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The experiences of a biochemist in the evolving world of G protein-dependent signaling

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PREFACE

This review describes how a biochemist and basic researcher (i.e. myself) came to make a career in the area of receptor-coupled signal transduction and the roles cellular signaling activities play both in normal physiology and in disease. Much of what has been the best part of this research life is due to the time I spent with Bob Lefkowitz (1982–1985), during an extraordinary period in the emerging field of G-protein-coupled receptors. Among my laboratory colleagues were some truly outstanding scientists including Marc Caron, the late Jeffrey Stadel, Berta Strulovici, Jeff Benovic, Brian Kobilka, and Henrik Dohlman, as well as many more. I came to Bob's laboratory after being trained as a physical biochemist and enzymologist. Bob and his laboratory exposed me to a research style that made it possible to connect the kinds of fundamental biochemical and mechanistic questions that I loved to think about with a direct relevance to disease. Indeed, I owe Bob a great deal for having imparted a research style and philosophy that has remained with me throughout my career. Below, I describe how this has taken me on an interesting journey through various areas of cellular signaling, which have a direct relevance to the actions of one or another type of G-protein

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1. The early days of G protein-coupled receptors (GPCRs): simplicity

I arrived in the Lefkowitz laboratory just after they had achieved the first purification of the β2-adrenergic receptor (HPLC) [1]. This was a heroic achievement, much of which was driven by Rob Shorr and Marc Caron, and soon moved to even greater heights by Jeff Benovic [2,3]. The purification of the putative β2-adrenergic receptor, comprised of just a single polypeptide chain, was based solely on the successful detergent-solubilization of a protein that exhibited the proper ligand-binding capability. Thus, the question remained as to whether this protein was capable of the other critical function that such a receptor needed to fulfill, namely, the ability to couple to its G-protein partner and transmit a signal. My goal upon arriving at the Lefkowitz laboratory in the summer of 1982 was to demonstrate that the newly purified β2-adrenergic receptor was capable of both hormone binding and signal

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propagation, by reconstituting the signaling interactions between the purified receptor, the purified Gs protein, and ultimately the purified effector protein, adenylyl cyclase. Eventually, I was able to do this but not without the help and efforts from others in the Lefkowitz laboratory (e.g. Caron, Benovic, Strulovici), as well as with the aid of some superb outside collaborators including Allen Spiegel, who at the time was an investigator at NIH (now the Dean of Albert Einstein Medical School), Lutz Birnbaumer, then at Baylor Medical College (now at the National Institute of Environmental Health), and the late Eva Neer of Harvard Medical School [4,5]. The reconstitution of the β2-adrenergic receptor signaling pathway showed us just how beautifully simplistic was the design of GPCR-signaling pathways (Fig. 1). Upon the binding of a hormone (agonist), the β2-adrenergic receptor (e.g. R* in Fig. 1) was able to associate with the heterotrimeric Gs protein, which had recently been purified by the laboratories of the late Al Gilman and Lutz Birnbaumer, stimulating the exchange of GDP for GTP on the alpha subunit of the G-protein (Gα). This resulted in the dissociation of the GTP-bound Gα subunit from its beta (Gβ) and gamma (Gγ) subunit partners, enabling its ensuing interaction with its effector protein, adenylyl cyclase (i.e. as depicted by E_1 in Fig. 1).

From this beginning came the discovery of additional layers of regulation. One critically important mode of regulation, as discovered by Jeff Benovic, results in the desensitization of the receptor-coupled signaling pathway as an outcome of receptor phosphorylation by G protein-coupled receptor kinases GRKs [6–8]. Receptor phosphorylation enabled members from a family of proteins called arrestins to directly bind to the receptor, resulting in a steric interference of G-protein coupling [9–11]. This not only shuts down receptor communication with its G-protein partner, but it also leads to receptor endocytosis and ultimately receptor ubiquitylation and degradation.

A second key mode of regulation was first discovered by Cassel and Selinger [12], who showed that GTP-hydrolysis by the Gs protein serves as a mechanism for halting the stimulation of adenylyl cyclase. Additional studies showed that Gα subunits were capable of an intrinsic GTP hydrolytic reaction that occurs within 30 s, although this shut-off mechanism can be greatly accelerated by members of the family of RGS (Regulators of G-protein signaling) proteins [13–15]. Any disruption of these regulatory mechanisms can have dire consequences. One classic example is cholera toxin-mediated pathogenesis, as this toxin catalyzes the modification of an arginine residue on the Gα subunit of the Gs protein that is essential for GTP-hydrolysis. Consequently, the cholera toxin-modified Gs-α subunit persists in a GTP-bound state, unable to shut-off, disrupting normal ion channel function in the intestine and resulting in severe diarrhea.

