# A Brief History of G Protein Coupled Receptors

Nobel Lecture, December 8, 2012

by Robert J. Lefkowitz

Howard Hughes Medical Institute, Duke University Medical
Institute, Durham, North Carolina 27710 USA.

The idea of receptors has fascinated scientists for more than a century and has certainly fascinated me and Brian throughout the entirety of our research careers. Today we know that the G protein coupled receptors, also known as seven transmembrane receptors, represent by far the largest, most versatile and most ubiquitous of the several families of plasma membrane receptors. They comprise almost a thousand genes which regulate virtually all known physiological processes in humans including the sensory modalities of vision, taste and smell. Moreover, these receptors are the targets for drugs accounting for more than half of all prescription drug sales in the world (1).

Despite the very central role that the study of receptors plays in biomedical research today, it is only in the last thirty years or so that there has been any general acceptance they even exist. Prior to that time, the notion of cellular receptors was highly controversial and associated with a great deal of skepticism. Perhaps the earliest explicit assertion concerning the existence of receptors was made by the British pharmacologist, J.N. Langley. In 1905 he wrote the following: "So we may suppose that in all cells two constituents at least are to be distinguished. The chief substance which is concerned with the chief function of the cell as contraction and secretion and receptive substances which are acted upon by chemical bodies and in certain cases by nervous stimuli. The receptive

substance affects or is capable of affecting the metabolism of the chief substance" (2).

Langley's statement thus explicitly postulates the two inter-linked functions of these hypothetical receptor structures: first, they interact with chemicals and stimuli, presumably by binding them in a specific way, and second, they act upon effectors within the cell to alter their function.

Langley's idea however was generally either ignored or derided. Exemplary of this is the following statement written some forty years later, ironically by his student Henry Dale who won the Nobel Prize for his studies on cholinergic neurotransmission. However, in 1943 he had this to say about his mentor's receptor idea: "It is a mere statement of fact to say that the action of adrenaline picks out certain such effector cells and leaves others unaffected; it is a simple deduction that the affected cells have a special affinity of some kind for adrenaline, but I doubt whether the attribution to such cells of "adrenaline receptors" does more than restate this deduction in another form" (3).
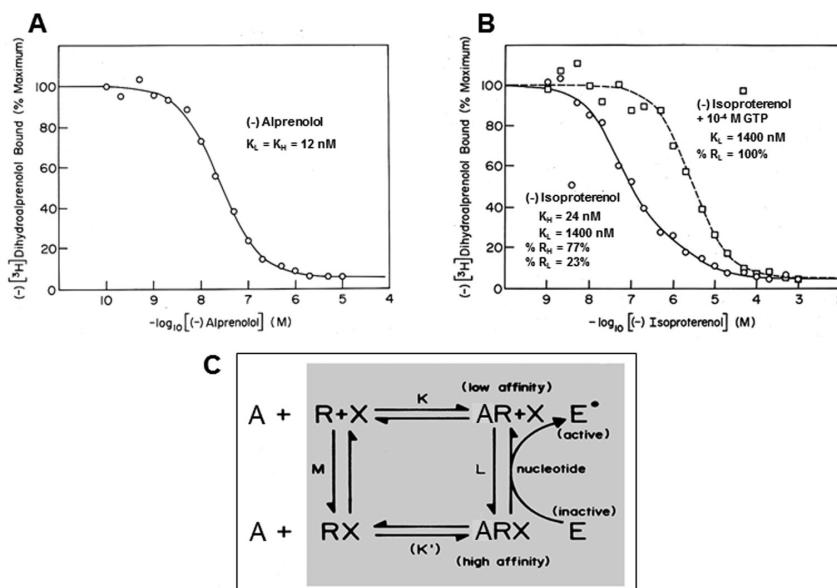


**FIGURE 1.** Radioligand binding to the $\beta_2$-adrenergic receptor from frog erythrocyte membranes.
A. Computerized curve fitting of binding data from displacement of [$^3$H] dihydroalprenolol by the antagonist (–) alprenolol. B. Displacement by the agonist (–) isoproterenol in the presence (□–□) or absence (○–○) of $10^{-4}$ M GTP. C. The ternary complex model. A and B are reproduced with permission from reference 11, C is reproduced from reference 12.

Perhaps even more ironic is this next quote from Raymond Ahlquist. He was a distinguished, classical pharmacologist of the mid-20th century who won the Lasker Prize for his paper in 1948 asserting that there were two types of receptors for adrenaline, which he called $\alpha$ and $\beta$ based on the differing abilities of various adrenergic agents to stimulate several physiological processes (4). Nonetheless, some twenty-five years later he wrote the following "This would be true if I were so presumptuous as to believe that $\alpha$ and $\beta$ receptors really did exist. There are those that think so and even propose to describe their intimate structure. To me they are an abstract concept conceived to explain observed responses of tissues produced by chemicals of various structure" (5).

So, it was against this background of skepticism that those of us who were interested in trying to bring these mythical receptors to life began the work some forty years ago. It was immediately clear that if we were to succeed it would be necessary to develop a whole suite of new technologies which did not then exist and that the first of these would have to be radioligand binding methods to study the receptors directly. And so together with student Rusty Williams and post doc Marc Caron I set out to develop such methods, initially for the $\beta$-adrenergic receptor (6) and then the $\alpha$-adrenergic receptor (7). The radioligand binding techniques that we developed in the early 70s immediately allowed us to study the regulation of the receptors by numerous factors (8), to discover previously unsuspected receptor subtypes (9), and to develop theories concerning the mechanisms of receptor action.

For example, we found that binding competition curves for antagonists such as the $\beta$-adrenergic antagonist alprenolol are steep and monophasic; whereas those for agonists, like isoproterenol or epinephrine, are shallow (10) showing two distinct binding states, one of high and one of low affinity (11) (Fig. 1A). The two states could be interconverted by the addition of guanine nucleotides which lead to a single population of receptors in the low affinity state (Fig. 1B). Together with Andre DeLean we developed the ternary complex model to explain this behavior (Fig. 1C) (12). It postulated that the low affinity form of the receptor, shown here as AR, is a complex of the agonist with the free receptor, whereas the high affinity form is a ternary complex of agonist, receptor and some other membrane component. The modulatory role of nucleotides on the affinity of agonist for the receptor immediately suggested that the component X in the scheme was the, at that time, very recently discovered guanine nucleotide regulatory protein (G protein). The ability of an agonist to drive the interaction of the receptor with the guanine nucleotide regulatory protein to form the ternary complex is essentially a measure of its efficacy to stimulate adenylate

cyclase. It can be simply approximated by the ratio of affinities of the agonist for the low (binary) and high (ternary) affinity forms of the receptor which can be easily obtained from these competition curves. These findings represent perhaps the earliest direct demonstration of the allosteric interactions of the receptor with agonists and effector G proteins, the binding of each to the receptor allosterically enhancing the binding affinity of the other. This approach to analyzing such ligand binding data has been universally applicable to the large family of GPCRs.

