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Review Article

Transmembrane Signaling and Animal Evolution

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CONTENTS

ABSTRACT

Ren-Jye Ho (1994) Transmembrane signaling and animal evolution. *Zoological Studies* **33**(1): 1-28. An explosive development in our transmembrane signaling (TMS) knowledge has occurred in recent years. Unquestionably, the most exciting recent advances relating to the structural identity of the components of TMS are molecular biological studies. These components include signal molecules, hormone membrane receptors, signal transducing G proteins, second messenger generating effectors, second messenger regulated protein kinases and protein phosphatases, discovered to have numerous subtypes and isoforms. For many of them, physiological importance is difficult to assign. This review lists briefly all these new advances with specific reference to animal evolution. The basic mechanism of TMS may be similar from low to high animal levels. But the number of TMS systems and the TMS components coupling methods show both convergent and divergent patterns. The time is right to focus on a comparative study of evolution of TMS. Clearly, a great deal of interesting research is waiting to be done. It is obvious that, to obtain a new level of understanding through a better informed evaluation, and minimize difficulties both functional and structural studies are equally important to make the knowledge complete along the phylogenetic path.

Key words: Second messengers, Adenylate cyclase, Phospholipases, Invertebrate, Hormones.

INTRODUCTION

All living cells have an inherited ability to respond to the changing environment by modifying their internal status to fulfill the survival principle of life. Only the higher animals, such as man, are capable of adjusting the environment. Any inappropriate response, may lead the cell to damage, disease or death.

In multicellular organism such as mammal, cells are differentiated to form organized tissues, organs and systems with specialized functions. Among these, there are specialized communicating systems to integrate the function of the whole body via different transmembrane signaling systems (TMS's). As the organisms become more and more complex along the evolutional path, the types of stimuli and types of responses as well as the ensuing relationships also become more complex. In these organisms, communication may be established not only between one differentiated organ to the other, but also between one organism to another, or between one sex and the other. Certain specialized chemical communication is therefore developed. Failure in integrated TMS are often found in many diseases, such as altered chloride channel in cystic fibrosis, deficiency of nicotinic cholinergic receptors at the neuromuscular junction in myathenia gravis symptoms, deficiency in β-adrenergic receptors in asthma and the loss of midbrain dopamine in Parkinson's disease among others. In the most severe cases the lives of the organisms are threatened.

The hormone action mechanism is beginning to be understood thanks to the introduction of the second messenger concept by Sutherland and others (1972). Following this direction, increasing numbers of cellular chemical modulators such as hormones, neurotransmitters and pheromones and their second messengers have been discovered. The question of how the second messenger and signal transduction system evolved along the evolutional path has come up among many scientists, even as early as the life time of Earl Sutherland. Several reviews focusing on signal transduction evolution have been written (De Loof and Schoofs 1990, Walker and Holden-Dye 1989, Jenssens 1988, Pertseva 1991, Vender 1984).

Due to the rapid progress and expansion of the scope of research, the present review will focus on the evolution of two signal transduction systems (TMS dealing with signal-activated adenylate cyclases and phospholipases) and crosstalk between these two systems. Recently, many excellent reviews have been written regarding the structures of the hormone receptors and the structure-functional relationship (Hollenberg 1991, Miller 1990, Hausdorff et al. 1990, Strader et al. 1989, Lefkowitz and Caron 1988), G proteins (Stryer 1991, Casey and Gilman 1988, Preissmuth et al. 1989, Birnbaumer 1990, Simon et al. 1991, Hepler and Gilman 1992) and effectors (Rhee and Choi 1992, Danchin 1993). The view that the response of cells as a resultant of crosstalk among different second messenger systems has become a new interest in recent years. The present article is written for the readers of Zoological Studies and reviews the most recent information. By reviewing the TMS of the entire animal kingdom and their evolutional relationship, it is my feeling that the structure of each component,

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Abbreviations used: ACA, adenylate cyclase from slim mold with adenylate cyclase activity; ACG, a protein from slime mold, with no or weak adenylate cyclase activity and has sequence homology with one type of guanylate cyclase; AKH, adipokinetic hormone; APK or PKA, cAMP-dependent protein kinase; AR, adrenergic receptor; cAMP, adenosine 3',5',-cyclic monophosphate; Cam, calmodulin; CCK, cholecystokinin; CD45, a T_4 lymphocyte membrane protein; cGMP, guanosine 3',5'-cyclic monophosphate; CLIP, corticotropin-like intermediate peptide; CRE, cAMP responsive element; CRF, corticotropin releasing hormone; CyA I, type 1 mammalian adenylate cyclase; DAG, diacylglycerol; E, effector; EGF, epidermal growth factor; ESAG and GRSAG, *Trypanosoma* genes encode different forms of adenylate cyclase; FMRFamide, phe-met-arg-phe-NH_2; G, G protein; GPK or PKG, cGMP-dependent protein kinase; HGH, hyperglycemic hormone; 5-HT, 5-hydroxyltryptamine; IP_3, or Ins(1,4,5)P_3, inositol 1,4,5-triphosphate; JH, juvenile hormone; MAchR, acetylcholine muscarinic receptor; PKC, protein kinase C; PIP_2, phosphatidylinositol 4,5-bisphosphate; PLC, PLA_2, and PLD, phospholipase C, A_2 and D respectively; PPase, protein phosphatase or phosphoserine phosphatase; PTPase, phosphotyrosine-specific protein phosphoatase; R, receptor or regulatory subunit; RPCH, red pigment concentrating hormone; *Rutabaga*, a *Drosophila* gene encode adenylate cyclase; TMS, transmembrane signaling; TPA, 12-O-tetradecanoylphorbol-13-acetate; TRE, TPA responsive element; TSHR, thyroid stimulating hormone receptor.

is key to the question, but the coupling of all the components in a TMS, both structurally and functionally, should ultimately be more informative. The limitations of space preclude citing many of the individuals who have contributed to our understanding of transmembrane signalling. I apologize for this unfortunate circumstance.

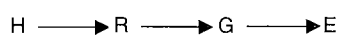
Regarding the second messenger and transmembrane signaling, it is not surprise only the mammalian subjects are extensively studied. Knowledge in the lower vertebrate and in invertebrate in this respect is rather scant. On the basis of the well-studied reports at the top of the evolutionary tree, TMS and their crosstalk can be summarized below in Fig. 1.

There may be six modes by which the signals of hormone (H) can be transmitted through receptor (R) via G protein (G) to effector (E).

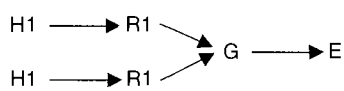
EVOLUTION OF HORMONES AND THEIR MEMBRANE RECEPTORS

It is clear from the literature that transmitter molecules evolved during the initial stages of biological evolution, that is, around one billion years ago. The receptor protein to recognize precise configurations of these transmitter ligands probably also begin to evolve at the same time. In addition, proteins developed around the primary receptor pro-

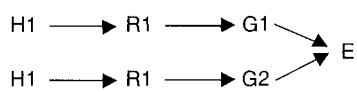
1. Signal of a hormone (H) transmitted by receptor (R) via one type of G-protein (G) to one type of effector (E).



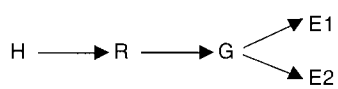
2. Signals of two types of hormones transmitted by two types of H via one type of G to one type of E.



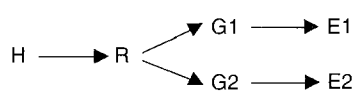
3. Signals of two types of hormones transmitted by two types of R via two types of G to one type of E.



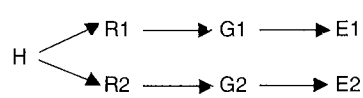
4. Signal of one type of hormone transmitted by one type of R via one type of G to two types of E.



5. Signal of one type of hormone transmitted by one type of R via two types of G to two types of E.



6. Signal of one type of hormone transmitted by two or more types of R via two or more types of G to two or more types of E.



* At all R, G, E levels along the TMS pathways, crosstalk between systems have been found.

Fig. 1. Six modes of transmembrane signals mediated through receptor via G proteins to effector. Six modes of hormone signals. H, R, G, and E represent hormone, receptor, G protein and effector, respectively.

tein and these provided further attachment for other molecules, such as modulators, effectors and ionophore proteins, to refine the transmitting processes and the physiological response.

The improvement of chromatographical, immunological and molecular biological techniques have greatly facilitated the purification and the determination of signal molecules and TMS components. Because of the very different morphology and anatomy of vertebrates and invertebrates, it has been assumed for many years that their physiology and endocrinology should also be quite different. Steroid hormones such as ecdysteroid and juvenile hormones (JH) the major hormones in arthropods are indeed absent in vertebrates. However similarities in neuropeptides suggest that the basic principles of invertebrate and vertebrate endocrine systems may be quite similar. We might find the origin in a common ancestor, in which these principles were probably already quite developed, although not much work has been done at this level. Unlike higher vertebrates, fish have a single gonadotropin acting on both testis and ovary and in both organs it is mediated by an adenylate cyclase/cAMP signal transduction system (Yu et al. 1991). It would be interesting to know whether the tropic hormone of gonads, ovary and testis, is the same in invertebrate. Therefore invertebrate signal transduction research has entered an era of fascinating fast progress. A few years ago, the number of neuro-peptide with hormone action was estimated at no more than a few dozens. Now the number of different neuropeptides, which might have a hormone or neurotransmitter or neuro-modulator function in invertebrates, is estimated to be in the order of a few hundred.

A. Invertebrate peptide hormones

Table 1 shows some major types of hormones and hormone-like molecules in lower forms of animals.

Table 1. Types of hormone and hormone-like molecules in lower forms of animals

Hormone
Peptide molecules
Adipokinetic hormone, hyperglycemic hormone and glucagon
FRMFamide family
APGW-amide and red pigment concentrating hormone
POMC family
Prothoracicotropic hormone
Tachykinin peptide and enkapharline
Arginine-vasotoin and diuretic hormone
Gastrin peptide/cholecystokinin family
Pheromone biosynthesis activating protein
Insulin-like protein and growth factor
Bombyxin
Egg laying hormone (ELH)
Neurotransmitters and biogenic amines.
Acetylcholine
Excitory amino acid
Inhibitory amino acid
Amines
Purines

References are cited in the text.

a. Adipokinetic hormone family and glucagon

Adipokinetic hormone-1 (AKH-1, isolated from locust corpora cardiaca by Stone et al. 1976), and hyperglycemic hormones I and II (HGH-I and HGH-II, isolated from cockroach corpora cardiaca by Witten et al, 1984, Scarborough et al. 1984) stimulate lipid and carbohydrate mobilization in insect. AKH and HGHs and another peptide, the red pigment concentrating hormone (RPCH, identified in crustaceans by Fernlund and Josefsson 1972), all have a partial sequence homology. They are hydrophobic peptides and do not contain acidic or basic residues. Each peptide begins with a pyroglutamic acid residue and the C-terminal residue is amidated. Phenylalanine and tryptophane are at residues 4 and 8 respectively. The peptides stimulate different responses in insects and crustaceans. Both AKH of *Manduca sexta* and HGH II of *Periplaneta aplysia* have sequence homologies and physiological actions in common with vertebrate glucagon (Ziegler et al. 1985, Acarborough et al. 1984). More than 10 peptides have been identified in this family all starting with a pyroglutamic acid residue and the C-terminal residue is amidated. (For review, see De Loof and Schoofs 1990.) Cyclic AMP as shown by Sutherland (1972) is the second messenger in mammalian liver glucagon action. No studies have clearly shown the mechanism of action of the hormones for the AKH-family.

b. FMRFamide family and POMC family

The tetrapeptide FMRFamide (Phe-Met-Arg-Phe-NH_2) is a cardio-excitatory agent in the venus clam *Macrocallista nimbosa* (Price and Greenberg 1977). Its distribution appears to be limited to many molluscans species (Price 1986). However, immunoreactivity to FRMFamide antibodies have been reported in all the major animal phyla and a number of FRMFamide-like peptides have been

isolated from coelenterates (Grimmelikehuijzen and Graff 1985), arthropods (Boer et al. 1980), and vertebrates (Dockray et al. 1983, Yang et al. 1985) indicating FMRFamide-like compounds are broadly distributed among invertebrates and that it is one of a family of related neuropeptides.

The FMRF precursor gene in *Aplysia* exhibits homologies with mammalian corticotropin releasing hormone (CRF), melanocytes stimulating hormone (MSH) and corticotropin-like intermediate peptide (CLIP). It is known that CRF, MSH and CLIP all act via cAMP (Sutherland and Robison 1966, Robison et al. 1971). Several vertebrate- and invertebrate-peptides, containing C-terminal identical or similar to FMRFamide, have been isolated and sequenced (Greenberg et al. 1988). Neuropeptides of *Drosophila*, which have structural features similar to FMRFamide have been described (Namber et al. 1988, Schneider and Taghert 1988). The function diversities of these peptides have been shown, but their action mechanisms have not been studied. The molluscan cardio-excitatory tetrapeptide FMRFamide and related peptide inhibits Na^+-Ca^{2+} exchange and such characteristics resemble the effect of opiates on Na^+-Ca^{2+} exchange. Endogenous FRMFamide may regulate intracellular calcium via Na^+-Ca^{2+} exchange (Khananshvili et al. 1993). Sodium current was initiated when the peptide was applied to the cell outside the patch pipette indicating that a second messenger is likely to be involved in the FRMFamide response. Recently the pheromone biosynthesis activating neuropeptide (PBAN) gene of the silkworm has been shown to encode, besides PBAN, diapause hormone and FMRFamide (Kawano et al. 1992). It indicates that the PBAN gene products not only regulate sexual behavior and diapause but also many biological processes in the silkworm.

FMRFamide also resembles the mammalian methionine enkephalin. The potential for the interaction of this neuropeptide with opioid receptor systems is recognized. In vertebrates FRMFamide-related peptides might be an endogenous opioid antagonist. In *Aplysia* FMRF gene (Taussig and Scheller 1986) contains FMRFamide and FLMFamide and other sequences that have homologies (about 30%) to MSH, CRF and CLIP. In vertebrates the POMC gene encodes these peptides. (for review see Keller 1992.)

c. APGWamide family and red pigment concentrating hormone (RPCH)

APGW-amide is a bioactive tetrapeptide (Ala-Pro-Gly-Trp-NH_2, Kuroki et al. 1990) which is closely related to the RPCH C-terminal. The action of APGWamide is similar to those of RPCH on molluscan muscle contraction or relaxation, but APGWamide is more active than RPCH (Minakata et al. 1991).