Perhaps even more remarkable was the realization of just how often this signaling architecture is used in biology [16]. A striking example is our ability to see in dim light through the phototransduction pathway operating in retinal rods [17,18]. In this case, the absorption of light by the photoreceptor, rhodopsin, stimulates GDP-GTP exchange on the G-protein transducin, which generates a GTP-bound Gα subunit that binds and activates an effector enzyme, the cyclic GMP phosphodiesterase, converting cyclic GMP to GMP. The reduction in the levels of cyclic GMP causes the closing of a sodium channel in retinal rods, resulting in a hyperpolarization of the rod membranes, which represents the signal that is sent to the optic nerve. Through the years, it became clear that similar types of GPCR-signaling systems were responsible for other sensory response systems, such as those that recognize different odorants and tastants (i.e. our senses of smell and taste), as well as for the regulation of smooth muscle contraction, platelet activation, various neurotransmitter activities and metabolic functions. In these various systems, both the Gα subunit bound to GTP and the Gβγ complex can engage and regulate effectors (E_1 and E_2 in Fig. 1).

2. A starting assumption: growth factor-dependent signal transduction utilizes similar signaling systems as those used by GPCRs

It was with this as a backdrop that I started to think about the similarities that might exist between GPCR-signaling systems and those stimulated by growth factors which control the normal growth of cells, and when de-regulated, give rise to cancer. Thus, upon starting my independent academic career at Cornell, I decided I would set out to reconstitute epidermal growth factor receptor (EGFR)-coupled signaling, with the idea that the EGFR would activate a G-protein, possibly through an EGFR-catalyzed phosphorylation of the G-protein. I assumed that the activated G-protein would then stimulate the activity of an effector protein, transmitting a signal to help drive cell cycle progression and mitogenesis. What made this idea particularly attractive was the existence of an obvious candidate for the G-protein functioning in EGFR-signaling, namely Ras.

The Ras (for *Rat sar*coma) protein was first identified as the causative agent in the rat sarcomas caused by the Harvey and Kirsten retroviruses. Ras was subsequently identified by a number of laboratories, including the Weinberg, Wigler, Barbacid, and Cooper groups, as the first human oncogene product [19–22]. Importantly, the Ras mutations driving tumorigenesis involved substitutions that prevented Ras from hydrolyzing GTP. Therefore, it seemed logical to assume that a normal mitogenic signaling pathway could start with the EGFR prompting the exchange of GDP for GTP on the Ras protein, with GTP-bound Ras then activating an effector protein for a defined period of time, before GTP-hydrolysis shuts down signal propagation. Those oncogenic mutations that prevent Ras from shutting off would give rise to uncontrolled cell growth and thereby represent an initial and important step in tumorigenesis.

Starting with this working model, my laboratory set out to reconstitute the functional coupling between highly purified preparations of the EGFR and Ras in liposomes, examining whether Ras was tyrosine

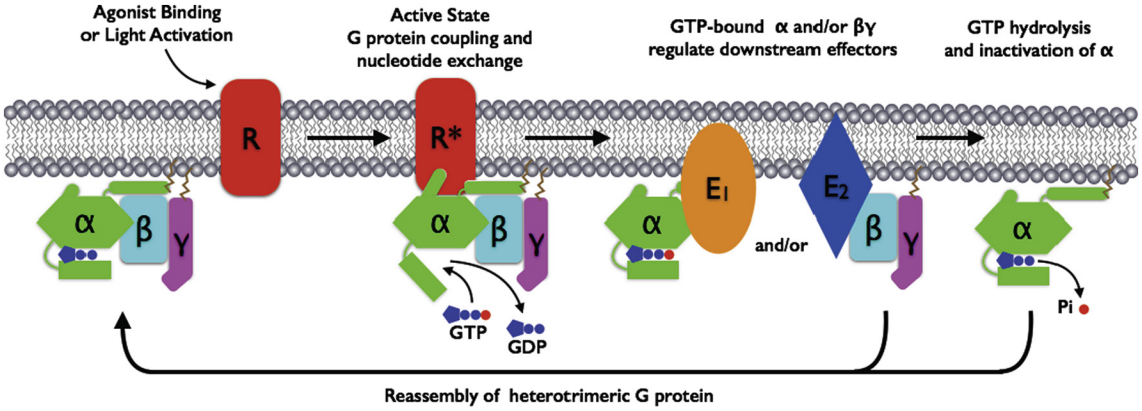

Fig. 1. A depiction of GPCR-G-protein-dependent signaling.

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