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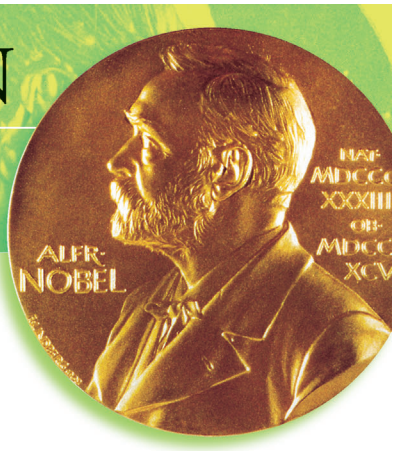

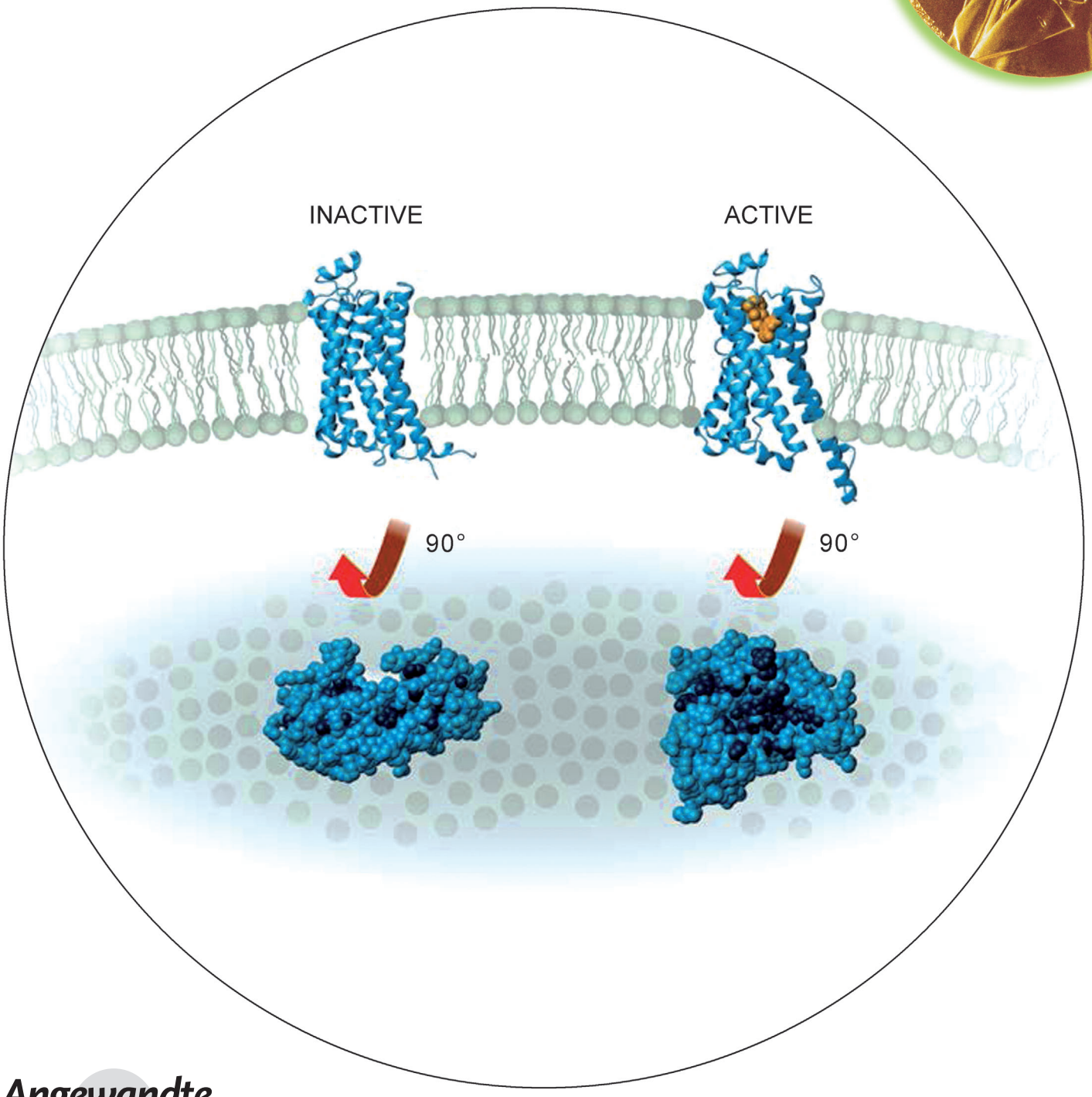

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G-Protein-Coupled Receptors

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A Brief History of G-Protein Coupled Receptors (Nobel Lecture)**