The insect prothoracicotropic hormone (PTTH) regulates the synthesis and release of juvenile hormones. There is evidence that the mode of action of this hormone is a membrane receptor-mediated effect and cAMP appears to be its second messenger (Smith et al. 1984). A Ca^{2+}/Cam sensitive-adenylate cyclase is involved (Meller et al. 1990, Allen et al. 1992). There are several factors which regulate its release; a hormone-sensitive phospholipase C (PLC) and Ca^{2+} mobilization may also be involved.

d. Arginine-vasotocin and diuretic hormone

The locust diuretic hormone monomer has 78% sequence identity with the vertebrate hormone arginine-vasotocin. It stimulates water transport by increasing the second messenger cAMP (Prouxa and Herault 1988).

e. Gastrin/cholecystokinin family

Leucophaea heads (Nachman et al. 1986a, b) and brain complexes (Schoofs et al. 1989) contain peptides which have sequence homologies with the active portion of the vertebrate gastrin/cholecystokinin. These hormones have been implicated as a satiety factor in mammals and has been demonstrated to inhibit feeding specific neurons in *Navanax*, a mollusk (Zimering et al. 1988). Two of the most important mammalian gastrointestinal hormones, gastrin and cholecystokinin (CCK), have long been known to share a common pentapeptide carboxyl terminal sequence. Gastrin, which is produced and released from endocrine cells in the gastric antrum, acts as a stimulant to gastric secretion and as a trophic factor on mucosal cells of the intestine. CCK produced and released by mucosal endocrine cells in the upper small intestine acts to stimulate pancreatic exocrine secretion, gallbladder contraction and inhibit gastric empting. The roles of these peptides were complicated when an immunoreactive gastrin was identified in the brain but was found to be CCK. The role of CCK in the central nervous system is still not clearly established. CCK is known to interact with distinct receptors in the periphery and brain. It is now termed CCK_A (for alimentary) and CCK_B (for brain), respectively. These receptors have distinct agonist and antagonist binding properties. The CCK_A receptor was known by functional criteria to be a member of the G

protein-coupled superfamily of receptors. Gastrin receptors have been characterized on the parietal cells and gastric smooth muscle cells and have been found to show binding characteristics similar to CCK_B. The sequence for the rat CCK_B receptor (Wank et al. 1992) and that for the canine gastrin receptor (Kopin et al. 1992) are 90% homologous. Human brain CCK_B/gastrin receptors are identical (Lee et al. 1993). The binding specificity studies by Vigna et al. (1986) suggest these receptors diverged in evolution at the same time as the peptide. Both gastrin and CCK use cAMP, IP_3 and Ca^{2+} as second messengers in mammals (see Table 5). CCK activates G_{i1}-, G_{i2}-, G_{i3}- and G_s-proteins in rat pancreatic acinar cells (Schnefel et al. 1990). The physiology of the invertebrate gastrin/CCK hormones as well as their receptor and mechanism of action, may be similar to their mammalian counter part, but remains to be studied.

f. Pheromone biosynthesis activating neuropeptide

It is known that pheromone biosynthesis activating neuropeptide (PBAN) stimulates biosynthesis and promotes the release of the male-attracting pheromone from the female gland via a adenylate cyclase/cAMP transmembrane signaling pathway. Excellent studies on the structures of insect pheromones and defensive secretions by several groups in the United States, Germany, Japan (for review see Mori 1989) and Taiwan, China (Chow and Lin 1986, Ho and Chow 1993) have been made over the last decade. Only recently the presence of odorant-sensitive phospholipase C in insect antennae (cockroach and locust) was reported by Boekhoff et al. (1990). The enzyme is shown to be present in high amounts and its activity is specifically enhanced by odorants and pheromones in a cell free system in a GTP dependent manner. In view of the type III adenylate cyclase (CyA III) is the effector in mammalian odorant TMS (Bakalyar and Reed 1990), whether there is also an odorant-sensitive adenylate cyclase involved in the insect system or a phospholipase Ca^{2+}/IP_3 system in mammals remains to be investigated.

g. Insulin-like protein and growth factor

Insulin is a life supporting polypeptide it is the first hormone isolated, crystallized and sequenced. And the legendary radioimmunoassay of Benson and Yalow was accomplished using this peptide. As a master regulator of metabolism and a factor for cell growth and differentiation it has been extensively studied, yet the mechanism of action is still not clear. Most knowledge about insulin has been obtained using mammalian subjects.

The existence of an Insulin-like growth factor in lower animal species other than vertebrates is suspected. Indeed, an insulin like peptide in an invertebrate (the flesh fly) was first found by immunocytochemistry by Duve and Thorpe (1979). Then a polypeptide of heterogeneous molecular forms was isolated from the head of *Bombyx*, named Bombyxin (Ishizaki and Suzuki 1984). This peptide family has striking homologies with mammalian insulin, especially in the A chain. Bombyxin is as insulin, the A and B chains have been proven to be connected to each other with disulfide bonds in the same manner. Bombyxin has even higher amino acid sequence homologies with insulin-like growth factor II. It is suggested that bombyxin plays significant roles in metabolism as well as growth regulation and cell differentiation, particularly in embryonic tissues (Fugo et al. 1989). The general organization of the preproinsulin gene is the same as that of the pre-probombyxin gene (Adachi et al. 1989). In another invertebrate species, the growth controlling molluscan neuron (snail), Smith et al. (1988) identified a similar insulin gene and the gene product is an insulin-related peptide. As predicted by Thorpe and Duve (1988) the insulin peptide family has a long evolutionary history, dating back to the pre-vertebrate era. A great many of studies regarding the effect of insulin on general and specific metabolism, second messenger cAMP, cGMP, inositol phosphate metabolism as well as cell growth and differentiation have been carried out over the last few decades. No consensus has been reached. One can be sure that the study of the mechanism of insulin action and insulin like factors of invertebrate origin and the evolution of insulin-like growth factors will be a great challenge in years ahead.

B. Evolution of hormone receptors

For an information carrier molecule such as a hormone or an agonist to activate a target cell, it must first bind in a highly specific manner to its receptor. The binding information must then be translated into an amplified cellular signal that ultimately leads to a biological response. In the second messenger model hypothesis of Sutherland (Sutherland and Robison 1966), a hormone molecule does not enter the cell but its signal activates the generation a second messenger within the target cell. In the last 10 years, tremendous progress has been made regarding identification receptors and receptor subtypes, charactering receptors in terms

of their ligand binding properties, ligand-receptor interactions and the mechanisms whereby they generate cellular signals such as receptor-G protein interactions. These studies have mostly been made with mammalian tissues and hormones. A great deal of work remains to be done in membrane receptors and their relation to hormones and transmembrane signaling of the lower forms of animals.

There are a number of compounds, either natural or synthetic which, have different abilities to bind hormone receptors. A compound that binds to receptors with selective specificity, reversibility and mimic the function of a hormone are called agonists. Compounds that have similar binding properties but do not mimic the function of hormones is called antagonists. An antagonist exhibits competitive inhibition for both binding and function of an agonist or natural hormone at the receptor level. Almost all antagonists were developed as tools for the study of hormone receptors using mammalian subjects. The extent to which an invertebrate receptor recognizes vertebrate antagonists varies greatly, indicating that possible proteins near the receptor protein are less similar than actual receptor proteins from one phylum to another. Therefore, the results of receptor studies from invertebrate and vertebrate tissues using the same antagonist are difficult to interpret.

The basic mechanisms whereby a receptor initiates a cellular transmembrane signal appear to be few. There are three distinctive super families of receptor structures. The receptor may be an ion channel, the receptor may be a transmembrane-regulated enzyme, or the receptor may be a coupled GTP-dependent ligand regulated manner with membrane associated guanine nucleotide binding proteins (or G proteins). The G proteins, in turn, modulate the activity of membrane associated enzymes, or channels.

It is a general feeling that the G-protein coupled hormone receptors usually have properties in common. These receptors are single chain glycoproteins with seven spanning helices. Table 2 is a list of these hormone receptors. The nature of the hormone, however, vary from lipid, amines to proteins. Each receptor is specific for its hormone ligand and may couple to more than one effector via G proteins.

There are certain domains in each specific of the seven membrane-spanning receptors.

a. Ligand-receptor binding domain for ligand recognition, (in the case of beta-adrenergic receptor, the second, third and seventh transmembrane spanning helices (MII, MIII, and MVII) may contribute to forming the ligand binding pocket).
b. A sequence for distinguishing agonist from antagonist
c. Sites for receptor glycosylation linked to the membrane-targeting properties (extracellular N-terminal sequence),
d. Transmembrane domain for anchoring the receptor in the plasma membrane (the membrane spanning domain MI to MVII),
e. A substrate or G protein binding domain (may be the cytosolic loop CIII),
f. A phosphate acceptor domain for enzymatic regulation of the receptor's activity (C-terminal sequence (the serine residues of the C-terminus and maybe CIII).
g. A sequence involved in receptor micro clustering,
h. A domain for interaction of other membrane proteins and to internalization process.
i. A catalytic domain for intracellular receptor enzyme in case the membrane receptor is also an enzyme.

Table 2. Receptors with Seven Membrane-Spanning Helices

Acetylcholine muscarinic (m1-m5)
Adenosine receptor
α-Adrenergic (α1, α2)
β-Adrenergic (β1, β2)
Angiotensin receptor
AVP receptor
Calcitonine receptor
cAMP receptor
Cholescystokinin receptor
Dopaminergic (D2)
Endothelin receptor
Follicle stimulating hormone receptor
Gastrin receptor
Glucagon receptor
Light receptor, rodopsin
Luteinizing hormone receptor
Octopaminergic receptor
Odorant receptor
Opioid peptide receptor
Parathyroid hormone receptor
Prostaglandin receptor
Purinergic (A1,A2)
Serotoninergic (5HT1a to 5HT1e)
Substance K receptor
Substance P receptor
Thyroid stimulating hormone receptor

Fig. 2 shows examples of the structural classification of TMS. There are three groups of

TMS's: A. distinct molecular complex consists of receptor, G protein and effector; B. single macromolecular transmembrane-regulated enzymes; and C. oligomeric ligand-gated ion channels. There is also the mixed group A-B and A-C. A total of 8 signal types, second messengers (cAMP, cGMP, IP_3, DAG and Ca^{2+}) and the second messenger-dependent protein kinases are listed.

C. Neurotransmitters and biogenic amines and their receptors

Neutrotransmitters and biogenic amines in invertebrates have been studied but the extent of the work is no match with that in mammals. The major ones (see also Table 1) are similar to that in mammals with varying degree of importance. The following two which appear to have more literature in terms of TMS are briefly reviewed here.

a. Acetylcholine and acetylcholine receptors

Acetylcholine occurs in tissues through out the animal phyla (Gardener and Warker 1982). Dale (1914) was responsible for the use of the classic terms of acetylcholine receptors into two major classes: 'Nicotinic' (stimulated by nicotine) and 'Muscarinic' (stimulated by muscarine). The two types of receptor proteins are different in size and oligomeric structure. Antibodies raised against one or other failed to cross-react (Frazer et al. 1983). Thus, they may have arisen separately during evolution. The nicotinic receptors are associated with excitatory and fast inhibitory responses and linked to ion channels, and the muscarinic receptors are more likely associated with slow inhibitory responses and linked to transducer proteins and second messenger systems.

Lee (1972) introduced a very useful tool into nicotinic pharmacology when he isolated-α-bungarotoxin from snake venom. This protein can bind irreversibly with the nicotinic receptor site. Nicotinic receptors have been demonstrated in most phyla. An α-bunagarotoxin protein from the insect which has a gated ion channel (Hanke and Breer 1986).

The ligand, quinuclidinyl benzilate (QNB), has been developed as an aid in the localization of muscarinic receptors (Birdsall and Hulme 1976). QNB binding sites have been found in many invertebrate species. The first report regarding inhibition of adenylate cyclase activity by acetylcholine was made by Murade et al. from Sutherland's laboratory (1962). This action of acetylcholine is not mediated by nicotinic receptors but by a muscarinic receptors. Recently, 5 subtypes of muscarinic receptors have been documented (m1 up to m5 AchR, Bonner et al. 1987). m1, m3 and m5 preferentially stimulate PIP_2 hydrolysis and m2 and m4 preferentially inhibits adenylate cyclase

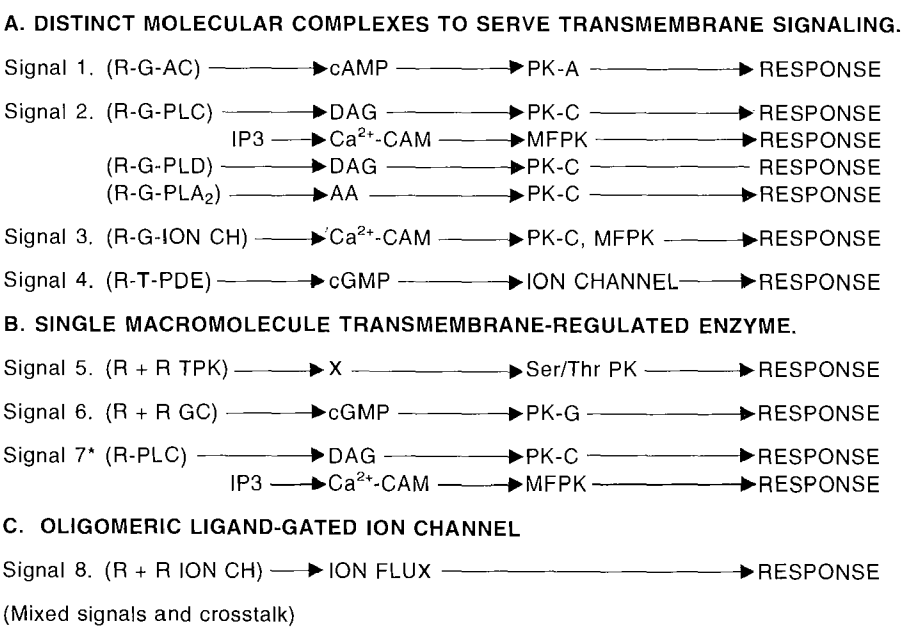


Fig. 2. Examples of transmembrane signal transduction: structural classification. Hormone transduction signals are classified on the basis of macromolecular structure of the receptor into three groups. The components of each type of transmembrane signal, second messenger, protein kinase are shown.

(Bonner et al. 1987, Wei and Hung 1989, Wei and Wang 1990, Wei et al. 1991, Yang et al. 1991, Liao et al. 1989, 1990). Further more, acetylcholine via m1 may stimulates adenylate cyclase and phospholipase C and muscle contraction-relaxation in a reciprocal manner in dog iris sphincter smooth muscle (Abdal-Latif et al. 1992). m1 may also mediate a stimulation of adenylate cyclase and phosphatidylinositol hydrolysis (Felder et al. 1989). It is certain that all m1 to m5 are mediated by G protein. m5 expressed in a cell type absence of proper G protein mediates no TMS (Huang et al. 1992).

This information was mainly obtained from mammalian species. There appear to be three different types of acetylcholine receptors in the insect: 1. broad-spectrum muscarinic and nicotinic receptors, 2. nicotinic receptors (bind α-bungarotoxin), 3. muscarinic receptors (bind quinuclidinyl benxilate). It is certain that more information from invertebrate studies will be reported in the near future.

b. Octopamine, octopamine receptors and other biogenic amines

Octopamine receptors from a number of invertebrates can be distinguished from catecholamine receptors interims of amine specificity. Only D(-) isomer for octopamine is the stereo specific agonist. The invertebrate receptor is similar to the alpha-adrenergic receptors invertebrates and is blocked by α-adrenergic blocking agent such as phentolamine. The former exhibit a preference of monophenolic amines with a single hydroxy group on the aromatic ring, while the latter prefer amines with 2 hydroxy groups on the ring. Considerable evidence indicates that octopamine functions as a neurotransmitter and neurohormone in invertebrates, where it has a physiological role analogous to that of norepinephrine in vertebrates (Harmar and Horn 1977, David and Coulon 1985, Evans 1987). A variety of biochemical and physiological data have supported the presence of octopamine receptor subtypes in different tissues and species (Roeder and Nathanson 1993). The octopamine receptors can be classified into 3 subtypes. Yet no unanimity of classification of the octopamine receptor subtypes is available.

It is not known if each of the three mediates different signal pathways. Octopamine sensitive adenylate cyclase has been found in invertebrate neural and non-neural tissues (De Prisco et al. 1991, David and Coulton 1985). The correlation between activation of adenylate cyclase and light emission from the light organ of the adult *Photinus pyralis* has been reported (Nathanson 1993). The disruption of *Manduca* feeding was also shown to be related to an increase in adenylate cyclase in response to this hormone (Nathason 1993). In the corpora allata *Diptoptera punctata*, Thompson et al. (1990) have shown that octopamine elevates cAMP levels and stimulates the release of allatostatin which then inhibits the release of JH. Lafton-Cazal and Baehr (1988) showed that octopamine elevates cAMP leading to an increase release of JH in *L. migratoria*. Recently the G protein coupled octopamine receptor gene from *Drosophila* has been expressed in mammalian cells, where it mediates the hormone by activating adenylate cyclase activity and exhibits a pharmacological profile consistent with an octopamine type 1 receptor. Sequence and pharmacological comparisons indicate the octopamine receptor is unique but closely related to mammalian adrenergic receptors, perhaps as an evolutionary precursor. If so, the octopamine receptor may have evolved before branching off from the main mammalian evolutionary path some 900 million years ago.