Undoubtedly one of the most important applications of our ligand binding techniques was to help us tag the putative receptor molecules through various stages of purification, so that we could isolate them. Purifying the $\beta$-adrenergic and other adrenergic receptors was truly a daunting task. The molecules are virtually trace contaminants of plasma membranes and required 100,000-fold purification. Moreover, we had to first figure out how to solubilize them from the membranes before we could even begin the purification work. Ultimately, the key to our success was our development of affinity chromatography matrices in which we were able to couple various $\beta$-adrenergic and then $\alpha$-adrenergic antagonists to solid supports (13–15) (Fig. 2A). Biospecific adsorption and elution from such columns with adrenergic ligands followed by other more conventional chromatographic steps ultimately led to our isolation of homogenous preparations of each of the four then known adrenergic receptors, $\alpha_1$, $\alpha_2$, $\beta_1$ and
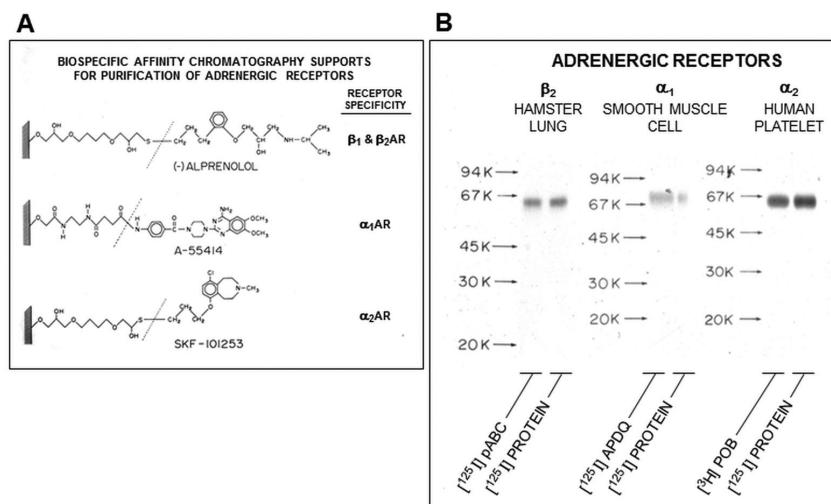


**FIGURE 2.** Isolation of the adrenergic receptors by affinity chromatography.

$\beta_2$ (reviewed in 16). Shown on this SDS polyacrylamide gel are such preparations for three of those four adrenergic receptors (Fig. 2B). This figure represents approximately one decade of work by a number of devoted students and postdocs, most notably Marc Caron and Jeff Benovic, among others. Each of the isolated putative receptor molecules is a glycoprotein of approximately 60,000–65,000 daltons molecular weight which bound specific alpha and $\beta$-adrenergic ligands with appropriate specificity and stereospecificity matching what would be expected from classical pharmacological experiments (16).

However, skepticism persisted as to whether these isolated molecules could also perform the companion function of a receptor, namely the ability to activate specific biological processes. That this was in fact the case was demonstrated by a talented postdoc, Rick Cerione. Initially, he reconstituted our purified $\beta$-receptor proteins into phospholipid vesicles and then fused these with erythrocytes from *Xenopus laevis*, the African clawed toad (17). These cells, while possessing the adenylate cyclase enzyme system and other GPCRs like the prostaglandin receptor, contained no $\beta$ receptors and hence $\beta$-adrenergic agonists did not stimulate adenylate cyclase activity. Once the receptor containing vesicles were fused with the cells, thus carrying the receptors into the cell membrane, the adenylate cyclase acquired responsiveness to $\beta$-adrenergic drugs. Within a year, and in collaboration with Lutz Birnbaumer and the late Eva Neer, we were able to achieve a complete reconstitution of a catecholamine sensitive adenylate cyclase from three proteins, the purified $\beta$-adrenergic receptor, the guanine nucleotide regulatory protein (Gs) and the catalytic unit of adenylate cyclase (18). These results conclusively proved that the isolated proteins were in fact the $\beta$-adrenergic receptor and were capable of carrying out both functions of a true receptor.

With highly purified, validated receptor proteins in hand we were able to obtain short stretches of amino acid sequence from cyanogen bromide fragments of the $\beta_2$-adrenergic receptor and to use these to design oligonucleotide probes to clone the cDNA and gene for the $\beta_2$-adrenergic receptor (19). This successful effort was done collaboratively with a team from Merck and my lab, and featured the first successful research effort of a young cardiology fellow who had been working in my lab for several years. His name was Brian Kobilka. In this first cloned sequence of a ligand binding GPCR we could observe all of the features now viewed as canonical for the family (Fig. 3): seven apparent transmembrane spanning hydrophobic domains, sites for regulatory phosphorylation in cytoplasmic domains and consensus sequences for glycosylation at the amino terminus. In what was at the time a remarkable surprise we observed sequence homology, shown here in blue (Fig. 3), with the visual light sensing protein
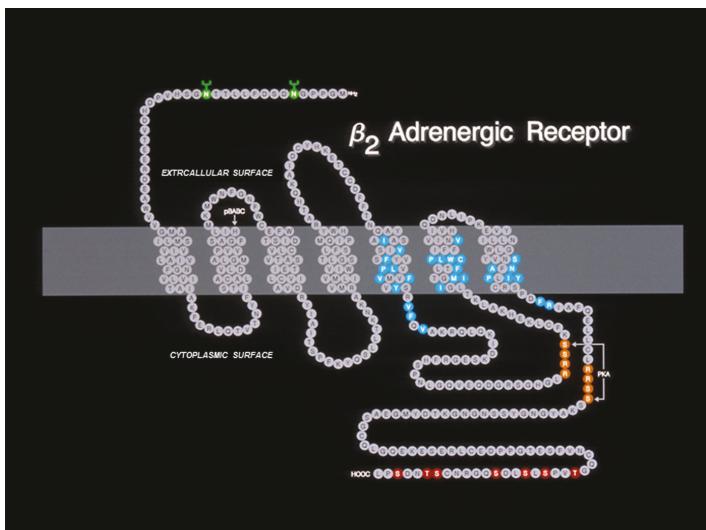
**FIGURE 3.** Cloning of the $\beta_2$-adrenergic receptor. Residues shaded in blue are homologous with rhodopsin; orange are consensus PKA phosphorylation sites; red, sites of GRK phosphorylation; green, consensus sites for N-linked glycosylation. Reproduced with permission from reference 65.

rhodopsin. Today, twenty-five years on, people find it hard to understand why this would come as a surprise. This was because rhodopsin, whose sequence had been determined a couple of years earlier by conventional protein sequencing (20,21), and bacteriorhodopsin (22), a light sensitive proton pump from archebacteria, were at the time the only two seven membrane spanning proteins known. And, since both were light-sensitive proteins, it had been speculated that seven membrane spans must be a signature feature of light sensitive molecules (20,21). Only with the cloning of the $\beta_2$-adrenergic receptor did it begin to emerge that it was instead the signature feature of G protein coupled receptors. Within a year we had cloned the highly homologous $\alpha_2$-adrenergic receptor (23) and within several years a total of eight adrenergic receptors (24), three of which were based on protein sequencing of the isolated molecules (19,23,25) and a serotonin receptor (26). All showed the conserved 7TM organization.

So by 1987 we were quite convinced that all of the then known G protein coupled receptors would likely be members of the superfamily of seven transmembrane receptors (27). Over the next several years the family grew rapidly as many laboratories cloned GPCRs almost invariably by homology techniques such as low stringency screening and then PCR. In fact, subsequently almost no other GPCR was ever purified prior to its cloning. Thus we always felt good about

the very difficult decade or more of work that went into the purification of the four adrenergic receptors which had provided the first sequences, the Rosetta Stone if you will, upon which the much larger superfamily could then be built.