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β_2 adrenergic receptor · G-protein-coupled receptors · Nobel lecture · protein structures · signaling

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 (GPCRs), 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]

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 α and β 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 α and β 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 postdoc Marc Caron I set out to develop such methods, initially for the β-adrenergic receptor[6] and then the α-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 β-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] (Figure 1 A). 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 (Figure 1 B). Together with Andre DeLean we developed the ternary complex model to explain this behavior (Figure 1 C).[12] It

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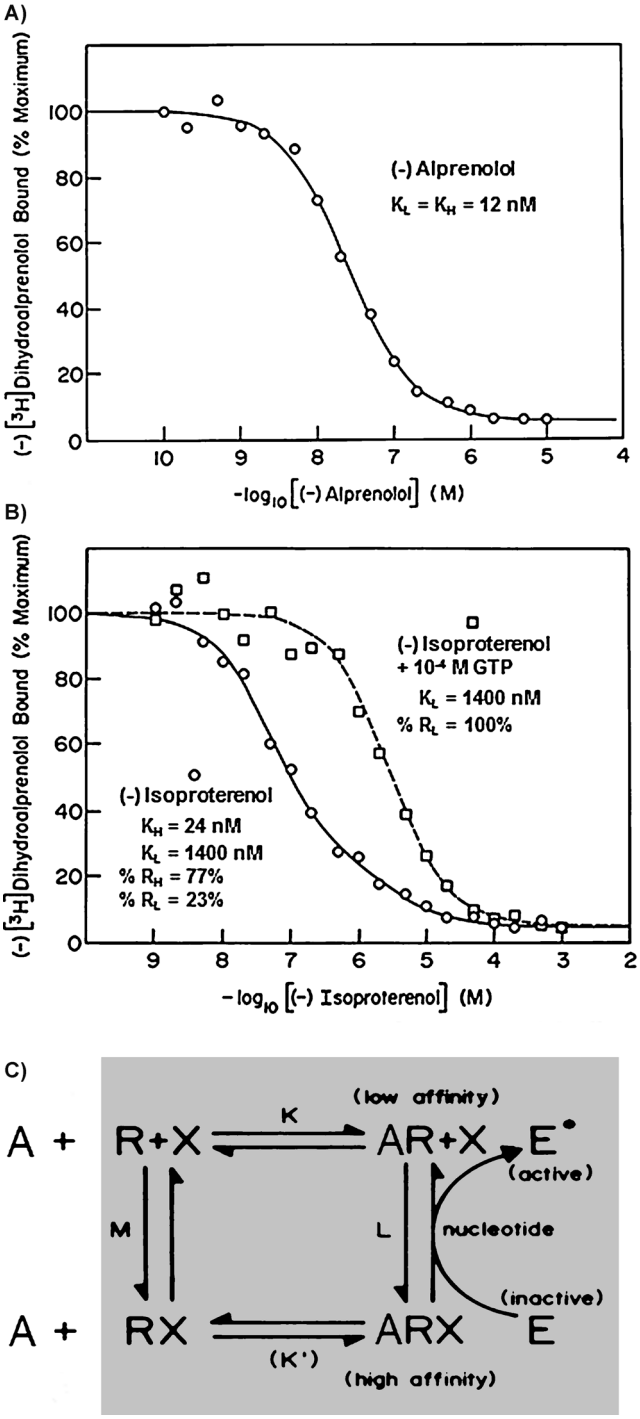

Figure 1. Radioligand binding to the β_2-adrenergic receptor from frog erythrocyte membranes. A) Computerized curve fitting of binding data from displacement of [^{3}H] dihydroalprenolol by the antagonist (−)-alprenolol. B) Displacement by the agonist (−)-isoproterenol in the presence (□) or absence (○) of 10^{-4} M GTP. C) The ternary complex model. (A) and (B) are reproduced with permission from Ref. [11], (C) is reproduced from Ref. [12].