MEDIATION OF TRANSMEMBRANE SIGNALING BY G PROTEINS

The majority of TMS receptors are coupled to an effector by G proteins. They belong to the family of heterotrimeric guanine nucleotide binding proteins that act as switches regulating the information flow from membrane receptors to a variety of effectors. The G protein is believed to be present in all eukaryotic cells, and they control metabolic, humeral, neural and developmental functions. (For recent reviews see: Birnbaumer 1990, Simon et al. 1991, Hepler and Gilman 1992)

There are two forms of signal transducing G proteins, the heterotrimeric G proteins that are made up of α, β and γ subunits, and the small G proteins that are single polypeptides composed of about 200 amino acids. The heterotrimeric G proteins are associated with signal transduction from cell surface receptors and are thought to act as switches that can exist in either of two states depending on the bound guanine nucleotide (GDP or GTP). A large family of transmembrane receptor proteins interact with G proteins during signal transduction process. Nearly all members in this family of receptors have seven membrane-spanning domains and show considerable amino acid sequence similarity. Fig. 3 is a model for receptor G protein-mediated signal transduction. It shows the

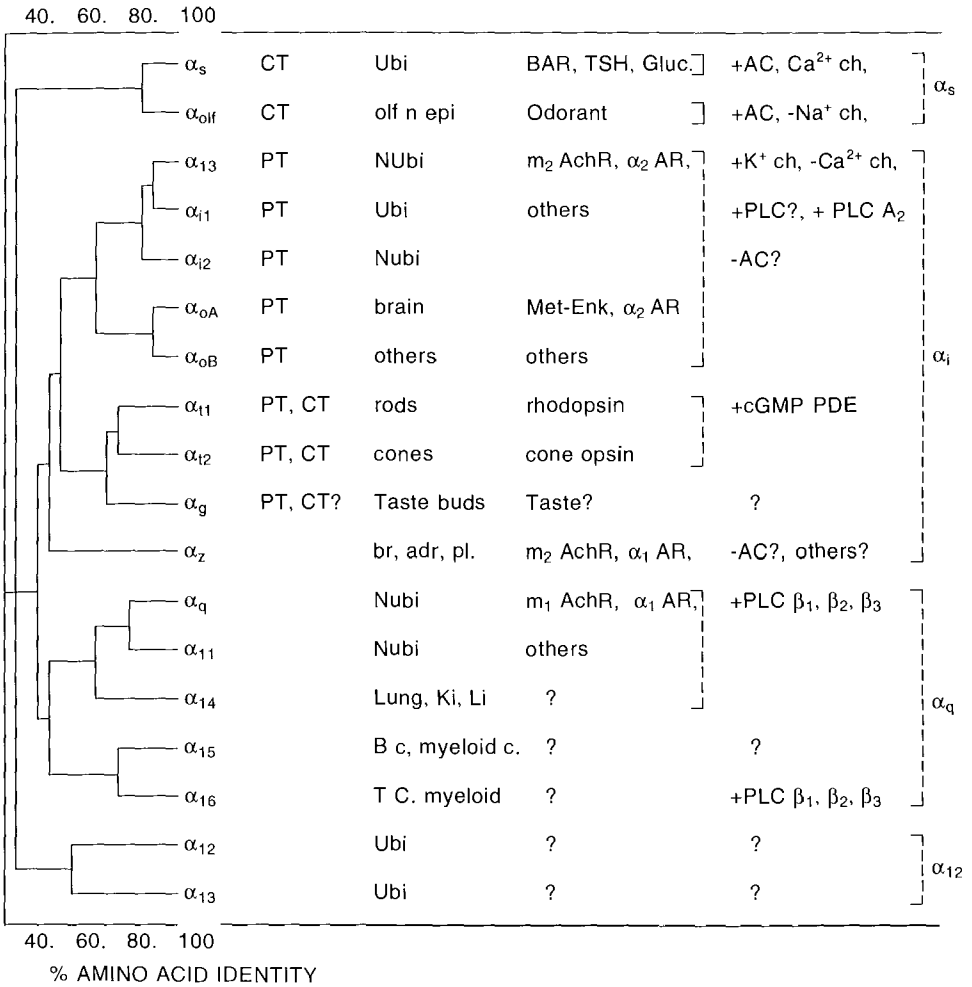


Fig. 3. Sequence relationships between mammalian Gα subunits and family groupings and G protein mediated transmembrane signaling. There are four groups of α_s shown on the right side of the graph, α_s, α_i, α_q and α_{12}. The left portion of this figure shows the % of amino acid identity of the isotypes of α-subunit of G proteins. Examples of tissue distribution, types of receptor and effector coupled to, and the kind of toxin may be affect by, all are listed. The abbreviations used are: C.T., cholera toxin; P.T., pertussis toxin; ubi, ubiquitous; nubi, nearly ubiquitous; olf n epi, olfactory neuroepithelium; br, brain; adr. adrenal; pl, platelets; ki, kidney; li, liver.

sequence relationships between mammalian Gα subunits, family groups and G protein mediated TMS. In which the coupling between G protein to receptor, and between G protein to effector are also shown. The type of hormones and the tissue distribution of hormone receptor and the sensitivity to cholera/pertussis toxin also exemplified. Gα subunit families are subdivided into α_s, α_i, α_q and α_{12}. The functions of many of the Gα isoforms are not known. A great deal of work remains to be done here. This chart is constructed based on information obtained from Hepler and Gilman (1992) and Simon et al. (1991).

A classification of the a subtype of α subunits found in mammals based on amino acid sequence similarity, is shown in Fig. 3. The family is made up of classes (denoted Gx with x designating the specific class), G_s, G_i, G_q and G_{12}. Each class is composed of specific isotypes (denoted $G_{\alpha x}$, with x designated the specific isotype) (see Fig. 3). Thus G_s class includes both the $G_{\alpha s}$ and $G_{\alpha olf}$ isotypes. Both $G_{\alpha s}$ and $G_{\alpha olf}$ are able to activate adenylate cyclase. $G_{\alpha i}$ class has 9 isotypes that are able to inhibit adenylate cyclase. $G_{\alpha q}$ class has 5 isotypes that are able to activate PLC_β or no known functions. 2 isotypes in $G_{\alpha 12}$ class have had no function assigned to them. The levels of G proteins vary with the hormonal states of the animal. Glucocorticoid-induced enhancement of adenylate cyclase activity in GH3 cells is mediated

at least in part, by increased expression of α_s. (Chang and Bourne 1987).

Diversity and Evolution of G_α subunit

There is no evidence for a cell surface receptor-coupled G protein in bacteria. In fungi, homologies of the subunits of heterotrimeric G proteins do exist. One of the heterotrimeric G proteins in yeast is coupled to the mating type receptor. The mechanism of G proteins action is different in yeast (*S. cerevisiae*) than in multicellular animals; it appears that the $\beta\gamma$ heterodimer rather than the α subunit interacts with the effector (Natsyniti et al. 1988). However mammalian proteins respond poorly to the yeast mating type receptor because they lack the appropriate receptor specificity. If the gene for the type receptor is replaced by the gene for the mammalian β-adrenergic receptor, catecholamines will trigger the yeast mating response (King et al. 1990).

Diversity and function of $\beta\gamma$ subunits

In mammals four distinct β subunit isotypes have been found. β_1, β_2, and β_3 are widely distributed, while β_4 is abundant in brain and lung tissue but is found at low levels in other tissues. The first 30-40 amino acids on the NH2-terminal of a β where γ subunits may interact. All the β subunits are made up of 8 segments of amino acid sequences.

There are four subtypes γ subunits have been demonstrated. $G_{\gamma 1}$ is expressed only in photoreceptors, while another G_γ is expressed at different levels in all tissues that were examined, $G_{\gamma 3}$ is expressed primarily in the brain and testis. The proteins are most divergent at their amino terminal sequence and they share considerable sequence homology at their carboxy terminal sequence. The amino acid sequence near the COOH-terminus of the γ subunits resembles the *ras* oncogene sequence with a characteristic cystein residue that is modified by carboxymethylation and isoprenylation or an all trans-geranylgeranyl moiety. This addition may be required to anchor the γ subunit in the membrane.

There are few examples of direct effects of $\beta\gamma$ subunits on purified components of mammalian signaling systems. Phospholipase A_2 and phospholipase C's are activated by distinct G proteins, the former is activated by a pertussis toxin sensitive G protein and the latter is activated by a G protein insensitive to this toxin (Burch et al. 1986). The $\beta\gamma$ subunit from the pertussis toxin-sensitive G protein is responsible for the activation of phospholipase A_2. Addition of $\beta\gamma$ subunit to rod photoreceptor outer segments apparently activate phospholipase A_2. Using antibodies to identify $G\beta$, Lin et al. (1992) reported that $\beta\gamma$ is associated with, besides plasma membrane, the mitotic spindle of tumor cells. $\beta\gamma$ may be also important in regulation of cell mitosis. $\beta\gamma$ subunit suppresses the activity of GTP activated α subunit. This led to the hypothesis that activation of $G_{\alpha i}$ frees $\beta\gamma$ subunits to interact with endogenous $G_{\alpha s}$, thus inactivating $G_{\alpha s}$. This effect might be due to that $\beta\gamma$ subunit directly act on adenylate cyclase (inhibitory or stimulatory) or indirectly. $\beta\gamma$ might act on calmodulin and inhibit the Ca^{2+}/calmodulin sensitive adenylate cyclase indirectly. Therefore, $\beta\gamma$ appears to have few functions: 1. stabilizing the interaction of α subunits with the receptor, 2. modulating the effects of activated α subunits and 3. regulating channel and phospholipase activity.

THE EFFCTORS AND GENERATION OF SECOND MESSENGERS

It is well established that both adenylate cyclase and phospholipase generate second messengers such as cAMP, DAG, IP_3 and Ca^{2+}.

A. Adenylate cyclase from unicellular organisms

a. *Dictyostelium*

Adenylate cyclase and its product cAMP were found in *Dictyostelium discoideum*, a unicellular organism. cAMP is either a chemotactic agent or an extra cellular messenger. Extra cellular cAMP, secreted by cells in a maturing aggregation center in an oscillatory fashion, initially served to organize the amoebae during aggregation (Devreoteds 1982). Surrounding cells respond both by chemotaxis moving towards the cAMP source and by synthesizing and secreting more cAMP from the rear end of its moving path, relaying the signal to cells further away. In this way a population of cells is able to relay cAMP signals over large distances. This process results in the formation of multicellular aggregates. Therefore, adenylate cyclase in *Dictyostelium* is an aggregation enzyme and it controls aggregation. Its activity and cAMP levels, low during growth, increase when aggregation begins (Klein 1976).

Within the resulting multicellular organism, cAMP continues to influence development. Cyclic cAMP acts as an important agent to influence differentiation of individual cells into stalk cells or

spore cells. During aggregation, the extra cellular cAMP functions analogously to a hormone whose effects are mediated by G protein-linked signal transduction pathways by stimulation of surface cAMP receptors, activating G protein(s) and eliciting a number of effects. These effects are such as chemotactic response, cAMP synthesis, cGMP synthesis (Jaconi et al. 1990, Schulkes et al. 1991), formation of Ins(1,4,5)P_3 (Van Haastert 1989) and early gene expression. Activation of adenylate cyclase may be linked to more than one G protein.

Furthermore, *Dictyostelium* contains two distinct adenylate cyclases (Pitt et al. 1992): a 12 transmembrane-span form (ACA), equivalent to adenylate cyclases identified in higher metazoans and single transmembrane-span form (ACG). The two genes are expressed at specific development stages and subject to different modes of regulation. ACA is expressed during aggregation, ACG is expressed only during germination and it is insensitive to guanine nucleotide. ACA is regulated by a surface cAMP receptor through a G protein which does not appear to be a *ras* gene product. The appearances of a form of enzyme in one of the earliest multicellular organisms that is similar to that in mammals may signify an evolutionary branching point in the function of adenylate cyclase/cAMP TMS.

b. *Trypanosoma*

A family of genes from the *Trypanosoma equiperdum* and *brucei* species (another group of unicellular organisms), homologous to ACG of *Distyostelium* in sequence and structure, has been identified (Ross et al. 1991). Like ACG, the members of this family appear to have a single transmembrane span separating an extra cellular domain from a cytoplasmic domain that shares homology with the catalytic domain of the adenylate and guanylate cyclase families. While expression of a *Trypanosoma equiperdum* gene in *S. cerevisiae* results in an increase of adenylate cyclase activity. The functions of these enzymes in the *Trypanosoma* life cycle have not yet been identified. Similar to ACG and an adenylate cyclase activity of mammalian sperm (Garbers and Kopt 1980, Ishikawa et al. 1992), this family of enzymes appears to be insensitive to guanine nucleotide.

Two distinct adenylate cyclase genes are present in *T. brucei*. The roles of these genes appear to be tissue specific or development-stage specific expression of adenylate cyclases. One transcribes only in the bloodstream form of a calcium-activated adenylate cyclase (about 140 KDa) which is devoid of the leucine-rich domain known to be involved in activation by Ras in yeast cells (Revelard et al. 1990), and the other is transcribed in both bloodstream and procyclic (about 150 KDa) forms. These enzymes can be found in the *Trypanosoma* membrane only at the surface of the flagellum (Paindavoine et al. 1992). The role of adenylate cyclases in trypanosomatids has not yet been defined, one of the functions may be related to cell growth.

B. Adenylate cyclase from the multicellular animals

Table 3 shows some well characterized adenylate cyclases from the animal kingdom. Classified forms, amino acid sequence, and molecular weight are listed as available. Tissue and animal sources, and whether it is stimulated, inhibited or no effected by Ca^{2+}/CAM, Mn^{2+}, forskolin, and adenosine as well as bicarbonate are also listed. All adenylate cyclases listed in Table 3 have their amino acid sequences known, except enzymes from bovine sperm. Enzyme I-VI, represents type I to VI adenylate cyclase (CyA I-VI), all are mammalian enzymes (Krupinski et al. 1989, Feinstein et al. 1991, Bakalya and Reed 1990, Gao and Gilman 1991, Ishikawa et al. 1992). Both CyA I and III depend on Ca^{2+}/calmodulin for maximum activity, and are also stimulated by forskolin, but not inhibited by adenosine. CyA II and IV both are Ca^{2+} insensitive, stimulated by forskolin, and not inhibited by adenosine. CyA V and VI both are adenosine inhibitable, Ca^{2+} insensitive and forskolin stimulatable. Bovine sperm adenylate cyclase has the smallest molecular weight among all, is insensitive to forskolin and activatable by bicarbonate (Okamura et al. 1991). 1991). Rutabaga cyclase is from *Drosophila melanogaster*. It is similar to bovine type I cyclase being Ca^{2+}/calmodulin sensitive and stimulated by forskolin and can also couple to Gs (Levine et al. 1992). ACA is from slime mold *Dictyostelium* (Pitt et al. 1992). ESAG AC is from the ESAG from the bloodstream forms of *T. brucei*, is Ca^{2+} stimulatable, and GRESAG from procyclic forms is Ca^{2+} inhibitable. (Paindavoine et al. 1992). Along the evolutional trait, all adenylate cyclase with amino acid sequence known are mammalian except one from slime mold and the other from *Drosophila*. It is expected that more reports concerning invertebrate adenylate cyclase will be published in the future.

C. Phospholipase C's from multicellular animals

A variety of hormones, such as neurotrans-

Table 3. Forms of adenylate cyclase from animal cells

Forms	a.a.	MW Kd	Source	Sensitive to				
				Ca^{++}	Mn^{++}	Forsk	Adeno	Bicarb
I	1134	124	Bovine brain	s	s	s	n	–
II	1090	123	rat brain, lung	n	s	s	n	–
III	1144	123	olfactory neuron	s	s	s	n	–
IV	1064	119	wildly distributed	n	s	s	n	–
V	1194		canine cardiac	i	s	s	i	–
VI	1165		canine cardiac	i	s	s	i	–
Sperm		46.3	bovine sperm	s	s	n	n	s
Rutabaga	2249		Drosophila melanogaster	s	–	s	–	–
ACA	1407		Dictyoslium	–	s	–	–	–
ESAG		150	T. brucei, bloodstream forms	s	–	–	–	–
GRESAG		140	T. brucei, procyclic forms	i	–	–	–	–

Forsk, forskolin; Adeno, adenosine; Bicarb, bicarbonate.
s. stimulation; i, inhibition; n, no effect; –, no report.
References are cited in the text.
I - VI, CyA I - VI; Sperm, bovine sperm cyclase; Rutabaga, cyclase from *Dropsophila melanogaster*; ACA, slime mold cyclase; ESAG, cyclase from the bloodstream forms of *Trypanosoma brucei*; GRESAG, cyclase from procyclic forms of *Trypanosoma brucei*.

mitters and growth factors, also activate phospholipase C, via the mediated membrane receptor, resulting the rapid hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP_2). Two second messengers are generated, diacylglycerol and inositol 1,4,5-triphosphate. Diacylglycerol binds and activates protein kinase C, and IP3 binds to specific intracellular receptors that promote the opening of calcium channels in vesicular storage sites associated with the endoplasmic reticulum. There are three classes of PLCs, PLC-β, PLC-γ and PLC-δ, in the literature, each has isoform 6, 5 and 5, respectively (for review see Rhee and Choi 1992). Twelve of them are found in mammalian cells and two from insect (*Drosophila*). None from any other species of the animal kingdom. The identity of the hormone-sensitive PLCs are known, the function of all known PLC isoforms are not assigned. However, hormone-sensitive PLC activity has been reported from tissues other than the species cited above (Litosch et al. 1985, Boekhoff et al. 1990).

