
HG GTP
cGMP
PDE·I
PPi
cyclase
GTP

FIGURE II

The molecular analogy between hormone-sensitive cyclase and light-sensitive phosphodiesterase systems. Agonist (a) binding produces conformational changes in the hormone receptor (HR) allowing it to activate a guanyl nucleotide (gn) binding protein composed of G, h, and 6 (K dalton) subunits by stimulating exchange of GTP for GDP. The G complex interacts with an inhibitory subunit (i) of adenylate cyclase (Cyc) to stimulate synthesis of cAMP. The G complex is subsequently inactivated by hydrolysis of bound GTP.

Light ($h\nu$) absorbed by 11-cis retinal of rhodopsin (R) isomerizes the chromophore to all-trans and produces conformation changes in the protein. Bleached rhodopsin (R*) activates the G complex which in turn activates phosphodiesterase. Components of the systems are experimentally interchangeable, suggesting that the G subunit and interface domains (receptor-G, G-catalytic output) are evolutionarily highly conserved.

FIGURE II



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