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 β-adrenergic and other adrenergic receptors was truly a daunting task. The molecules are virtually trace contaminants of plasma membranes and required 100000-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 β-adrenergic and then α-adrenergic antagonists to solid supports (Figure 2 A).[13–15] 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, α_1, α_2, β_1 and β_2 (reviewed in Ref. [16]). Shown on this SDS polyacrylamide gel are such preparations for three of those four adrenergic receptors (Figure 2 B). This figure represents approximately one decade of work by a number of devoted students and postdocs, most notably Marc Caron and Jeff Benovic, amongst others. Each of the isolated putative receptor molecules is a glycoprotein of approximately 60000–65000 daltons molecular weight which bound specific α- and β-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 β-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 β receptors and hence β-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

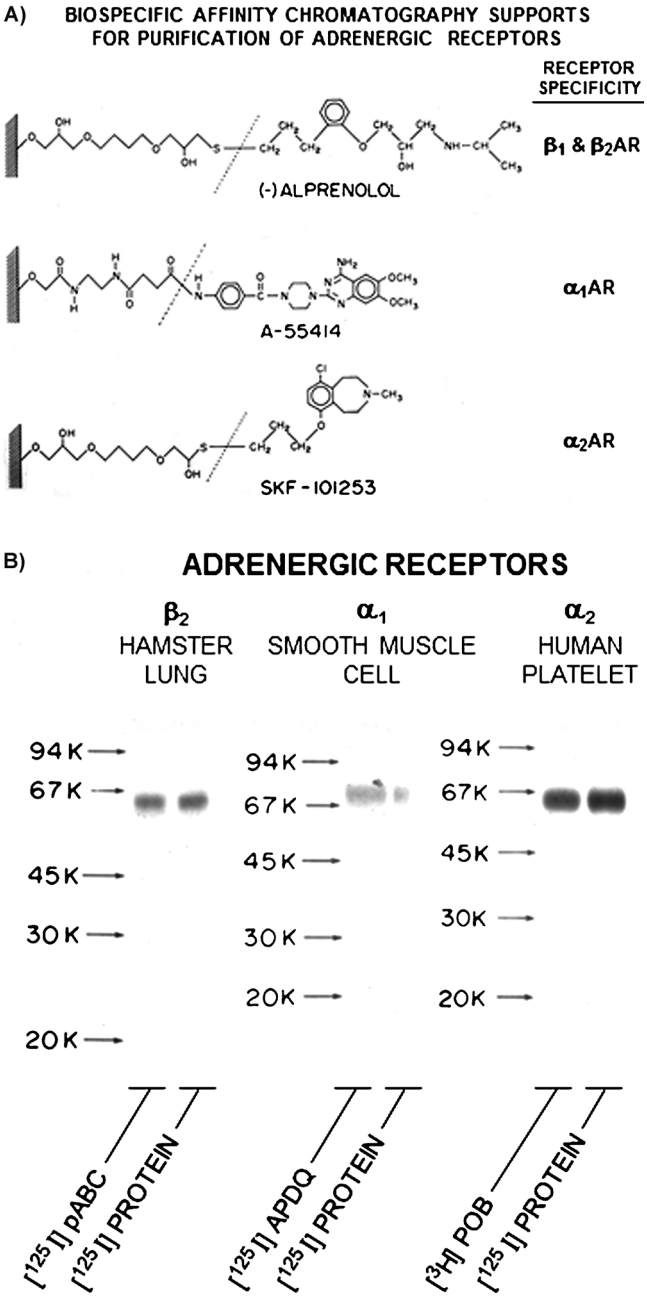

Figure 2. Isolation of the adrenergic receptors by affinity chromatography.

responsiveness to β-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 β-adrenergic receptor, the guanine nucleotide regulatory protein (G_s) and the catalytic unit of adenylate cyclase.[18] These results conclusively proved that the isolated proteins were in fact the β-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 β_2-adrenergic receptor and to use these to design oligonucleotide probes to clone the cDNA and gene for the β_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 (Figure 3): seven apparent transmembrane spanning hydrophobic domains, sites for regulatory phosphorylation in cytoplasmic domain, 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 (Figure 3), with the visual light-sensing protein 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 β_2-adrenergic receptor did it begin to emerge that it was instead the signature feature of GPCRs. Within a year we had cloned the highly homologous α_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 GPCRs 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 polymerase chain reaction (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 α_2 and β_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 α_2 receptor inhibiting adenylate cyclase through G_i and the β_2-adrenergic receptor stimulating it through G_s (Figure 4A). By creating these chimeras we were able to demonstrate that the G protein coupling specificity and hence the function of the α_2-receptor could be converted from G_i inhibitory to G_s stimulatory simply by replacing its third cytoplasmic loop with that of the β_2-adrenergic receptor (Figure 4B). These