Isoforms of the PLC-β group are activated via a G protein dependent manner. G proteins of Go, Gi and Gq are involved in these processes. The activation by Go and Gi are inhibied by pertussis toxin (pertussis toxin-sensitive) and that by Gq are not inhibited by this toxin (pertussis toxin-insensitive). In the later case, PLC activation is observed only in the presence of AlF_4^- and not with the nonhydrolyzable GTP analog GTPγS. Stimulation of PLC by α_q was observed over a large range of Ca^{2+} (Smrcka et al. 1991).

Isoforms of the PLC-γ group are activated through tyrosine phosphorylation by growth factor receptor tyrosine kinase or by non receptor tyrosine protein kinase (TPK). This group of isoforms, differs from members of PLC-β and PLC-δ, each has an additional SH domain between the X and Y domains. Hormones acting via this way are listed in Table 4. These pathways do not use G protein as a transducer. The activation of PLC-γ by polypeptide growth factors, such as PDGF, EGF, FGF, colony-stimulating factor and insulin, are mediated by binding and activation of their specific membrane receptors itself is a tyrosine protein kinase. This enzyme activates by phosphorylation of the effector PLC-γ. The activation of PLC-γ by the membrane IgM in B lymphocytes and the T cell antigen receptor (TCR) may be via a different pathway in which a non receptor tyrosine kinase is involved.

The mechanism of the receptor-mediated activation of PLC-δ is not known. Neither the receptor nor G protein that coupled to any of the PLC-δ members has been demonstrated.

A number of receptors may activate PLC-β via pertussis toxin-sensitive G proteins (such as members in $G_{\alpha i}/G_{\beta o}$ group). These receptors include those for muscarinic (m2, m4), α1-adrenergic, H1-histaminergic, 5-HT1C and 5-HT2, serotonergic, P2-purinergic, D1-glutaminergic, adenosine (none A1, none A2), thrombin, and neuropeptide Y, A number of receptors may activate PLC-β via pertussis toxin-insensitive G proteins (such as members in $G_{\alpha q}$ group). These receptors include those for

Table 4. Hormones activation of PLC_γ via receptor or nonrecreptor tyrosine protein kinases[1]

Hormone
EGF
FGF
NGF
Interluekin-1, -4, -7
TCR-receptor CD3
Membrane bound IgM,
High-affinity IgE receptor
IgD
IgG receptor
ANF
Endothelin
Manose 6-phosphate-containing peptide
Sperm
1,25-dihydroxyvitamin D3
Glucose
Bitter taste
Light, plc
Membrane depolarization, plc
PAF
Bradykinin
Substance P
f-met-leu-phe

[1]PLC activation is not mediated by a G protein. References are cited in the text.

thromboxane A2, bradykinin, angiotensin, histamine, vasopressin, muscarinica (m1, m3, m5), cholecystokinin and TRH. They appear to be specific in their interaction with different members of the Gq subfamily with different PLC-β effectors. This specificity may be important in generating tissue or receptor-specific in vivo response.

In addition, receptors that use the pertussis toxin-sensitive pathway in one cell type can use the pertussis toxin-insensitive G proteins in other cell types, or use both pathways in a given cell type. (for a recent review see Rhee and Choi 1992). A growing number of reports have shown that phospholipase D and phospholipase A2 may be the TMS effectors. These recent findings are also listed in Table 4.

REGULATION OF PROTEIN PHOSPHORYLATION AND DEPHOSPHORYLATION BY SECOND MESSENGERS

A. Protein kinases

The relative activity of any regulatory protein molecule depends on its level of phosphorylation, which depends on the relative activity of both protein kinases and protein phosphatases. The second messenger-dependent protein kinases have at least two separate chains or portions within a single chain, regulatory and catalytic subunit or regulatory and catalytic domain. The discoveries of a cAMP-dependent protein kinase provide the first clues about protein phosphorylation and its role in regulation (for recent review see Taylor 1989, Kennelly and Krebs 1991, Shabb and Corbin 1992).

It is now known that all second messengers mediate the actions of agonists by modulating the activities of protein kinases and protein phosphatases. Phosphorylation (or dephosphorylation) of Ser/Thr and (occasionally) Tyr residue triggers conformational changes in regulated proteins that alter their properties, leading to the physiological responses that are evoked by particular agonists. The major second messengers that operate in eukaryotic cells and the protein kinases that they activate are summarized in Fig. 2.

Like most protein kinases, the cAMP-dependent protein kinase is tightly regulated and maintained in an inactive form without cAMP, cAMP binds to a distinct regulatory (R) subunit inducing conformational changes that lead to dissociation of the holoenzyme. The active dissociated catalytic (C) subunit shares extensive sequence similarities with all eukaryotic protein kinases. Cyclic AMP nearly exerts all its effects by this type of enzymes. Many other protein kinases all have similar regulatory and catalytic counter parts. A few examples follow: The second messenger cGMP-dependent protein kinase has regulatory domains as part of a continuous polypeptide chain together with its catalytic domain. It is likely to have a more restricted role, since it is located predominantly in smooth muscle and the cerebella region of the brain. The second messenger Ca^{2+} ion, binds to calmodulin, triggering conformational changes in this protein that allow it to activate many enzymes, including a number of protein kinases. Calmodulin-dependent protein kinases can be divided into two classes, termed "multifunctional" (such as the calmodulin-dependent protein kinase II) and "dedicated" (such as myosin light chain kinase, phosphorylase kinase, etc.). The myosin light chain kinase has a Ca^{2+}/calmodulin binding domain carboxyl-terminal to the catalytic core, and binding of these ligands activates the kinase. In contrast, protein kinase C is activated by Ca^{2+}, diacylglycerol or phorbol ester, and phospholipids, and the recognition site for these ligands lies amino-terminal to the catalytic core.

This enzyme is likely to have multiple functions within cells. Myosin light chain kinase is cytoplasmic, while the protein kinase C is transiently associated with the plasmic membrane. The transforming protein from *Rous sarcoma* virus, $pp60^{v\text{-}src}$, was the first recognized protein tyrosine kinase. The carboxyl-terminal region of $pp60^{v\text{-}src}$ is important for regulation, and removal of a phosphorylation site from this region is sufficient to convert the proto-oncoprotein into a transforming protein. Myristylation at the amino terminus localizes $pp60^{v\text{-}src}$ at the plasma membrane. Growth factor receptors, such as the EGF[1] receptor, actually span the membrane via a single membrane-spanning segment. Binding of EGF to the extra cellular domain activates the cytoplasmic kinase domain.

a. The catalytic subunit

The catalytic subunit of cAMP protein kinase has three major functional sites, ATP binding, peptide binding and catalytic sites. By affinity labeling with analog of ATP, the Lys^{72} is covalently modified. This Lys is invariant in every protein kinase. Replacement of the Lys invariably leads to activity loss. This result provided the first clue that ATP binding was localized near the amino terminus. Carboxyl groups in the C-subunit may be important both for catalysis and for peptide recognition. Dicyclohexylcarbodiimide (DCCD) inhibits the C-subunit and MgATP affords protection. The major carboxyl group reacts with DCCD is Asp^{184}, which is also invariant in all protein kinase. Modification of the apoenzyme with DCCD leads to covalent cross-linking of Lys^{72} and Asp^{184}, thus placing both invariant residues in close proximity at the active site. Different protein kinases recognize different peptide substrata. Information regarding the consensus sequences showing the sequence feature surrounding the phosphorylation sites on protein substrate is useful. As models of critical substrate recognition determinants they presumably form reflected images of the corresponding substrate binding domains. They have been used to identify auto-inhibitory domains involved in the regulation of a number of protein kinases and phosphatases. The C-subunit cAMP-kinase, for example, requires the basic amino acids precede the site of phospho-transfer with the consensus sequence for recognition being Arg-Arg/Lys-X-Ser/Thr > Arg-X_2-Ser/Thr = Arg-x-Ser/Thr. Affinity labeling with a peptide analog established that Cys^{199} is close to the peptide binding site. Modification of Cys^{199} also inactivates the enzyme. The acidic Glu^{170} was found to be the recognition site for the basic Arg in the peptide substrate. EDC treatment of apoenzyme leads to loss of activity that is partially protected by MgATP and fully protected by MgATP plus an inhibitor peptide. Glu^{170} is partially protected by MgATP and fully protected in the presence of both MgATP and peptide. This substrate is conserved in those Ser/Thr-specific kinases having a requirement for basic residues preceding the phosphorylation site, such as protein kinase C and myosin light chain kinase, but is replaced with a basic residue in casein kinase II which requires acidic groups flanking the phosphorylation site. The consensus sequences recognized by protein kinase C is ($Arg/Lys_{1\text{-}3}$, $X_{2\text{-}0}$)-Ser/Thr-($X_{2\text{-}0}$, $Arg/Lys_{1\text{-}3}$) > Ser/Thr-($X_{2\text{-}0}$, $Arg/Lys_{1\text{-}3}$) = ($Arg/Lys_{1\text{-}3}$, $X_{2\text{-}0}$)-Ser/Thr. Protein tyrosine kinases, which typically require that acidic groups precede the site of phosphorylation, also frequently have a positive charged amino acid at that position. The consensus sequences most frequently recognized by calmodulin kinase II is Arg-X-X-Ser/Thr. Consensus sequences have many other useful applications. They have been used to identify auto-inhibitory domains involved in the regulation of a number of protein kinases and phosphatases. They also have served as guides for the design of synthetic peptide substrates of great utility.

b. The regulatory subunit

The major known function of the R-subunit is to bind to and inhibit the C-subunit in the absence of cAMP. The inactive holoenzyme dissociates in the presence of cAMP into a R-subunit dimer and two active C-subunits. Two major classes of R-subunits and their corresponding holoenzymes exist. Type II holoenzymes can be distinguished readily by autophosphorylation of the R subunits, while type I holoenzymes have a high affinity binding site for MgATP. All R-subunits share the common domain structure. Two protomers in the asymmetric dimer interact at the amino terminus of the R-subunits. Within the amino-terminal region there is a proteolytic sensitive "hinge" region that contains a substrate-like sequence that is essential for interacting with the C-subunit. Two cAMP-binding domains at the carboxyl terminus are highly conserved in all R-subunits, the two sites are not equivalent. Analogs of cAMP can discriminate between the two sites with N^6-substituted analogs showing a preference for site A and C-8-substituted analogs preferring site B. The off-rates for cAMP also differ with site showing a relatively fast off rate and site B a very slow off rate. Cyclic AMP

binding and activation both show positive cooperativity. The major cooperativity is between site A and site B. Site B of the R^1-subunit can be photoaffinity labeled at a single site, Tyr^{371} subunit. Two residues appear to interact directly with the bound cAMP in Site B. Arg^{333} interacts with the negative charge on the cyclic phosphate ring, and Glu^{324} hydrogen bonds to the 2'-OH of the ribose ring. These 2 residues are invariant in every R-subunit. The hinge region of each R-subunit contains either a phosphorylation site (R^2) and pseudo-phosphorylation site (R^1, and protein kinase inhibitor). The molecular anatomy of this enzyme has been carried out mainly by Taylor and associate (1989 1991).

c. Molecular phylogeny of cyclic nucleotide-binding proteins

The too simplistic view that the protein kinases mediated all the effects of cAMP and cGMP in eukaryotic tissues has been amended with the discovery of new types of cyclic nucleotide receptors. These include cyclic nucleotide-gated cation channels, cGMP-bindng cyclic nucleotide phosphodiesterases (PDEs) and extracellular cAMP receptors (cARs) from slime mold. It is now believed that cyclic nucleotides have a diversified portfolio of binding proteins including the catabolite gene activator protein (CAP) through which a wide range of cellular processes can be regulated.

The phylogenetic tree of CAP-related cyclic nucleotide binding domain in proteins is constructed (Shabb and Corbin, 1992). As shown in Fig. 4, the cyclic nucleotide-binding domains of CAP, protein kinases, and ion channels comprise three distantly related groups. All protein kinases A domains are more like each other than they are like their corresponding B domains. R subunits from slim mold (—), nematodes (CE) and fruit flies (DM) all appear to be more related to the mammalian type I R than to the type II R subunit. This suggests either that a type II homologue has yet to be discovered in these organisms or that this type of gene has been lost in lower eukaryotes while remaining maintained in mammalian organisms. Mammals have undergone more recent diversification of R subunits to generate α and β subclasses (and perhaps yet other undiscovered subclasses).

Through the course of evolution, cyclic nucleotide-binding domains have been recruited by proteins that perform a variety of functions, including protein phosphorylation, ion conductance, and regulation of gene transcription. The molecular mechanisms by which the cyclic nucleotide-binding domain regulate these protein functions are not

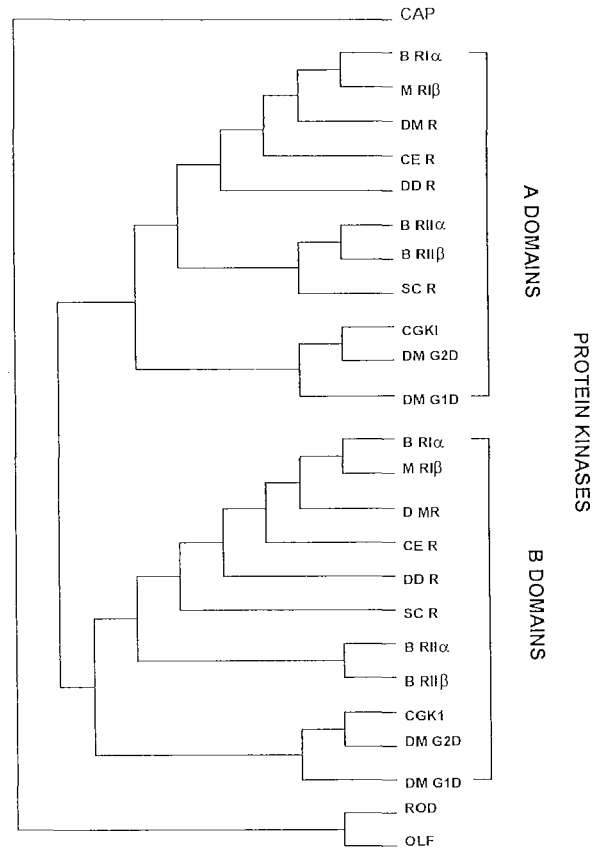


Fig. 4. Phylogenetic tree of cyclic nucleotide-binding proteins. The graph is an unrooted parsimony tree (see review by Shabb and Corbin, 1992). cAMP kinase R subunits: B RIα, bovine RIα; M Rβ, murine RIβ; DM R, *Drosophila melanogaster* R; Ce R, Caenorhabditis elegans R; DD R, D. discoideum R; B RIIα, bovine RIIβ; SC R, Sacharomyces cerevisiae R. cGMP kinase: B GKI, bovine cGMP kinase I; DM G1D and DM G2D, D. melanogaster G1D and G2D. Ion channels: ROD is bovine rod photoreceptor cGMP-gated channel, and OLF is bovine olfactory epithelium cyclic nucleotide-gated channel. CAP is catalytic gene activator protein.

clearly understood, although they will share some common features. Several cyclic nucleotide-binding proteins, including some phosphodiesterase and extracellular cAMP receptors, are probably unrelated to CAP. Each may acquire the cyclic nucleotide binding ability independently.

B. Protein phosphatases

a. The serine/threonine protein phosphatases

Protein phosphatases, like protein kinases, are controlled by second messenger systems. The interactions between TMS's for regulation of protein kinase activities have also been seen in the protein phosphatase systems.

As protein kinases, protein phosphatases are also classified into two major types: serine/threonine-specific and tyrosine-specific protein phosphatases. Unlike most protein kinases, the serine/threonine-specific protein phosphatases show broad and overlapping substrate specificities in vitro and their classification requires the use of specific inhibitors and activators. Using this criteria for example, four major classes of protein phosphatases (PP) catalytic subunits have been identified in eukaryotic cells. By using phosphorylase kinase as substrate, Type I phosphatases specifically dephosphorylate the β-subunit and Type II phosphatases preferentially dephosphorylate the α-subunit. Type I phosphatases are inhibited by the thermostable inhibitor-1 and inhibitor-2 and Type II phosphatases are unaffected by the protein inhibitors and affected by okadaic acid at high concentrations. Type II protein phosphatases comprise three subtypes (PP2A, PP2B and PP2C) that can be distinguished by their requirement for cations. PP2A, like PP1, does not have an absolute requirement for bivalent cations, whereas PP2B and PP2C are Ca^{2+}/calmodulin- and Mg^{2+}-dependent, respectively. Okadaic acid can be used as a more sensitive method to identify these subtypes. It completely inhibits PP2A at 1 nM in the presence of both inhibitors 1 and 2. High concentrations of okadaic acid are required for inhibition of PP1 (I_{50} = 10-15 nM), while PP2B is far less sensitive to okadaic acid than PP1, and PP2C is resistant (Cohen and Cohen 1989).