We next used several techniques to try to understand how the unique and highly conserved seven transmembrane-spanning receptor structure determined the two core functions of ligand binding and G protein activation. We relied primarily on site directed mutagenesis (28) and the creation of the first chimeric receptors, in this case chimeras of the $a_2$ and $\beta_2$-adrenergic receptor (29). Again Brian took the lead in this work. While closely related in structure (these two receptors showed 50% sequence identity) they perform diametrically opposite biochemical and physiological functions with the $a_2$ receptor inhibiting adenylate cyclase through Gi and the $\beta_2$-adrenergic receptor stimulating it through Gs (Fig. 4A). By creating these chimeras we were able to demonstrate that the G protein coupling specificity and hence the function of the $a_2$-receptor could be converted from Gi inhibitory to Gs stimulatory simply by replacing its third cytoplasmic loop with that of the $\beta_2$-adrenergic receptor (Fig. 4B). These and many other such studies would confirm that the membrane spans and extracellular loops were responsible for the ligand binding specificity of the receptors whereas the regions shown in blue here in the cytosol were responsible for determining the specificity of G protein coupling (30,31) (Fig. 4C).
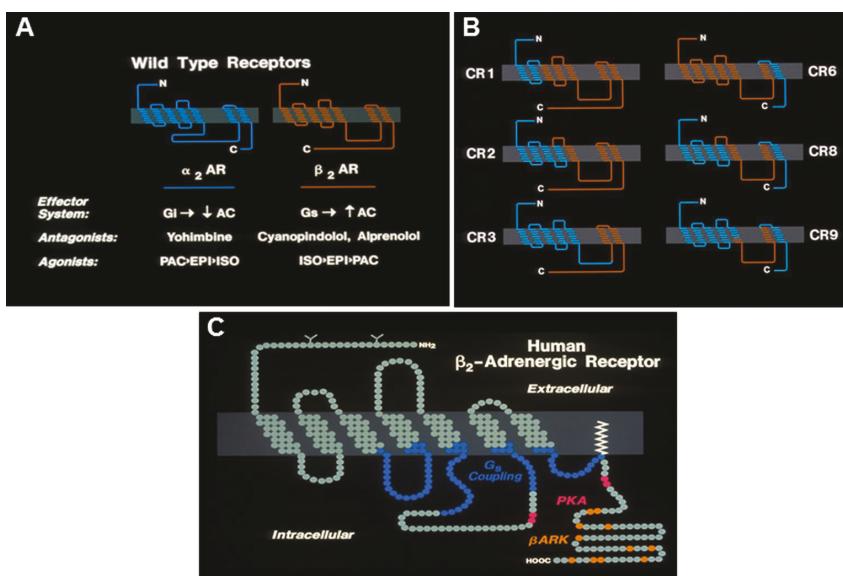


FIGURE 4.  Chimeric $a_2$-$\beta_2$-adrenergic receptors. Adapted from reference 29 and 65.
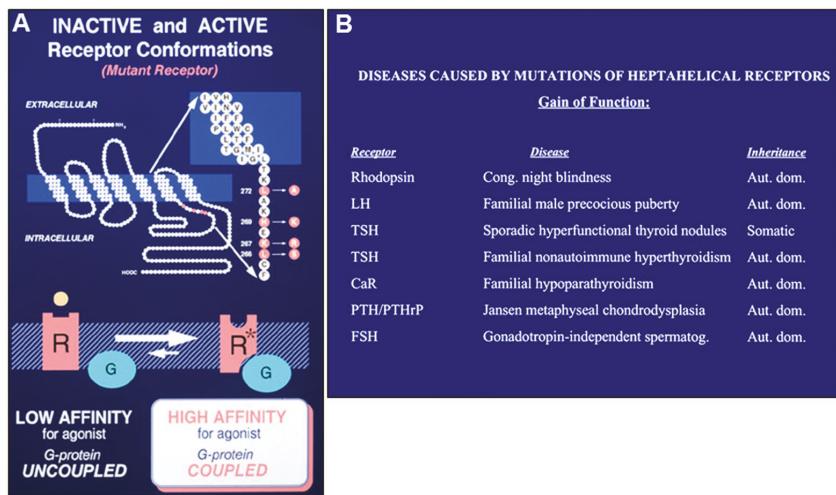
**FIGURE 5.** Constitutively active mutant adrenergic receptors.

In the course of performing further mutagenesis Susanna Cotecchia, a fellow in the lab, serendipitously discovered, much to our surprise, that some mutations in the distal part of the third cytoplasmic loop of various adrenergic receptors led to constitutively active mutant receptors. These are receptors which are active even in the absence of ligand (32–34) (Fig. 5A). At the time we conceptualized this as being due to their abrogation of certain crucial intramolecular interactions which would normally keep the receptor in its inactive conformation, shown as R and hence mimic the conformational changes normally produced by agonists leading to the active form of the receptor able to couple to G shown as R* (Fig. 5A). Shortly Brian Kobilka will explain how x-ray crystal structures of the receptors have revealed the nature of the intramolecular constraints which are ruptured by agonist-induced activation of the receptors. Interestingly, a growing list of human diseases have now been shown to be due to such activating mutations of various GPCRs (Fig. 5B) (35).

Contemporaneous with this work on the structure of the receptors, my laboratory had focused on trying to understand the virtually universal phenomenon of receptor desensitization. This phenomenon is illustrated in Fig. 6 for the β-adrenergic receptor expressed in a cultured cell system. When an agonist, such as isoproterenol, which is a synthetic congener of epinephrine is added to the cells, it stimulates the receptors and cAMP is elevated in response. But within a few minutes the levels return essentially to the unstimulated state despite the continued presence of the drug (36). From the earliest days of my
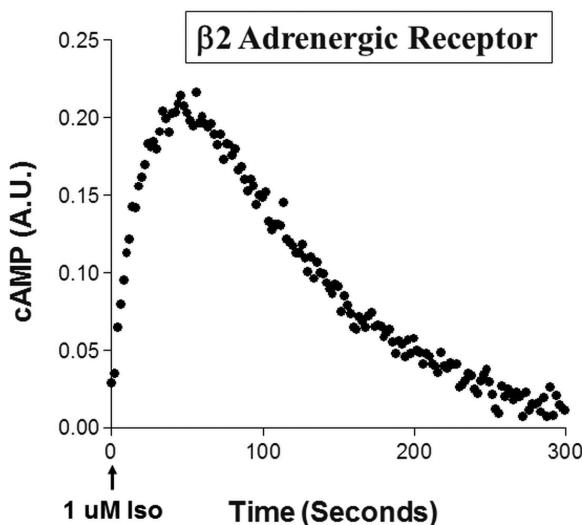
**FIGURE 6.** Desensitization of cAMP production after stimulation of the $\beta_2$-adrenergic receptor. Reproduced with permission from reference 36.

career, I had always been fascinated by this phenomenon perhaps because it represents such a clear example of what is one of the most pervasive principles of physiology, termed homeostasis. Thus, cells and tissues when stimulated in almost any way have myriad mechanisms which tend to return them to their basal unstimulated state.

Let me tell you how we came to discover the major biochemical mechanism responsible for such desensitization. In about 1980 when we had just developed photoaffinity probes for the $\beta$-adrenergic receptor (37), Jeff Stadel, a fellow in the laboratory, used this probe to label $\beta$-adrenergic receptors in cells which had been desensitized by prior exposure to the agonist isoproterenol (38). When we subjected these photoaffinity labeled desensitized receptors to SDS Page we observed that their mobility in the gels was retarded (Fig. 7). It had recently been described that phosphorylation of membrane proteins often led to such changes in electrophoretic mobility. Accordingly, we labeled the cells with in-organic phosphate and were able to demonstrate that the catecholamine in-duced desensitization was associated with phosphorylation of the $\beta$-adrenergic receptor (38).

Over the next several years a talented graduate student, Jeff Benovic, was able to first identify the novel kinase responsible for the phosphorylation (39), to purify it to homogeneity from bovine brain (40) and to clone its cDNA (41). It was a novel cAMP independent kinase which we called $\beta$ARK, for $\beta$-adrenergic
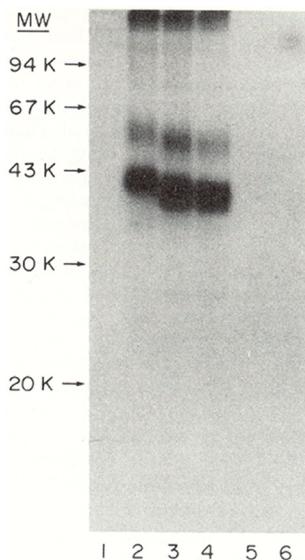
**FIGURE 7.** Desensitization involves receptor phosphorylation. SDS-PAGE of $\beta_2$-adrenergic receptors from turkey erythrocyte membranes covalently labeled with the photoaffinity probe [^{125}I]-p-azidobenzylcarazolol. Lane 2, cells incubated with isoproterenol, 1 $\mu$M; lane 3, isoproterenol, 1 $\mu$M in the presence of 10 $\mu$M propranolol (an antagonist); lane 4, control cells incubated with buffer alone. Reproduced with permission from reference 38.
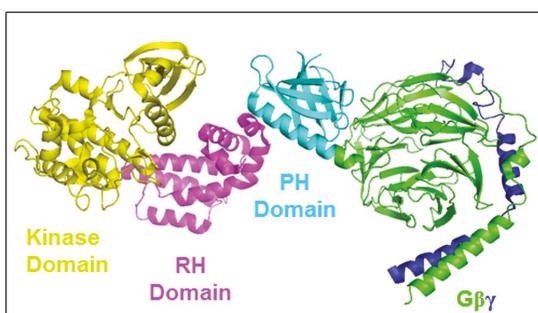