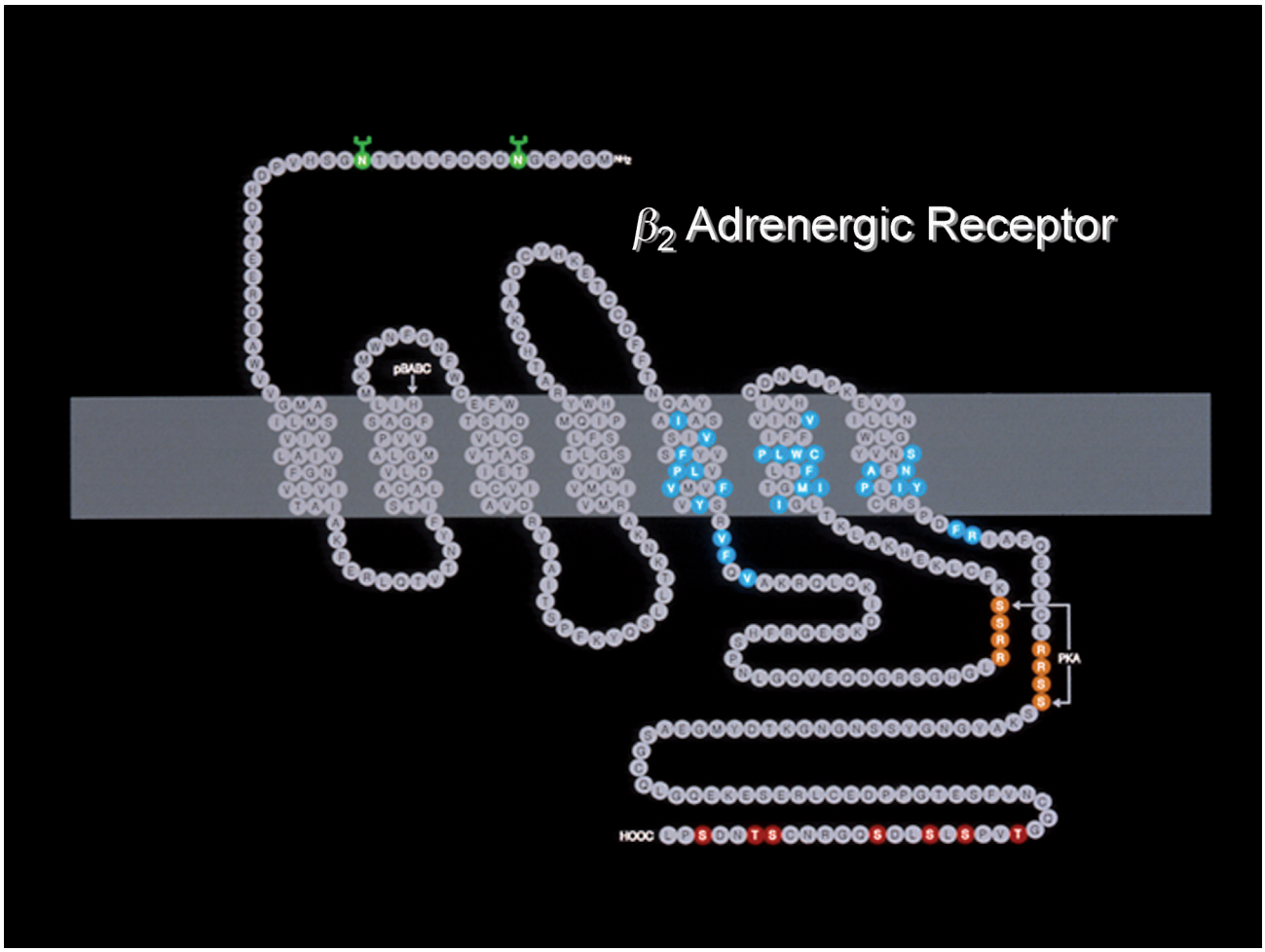

Figure 3. Cloning of the β_2-adrenergic receptor. Residues shaded in blue are homologous with rhodopsin; orange are consensus PKA phosphorylation sites; red, sites of GRK phosphorylation; green, consensus sites for N-linked glycosylation. Reproduced with permission from Ref. [65].