Because PP1 and PP2A are likely to be the chief enzymes that reverse the protein kinase C action. It is not surprising that okadaic acid should be as potent a tumor promoter as the phorbol esters which activate protein kinase C. Tumor promotion presumably stems from increased phosphorylation of one or more proteins that are substrates for protein kinase C and are dephosphorylated by PP1/PP2A. The tumor promoting effect of okadaic acid implies that one or more members of the PP1/PP2A family must function as tumor suppressors in normal cells.

Remarkably, the sensitivity of PP1 and PP2A to okadaic acid, is virtually identical in organisms as diverse as mammals, fruit flies, starfish, yeast and higher plants. (The exception is *Paramecium*, where PP2A-like activity is resistant to okadaic acid.) PP2B has also been identified in invertebrates and lower eukaryotes. Complementary DNA of PP1 from mammalian tissues, *Drosophila*, yeast, and PP2A from mammalian tissues and *Drosophila* have revealed extreme conservation of these enzymes throughout evolution. Peptide sequencing and cDNA cloning have failed to reveal any similarity between PP2C and PP1/PP2A. Thus the isoforms of PP2C represent a second and quite distinct protein phosphatase gene family. (for review see Cohen and Cohen 1989, Asaoka et al. 1992.)

b. Protein-tyrosine-specific phosphatases

Research concerning protein-tyrosine phosphatase (PTPase) is a recent development. This type of enzyme has specificity for phosphotyrosine. Interest in studying PTPases was sparked by Tonks, Fisher and associates (1988), who purified a 35 kd soluble PTPase from placenta, PTPase 1B. Its sequence (Charbonneau et al. 1988) showed no relationship to any of the protein-serine phosphatases catalytic subunits. However, it was similar to the cytoplasmic domain of CD45 (a leukocyte membrane protein), which also had PTPase activity (Cool, Krebs and associates 1989). Similar to multiple PTKs, both receptor-PTPase and non-receptor-PTPase are documented. Little is known about the regulation of PTPase activity. CD45 is known to be a phosphoprotein, containing predominantly phosphoserine at several sites. It is a substrate for PKC, but is not known whether any of these phosphorylations regulate CD45 PTPase activity. A number of oncogenes encode PTKs and transform by virtue of elevated tyrosine phosphorylation. One would predict that overexpression of a PTPase should reverse the transformed phenotype of cells transformed by such oncogenes. The existence of PTPase genes in *Drosophila* coupled hints that there are PTPase-related genes in other simple organisms. Recently, PTPase from shrimp (*Penaeus japonicus*) hepatopancreas has been isolated and characterized with a relative mass of 28 kd (Chuang and Wan 1993). The 70 kd-subunit of insulin receptor of the same tissue as autophosphorylated after addition of insulin which is dephosphorylated by the isolated PTPase from shrimp (Lin et al. 1993). The discovery of PTPases including CD45 has revealed a new transmembrane signal transduction mechanism. (for review see Hunter 1989.)

TERMINATION, DESENSITIZATION, CROSSTALK AND MODIFICATION OF SIGNALS

There is an increasing number of hormones that trigger more than one TMS. These TMS may cooperate with each other to control a whole host of cellular processes. Table 5 lists all hormones

Table 5. Examples of hormone and extracellular messenger that activate more than one transmembrane signaling system

Hormone	References
ACTH	20, 18.
ADH	16,
Angiotensin	16, 18.
Auricle natriuretic factor	18, 9.
Calcitonin	2.
Cholecystokinin	18, 13.
Cortictropin releasing factor	18. 9.
Endothelin	18, 6. 23. 10.
Epidermal growth factor	25.
Gastrin	16, 18. 17. 13.
Luteinizing hormone	20. 8. 9.
Melanocyte stimulating hormone	16. 11.
Opioid peptide	4, 14.
Oxytocin	18,
PTH	16, 18, 15.
PAF	18, 12.
Secretin	20, 18,
Somatostatin	18.
Thrombin	18.
TSH	20, 21.
Vasopressin	20, 18, 7.
Acetylcholine	20, 18. 1.
Adenosine	18. 19.
ATP	24.
cAMP	22.
Dopamine	18,
Epinephrine	20, 18.
Glutamine	18,
Histamine	20, 18. 5.
Norepinephrine	20, 18.
Prostaglandin	16, 18.
Serotonine	18.

1. Abedel-Latif et al. 1992. 2. Alam et al. 1993. 3. Castro et al. 1989. 4. Cruciani, et al. 1993. 5. Dawson et al. 1993. 6. Eguchi et al. 1993. 7. Gary et al. 1988. 8. Guderman et al. 1992a. 9. Guderman et al. 1992b. 10. Henry et al. 1992. 11. Kapas et al. 1992. 12. Kester et al. 1992. 13. Lee et al. 1993. 14. Mangoura and Dawson, 1993. 15. Resshkin et al. 1991. 16. Robison et al. 1971. 17. Roche et al. 1990. 18. Rhee and Choi 1992. 19. Sho et al. 1991. 20. Sutherland and Bobison, 1966. 21. van Sande et al. 1990. 22. van Dujin and van Haastert, 1992. 23. Zhang et al. 1992. 24. Sato et al. 1992. 25. Yang et al. 1993.

with this multiple signaling nature, including cAMP. The properties of these hormones are tissue specific. All concern multiple second messengers, cAMP, cGMP, DAG, IP_3 and Ca^{2+} that lead to complex cross-regulation. In many cells the multiple pathways appear to act reciprocally or synergistically.

A. Termination and desensitization of hormone signals

In adenylate cyclase transmembrane signaling system, there is more than one cellular mechanism to switch off the stimulatory action initiated by the extra cellular hormone or transmitter. The incoming signal is membrane receptor mediated via the heterotrimeric G protein following the binding by GTP that lead to dissociate and activate $G_{\alpha s}$. Within the α subunit itself there is an internal switch, a GTPase. This switch is turned on after binding and activation of adenylate cyclase. Thus the bound GTP on the α subunit is hydrolyzed to GDP, and the GDP bound $G\alpha$ dissociates from adenylate cyclase thereby terminating the G protein activation process. Presumably the same mechanisms terminate other $G\alpha$-activated effectors including the PLC-β, adenylate cyclase (the inhibitory path carried out by the $G\alpha i$), ion channels and cGMP specific phosphodiesterase.

The second method for modulation or termination of the G protein-mediated TMS is that the action of α subunit may be inhibited directly by the $\beta\gamma$ heterodimeric subunit and certain effectors (ion channels or adenylate cyclase) may be activated by the $\beta\gamma$ of G proteins. The actions of $\beta\gamma$ can not be terminated by this GTPase.

The other method for modulation or termination of the G protein-mediated TMS is the feed back mechanism. Second messengers, such as cAMP, diacylglycerol, inositol 1,4,5 triphosphate, and cytoplasmic Ca^{2+}, generated by the hormonally activated adenylate cyclase or phospholipase C's or phospholipase D may modify the activity of the same or other TMS system directly or indirectly. These second messengers in turn activate certain protein kinases (cAMP-dependent protein kinase or protein kinase C) which phosphorylate a given protein component of the TMS. This type of phosphorylation reaction might lead to cross-regulation of signaling pathways. This is the so called 'heterogous' desensitization, in which many types of receptors and responses are modulated simultaneously. Phosphorylation of β-adrenergic receptors by cAMP dependent protein kinase or β-adrenergic receptor kinase depends on the agonist occupancy of the receptor. This mechanism of phosphorylation may function to inactivate the receptor in some forms of agonist specific or 'homologous' desensitization (Lefkowitz and Caron 1989).

B. Crosstalk of transmembrane signals

a. Single type of hormone leads to multiple TMS through different types of receptors

α- and β- adrenergic receptors couple to two different G proteins, $G\alpha i$ and $G\alpha s$, and activate two

opposite pathways, one decreases cAMP and the other increases it. These two TMS can be activated by one single hormone, norepinephrine for example, in many tissues include adipocytes. The effect of norepinephrine is readily modified towards a single TMS effect by either an α- or α-adrenergic blocking agent. Many hormones listed in Table 5 show activation of multiple TMS such as DAG/IP_3 and calcium mobilization versus cAMP synthesis. These two systems are often antagonistic or synergistic.

b. Activation of single type receptor leads to multiple TMS's

There are clear examples of TMS crosstalk at the receptor levels. In this case, the signal of one type of hormone transmitted by one type of receptor via two types of G proteins to two types of effectors (Fig. 2). The thyrotropin receptor (TSHR) is coupled to the PIP2 and the cAMP signal system in FRTL-5 rat thyroid cells. The rat TSHR, similar to α_1-AR has seven transmembrane domains. It is an example of a single receptor coupled to two or more TMS mediated by more than one G proteins (Thompson 1992). Alanine 623 is related to TSHR-G interactions (Kosugi et al. 19992). Mutation of Ala^{625} in the carboxyl end of the third cytoplasmic loop of the TSH-receptor alters Graves' IgG-stimulated inositol phosphate formation but not in stimulated cAMP formation. This interesting phenomenon of one receptor mediating two TMS has been shown in other systems (Table 5). TSH and other trophic hormones from several sources including fish and other lower form of vertebrates activates the function of their own thyroid gland via a cAMP mediated mechanism (Yu and associates 1990). The TSH receptors also activate PLC and generate DAG and IP3. α1-AR, α1b-AR, α_2-AR β-AR all have multiple actions. Mutation of a specific amino acid residue or deletion a short length of the peptide chain within the same location as of TSHR receptor lead to loss of one function but not the other. Further, more cell surface cAMP receptors (cARs) of *Dictyostelium* have been implicated in multiple aspects of development. Strong evidence shown by Sun, et al (1990) shows that antisence mutagenes of cAMP receptor block cAMP binding, chemotactic response and cAMP and cGMP synthesis, cells fail to aggregate and undergo further differentiation. The same receptor probably coupled to different G proteins and leading to different signaling pathways.

c. Crosstalk at the levels of G protein

Transmembrane signaling at the levels of G proteins can be very complex. If all the subunits of G protein combined at random, there would be almost more than one hundred different kinds of heterotrimers. (Fig. 3). Different combination could have different affinities for individual receptors. There may be a mechanism that assembles the herterotrimer in a specific manner and transports specific assembles to intracellular compartments that are enriched for the presence of appropriate receptors or effectors. Specific cellular location of isotypes of G protein together with their effectors in certain cells may increase their ability to crosstalk or interact. Specificity can be controled by feedback processes. Activation of a particular G protein-coupled pathway can open the Ca^{2+} channels and generate second messengers that regulate protein kinase. The kinases in turn can influence the information processing system.

There are also a number of examples where the addition of ligand leads to rapid phosphorylation and inactivation of the $G_{\alpha s}$ subunit (in the case of *Dictyostelium* the phosphorylation of $G\alpha_2$). These reflect a desensitization or adaptation process. Other modifications including myristylation, isoprenylation, carboxymethylation and ADP-ribosylation that could also be regulated to modulate the activity of different G proteins. Furthermore, cholera toxin induces cAMP-dependent degradation of G_s in GH_3 cell line, in wild type S49 lymphoma cells, in S49 kin^- mutants and in S49 H_{21a} mutants suggesting that the cholera toxin-induced covalent modification of α_s marks the protein for accelerated degradation (Chang and Bourne 1989). Thus G protein similarities can generate crosstalk between circuits, resulting in signal integration.

d. Crosstalk at the levels of effector

Two different hormones through two TMS pathways may act antagonistically on a single effector, adenylate cyclase. One of the earliest examples is the effects of glucagon and norepinephrine on adipocytes metabolism. In this experiment norepinephrine is an α-adrenergic agonist which norepinephrine inhibits glucagon stimulated elevation of cAMP. Many hormone pairs exert antagonistic actions this way.

Signal stimulated formation of adenosine is a potent inhibitor of adenylate cyclase. The second messenger calcium ion is also an important regulator of a number of effectors and other enzymes. There are Ca^{2+}/calmodulin-sensitive adenylate cyclases (CyA I and III), Ca^{2+}-inhibitable adenylate cyclase (CyA V and VI). Ca^{2+} is also required for maximum activity of phospholipase D, A2 and cer-

tain type phospholipase C and several protein kinases, including protein kinase C. Multiple TMS pathways lead to increased phospholipids hydrolysis and increase in cytosol Ca^{2+} concentration. An important action of the DAG/C-kinase pathway is to inhibit calcium signaling by stimulating the removal of calcium from the cytoplasmic compartment by activating the calcium pump. Protein kinase C may also act to reduce calcium signaling by stimulating the enzyme that hydrolyzes Ins1,4,5P_3. There are at least three non-mitochondrial intracellular calcium pools (Chueh et al. 1990). Plasma membrane is also a major regulator of calcium fluxes. Such as the Na-dependent Ca^{2+} exchanger and ATP-dependent Ca^{2+} pump in the plasma membrane (of bovine chromaffin cells) (Kao and Cheung 1990). Multiple control mechanisms regulate the cellular Ca^{2+} homeostasis. Again, there is a lack invertebrates knowledge of this aspects.

e. Modification of transduced signals by inhibitors and activators of protein kinases and protein phosphatases

Both protein inhibitor-1 and inhibitor-2 of protein phosphatases are interconvertable and regulated by phosphorylation and dephosphorylation. Calcineurin is a Ca^{2+}/calmodulin-dependent phosphatase, it dephosphorylates and inhibits protein inhibitor-1. The phosphorylated inhibitor-1 is the active form. Therefore dephosphorylation of inhibitor-1 by calcineurin leads to inactivation of this inhibitor, and the inhibition is reversed and the phosphatase is activated (Yang et al. 1982). The protein phosphatase activator is a protein kinase. It phosphorylates protein inhibitor-2 and the phosphorylated inhibitor-2 is inactive. Therefore FA activates the inhibitor-2 inhibited protein phosphatase by phosphorylating the inhibitor (Jurgensent et al. 1984). The existence of FA kinase is in many mammalian tissues, including skeletal muscle, brain, liver, heart, human platelet and adipocyte. Yang and coworkers also reported that EGF or insulin induces the kinase activity FA (Yang et al. 1989) and showed dysfunction of kinase FA activity in patients with non-insulin-dependent diabetes mellitus (Yang et al. 1992). No attention has yet given to studies on these invertebrate modulators.

SPATIOTEMPORAL ASPECTS OF TRANSMEMBRANE SIGNALING

As more is learned about second messenger systems, it becomes apparent that we have to pay more attention to spatial and temporal aspects of signaling (Brown et al. 1984). Spatial aspects concern the non-uniform spatial distribution of second messengers, whereas temporal aspects deal with second messenger levels that may oscillate. Many examples can be cited for the spatiotemporal aspects of signaling. The cAMP and PKA-activated chloride channel localized in the apical membrane domain of the polarized human airway epithelium cells isone (Frizzell 1993). Odorant receptor and adenylate cyclase CyA III is localized in the cilia of olfactory neuron (Bakalyar and Reed 1990) is another. The muscarinic receptors that generate Ins(1,4,5)P_3 are localized at the animal pole of *Xenopus* oocytes (Kusano 1982) is yet another. Examples for the periodic release of cAMP are also shown from the slime mold, pacemaker neuron in *Aplysia*, oscillations in membrane potential in insulin-secreting β-cells, anterior pituitary and the salivary gland, and calcium signaling in exocrine acinar cells (Dissing et al. 1993, Habara and Kanno 1991, Kasai and Augustine 1990, Pepersen and Wakui 1990, Berridge 1987 1990). The control might be exercised through a frequency-dependent rather than an amplitude-dependent mechanism. What might be important therefore is not so much the absolute level of second messengers but rather the rate at which their concentrations fluctuate.