**FIGURE 8.** G Protein Coupled Receptor Kinases. Reprinted with permission from reference 44.

receptor kinase. It is now known as G protein coupled receptor kinase 2, or GRK2. Contemporaneously, it had been found that rhodopsin was phosphorylated by a novel kinase termed rhodopsin kinase, when it was bleached by light (42). Similarly, this seemed to be associated with a reduction in its function. We were able to clone the cDNA for rhodopsin kinase (42) and show that it and $\beta$ARK were

the first two members of a novel kinase subfamily, now referred to as G protein coupled receptor kinases. Today we know this family contains seven enzymes, two, GRKs 1 and 7 are limited to the retina. GRKs 2, 3, 5 and 6 are ubiquitously expressed (43) (Fig. 8). Thanks to the work of John Tesmer's lab, crystal structures for several of these are available, the first being the structure that he solved in collaboration with my laboratory about 10 years ago for GRK2 in complex with the $\beta\gamma$ subunits of the G proteins, with which it interacts in the cytosol to facilitate its translocation to the plasma membrane bound receptors (44) (Fig. 8). All members of the family share a conserved tripartite domain structure with a central conserved catalytic kinase domain flanked by two more divergent regulatory domains.

But it turned out there was more to the story than just these GRKs phosphorylating the receptor. In the course of purifying GRK2 we found that even as its specific activity for phosphorylating receptor preparations increased, its ability to desensitize the isolated receptor, assayed in a reconstituted system, declined. This suggested to us that we might be losing some other required element or cofactor (45).

At about this time the late Hermann Kuhn described his observation that an abundant retinal protein, then only known as 48K protein or S-antigen, somehow worked together with rhodopsin kinase to deactivate rhodopsin (46). Accordingly, the protein was renamed arrestin, because it "arrested" rhodopsin function. We immediately speculated that this protein might be similar to what we were losing during our GRK2 purification. Granted, its restricted expression to only the retina meant that it could not be the very protein we were seeking. Calling Kuhn, I arranged for him to ship us some of this 48K protein, which Benovic was, in short order, able to demonstrate restored the ability of $\beta$ARK or GRK2 to desensitize the $\beta$receptor in vitro, albeit at high concentrations (45).

Very shortly thereafter, Toshimichi Shinohara at the NIH cloned the cDNA for this retinal protein (47). Reasoning that what we might be losing during our enzyme preparations might not only be functionally analogous with the retinal arrestin or 48K protein, but in fact structurally homologous with it, we obtained Shinohara's clone and, using low stringency screening techniques, Martin Lohse, then a fellow in the laboratory, was able to clone the cDNA for a 70% sequence identical molecule which we named $\beta$-arrestin (48). A year or two later another fellow, Håvard Attramadal, was able to clone another similar molecule which we termed $\beta$-arrestin2 (49). Now, with all three authentic recombinant arrestin molecules in hand, visual arrestin and the two $\beta$-arrestins 1 and 2, we could compare their abilities to desensitize rhodopsin and the $\beta$-receptor *in vitro* in reconstituted systems (Fig. 9). When we used rhodopsin kinase phosphorylated
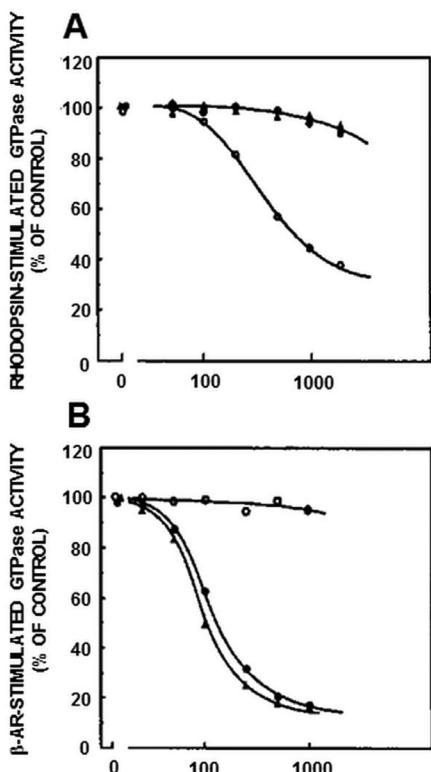
**FIGURE 9.** Inhibition of $\beta_2$-adrenergic receptor and rhodopsin function by $\beta$-arrestin 1, $\beta$-arrestin 2, and arrestin in reconstituted systems. (A) rhodopsin was phosphorylated with rhodopsin kinase. (B) $\beta_2$-adrenergic receptors were phosphorylated with GRK2. Rhodopsin-stimulated transducin GTPase was most potently inhibited by visual arrestin (○). In contrast, $\beta_2$-adrenergic receptor-stimulated Gs GTPase was much more potently inhibited by $\beta$-arrestin 1 (●) or $\beta$-arrestin 2 (▲). Reproduced with permission from reference 49.

rhodopsin, visual arrestin was quite potent in inactivating signaling, whereas the two $\beta$-arrestins were quite weak (Fig. 9A). Conversely, for GRK2 phosphorylated $\beta$-receptor, the two $\beta$-arrestins were highly potent and arrestin was very weak (Fig. 9B). These findings established the commonality of desensitization mechanisms for both rhodopsin and the $\beta$-receptor, albeit with great specificity for the arrestin molecule involved (49).

Today we know that there are four arrestins. Two of them, arrestin1 and X arrestin, are limited to the retina. $\beta$-Arrestins 1 and 2, also known as arrestin 2 and 3, are ubiquitously expressed. Crystal structures of all have been solved and show, in each case, a two domain protein consisting almost entirely of antiparallel $\beta$ sheets connected by a hinge region and stabilized by a polar core (50) (Fig. 10).

| | **AKA** | **Distribution** | **7MSR** |
|---|---|---|---|
| **Arrestin 1** | (Visual Arrestin) | Retinal rods | Rhodopsin |
| **β-Arrestin 1** | (Arrestin 2) | Ubiquitous | Most |
| **β-Arrestin 2** | (Arrestin 3) | Ubiquitous | Most |
| **X Arrestin** | (Arrestin 4) | Retinal cones | Opsins |
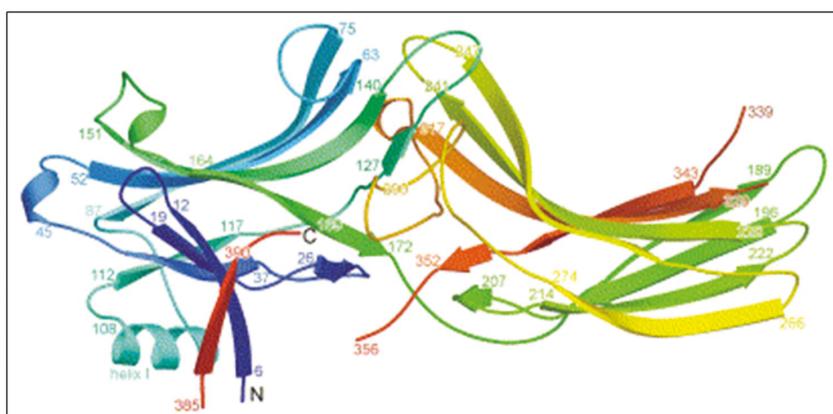


**FIGURE 10.**  The arrestins. Adapted from reference 50 with permission.

So where this all brought us to by the mid 90s is depicted in Fig. 11. It illustrates the two universal paradigms which govern the function of the GPCRs, and is based on the understanding that three families of proteins share the remarkable property of being able to interact almost universally with the receptors in an entirely agonist or stimulus dependent fashion. These proteins are the heterotrimeric G proteins, the G protein coupled receptor kinases and the β-arrestins. After stimulation, the activated receptors interact with the G proteins to lead to cell signaling via a cascade of phosphorylations. However, the activated receptors are recognized and phosphorylated by GRKs leading to the binding of a second protein, β-arrestin, which then sterically interdicts stimulation of the G protein by the receptor, thus leading to desensitization and waning of physiological responses (51). We also discovered other feedback mechanisms which operate to reduce receptor signaling activity, such as phosphorylation of the receptors by second messenger kinases like PKA and PKC. But they are generally not receptor specific (52).

