SIGNAL TRANSDUCTION; A TWENTY YEAR HISTORY OF G-PROTEINS

It is now recognized that large sets of membrane receptors are linked to proteins that bind and degrade GTP. The GTP binding proteins are regulatory proteins. They regulate the activities of membrane processes such as enzymes, ion channels, and transport systems that generate signals responsible for mediating the actions of external signals (hormones, neurotransmitters, light, odorants,etc.) on their target cells. This knowledge evolved largely from studies on the regulation of adenylyl cyclase by hormones and involved research laboratories around the globe.The purpose of this article is to provide an overview of the key findings and of the thoughts that provided fundamental insights in the step by step unraveling of this still-evolving story.

Research in this field was spawned by the discovery of a hormone-stimulated adenyl (now adenylyl) cyclase system by Sutherland and co-works in the late 50's . Sutherland determined that the enzyme system is located on the cell surface (plasma membrane) and that many hormones stimulate cAMP formation in a variety of cell types. This led to a minimalist model, analogous to a multisubunit enzyme, of a multi-subunit enzyme formed of a regulatory subunit responsible for hormone binding and a catalytic subunit responsible for the production of cAMP from the substrate ATP. This concept was summarized by Robision, Butcher, and Sutherland in 1967 (Fig1).

Today, we essentially know of two additional facts (and a multitude of details) . Receptors are distinct molecules that form linkages in the plasma membrane to the GTP-regulatory proteins (G-proteins). G-proteins are a family of structurally related proteins that functionally link activation of receptors to the regulation of a host of signal generating (or effector) systems, the number of which is still expanding.

That the hormone-sensitive adenylyl cyclase must be a multimeric enzyme complex formed of receptor molecules that exist independently from the adenylyl cyclase molecule was inferred by Birnbaumer et al (1969) and Rodbell et al (1970a) . Their studies with isolated fat cells showed that five different hormones acted at independent binding sites to stimulate a common adenylyl cyclase molecule. A common feature of the hormone stimulatory process was shown to be a dramatic increase in the apparent affinity of the system for Mg ions. (Fig 2).

A complex and as yet not fully understood signal transduction process was envisioned by Rodbell and coworkers (1969) as a key step in the conversion of the hormone binding signal into increased adenylyl cyclase activity. Borrowing from information transfer theory, they coined the terms discriminator for the role played by receptor, amplifier for the role of the cAMP forming enzyme, and transducer for the role of the intervening coupling process (Fig 3).

The actual physical separateness of receptors from adenylyl cyclase was shown by Orly and Schram (1976) when they transferred a β-adrenergic receptor from a cell in which the enzyme had been inactivated to a cell with active enzyme but defective in this receptor, and obtained functional stimulation of the acceptor adenylyl cyclase by the receptor from the donor cell. The first purification of the β-adrenergic receptor came from the laboratory of Lefkowitz, Caron, and associates (Shorr et al, 1982) and that of adenylyl cyclase was that of Pfeuffer (Pfeuffer and Metzger, 1982; Pfeuffer et al., 1985).

The role of GTP in hormonal stimulation of adenylyl cyclase was discovered by Rodbell's group (Rodbell et al., 1971a) as a consequence of findings that GTP regulates the specific binding of glucagon to liver membranes (Rodbell et al., 1971b). The basic findings were that without GTP in the assay medium glucagon exerted little effect on adenylyl cyclase activity, and that GTP dramatically increased the binding kinetics of the hormones to its receptor at the same concentrations required for hormonal stimulation of the enzyme. These findings suggested that the transducer is the site of GTP action, as conceptualized in Fig 4 (Rodbell, 1971c)

The first studies showing that the actions of GTP on receptors and adenylyl cyclase were not restricted to the liver came from findings that stimulation of human platelet adenylyl cyclase required GTP (Krishna et al, 1972) and that GTP exerted the same effects on receptors for angiotensin II in adrenal glomerulosa cell membrane as were seen for the glucagon receptor in liver. Later, independent studies from the laboratories of Lefkowitz (Lefkowitz et al, 1976) and Gilman (Maguire et al. , 1976) revealed that the effects of GTP on the β–adrenergic receptor were restricted to the binding of agonists.Many studies on different types or classes of receptors have shown that receptor binding affinities for agonists are regulated by guanine nucleotides. Such findings are now employed as an important means for ascertaining that receptors are linked to G-proteins.

Sutherland and his colleagues showed during the 60's that adenylyl cyclase activity is inhibited by catecholamines in some tissues (Butcher et al,1965). In 1973, Rodbell and collaborators (Harwood et al, 1973) reported that GTP both stimulated and inhibited adenylyl cyclase activity in fat cell membranes depending on the nucleotide's concentration. Fluoride ion, first shown by Sutherland and al (Sutherland and Rall, 195) to stimulate adenylyl cyclase through a process unrelated to receptors, was then found to both stimulate the enzyme and to inhibit the hormonal response of the enzyme in fat cells (Harwood and Rodbell, 1974). Stimulation and inhibition of adenylyl cyclase were later proposed to be due to separate processes based on kinetic and chemical modification studies (Yamamura et al., 1977) . Collectively, these findings set the stage for the concept developed later that adenylyl cyclase is dually regulated by separate sets of receptors coupled to different types of G-proteins (Rodbell, 1980).