The other aspect of spatiotemporal transmembrane signaling is the programed developmental appearance or disappearance of TMS components. TMS is either on-set-, up-regulated or down-regulated due to the active participation of a component in a given TMS. Development of gastrin initiated TMS in HCl secretion in the new born and down regulation of parathyroid hormone and β-adrenergic activities in the aging (Hanai et al. 1990, Jiang et al. 1993) are examples. The other example is that in developing embryonic muscle cells of 1-day-old *Xenopus* cultures, cAMP analogues or forskolin increased the mean open durtion of the low-conductance of Ach channels of the postsynaptic membrane. This effect disappeared in myocytes of 3-day-old cultures suggesting the sensitivity of Ach channels to modulation by adenylate cyclase mediated process was related to the age and restricted during the early period of development (Fu 1993). Fu and Lin (1993) also have shown that signals activating protein kinase C pontentiates postsynapic acetylcholine respond at the same early stage. All indicate that phosphorylation by both cAMP PK and PKC may involve in such modulation. These TMS's exhibit

spatiotemporal properties. Therefore, initiation or loss of specific functions during development or aging are closely related to the activity of TMS.

LONG TERM ASPECTS OF TRANSMEMBRANE SIGNALING

The short term hormonal effects described above are switched on and off quickly leaving the cell largely unchanged. The transient signals generated by stimulation of cell surface receptors are converted into long-term changes in gene expression by signal-related transcription factors that mediate the effects of polypeptide hormones, cytokines, growth factors and neurotransmitters. In both cases there are early second messenger events leading to rapid changes in ion fluxes and protein phosphorylation. In long-term regulation, extracellular signals modulate the activity of many different types of transcription factors. These factors have a modular structure consisting of distinct and separable DNA binding, dimerization and transcriptional activation domains. Studied members of the superfamily are the AP-1 (Jun/Fos) and CREB/ATF proteins that control gene expression by binding to the TPA (12-o-tetradecanoylphorbol-13-acetate) response element (TRE and cyclic AMP response element (CRE) (Karin and Smeal 1992).

All tropic hormones and growth factors described above have both short- and long-term effects. Insulin has both short- and long-term effects on cellular metabolism. The short-term effects are directly related to or below the insulin-receptor binding relationship with an effective dosage in the range of 10^{-10} M. And the long-term effects are cell growth promotion. The mechanism of the long-term effect is not clearly understood. In cell lines of transfected hepatoma hepatitis B virus, virus antigen production is inhibited by insulin in a dose dependent manner way above nM range (Chou et al. 1989, Chou 1990). Lin and associates (1992) have reported that tumor promoters and tumor promoter inhibitors alter TMS during tumor formation (Huang et al. 1991, Wang et al. 1993, Wu et al. 1992). And therefore certain TMS activities are different between normal and tumor cells. The adrenergic receptor activity in Chang's hepatoma cell membrane is altered from that of normal rat hepatocyte. Recently Levi et al. (1993) reported that the coat protein of a virus inhibits the beta-adernergic function. Such long-term regulation in TMS-related cellular activity and changes in tumorgenesis represent a major challenge in the years ahead.

EVOLUTION OF ADENYLATE CYCLASE AND TRANSMEMBRANE SIGNALING SYSTEMS

Phylogeny of cyclases has been recently reviewed by Danchin (1993, with 323 references). In which (Fig. 9), a tentative phylogenetic tree is proposed. Where all cyclases may derive from an ancestral nucleotide-binding protein, made of small independent modules as seen in protein kinases (Fig. 4, and Shabb and Corbin, 1991). The different adenylate classes may have evolved independently, with the oldest activity corresponding to class III enzymes (Danchin's classification, see also Fig. 3 in that reference), and being derived from an ancestral nucleotide triphosphate synthesizing enzyme. The appearance of the multicellular organism along the evolutional path has created a new regulatory constraint, which requires cell communication. Hormones are the mediators of such an integrated pattern. How then are hormone-mediated interactions integrated into a physiological and behavioral pattern that can accommodate the coordinated functioning of every single specialized cell into appropriate grouping remains unclear. It is generally accepted that such integration proceeds through a cascade of TMS events within each cell. Adenylate cyclase must, accordingly, have regions that permit regulation by specific G proteins, and or by other modulator proteins or non-protein factors. This explains the wide variety of noncatalytic domains found in the proteins whose genes have thus far been characterized. Amino acid similarities of adenylate cyclases from mammalian sources have been compared (Katsushika et al. 1992, Krupinski et al. 1992). The dendrogram of the adenylate cyclase family members of the animal kingdom is shown in Fig. 5 (Tsaur et al.). A total of 9 available enzymes with complete amino acid sequences are used for construction this tree. The ATP binding and cyclase domains are the most conserved regions. Through the course of evolution, the cyclase domains have recruited other proteins to regulate this catalytic activity. The differences among the cyclases are their sensitivity to stimulatory effects of calmodulin (types I and III), and their capacity to be inhibited (type I), unaffected (type III), or stimulated (in the presence of $G\alpha s$) (types II and VI) by the G protein βr subunit complex. Types II and IV adenylate cyclases also show potentiative interactions between forskolin and $G\alpha s$, where type I does not (Gao and Gilman 1991). All types II, IV, V and VI do not have the capacity to be stimulated by cal-

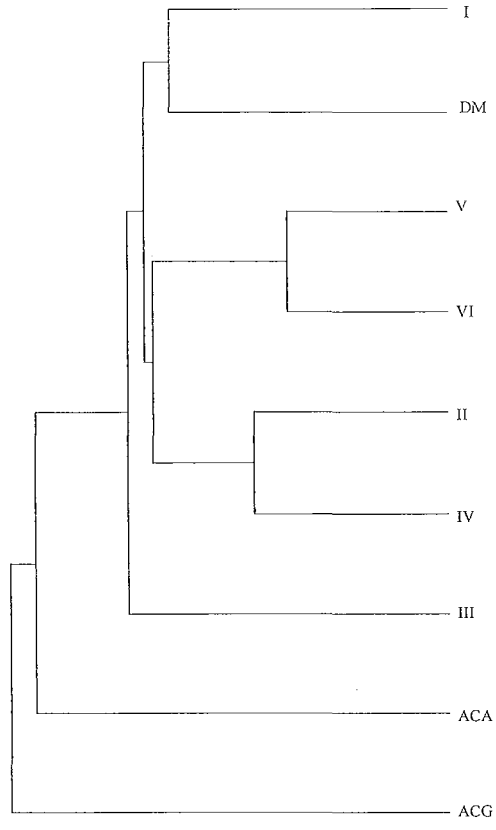


Fig. 5. Dendrogram of adenylate cyclase family of animal kingdom. A nine members of the animal kingdom adenylate cyclase family (Krupinski et al. 1989, Feinstein et al. 1991, Bakalyar et al. 1990, Gao and Gilman 1991, Ishikawa et al. 1992, Katsushika et al. 1992, Pitt et al. 1992, Levin et al. 1992) were analyzed for their amino sequence homology by using the multiple sequence analysis program PILEUP (Devereus et al. 1984) and used to construct a dendrogram with the aid of GCG computer package. Six enzymes, I to VI, are mammalian cyclase type I to VI. DM is cyclase Rutabaga of *Drosophila melanogaster*. ACA and ACG are enzyme proteins of *Dictyostelium*.

modulin, and types V and VI are Ca^{2+}-inhibitable while types II and IV are not. Type V and VI are also inhibitable by adenosine. The *Drosophila* enzyme is most similar to type I, and the slime mold enzymes ACA and ACG show more difference from other enzymes. ACG is a cyclase gene product but does not have adenylate cyclase activity as ACA. On the basis of the evolutional position of the animal source of these enzymes, this dendrogram may have a meaning of evolutional relationship. Since the *Drosophila* cyclase is similar to type I enzyme of bovine brain, the ancestor genes of these types of cyclases may occur prior to the branching age of the evolutional tree approximately 900 million years ago. The existence of the ancestor genes for types II, IV, V and VI cyclases may be even earlier. The appearance of ACA and ACG genes of DM slime mold should be farther earlier. But the divergent regulatory domains of these enzymes may be recruited at the later stages on the evolution path. Adenylate cyclase is a component of TMS, each type is coupled to and activated or inhibited by G protein. The challenge in the study of TMS evolution is great.

THE CONCLUDING REMARKS

The functional studies of TMS triggered the search for the mechanism of action and structural identity of the components of TMS. Molecular biology studies approach have made tremendous progress alone this line. These excellent tools are essential in this research field. The advancements of the structural studies raise a number of new questions regarding the physiological role of these new isoform proteins. These questions are urgently waiting to be answered. Sutherland applied the Sutherland criteria (1972) and proved cAMP is a second messenger in mediation of the action of many hormones in their specific target tissues. These criteria for cAMP may be extended and applied to other second messenger systems. The Krebs criteria (1972) for cAMP dependent protein kinase can be extended to the protein kinases depending on the other second messengers such as diacylglycerol dependent protein kinase or protein kinase C. These criteria are linking hormone and second messenger dependent protein kinase to specific function in their target cells. The scope of the mechanism of hormone action of many extra cellular mediators via a single type of second messenger cAMP (1972) has grown into a multiple TMS (Fig. 2). There are several different second messengers, each may involve several different G proteins and coupled to several different effectors. Many isoforms of G protein subunits and effectors have been recently discovered. Each of the different proteins may line up to form a different TMS. The functions of these TMS have not been assigned. A set of additional criteria, using the criteria of Sutherland and that of Krebs as guidelines, may be useful to evaluate the existence physiologically or pathologically of these TMS. Furthermore the crosstalk of these TMS and their distribution in the target cells is yet to be studied. This immense task may require a joint effort to be made by zoologists, physiologists, biochemists, pharmacologists as well as molecular biologists.

Table 6. Components of protein-mediated transmembrane signaling systems and time of phylum divergence from mammals

M-years[1]	Species	TMS	Total number of each TMS components (amino acid sequence reported)			
		activity reported	H	R	G $\alpha\ \beta\ \tau$	E
Present	Mammalia	yes[2]	>100	>100	16+	10
	Echinodermata	yes[3]	?	?	?	?
	Polychaeta	yes[4]	?	?	?	?
900	Insecta	yes[5]	<100	1	?	1
	Crustacean	yes[6]	<	?	?	?
	Mullusca	yes[7]	<100	?	?	?
	Annelida	yes[8]	?	?	?	?
	Plathelminthes	yes[9]	?	?	?	?
1,500	Protozoa	yes[10]	1	?	?	4
	Tetreahymena	?	?	?	?	?
	T. equiperdum	?	?	?	2	?
	D. discoidem	yes	?	?	2	?

[1]Time of divergence from mammalian line.
[2]All mammalian species, many hormones, AC, GC, PLC, PLA2, PLD.
[3]Sea urchin, sea cucumber, Ca^{2+}/CAM-AC, GC, dopamine D1 and D2, PKA FMRFamide and CCK-like peptide.
[4]S. magnifica, FMRFamide, substance P, catecholamines, 5HT, acetylcholine.
[5]Many species. sprum bud worm, manduca sexta, clayfish, moth, silkworm, grasshopper, tabacco hornworm, *Drosophila*. Octopamine, 5HT, serotonine, dopamine, acetylcholine, PDH, PBAN, pheromone, PTTH, HGH, FMRFamide.
[6]Shrimp, crab, lobster. PCH, PDH, GRH, Molt inhibiting H, RPCH, HGH, Hormone binding receptor.
[7]*Rapana thomasiana*, snail, clam, αBCP, AC, PKA, cAMP ELH FMRFamide, acetylcholine, CARP, samall cardia, peptide, 5HT, CHH.
[8]Earth worm, leech, 5-HT, FMRFamide-like peptide, AC, PKA.
[9]Liver fluke *Fasciola hepatica*, 5-HT, cAMP, AC.
[10]Slime mold, *Trypanosoma, Tetrahymena*. AC, PLC, oxytocine, vassopressin, PDE, PKA.

A comparative study of evolution and cross-talk of TMS in the animal kingdom has revealed that the basic TMS mechanism may be similar from low to high animal levels. But the number of TMS systems and the way of coupling of the components of TMS show both convergent and divergent patterns. The famous word for cAMP 'What is in *E. coli* is what is in elephant' needs to be amended. We now know much more TMS is in mammals than all other animals combined. It is due to our research efforts that are mainly focused on the mammals. We have to study more invertebrate systems along this line of thinking. It is obvious that an *Amoeba* or an earth worm may not have the same number of specialized tissues, hormones and hormone receptors, G proteins and effectors (Table 6). It appears that the coupling between these components in lower animals is not as clear as that in mammals. The distinction as a transmembrane signal pathway, and the interaction between different TMS form a meaningful regulatory network is yet to be worked out. Furthermore, as a result of morphological and physiological differentiation in evolution, spatiotemporol variations in TMS occur. Clearly a great deal of interesting research is waiting to be done. Both functional and structural studies are required to make the knowledge complete along the evolutional path in both mammals and non mammals (including invertebrate).

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REFERENCES

Abdel-Ladif AA, SPK Yousufzai, S De, SD Tachado. 1992. Carbachol stimulates adenylate cyclase and phospholipase C and muscle contraction-relaxation in reciprocal manner in dog iris sphicter smooth muscle. Europ. J. Pharmacol. **226:** 351-361.

Abou-Samra AB, H Juppner, T Force, MW Freeman, XF Kong, E Schipani, P Urena, J Richards, JV Bonventre, JT Potts Jr. et al. 1992. Expression cloning of a common receptor for parathyroid hormone and parathyroid hormone-related peptide from rat osteoblast-like cells: a single receptor stimulates intracellular accumulation of both cAMP and inositol triphosphates and increases intracellular calcium. Proc. Natl. Acad. Sci. USA **89:** 2732-2736.

Adachi T, S Takiya, Y Suzuki, M Iwami, A Kawakami, A Takahashi. 1989. cDNA structure and expression of Bombyxin, an insulin-like brian peptide of the silkworm *Bombyx mori*. J. Biol. Chem. **264:** 7681-7685.

Alam AS, CM Bax, VS Sharke, BE Bax, RJ Bevis, CL Huang, BS Moonga, M Pazians, M Zaldi. 1993. Further studies on the mode of action of calcitonin on isolated rat osteoclasts: Pharmacological evidence for a second site mediating intracellular Ca^{2+} mobilization and cell retraction. J. Endocrin. **136:** 7-15.

Allen CU, WP Janzen, NA Granger. 1992. Manipulation of intracellular calcium affects in vitro juvenile hormone synthesis by larval corpora allata of *Manduca sexta*. Mol. and Cell. Endocr. **84:** 227-241.

Arakawa S, JD Gocayne, WR McCombie, DA Urquhart, LM Hall, CM Fraser, JC Venter. 1990. Cloning, localization, and permanent expression of a *Drosophila* octopamine receptor. Neuron **4:** 343-354.

Asaoka Y, S-i Nakamura, K Yoshida, Y Nishizuka. 1992. Protein kinase C, calcium and phospholid degradation. TIBS **17:** 414-417.

Bakalyar HA, RR Reed. 1990. Identification of a specialized adenylyl cyclase that may mediate odorant detection. Science **250:** 1403-1406.

Baudry M, J Evans, G Lynch. 1986. Excitatory amino acids inhibits stimulation of phosphatidylinositol metabolism by aminergic agonists in hippocampus. Nature **319:** 329-331.

Berridge M. 1987. Inositol triphosphate and diacylglycerol: two interacting second messengers. Ann. Rev. Biochem. **56:** 159-193.

Berridge M. 1990. Calcium oscillations. J. Biol. Chem. **265:** 9583-9586.

Biden TJ, CB Wollheim, W Schlegel. 1986. Inositol 1,4,5-triphosphate and intracellular Ca^{2+} homeostasis in clonal pituitary cells (GH_3). J. Biol. Chem. **261:** 7223-7229.

Birnbaumer L. 1990. Transduction of receptor signal into modulation of effector activity by G protein: the first 20 years or so. FASEB J. **4:** 3068-3078.

Blackmore PF, SB Bocckino, LE Waynick, JH Exton. 1985. Role of guanine nucleotide-binding regulatory protein in the hydrolysis of hepatocyte phosphatidylinositol 4,5bisphosphate by calcium-mobilizing hormones and control of cell calcium. Studies utilizing aluminum floride. J. Biol. Chem. **260:** 14477-14483.

Boer HH, LPC Schot, JA Vennstra, D Reichelt. 1980. Immunocytochemical identification of neural elements in the central nervous system of a snail, some insects, a fish, and a mammal with antiserum to the molluscan cardioexctitatory tetrapeptide FRMFamide. Cell Tissue Res. **213:** 21-27.

Boekhoff I, J Strotmann, K Raming, E Tareilus, H Breer. 1990. Odorant-sensitive phospholipase C in insect antennae. Cellular Signalling **2:** 49-56.

Bonner TI, NJ Buckley, AC Young, MR Brann. 1987. Identification of a family of muscarinic acetlycholine receptor genes. Science **237:** 527-528.