Over the past ten years, however, an entirely new paradigm has emerged as we have realized that the β-arrestin-GRK system is actually multifunctional (Fig. 12). Thus it not only desensitizes G protein-mediated signaling but simultaneously serves as a signal transducing system in its own right. β-Arrestins act as adaptors or scaffolds which link the receptors to an ever growing number of
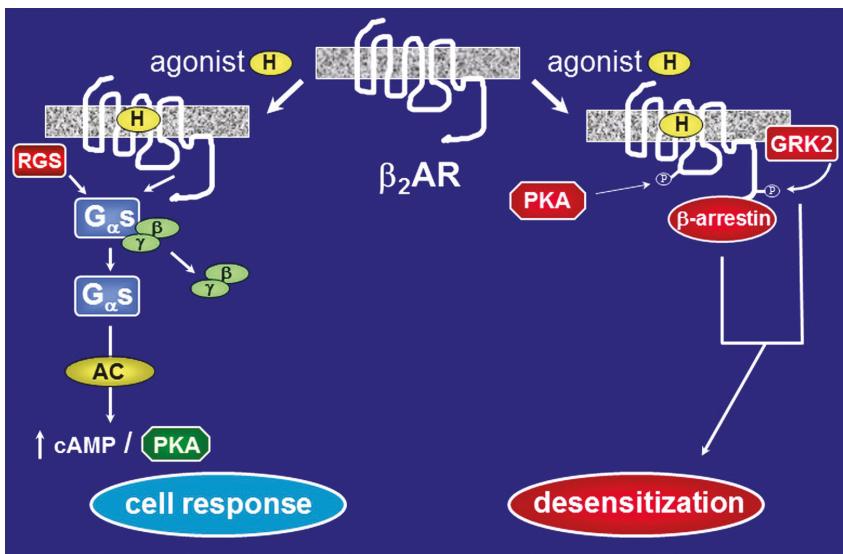
**FIGURE 11.** Activation and desensitization of GPCRs.
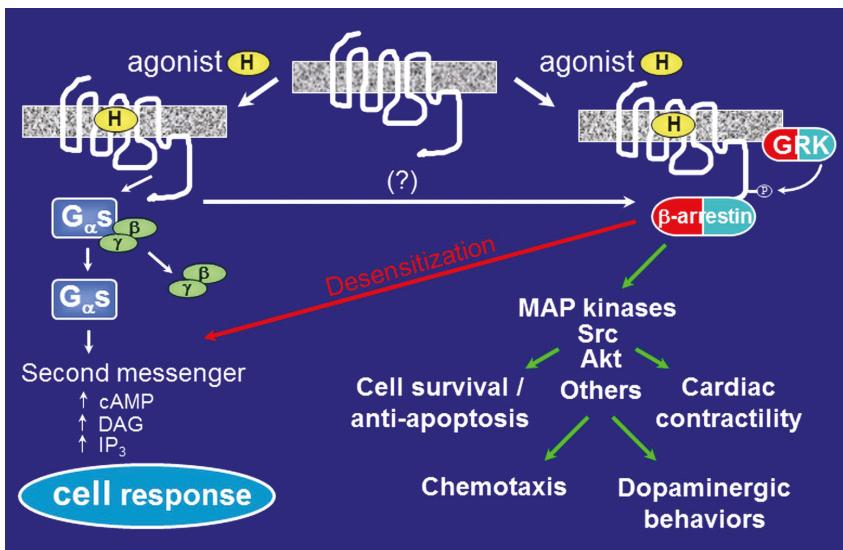


**FIGURE 12.** New paradigm for multiple β-arrestin-mediated functions. Reproduced with permission from reference 65.

intracellular molecules (53,54). Some of the pathways which have been demonstrated over the past few years are shown here, as are the resulting cellular physiological consequences. Most thoroughly studied have been the MAP kinase enzymes. $\beta$-Arrestins also mediate clathrin coated pit endocytosis by interacting with a growing list of elements of the endocytic machinery (55). Thus, the $\beta$-arrestins mediate three types of function, desensitization, receptor internalization and signaling.

In the course of studying $\beta$-arrestin mediated signaling, we made an interesting discovery which has greatly facilitated this work and which may also have significant therapeutic implications. This was of so-called biased agonists (reviewed in 56). A biased agonist is a ligand which stabilizes a particular active conformation of a receptor, thus stimulating some responses but not others. Seven transmembrane receptor ligands, for example, can be biased toward a particular G protein or $\beta$-arrestin. Mutated receptors can also be biased.

In the classical two state model of receptor activation, receptors can exist as either an inactive receptor R or an active receptor R*, with agonists stabilizing the active conformation which promotes cellular effects. In this model all the effects of the receptor are a consequence of the single activated R* form of the receptor. However, the existence of biased agonists which can lead to stimulation of exclusively $\beta$-arrestin- or G protein-mediated signaling implies that there must be multiple active conformations of the receptor (57).

By way of illustration, Fig. 13 shows some data comparing the ability of three ligands to promote interaction of the angiotensin $AT_{1A}$ receptor with G proteins ($G_q$) or $\beta$-arrestin in a cell line expressing the receptor (58). G protein activation is being assayed by a typical second messenger assay and is shown in red, and $\beta$-arrestin recruitment is shown in black. The first panel shows the dose response curves for a typical unbiased full agonist, angiotensin itself. You can see that the
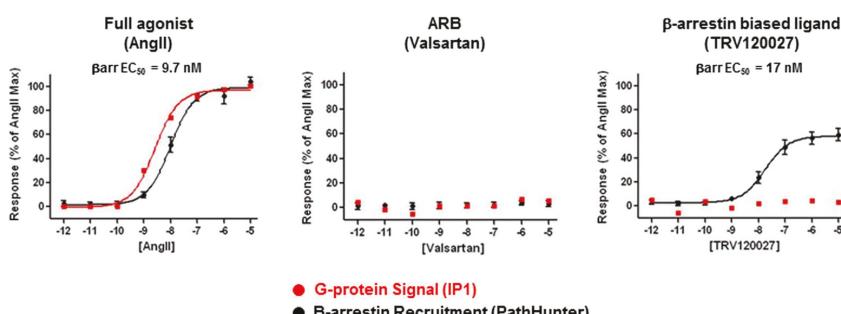


**FIGURE 13.** Activation of $G_q$ and $\beta$-arrestin by the angiotensin $AT_{1a}$ receptor stimulated by various ligands. See text for details. Reproduced with permission from reference 58.

curves for G protein and *β*-arrestin interaction are very similar. This middle panel depicts the situation for a classical competitive antagonist which has no activity in either assay. The third panel shows the data for a completely *β*-arrestin biased ligand, in this case TRV120027, which is an octapeptide analog of angiotensin. As you can see, this ligand has absolutely no ability to activate G protein signaling, thus it is a classical competitive antagonist for G protein activation, but it has substantial activity for *β*-arrestin recruitment.

As I noted, one consequence of this *β*-arrestin recruitment is to stimulate signaling pathways such as the ERK map kinase in a fashion parallel to and in some cases completely independent of G-proteins. In order to gain a more global view of the consequences of *β*-arrestin-mediated signaling we have used mass spectrometry to quantify all the phosphorylation sites present in cells before and after stimulation of the angiotensin receptor with a *β*-arrestin biased agonist which does not activate the G proteins (59).