In 1974, Rodbell's group emphasized that the actions of hormone and GTP in stimulating adenylyl cyclase were interdependent (Rodbell et al., 1974) and thus conceptually led to the realization that hormones act to regulate the effect of GTP on adenylyl cyclase as opposed to GTP inititating its action at the receptor. This concept was solidified by Rodbell's introduction of the GTP analog GMP-P(NH)P (Londos et al, 1974) which proved to be a potent activator of adenylyl cyclase in eukaryotic cells.Activation by GMP-P(NH)P was a slow process that was greatly accelerated by hormones (Salomon et al, 1975).

Not only was activation by GMP (NH)P slow in onset, its effects were persistent (Schramm and Rodbell, 1975). Because GMP P(NH)P is a poorly hydrolyzed substrate for GTPases, this raised the possibility that the GTP regulatory site might also be the active site of a GTPase present in the hormone-excited state of the GTP-regulatory process (Rendell et al, 1976). This was proven in turkey erythrocyte membranes by Cassel and Selinger (1976) who were the first to measure hormonal stimulation of GTP hydrolysis. They also showed that activation of adenylyl cyclase by cholera toxin was accompanied by inhibition of hormone-induced stimulation of GTP hydrolysis (Cassel and Selinger, 1977), and that receptor occupancy caused release of GDP, the product of GTP hydrolysis (Cassel and Selinger, 1978). They proposed a GTPase regulatory cycle as the controlling element of adenylyl cyclase activity. In this cycle GTP hydrolysis terminated stimulation; hormones activate the GTP-regulatory process by stimulating the exchange of bound GDP with GTP. Fig 5 illustrates the proposed regulatory cycle.

That nucleotide exchange is not the only step involved in hormone action on G-proteins was shown by Birnbaumer's group who noticed that, though hormones accelerate the actions of two non-hydrolyzable guanine nucleotides, GMP-P(NH)p and GTPγS, the analogs activate adenylyl cyclase at different rates (Birnbaumer et l., 1980; Iyengar et al., 1980).

Evidence that the site of action of GTP resides on a protein separate from adenyl cyclase and receptor was first published in 1975 (Pfeuffer and Helmreich, 1975). Resolution of a GTP-binding factor from adenylyl cyclase and its reconstituion to give ~~give~~ a stimulatable system was published in full by Pfeuffer in 1977. Simultaneously Ross and Gilman (1977) reported that membranes from a mutant lymphoma cell line thought to lack adenylyl cyclase (cyc-) acquired adenylyl cyclase activity upon addition of a detergent extract derived from wild type membranes. This cell line was isolated in Gordon Tomkin's laboratory as a result of its resistance to the killing effect of cyclic AMP (Tompkins,197) and which became part of a large number of powerful model cell systems for exploring the pathways of hormone action (Bourne et al., 1975).

Using the cyc- mutant, which turned out to contain adenylyl cyclase, Gilman and collaborators established that the reconstituting activity in detergent extracts was responsible for the stimulatory actions of GTP and fluoride ion, and for obtaining GTP-dependent hormonal stimulation of the system (Ross et al., 1978). This factor was thus the functional equivalent to the GTP-binding protein isolated earlier by Pfeuffer (1977).

The GTP binding/regulatory component of adenylyl cyclase was called N (for nucleotide binding) by Rodbell, and G/F(for mediation of effects of guanine nucleotides and fluoride ion) by Gilman. Today it is generally called Gs. It has been studied extensively in several laboratories. It is the site of action of cholera toxin (Johnson et al., 1978) and is the site at which GMP-P(NH)P acts persistently (Howlett et al, 1979). Importantly, it was shown to be the true carrier of information. Studies by Citri and Schramm (1980) showed that receptor-mediated activation of adenylyl cyclase could be separated into receptor-mediated activation of the G-protein by GMP-P(NH)P independently of the presence of adenylyl cyclase; the activated material was then transferred to another membrane containing adenylyl cyclase with resultant activation of the enzyme. The activated GTP -regulatory protein was shown to transfer from a membrane lacking adenylyl cyclase (human erythrocytes) to cyc-membranes without prior detergent

extraction (Nielsen et al, 1980), thus giving rise to the idea that activation of Gs may involve release of the protein from its membrane mooring.

Mg^{2+} is required for activation of Gs (Iyengar, 1981). By using a two-step assay modeled after that of Citri and Schramm, Iyengar and Birnbaumer (1982) determined that the mechanism by which hormone receptors promote the activation of Gs by guanine nucleotides involves a drastic reduction in the level of Mg^{2+} · These findings supported fully the earlier model proposed by Rodbell and Birnbaumer that Mg ions are fundamentally involved in the transduction process leading to stimulation of adenylyl cyclase activity by hormones (Fig 2). GTP and Mg ions act in concert on Gs in this process. It was clear by 1980 that the earlier concept that adenylyl cyclase systems are vectorial information transfer systems (Rodbell, 1974) involving at least three distinct, but interactive proteins (receptor, G protein, and enzyme) is correct.

The intimation that adenylyl cyclase may be regulated by two independent vectorial systems involving separate G proteins was provided by the biphasic actions of guanine nucleotides and fluoride ion on the fat cell adenylyl cyclase system, summarized earlier. but by a series of events in the late 70's and early 80's that led ultimately to the current complexity of structures and functions of G proteins.