Bonner TI, AC Young, MR Brann, NJ Buckley. 1988. Cloning and expression of the human and rat M5 muscarinic acetylcholine receptor genes. Neuron **1:** 403-XXX.

Breer H, I Boekhoff, E Tareilus. 1990. Rapid kinetics of second messenger formation in olfactory transduction. Nature **345:** 65-68.

Brown RD, KD Berger, P Taylor. 1984. α_1-adrenergic receptor activation mobilizes cellular Ca^{2+} in a muscle cell line. J. Biol. Chem. **259:** 7554-7562.

Castro MG, F Gusovsky, YP, Loh. 1989. Transmembrane signaling mediating adrenocortictropin relasease from mouse anterior pituitary cell. Mol. Cell. Endocrin. **65:** 165-173.

Change FH, HR Bourne. 1987. Dexamethasone incrase adenylyl cyclse activity and expression of the α-subunit of G_s in GH_3 cells. Endocrin **121:** 1711-1715.

Chang FH, HR Bourne. 1989. Cholera toxin induces cAMP-indenpendt degradation of G_s. J. Biol. Chem. **264:** 5352-5357.

Charbonneau H, NK Tonks, S Kumar, CD Dilts, M Harrylock, DE Cool, EG Krebs, EH Fisher, KA Walsh. 1989. Human placenta protein-tyrosine-phosphase: amino acid sequence and relationship to a family of receptor-like proteins. Proc. Natl. Acad. Sci. USA **86:** 5252-5256.

Cheuh SH, JA Lie, LS Kao, 1990. Intracellular calcium pool in neuroblastoma x glioma hybrid NG108-15 cells. J. Neuro-Chem. **55:** 1281-1286.

Chou CK. 1990. The role of receptor kinase in insulin action and the effects of insulin on human hepatoma cells. Clin. Biochem. **23:** 37-41.

Chou CK, TS Su, C Change, CO Hu, MY Huang, CS Suen, NW Chou, LP Ting. 1989. Insulin suppresses hepatitis B surface antigene expression in human hepatoma cell. J. Biol. Chem. **264:** 15304-15308.

Chow YS, YM Lin. 1986. Actinidine, a defensive secretion of stick insect, *Megacrania alpheus* Westwood (Orthoptera: Phasmatidae). J. Entomol. Sci. **21:** 97-101.

Christensen TA, H Itagaki, PEA Teal, RD Jasensky, JH Tumlinson, JG Hildebrand. 1991. Innervation and neural regulation of the sex pheromone gland in female *Heliothis* moths. Pro. Natl. Acad. Sci. USA **88:** 4971-4975.

Cohen, P. 1992. Signal integration at the level of protein kinases, protein phosphatases and their substrates. TIPS **17:** 408-413.

Chuang NN, PC Wang. 1993. Characterization of phosphotyrosyl protein phosphatase from the hepatopancras of the shrimp *Penaeus japonicus* (Crustacea: Decapoda). J. Exp. Zool. **266:** 181-187.

Cool DE, NK Tons, H Charbonneau, KA Walsh, EH Fisher, EG Krebs. 1989. cDNA isolated from human T-cell library encodes a member of the protein-tyrosine-phosphatase family. Proc. Natl. Acad. Sci. USA **86:** 5257-5261.

Cruciani RA, B Dvorkin, SA Morris, SM Crain. 1993. Direct coupling of opioid receptors to both stimulatory and inhibitory guanine nucleotide-binding proteins in F-11 neuroblastoma-sensory neuron hybrid cells. Proc. Natl. Acad. Sci. USA **90:** 3019-3023.

Danchin A. 1993. Phylogeny of adenylate cyclases. Advances in Second Messenger and Phosphoprotein Research **27:** 109-162.

Davis MTB, VN Vakharia, J Henry, TG Kempe, AK Raina. 1992. Molecular cloning of the pheromone biosynthesis-activating neuropeptide in *Helicoverpa zea*. Proc. Natl. Acad. Sci. USA **89:** 142-146.

De Prisco P, B Depetrocellis, E Parisi. 1991. A dopamone- and octopamine-sensitive adenylate cyclase in the nervous system of *Octopus vulgaris*. Comp. Biochem. Physiol. B: Comp. Biochem. **100:** 805-808.

De Loof A, L Schoofs. 1990. Homologies between the amino acid sequences of some vertebrate peptide hormones and peptides isolated from invertebrate sources. Comp. Biochem. Physiol. **95B:** 459-468.

Devereux J, P Haeberli, O Simities. 1984. A comprehensive set of sequences analysis programs for VAX. Nucleic Acids **12:** 387-395.

Dissing S, JL Gromada, NK Jorgensen, J Frokjaer-jensen, B Nauntofte. 1993. Spatiotemporal aspects of Ca^{2+} signaling in exoxrine acinar cells. NIP **8:** 103-107.

Dockray GJ, KR Teeve, J Shively, RJ Gayton, CS Barnard. 1983. A novel active pentapeptide from chicken brain identified by antibodies to FRMFamide. Nature **305:** 328-330.

Duve H, A Thorpe. 1979. Immunofluorescent localization of insulin-like materials in the median neurosecretory cells of the blowfly *Calliphora vomitoria* (Diptera). Cell Tiss. Res. **200:** 187-191.

Eguchi S, Y Hirata, T Imai, F Marumo. 1993. Endothelin receptor subtypes are coupled to adenylate cyclase via different guanyl nucleotide- binding proteins in vasculature. Endocrin **132:** 524-529.

Enjalbert A, F Sladerczek, G Guillon, P Betrand, C Shu, J Epelbaum, A Garcia-Sainz, S Jard, C Lombard, C Kordon, J Bockaert. 1986. Angiotensin II and Dopamine modulate both cAMP and inositol phosphate production in anterior pituitary cells. J. Biol. Chem. **261:** 4071-4075.

Europe-Finner GN, PC Newell. 1986. Inositol 1,4,5-triphosphate and calcium stimulate actin polymerization in *Dictyostelium discoideum*. J. Cell Sci. **82:** 41-51.

Evans PD. 1987. Phenyliminoimidazolidine derivatives activates both octopamine1 and octopamine 2 receptor subtypes in locust skeletal muscle. J. Exp. Biol. **129:** 239-250.

Exton JH. 1990. Signalling through phosphatidylcholine breakdown. J. Biol. Chem. **265:** 1-4.

Fain JN, MJ Berridge. 1979. Relationship between phosphatidylinositol synthesis and recovery of 5-hydroxytryptamine-responsive Ca^{2+} flux in blowfly salivary gland. Biochem. J. **180:** 655-661.

Feinstein, PG, KA Schrader, HA Bakalyar, WJ Tang, J Krupinski, AG Gilman, RR Reed. 1991. Melecular cloning and characterization of a Ca^{2+}/calmodulin insensitive adenylyl cyclase from rat brain. Proc. Natl. Acad. Sci. USA **88:** 10173-10177.

Frizzel RA. 1993. The molecular physiology of cystic fibrosis. News in Physiol. Sci. **8:** 117-120.

Fu WM, JL Lin. 1993. activation of protein kinase C potentiates postsynaptic acetylcholine response at developing neuromuscular synapses. Br. J. Pharmacol. **110:** 707-712.

Fu WM. 1993. Potentiation of acetylcholine responses in xenopus embryonic muscle cells by dibutyryl cAMP. Pflugers Arch. (in press).

Fugo H, J Hua, M Nakijima, H Nagasawa, A Suzuki. 1987. Neurohormone in developing embrios of the silk worm *Bombyx mori*: the presence of characteristics of prothoracicotripic hormone. J. Insect Physiol. **33:** 243-248.

Gao B, AG Gilman. 1991. Cloning and expression of a didely distributed (type IV) adenylyl cyclase. Proc. Natl. Acad. Sci. USA **88:** 10178-10182.

Garbers DL, GS Kopt. 1980. The regulation of spermatozoa by calcium and cycolic nucleotide. *In* Advanced Cyclic Nucleotid Research, eds. P. Greengard, A. Robinson. New York: Reven Press, pp. 251-306.

Garg LC, E Kapturczak, M Steiner, MI Philips, 1988. Vasopressin stimulates phosphoinositide hydrolysis in LLC-PK1 cells. Am. J. Physiol. **255:** C502-507.

Greenberg MJ, Payza K, Nachman RJ, Holman GM, Price DA. 1988. Relationships between the FMRF amide-related peptides and other peptide families. Peptides 9 Suppl **1:** 125-135.

Grimmelikehuijzen CDP, D Graff, 1985. ARG-PHE-amide-like peptides in the primitive nervous system of coelenterates. Peptide **6:** 477.

Guderman T, M Birnbaumer, L Birnbaumer. 1992a. Evidence for Dual coupling of the murine luteinizing hormone receptor to adenylate cyclase and phosphoinositide breakdown and Ca^{2+} mobilization. J. Biol. Chem. **267:** 4479-4488.

Guderman T, C Nichols, FO Levy, M Birnbaumer, L Birnbaumer. 1992b. Ca^{2+} mobilization by the lutinizing hormone receptor expressed in Xenopus oocytes independent of 3',5'-cyclic adenosine monophosphate formation: evidence for parallel activation of two signaling pathways. Mol. Endocrin. **6:** 272-278.

Habara Y, T Kanno. 1991. Dose-dependency in spatial dynamics of[Ca^{2+}] in pancreatic acinar cells. Cell. Calcium **12:** 533-542.

Hanai H, DP Brennan, L Cheng, ME Goldman, M Chorev, MA Levine, B Sacktor, CT Liang. 1990. Downregulation of parathyroid homone receptors in renal membrane from aged rats. J. Am. Physiol. **259:** F444-450.

Harmar AJ, AS Houn. 1977. Octopamine-sensitive adenylate cylase in cockroach brain: effect of agonists, antagonists and guanylyl nucleotides. Mol. Pharmacol. **13:** 512-520.

Henry PJ, PJ Rigby, GJ Self, JM Preuss, RG Goldie, 1992. Br. J. Pharmacol. **105:** 135-141.

Hepler JR, AG Gilman. 1992. G proteins. TIBS, **17:** 383-388.

Ho HY, YS Chow. 1993. Chemical identification of defensive secretion of stick insect *Megacrania tsudai* Shiraki. J. Chem. Ecol. **19:** 39-46.

Hollingsworth EB, JW Daly. 1985. Accumulation of inositol phosphates and cyclic AMP in guinea-pig cerebral cortical preparations. Effect of norepinephrine, histamine, carbamylcholine and 2-chloroadenosine. Biochim. Biophys. Act. **847:** 207-216.

Huang HC, CR Lee, YI Weng, MC Lee, YT Lee. 1992. Vasodilator effec of scoporone (6,7-dimethoxycoumarin) from a Chinese herb. Europ. J. Pharmacol. **218'** 123-128.

Huang H-j, C-f Liao, B-c Yang, Ft Kuo. 1992. Functional expression of rat M5 muscarinic acetylcholine receptor in yeast. Biochem. Biophys. Res. Com. **182:** 1180-1186.

Hunter T, Protein-tyrosine phosphatases: the other side of the coin. Cell **58:** 1013-1016.

Ishikawa Y, S Katsushika, L Chen, NJ Halnon, J-I Kawabe, CJ Homcy. 1992. Isolation and characterization of a novel cardiac adenylylcyclase cDNA. J. Biol. Chem. **267:** 13553-13557.

Ishizaki H, A Mizoguchi, M Hatta, A Suzuki, H Nagasawa, H Kataoka, A Isogai, S Tamura, M Fujino, C Kitada. 1987. Prothoracicotropic hormone (PTTH) of the silkmoth, *Bombyx mori*: 4k-PTTH. In Molecular Entomology (Edited by Law JH) pp 119-128. Alan R. Liss, New York.

Janssens PMW. 1988. The evolutionary origin of eukaryotic transmembrane signal transduction. Comp. Biochem. Physiol. **90A:** 209-223.

Jiang MT, MP Moffat, N Narayama. 1993. Age-related alterrations in the phosphorylation of sarcoplasmic reticulum and myofibrillar proteins and diminished contractile response to isoproterenol in rat ventricle. Cir. Res. **72:** 102-111.

Jurgensen S, E Shacter, CY Huang, PB Chock, SD Yang,

JR Vandenheede, W Merlevelde. 1984. On the mechanism of activation of the ATP.Mg(II)-dependent phosphoprotein phosphatase by kinase F_A. J. Biol. Chem. **259:** 5864-5870.

Kao LS, NS Cheung. 1990. Mechanism of calcium transport across the plasma membrane of bovine chromaffin cells. J. Neurchem. **54:** 1972-1979.

Kapas S, CD Orford, S Barker, GP Vinson, JP Hinson, 1992. Studies on the intracellular mechanism of alpha-melanocyte-stimulating hormone on rat adrenal zona glumerulosa. J. Mol. Endocrin. **9:** 47-54.

Kasai H, GJ Augustine. 1990. Cytosolic Ca^{2+} gradient trigering unidirectional fluid secretion from exocrine pancreas. Nature **348:** 735-7380.

Katsushika S, L Chen, J-i Kawabe, R Nilakantan, NJ Halnon, CJ Homcy, Y Ishikawa. 1992. Cloning and characterization of a sixth adenylyl cyclase isoform: Type V and VI constitute a subgroup within the mammalian adenylyl cyclase family. Proc. Natl. Acad. Sci. USA **89:** 8774-8778.

Kawano T, H Kataoka, H Nagasawa, A Isogai, A Suzuki. 1992. cDNA cloning and sequence determination of the pheromone biosynthesis actviating neuropeptide of the silkworm, *Bombyx Mori*. Biochem. Biophys. Res. Comm. **189:** 221-226.

Keller R. 1992. Crustacean neuropeptides: structure, function and comparative aspects. Experientia **48:** 439-448.

Kennelly PJ, EG Krebs. 1991. Consensus sequences as substrate specificity determinants for protein kinases and protein phosphatases. J. Biol. Chem. **266:** 15555-15558.

Kester M, CP Thomas, J Wang, MJ Dunn. 1992. Platelet-activating factor stimulates multiple signaling pathways in culture rat mesangial cells. J. Cell. Physiol. **153:** 244-255.

Khanashvili D, Price DC, Greenberg MJ, Sarne Y. 1993. Phe-Met-Arg-Phe-NH_2 (FMRFa)-related peptides inhibit Na^+-Ca^{2+} exchange in cardiac sarcolemma vesciels. J. Biol. Chem. **268:** 200-206.

King K, HG Golman, J Thorner, MG Caron, RJ Lefkowitz. 1990. Control of yeast mating signal transduction by a mammalian β_2-adrenergic receptor and G_s α subunit. Science **250:** 121-123.

Kitamura A, H Nagasawa, H Kataoka, T Inoue, S Matsumoto, T Ando, A Suzuki. 1989. Amino acid sequence of pheromone biosynthesis-activating neuropeptide (PBAN) of silkworm, *Bombyx mori*. Biochem. Biophys. Res. Com. **163:** 520-526.

Kopin AS, Y-M Lee, EW McBride, LJ Miller, M Lu, HY Lin Jr., LF Kolakowski, M Beinborn. 1992. Expression cloning and characterization of the canine parietal gastrin receptor. Proc. Natl. Acad. Sci. USA **89:** 3605-3609.

Kosugi S, F Okajima, T Ban, A Hidaka, A Shenker, LD Kohn. 1992. Mutation of a Alanine 623 in the third cytoplasmic loop of the rat thyrotropin (TSHR) receptor results in a loss in the phosphatide but not cAMP signal induced by TSH and receptor autoantibodies. J. Biol. Chem. **267:** 24153-24156.

Krupinske, J, F Coussen, HA Bakalyar, WJ Tang, PG Feinstein, K Orth, C Slaughter, RR Reed, AG Gilman. 1989. Adenylyl cyclase amino acid sequence: possible channel- or transporter-like structure. Science **244:** 1558-1564.

Krupinski J, TC Lehman, CD Frankenfield, JC Zwaagstra, PA Watson. 1992. Molecular diversity in the adenylyl-cyclase family. J. Biol. Chem. **267:** 24858-24862.

Kusano K, R Melidi, M Stinnakre. 1982. Cholinergic and catecholaminergic receptors in the xenopus oocyte membrane. J. Physiol. **328:** 143-170.

Lee YM, M Beinborn, EW McBride, M Lu, LF Kolakowski Jr., AS Kopin. 1993. The human brain cholescystokinin-B/gastrin receptor. Cloning and characterization. J. Biol. Chem. **268:** 8164-8169.

Lefkowitz RJ, MG Caron. 1988. Adrenergic receptors, models for the study of receptors coupled to guanine nucleotide regulatory proteins. J. Biol. Chem. **263:** 4993-4996.