As shown in Fig. 14, a number of signaling networks lit up with phosphorylation when *β*-arrestin, but not G protein, mediated signaling was activated in this way. These included various elements of MAP kinase signaling, PI3 kinase-AKT signaling, DNA repair mechanisms, cell cycle and development pathways and an extensive network of molecules involved in cytoskeletal reorganization
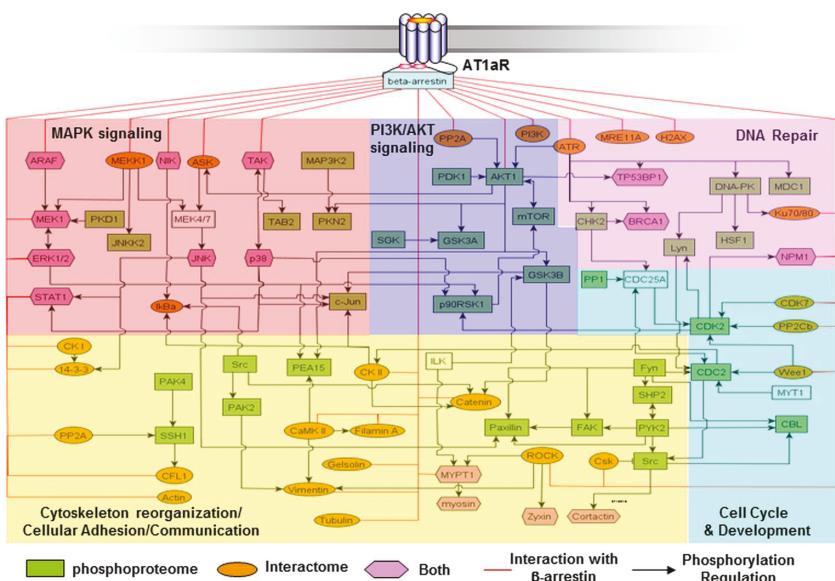


**FIGURE 14.** A *β*-arrestin dependent kinase network downstream of the angiotensn AT$_{1a}$ receptor reproduced with permission from reference 59.

and actin dynamics. These results suggest that β-arrestin-mediated signaling is extremely diverse and involves activation of many of the same pathways which can be activated through G proteins. However, often with very distinct cellular consequences.

Results such as these likely have implications for the development of new therapeutic agents. Let me give you several examples. Angiotensin acting through G protein-mediated effects is one of the most potent vasoconstrictors known and can lead to increases in blood pressure. In contrast, its β-arrestin-mediated effects include several that are potentially beneficial such as cyto-protection and anti-apoptotic effects (60). Angiotensin receptor blockers, so called ARBs, are among the most important drugs used in the treatment of cardiovascular illnesses specifically because they block the potentially harm-ful G protein-mediated hypertensive effects of angiotensin. However, they also block potentially beneficial β-arrestin-mediated effects. We hypothesized that a β-arrestin biased angiotensin receptor ligand that blocks G protein mediated signaling while at the same time stimulating potentially beneficial β-arrestin-mediated effects might represent a novel and uniquely effective type of thera-peutic agent. In fact, a compound with just such properties, Trevena 120027, slows the progression of heart failure in animals, lowers blood pressure (61), and is anti-apoptotic (62).

Ligands can also be biased toward a G protein and such agents also may have therapeutic potential. For example, the therapeutic utility of opiates, the most potent pain relieving medications available, are mediated through stimu-lation of Gi proteins through the μ-opioid receptor (63,64), whereas the distress-ing side effects of constipation, respiratory depression and tolerance, necessi-tating larger and larger doses, are all mediated through β-arrestin2-mediated signaling and are lost in β-arrestin2 knockout mice (63,64). Thus, G protein bi-ased ligands for the μ-opioid receptor should relieve pain while having mark-edly reduced adverse effects.

These examples of β-arrestin and G protein biased ligands demonstrate how our new understanding of these two types of signaling pathways, gained initially at a biochemical level, can potentially be harnessed for therapeutic benefit.

I have been remarkably fortunate to have trained more than 200 students and fellows during my career. Rather than simply display a list of their names I have tried to indicate some of the most important contributors during my lec-ture. However, as a representation of the full group I'd like to show you a photo-graph (Fig. 15) taken about ten years ago at the time of my 60th Birthday when

**FIGURE 15.**  Former and current members of the author's laboratory celebrating at his 60th birthday party in 2003. Inset: Dr. Brian Kobilka, co-recipient of the 2012 Nobel Prize in Chemistry.

many of these former associates returned to Duke for a celebration and let me call out one former fellow in particular hidden away in the back row . . . he is your next speaker, Dr. Brian Kobilka.

## ACKNOWLEDGMENT

## REFERENCES

1.  Pierce, K.L., Premont, R.T. and Lefkowitz, R.J., "Seven Transmembrane Spanning Receptors," *Nature Rev. Molec. Cell. Biol.* 3:639–650, 2002. PMID 12209124
2.  Langley, J.N., "On the reaction of cells and of nerve-endings to certain poisons, chiefly as regards the reaction of striated muscle to nicotine and to curare," *J Physiol.* 33: 374–413, 1905. PMID 16992819
3.  Dale, H.H. Modes of Drug Action, "General Introductory Address," *Transactions of the Faraday Soc.* 39: 319–322, 1943.
4.  Ahlquist, R.P., "A study of the adrenotropic receptors," *Am. J. Physiol.* 153:586–600, 1948. PMID 18882199

5. Ahlquist, R.P., "Adrenergic receptors: a personal and practical view," *Perspect. Biol. Med.* 17:119–122, 1973. PMID 4148041

6. Mukherjee, C., Caron, M.G., Coverstone, M. and Lefkowitz, R.J., "Identification of β-adrenergic receptors in frog erythrocyte membranes with [3H](–)alprenolol," *J. Biol. Chem.* 250: 4869–4875, 1975. PMID 238972

7. Williams, L.T. and Lefkowitz, R.J., "Identification of α-adrenergic receptors by [H3]di-hydroergocryptine binding," *Science* 192: 791–793, 1976. PMID 4894

8. Stiles, G.L., Caron, M.G. and Lefkowitz, R.J., "The β-adrenergic receptor: Biochemical mechanisms of physiological regulation," *Physiol. Rev.* 64: 661–743, 1984. PMID 6143332

9. Lorenz, W., Lomasney, J.W., Collins, S., Regan, J.W., Caron, M.G. and Lefkowitz, R.J., "Expression of three α-2 adrenergic receptor subtypes in rat tissues: Implications for α-2 receptor classification," *Mol. Pharmacol.* 38:599–603, 1990. PMID 2172770

10. Lefkowitz, R.J., Mullikin, D. and Caron, M.G., "Regulation of β-adrenergic receptors by 5'guanylylimidodiphosphate and other purine nucleotides," *J. Biol. Chem.* 251: 4688–4692, 1976. PMID 947904

11. Kent, R.S., De Lean, A. and Lefkowitz, R.J., "A quantitative analysis of β-adrenergic receptor interactions: Resolution of high and low affinity states of the receptor by computer modeling of ligand binding data," *Mol. Pharmacol.* 17: 14–23, 1980. PMID 6104284

12. De Lean, A., Stadel, J.M. and Lefkowitz, R.J., "A ternary complex model explains the agonist-specific binding properties of the adenylate-cyclase coupled β-adrenergic receptor," *J. Biol. Chem.* 255: 7108–7117, 1980. PMID 6248546

13. Caron, M.G., Srinivasan, Y., Pitha, J., Kiolek, K. and Lefkowitz, R.J., "Affinity chromatography of the β-adrenergic receptors," *J. Biol. Chem.* 254: 2923–2927, 1979. PMID 218957

14. Regan, J., Barden, N., Lefkowitz, R.J., Caron, M.G., DeMarinis, R.M., Krog, A.J., Holden, K.G., Matthews, W.D. and Hieble, J.P., "Affinity chromatography of human platelet $α_2$-adrenergic receptors," *Proc. Natl. Acad. Sci. USA* 79: 7223–7227, 1982. PMID 6130523

15. Lomasney, J.W., Leeb-Lundberg, L.M.F., Cotecchia, S., Regan, J.W., DeBernardis, J.F., Caron, M.G. and Lefkowitz, R.J., "Mammalian α1-adrenergic receptor: Purification and characterization of the native receptor ligand binding subunit," *J. Biol. Chem.* 261: 7710–7716, 1986. PMID 3011796

16. Dohlman, H.G., Thorner, J., Caron, M.G. and Lefkowitz, R.J., "Model systems for the study of seven-transmembrane-segment receptors," *Annu. Rev. Biochem.* 60:653–688, 1991.PMID 1652922

17. Cerione, R.A., Strulovici, B., Benovic, J.L., Lefkowitz, R.J. and Caron, M.G., "The pure β-adrenergic receptor: A single polypeptide confers catecholamine responsiveness to an adenylate cyclase system," *Nature* 306: 562–566, 1983. PMID 6316161

18. Cerione, R.A., Sibley, D.R., Codina, J., Benovic, J.L., Winslow, J., Neer, E.J., Birnbaumer, L., Caron, M.G. and Lefkowitz, R.J., "Reconstitution of a hormone-sensitive adenylate cyclase system: The pure β-adrenergic receptor and guanine nucleotide regulatory protein confer hormone responsiveness on the resolved catalytic unit," *J. Biol. Chem.* 259: 9979–9982, 1984. PMID 6088509

19. Dixon, R.A.F., Kobilka, B.K., Strader, D.J., Benovic, J.L., Dohlman, H.G., Frielle, T., Bolanowski, M.A., Bennett, C.D., Rands, E., Diehl, R.E., Mumford, R.A., Slater, E.E., Sigal,

I.S., Caron, M.G., Lefkowitz, R.J. and Strader, C.D., "Cloning of the gene and cDNA for mammalian $\beta$-adrenergic receptor and homology with rhodopsin," *Nature* 321: 75–79, 1986. PMID 3010132

20. Hargrave, P.A., McDowell, J.H., Curtis, D.R., Wang, J.K., Juszcak, E., Fong, S.L., Rao, J.K., and Argos, P., "The structure of bovine rhodopsin," *Biophys Struct Mech.* 9, 235–244, 1983. PMID 6342691

21. Ovchinnikov, Y.A., "Rhodopsin and bacteriorhodopsin: structure-function relationships," *FEBS Lett.* 148, 179–191, 1982. PMID 6759163

22. Engelman, D.M., Henderson, R., McLachlan, A.D., and Wallace, B.A., "Path of the polypeptide in bacteriorhodopsin," *Proc. Natl. Acad. Sci. USA* 77, 2023–2027, 1980. PMID 6929535

23. Kobilka, B.K., Matsui, H., Kobilka, T.S., Yang-Feng, T.L., Francke, U., Caron, M.G., Lefkowitz, R.J. and J.W. Regan, "Cloning, sequencing, and expression of the gene coding for the human platelet $a_2$-adrenergic receptor," *Science* 238:650–656, 1987. PMID 2823383

24. Lefkowitz, R.J. and Caron, M.G., "The Adrenergic Receptors," in Advances in Second Messenger and Phosphoprotein Research, in: *The Biology and Medicine of Signal Transduction*. (Eds. Yasumatoma Nishizuka et al.,) Raven Press, N.Y. Volume 24: 1–8, 1990.

25. Cotecchia, S., Schwinn, D.A., Randall, R.R., Lefkowitz, R.J., Caron, M.G. and Kobilka, B.K., "Molecular cloning and expression of the cDNA for the hamster $a_1$-adrenergic receptor," *Proc. Natl. Acad. Sci. USA* 85:7159–7163, 1988. PMID 2845398

26. Fargin, A., Raymond, R.J., Lohse, M.J., Kobilka, B.K., Caron, M.G. and Lefkowitz, R.J., "The genomic clone G-21 encodes the serotonin (5-HT1A) receptor," *Nature* 335:358–360, 1988. PMID 3138543

27. Dohlman, H.G., Caron, M.G., and Lefkowitz, R.J., "A family of receptors coupled to guanine nucleotide regulatory proteins," *Biochemistry* 26: 2657–2664, 1987. PMID 3038163

28. O'Dowd, B.F., Hnatowich, M., Regan, J.W., Leader, W.M., Caron, M.G., and Lefkowitz, R.J., "Site directed mutagenesis of the cytoplasmic domains of the human $\beta_2$-adrenergic receptor. Localization of regions involved in G protein-receptor coupling," *J Biol. Chem.* 263: 15985–15992. PMID 2846532

29. Kobilka, B.K., Kobilka, T.S., Daniel, K., Regan, J.W., Caron, M.G. and Lefkowitz, R.J., "Chimeric $a_2$-,$\beta_2$-adrenergic receptors: Delineation of domains involved in effector coupling and ligand binding specificity," *Science* 240:1310–1316, 1988. PMID 2836950

30. Ostrowski, J., Kjelsberg, M.A., Caron, M.G. and Lefkowitz, R.J., "Mutagenesis of the $\beta_2$-adrenergic receptor: how structure elucidates function," *Annu. Rev. Pharmacol. and Toxicol.* 32:167–183, 1992. PMID 1318669

31. Strader, C.D. and Dixon, R.A., "Genetic Analysis of the $\beta$-adrenergic receptor," *Adv. Exp. Med. Biol.* 287: 209–220, 1991.

32. Cotecchia, S., Exum, S., Caron, M.G. and Lefkowitz, R.J., "Regions of the $a_1$-adrenergic receptor implicated in coupling to phosphatidylinositol hydrolysis and enhanced sensitivity of biological function," *Proc. Natl. Acad. Sci. USA* 87:2896–2900, 1990. PMID 2158097

33. Kjelsberg, M.A., Cotecchia, S., Ostrowski, J., Caron, M.G. and Lefkowitz, R.J., "Constitutive activation of the $\alpha_1$B-adrenergic receptor by all amino acid substitutions at a single site," *J. Biol. Chem.* 267:1430–1433, 1992.PMID 1346134

34. Samama, P., Cotecchia, S., Costa, T. and Lefkowitz, R.J., "A mutation-induced activated state of the $\beta_2$-adrenergic receptor-extending the ternary complex model," *J. Biol. Chem.*, 268:4625–4636, 1993. PMID 8095262

35. Spiegel, A.M., in *G Proteins, Receptors and Disease* (ed. Spiegel, A.M.) 1–21 (Humana Press, Totowa, New Jersey, 1998.

36. Violin, J.D., DiPilato, L.M., Yildirim, N., Elston, T.C., Zhang, J. and Lefkowitz, R.J., "$\beta_2$-Adrenergic receptor signaling and desensitization elucidated by quantitative modeling of real-time cAMP dynamics," *J. Biol. Chem.* 283: 2949–2961, 2008. PMID 18045878

37. Lavin, T., Heald, S., Jeffs, P., Shorr, R., Lefkowitz, R.J. and Caron, M.G., "Photoaffinity labeling of the $\beta$-adrenergic receptor. *J. Biol. Chem.* 256: 11944–11950, 1981. PMID 6271767

38. Stadel, J.M., Nambi, P., Lavin, T.N., Heald, S.L., Caron, M.G., and Lefkowitz, R.J., "Catecholamine-induced desensitization of turkey erythrocyte adenylate cyclase. Structural alterations in the $\beta$-adrenergic receptor revealed by photoaffinity labeling," *J. Biol. Chem.* 257:7242–9245, 1982. PMID 6125504

39. Benovic, J.L., Strasser, R.H., Caron, M.G., and Lefkowitz, R.J., "$\beta$-adrenergic receptor kinase: identification of a novel protein kinase that phosphorylates the agonist-occupied form of the receptor," *Proc. Natl. Acad. Sci. USA* 83:2797–2801, 1986. PMID 2871555