Levi G, M. Patrizio, A Bernardo, TC Petrucci, C Agresti. 1993. Human immunodeficiency virus coat protein gp120 inhibits the β-adrenergic regulation of astroglial and microglical functions. Proc. Natl. Acad. Sci. USA **90:** 1541-1545.

Levin LR, PL, Han, PM Hwang, PG Feinstein, RL Davis, RR Reed. 1992, The *Drosophila* learning and memory gene rutabaga encodes a Ca^{2+}.calmodulin-responsive adenylyl cyclase. Cell **68:** 479-489.

Liao CF, APN Themen, R Joho, C Barberies, M Birnbaumer, L Birnbaumer. 1989. Molecular cloning and expression of a fifth muscarinic acetylcholin receptor. J. Biol. Chem. **264:** 7328-7337.

Liao CF, WP Schilling, M Birnbaumer, L Birnbaumer. 1990. Stimulation of the M5 muscarinic acetylcholin receptor as seen through stable expression in muline L cells. J. Biol. Chem. **265:** 11273-11284.

Lin CL, PC Wang, NN Chuang. 1993. Specific phosphorylation of membrane proteins of M_r 44,000 and M_r 32,000 by the autophosphorylated insulin receptor from the hepatopancreas of the shrimp *Penaeus monodon* (Crustacea: Decapoda). J. Exp. Zool. **267:** 113-119.

Lin CT, HC Wu, HF Cheng, JT Chang, KJ Chang. 1992. Identification of β-subunit of GTP-binding regulatory protein in mitotic spindle. Lab. Invest. **67:** 770-778.

Litosch I, C Wallis, JN Fain. 1985. 5-hydroytryptamine stimulates inositol phosphate production in a cell-free system from blowfly salivary glands. J. Biol. Chem. **260:** 5464-5471.

Mangoura D, G Dawson. 1993. Opioid peptides activate phospholipase D and protein kinase C-e in chick embrio cultures. Proc. Natl. Acad. Sci. USA **90:**2915-2919.

Matsumoto S, A Isogai, A Suzuki. 1985. N-terminal amino acid sequence of an insect neurohormone, melanization and reddish coloration hormone (MCRH): heterogeneity and sequence homology with human insulin-like growth factor II. FEBS Lett. **189:** 115-118.

Matsumoto S, A Kitamura, H Nagasawa, H Kataoka, C Orisakasa, T Mitsui, A Suzuki. 1990. Functional diversity of a nuerohormone produced by suboesophargeal ganglion: odenttity of malanization and redish colouration homone and pheromone biosynthesis activating nueropeptide. J. Insect. Physiol. **36:** 427-432.

Meller V, S Sakural, LI Gilbert. 1990. Developmental regulation of calmodulin-dependent adenylate cyclase activity in an insect endocrine gland. Cell Regulation, **1:** 771-780.

Mori K. 1989. Synthesis as an indispensable tool for the study of chemical cmmunication. Chemic Scripta **29:** 395-400.

Nambu JR, C Murphy-Erdosh, PC Andrew, GJ Feistner, RH Scheller. 1988. Isolation and characterization of a drosophila neuropeptide gene. Neuron **1:** 55-61.

Nagasawa H, H Kataoka, A Isogai, S Tamura, A Suzuki, H Ishizaki, A Mizoguchi, Y Fujiwara, A Suzuki. 1984. Amino-terminal amino acid sequence of the silk-worm prothoracicotropic hormone: homology with insulin. Science **226:** 1344-1345.

Nagasawa H, H Kataoka, A Isogai, S Tamura, A Suzuki, A Quzoguchi, Y Fujiwara, A Suzuki, SY Takahashi, A Ishizaki. 1986. Amino acid sequence of a prothoracicotropic hor-

mone of silk-worm *Bombyx mori*. Proc. Natl. Acad. Sci. USA **83:** 5840-5843.

Nishizuka Y. 1984. The role of protein kinase C in cell surface signal transduction and tumor promotion. Nature, **308:** 693-698.

Nishizuka Y. 1986. Studies and perspectives of protein kinase C. Science **233:** 305-312.

Okamura N, Y Tajima, S Onoe, Y Sugita. 1991. Purification of biocarbonate-sensitive sperm adenylyl cyclase by 4-acetamido-4′-isothiocyanostibene-2,2′-disulfonic acid-affinity chromatography. J. Biol. Chem. **266:** 17754-17759.

Paindavoine P, S Rolin, S Van Assel, M Geuskens. 1992. A gene from the variant surface glycoprotein expression site encodes one of several trandmembrane adenylate cyclases located on the flagellum of *Trypanosoma brucei*. Molec. Cell. Biol. **12:** 1218-1225.

Pertseva M. 1991. The evolution of hormone signalling systems. Comp. Biochem. Physiol. **100A:** 775-787.

Petersen OH, M Wakui. 1990. Topical review. Oscillating intracellular Ca^{2+} signals evoked by activation of receptors linked to inositol lipid hydrolysis: mechanism of generation. J. Memb. Biol. **118:** 93-105.

Pitt GS, N Milona, J Borleis, KC Lin, RR Reed, PN Devreotes. 1992. Structurally distinct and stage specific adenylyl cyclase genes play different roles in *Dictyostelium* development. Cell **69:** 305-315.

Price DA. 1986. Evolution of a mollusca cardioregulatory neuropeptide. Am. Zool. **26:** 1007-1015.

Price DA, MJ Greenberg. 1977. Structure of molluscancardioexcitory neuropeptide. Science **197:** 670-671.

Qian JM, WH Rowley, RT Jensen. 1993. Gastrin and CCK acctivate phospholipase C and stimulate pepsinogen release by interacting with two distinct receptors. Am. J. Physiol. **264:** G718-727.

Raina AK, H Jeffe, TG Kempe, P Keim, RW Blacher, HM Fales, CT Riley, JA Klum, RL Ridway, DK Hayes. 1989. Identification of a neuropeptide hormone that regulates sex pheromone production in female moth. Science **244:** 796-798.

Randall RW, RW Bonser, NT Thompson, LG Garland. 1990. Aspinrin activates phospholipase D in collagen-stimulated rabbit platelets. FEBS Lett. **264:** 87-90.

Reshkin SJ, J Forgo, H Murer. 1991. Apical and basaolateral effects of PTH in OK cells: transport inhibition, messenger production, effects of pertussis toxin, and interaction with a PTH analog. J. Memb. Biol. **124:** 227-237.

Rhee SG, KD Choi. 1992. Multiple forms of phospholipase C isozymes and their activation mechanism. Adv. Second Messenger and Phosphoprotein Res. **25:** 35-61.

Rhee SG, KD Choi. 1992. Regulation of inositol phospholipid-specific phospholipase C isozymes. J. Biol. Chem. **267:** 12393-12396.

Robison GA, RW Butcher, EW Sutherland. 1968. Cyclic AMP. Ann. Rev. Biochem. **37:** 149-174.

Roche S, JP Bali, R Magous. 1990. Involvement of a pertussis toxin-enesitive G protein in the action of gastrin on gastric parietal cells. Biochim. Biophys. Act. **1055:** 287-294.

Roeder T, JA Nathanson. 1993. Characterization of neuronal octopamine-3 receptorts. Neurochem. Res. **18:** 921-925.

Scarborough RM, GC Jamieson, F Kalish, SJ Kramer, CA Miller, DA Schooley. 1984. Isolation and primary structure of two peptides with cardioacceleratory and hyperglycemic activity from the corpora cadiaca of *Periplaneta americana*. Proc. Natl. Acad. Sci. USA **81:** 5575-5579.

Schleicher S, I Boekhoff, J Arriza, RJ Lefkowits, H Breer. 1993. A β-adrenergic receptor kinase-like enzyme is involve in olfactory signal termination. Proc. Natl. Acad. Sci. USA **90:** 1420-1424.

Schnefel S, A Profrock, KD Hinsch, I Schulz. 1990. Cholecyctokinin activates Gi1-, Gi2-, Gi3- and several Gs-proteins in rat pancreatic acinar cell. Biochemical J. **269:** 483-488.

Schneider LE, PH Taghert. 1988. Isolation and characterization of a drosophila gene that encodes multiple neuropeptide related to Phe-Met-Arg-Phe-NH_2 (FMRFamide). Proc. Natl. Acad. Sci. USA **88:** 1993-97.

Shabb JB, JD Corbin. 1992. Cyclic nucleotide binding domain in proteins having diverse functions. J. Biol. Chem., **267:** 5723-5726.

Sho KM, F Okajima, M Abdul Majid, Y Kondo. 1991. Thyrotropin actions by P1-purinergic agonist in FRTL-5 thyroid cells. Inhibition of cAMP pathway and stimulation of phospholipase C-Ca^{2+} pathway. J. Biol. Chem. **266:** 12180-12184.

Simon MI, MP Strathmann, N Gautam. 1991. Diversity of G protein in signal transduction. Science **252:** 802-808.

Smit AB, E Vreugdenhil, RHM Ebberink, VPM Geraerts, J Klootwijk, J Joose. 1988. Growth controling molluscan neurons produce the precusor of an insulin-related peptide. Nature **331:** 535-539.

Smith WA, LI Gilbert, WE Bollenbacher. 1984. The role of cyclic AMP in the regulation of ecdysone synthesis. Molecular and Cellular Endocr. **37:** 285-294.

Smrck AV, JR Helper, KO Brown, PC Sternweis. 1991. Regulation of ployphosphoinosittide-sepcific phospholipase C activity by purified Gq. Science **251:** 804-807.

Sun TJ, PJM Van Haastert, PN Devreotes. 1990. Surface cAMP receptors mediate multiple responses during development in Dictyostelium: Evidence by antisense mutagenesis. J. Cell Biol. **110:** 1549-1554.

Suryanarayana S, M von Zastrow, BK Kobilka, 1992. Identification of Intramolecular interactions in adrenergic receptors. J. Biol. Chem. **267:** 21991-21994.

Sutherland EW, GA Robison. 1966. The role of cyclic -3', 5'-AMP in response to catecholamines and other hormones. Pharmacological Rev. **18:** 145-161.

Taylor SJ, HZ Chae, SG Rhee, JH Exton. 1991. Activation of the β_1 isozyme of phospholipase C by α subunits of the G_q class of G Proteins. Nature **350:** 516-518.

Taylor SS, 1989. cAMP-dependent protein kinase, model for an enzyme family. J. Biol. Chem. **264:** 8443-8446.

Thompson EB. 1992. Mole. Endocrinol. **6:** 501-xxx.

Thompson NT, RW Bosner, L Garland. 1991. Receptor coupled phospholipase D and its inhibition. TIPS **12:** 404-408.

Thorpe A, Duve H. 1988. Insulin, found at last? Nature **331:** 483-484.

Tonks NK, CD Dilts, EH Fisher. 1988. Purification of the major-protein-tyrosine-phosphatases of human placents. J. Biol. Chem. **263:** 6722-6730.

Tsaur SC, FJ Lin, JL Wu, RJ Ho. Evolution of adenylate cyclases in animal kingdom. In preparation.

Van Duijin B, PJM Van Haastert. 1992. J. Cell Sci. **102:** 753-768.

Van Haastert PJM. 1989. Determination of inositol 1, 4, 5-triphosphate levels in *Dictyostelium* by isotopic dilution assay. Anal. Biochem. **177:** 115-119.

Van Sande J, E Raspe, J Perret, C Lejeune, C Malhaut, G Vassart, JE Dumont. 1990. Thyrotropin activates both the cyclic AMP and PIP2 cascades in CHO cells expressing the human cDNA of TSH receptor. Mol. Cell. Endorin. **74:** R1-6.

Venter JC. 1984. Evolution and structure of neurotransmitter

receptors. in Monoclonal and anti-idiotyoic antibodies: probes for receptor structure and function, New York: Alan R. Liss, Inc. pp 117-139.
Vign SR, MC Thorndyke, JA Williams. 1986. Evidence for a common evolutionary origin of brain and pancreas cholecystokinin receptors. Proc. Natl. Acad. Sci. USA **83:** 4355-4359.
Vizi ES, J Kiss, IJ Elenkov. 1991. Presynaptic modulation of cholinergic and noradrenergic neurotransmission: interaction between them. NIPS **6:** 119-123.
Walker RJ, L Holden-Dye. 1989. Commentary on the evolution of transmitters, receptors and ion channels in invertebrates. Comp. Biochem. Physiol. **93A:** 25-39.
Wank SA, JR Pisegna, A Deweerth. 1992. Brain and gastrointestinal cholecystokinin receptor family: structure and functional expression. Proc. Natl. Acad. Sci. USA **89:** 8691-8695.
Wei JW, Hung. 1989. Evidence for the coupling of muscarinic M1 receptors to polyphosphoinositide turnover in rat cortical synapsomes. Chinese J. Physiol. **32:** 103-114.
Wei JW, MC Wang. 1990. Muscarinic M2 receptors coupled to inhibition of adenylate cyclase in the rat hear. Chinese J. Physiol. **33:** 315-327.
Wei WJ, SR Yeh, EK Wang. 1991. Evidence for the coupling of muscarinic M3 receptor to cyclic AMP formation and polyphosphatidyl inositol turnover in rat salivary glands. Chinese J. Physiol. **34:** 301-312.
Yang CM, S-P Chou, TC Sung. 1991. Muscarinic receptor subtypes coupled to generation of diffeent second messengers in isolated tracheal smooth muscle cells. Br. J. Pharmacol. **104:** 613-618.
Yang H-Y, W Fratta, EA Majane, E Costa. 1985. Isolation, sequencing, synthesis and pharmacological characterization of two brain neuropeptide that mpodulate the action of morphine. Proc. Natl. Acad. Sci. USA **82:** 7757-7761.
Yang SD, EA Tallant, WY Cheung. 1982. Calcineurin is a calmodulin-dependent protein phosphatase. Biochem. Biophys. Res. Com. **106:** 1419-1425.
Yang, SD, CK Chou, M Huang, JS Song, HC Chen. 1989. Epedermal growth factor induces activation of protein kinase FA and ATP. Mg-dependent protein phosphatase in A431 cells. J. Biol. Chem. **264:** 5407-5411.
Yang SD, HS Hwang, TL Ha, YG Lai, YJ Jean. 1992. Dysfunction of insulin mediator protein kinase FA in lymphocytes of patients with NIDDM. Diabetes **41:** 68-75.
Yoshimura M, DMF Cooper. 1992. Cloning and expression of a Ca^{2+}-inhibitable adenylyl cyclase from NCB-20 cells. Proc. Natl. Acad. Sci. USA **89:** 6716-6720.
Yu JYL, ST Shen, YC Wu, SH Chen, CT Liu. 1991. Gonadotropin specficity and species diversity of gonadal steroid hormone formation in Fish. Bull. Inst. Zool. Acad. Sini. **16:** 61-68.
Zhang Y, AA Abdel-Latif. 1992. Acivation of phospholipase D by endothelin-1 and pharmacological agents in rabbit iris sphincter smooth muscle. Cell. Signaling **4:** 777-786.
Ziegler R, K Ekhart, H Schartz, R Keller. 1985. Amino acid sequence of *Manduca sexta* adipokinetic hormone elucidated by combined fast atom bombardment (FAB)/tandem mass spectrometry. Biochem. Biophys. Res. Comm. **133:** 337-342.

從動物進化看訊息傳遞

何 仁 傑

近年來由於分子生物學技術之引進，使得穿越細胞膜訊息傳遞（膜訊傳）之研究大有進展。膜訊傳系統之成員，包括訊息分子、細胞膜受體、訊息傳遞G蛋白、調控第二訊使之效應物、及第二訊使調控之蛋白激酶與磷酸蛋白磷酸酶，都具有多種亞型及同亞型分子之報告，但這些分子之基本生理功能大都尚不清楚。

本篇論述，依據動物進化之遠近，列出膜訊傳系統成員之最新進展，膜訊傳之基本機轉由低等至高等動物可能相似，但膜訊傳系統之分化數目與各成員間之偶聯方式，則可能是以聚合型式或分歧型式進化。目前顯然有許多具有學術意義之問題仍等待深入研究與解答，此時，正是作膜訊傳進化及比較研究之恰當時機，如同時由膜訊傳之功能與膜訊傳成員之分子結構著手研究，則將使膜訊傳系統之進化關係及既得之研究成果之生理意義更加確定，必將使今後在研究上可能遇到的困難大幅降低，展進更快。

關鍵詞：第二訊息，腺苷酸環化酶，磷脂酶，無脊椎動物，激素。