40. Benovic, J.L., Mayor, F., Jr., Staniszewski, C., Lefkowitz, R.J., and Caron, M.G., "Purification and characterization of the $\beta$-adrenergic receptor kinase," *J. Biol. Chem.* 262, 9026–9032, 1987. PMID 3036840

41. Benovic, J.L., DeBlasi, A., Stone, W.C., Caron, M.G., and Lefkowitz, R.J., "$\beta$-adrenergic receptor kinase: primary structure delineates a multigene family," *Science* 246:235–240, 1989. PMID 2552582

42. Lorenz, W. Inglese, J., Palczewski, K., Onorato, J.J., Caron, M.G., and Lefkowitz, R.J., "The receptor kinase family: primary structure of rhodopsin kinase reveals similarities to the $\beta$-adrenergic receptor kinase," *Proc. Natl. Acad. Sci. USA* 88:8715–8719, 1991. PMID 1656454

43. Pitcher, J.A., Freedman, N.J., and Lefkowitz, R.J., "G protein-coupled receptor kinases," *Annu. Rev. Biochem.* 67:653–692, 1998. PMID 9759500

44. Lodowski, D.T., Pitcher, J.A., Capel. W.D., Lefkowitz, R.J. and Tesmer, J.J.G., "Keeping G Proteins at Bay: A Complex Between G Protein-Coupled Receptor Kinase 2 and G$\beta\gamma$," *Science* 300:1256–1262, 2003.

45. Benovic, J.L., Kuhn, H., Weyand, I., Codina, J., Caron, M.G. and Lefkowitz, R.J., "Functional desensitization of the isolated $\beta$-adrenergic receptor by the $\beta$-adrenergic receptor kinase: potential role of an analog of the retinal protein arrestin (48K protein)," *Proc. Natl. Acad. Sci. USA* 84:8879–8882, 1987. PMID 2827157

46. Kuhn, H. and Wilden, U., "Deactivation of photoactivated rhodopsin by rhodopsin-kinase and arrestin," *J. Recept. Res.* 7:283–298, 1987.

47. Shinohara, T, Dietzschold, B, Craft, C.M., Wistow, G., Early, J.J. Donoso, L.A., Horwitz, J., and Tao, R., "Primary and secondary structure of bovine retinal S antigen (48-kDa protein)," *Proc. Natl. Acad. Sci. USA* 84:6975–6979, 1987. PMID 3478675

48. Lohse, M.J., Benovic, J.L. Codina, J., Caron, M.G., and Lefkowitz, R.J., "β-arrestin: a protein that regulates β-adrenergic receptor function," *Science* 248:1547–1550, 1990. PMID 2163110

49. Attramadal, H., Arriza, J.L., Aoki, C., Dawson, T.M., Codina, J., Kwatra, M.M., Snyder, S.H., Caron, M.G., and Lefkowitz, R.J., "β-arrestin2, a novel member of the arrestin/β-arrestin gene family," *J. Biol. Chem.* 267:17882–17890, 1992. PMID 1517224

50. Han, M., Gurevich, V.V., Vishnivetskiy, S.A., Sigler, P.B., and Schubert, C., "Crystal structure of β-arrestin at 1.9 A: possible mechanism of receptor binding and membrane translocation," *Structure* 9:869–880, 2001. PMID 11566136

51. Hausdorff, W.P., Caron, M.G. and Lefkowitz, R.J., "Turning off the signal: Desensitization of β-adrenergic receptor function," *FASEB J.* 4:2881–2889, 1990. PMID 2165947

52. Bouvier, M., Leeb-Lundberg, L.M., Benovic, J.L., Caron, M.G. and Lefkowitz, R.J., "Regulation of adrenergic receptor function by phosphorylation: Effects of agonist occupancy on phosphorylation of $a_1$- and $β_2$-adrenergic receptors by protein kinase C and the cyclic AMP-dependent protein kinase," *J. Biol. Chem.* 262:3106–3113, 1987. PMID 3029101

53. DeWire, S.M., Ahn, S., Lefkowitz, R.J., and Shenoy, S.K., "β-arrestins and Cell Signaling," *Annu Rev. Physiology* 69:483–510, 2007.

54. Shukla, A.K., Xiao, K., and Lefkowitz, R.J., "Emerging paradigms of β-arrestin-dependent seven transmembrane receptor signaling," *Trends in Biochemical Sciences* 36: 457–469, 2011. PMCID:PMC3168679

55. Shenoy, S.K. and Lefkowitz, R.J., "β-Arrestin-mediated receptor trafficking and signal transduction," *Trends in Pharmacological Sciences* 32: 521–533, 2011. PMCID:PMC3159699

56. Violin, J.D., and Lefkowitz, R.J., "β-arrestin-biased ligands at seven-transmembrane receptors," *Trends in Pharm. Sci.* 28:416–422, 2007. PMID 17644195

57. Rajagopal, S., Rajagopal, K., Lefkowitz, R.J., "Teaching old receptors new tricks: biasing seven-transmembrane receptors," *Nature Reviews Drug Discovery* 9, 1–12, 2010. PMCID: PMC2902265

58. Violin, J.D., Dewire, S.M., Yamashita, D. Rominger, D.H., Nguyen, L., Schiller, K. Whalen, E.J., Gowen, M., and Lark, M.W., "Selectively engaging β-arrestins at the angiotensin II type I receptor reduces blood pressure and increases cardiac performance, " *J. Pharmacol. Exp. Ther.* 335:572–579, 2010. PMID 20801892

59. Xiao, K., Sun, J., Kim, J., Rajagopal S., Zhai B., Villen, J., Haas, W., Kovacs, J.J., Shukla, A.K., Hara, M.R., Hernandez, M., Lachmann, A., Zhao, S., Lin Y., Cheng,Y., Mizuno, K., Ma'ayan, A., Gygi, S.P., and Lefkowitz, R.J., "Global phosphorylation analysis of β-arrestin-mediated signaling downstream of seven transmembrane receptor (7TMR), " *Proc. Natl. Acad. Sci. USA* 107: 15299–15304, 2010. PMID 20686112

60. Ahn, S., Kim, J., Hara, M.R., Ren, X-R. and Lefkowitz, R.J., "β-arrestin-2 mediates anti-apoptotic signaling through regulation of BAD phosphorylation," *J. Biol. Chem.* 284: 8855–8865, 2009. PMCID:PMC2659243

61. Boerringter, Lark, M.W., Whalen, E.J., Soergel, D.G., Violin, J.D., and Burnett, J.C., Jr., "Cardiorenal actions of TRV120027, a novel β-arrestin-biased ligand at the angiotensin II type I receptor, in healthy and heart failure canines: a novel therapeutic strategy for acute heart failure," *Circ. Heart Failure* 6: 770–778, 2011. PMID 21835984

62. Kim, K.S., Abraham, D., Williams, B., Violin, J.D., Mao, L., and Rockman, H.A., "β-arrestin-biased AT1R stimulation promotes cell survival during acute cardiac injury," *Am. J. Physiol. Heart Circ. Physiol.* 303:H1001–H1010, 2012. PMID 22886417

63. Raehal, K.M., Walker, J.K., and Bohn, L.M., "Morphine side effects in β-arrestin 2 knockout mice," *J. Pharmacol. Exp. Ther.* 314:1195–1201, 2005. PMID 15917400

64. Bohn, L.M., Gainetdinov, R.R., Lin, F.T., Lefkowitz, R.J., and Caron, M.G., "Mu-opioid receptor desensitization by β-arrestin-2 determines morphine tolerance but not dependence," *Nature* 408:720–723, 2000. PMID 11130073

65. Lefkowitz, R.J., "Seven Transmembrane Receptors—A Brief Personal Retrospective," *Biochemica et Biophysica Acta* 1768:748–755, 2007. PMID 17173855

Portrait photo of professor Lefkowitz by photographer Ulla Montan.