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] (Figure 4C).

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] (Figure 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* (Figure 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 (Figure 5B).[35]

Contemporaneous with this work on the structure of the receptors, my laboratory had been focused on trying to understand the virtually universal phenomenon of receptor desensitization. This phenomenon is illustrated in Figure 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 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 β-adrenergic receptor,[37] Jeff Stadel, a fellow in

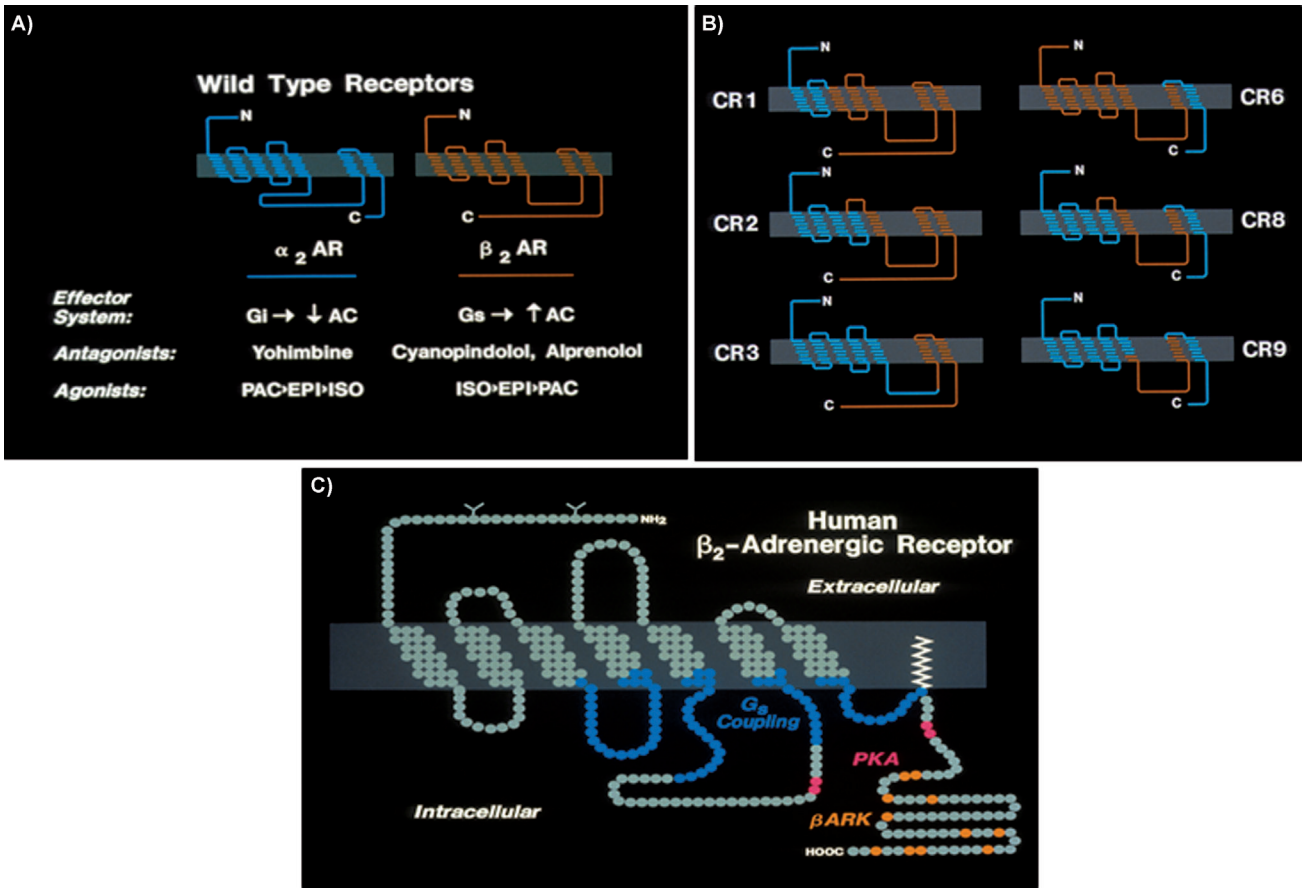

Figure 4. Chimeric α_2-β_2-adrenergic receptors. Adapted from Ref. [29] and Ref. [65].

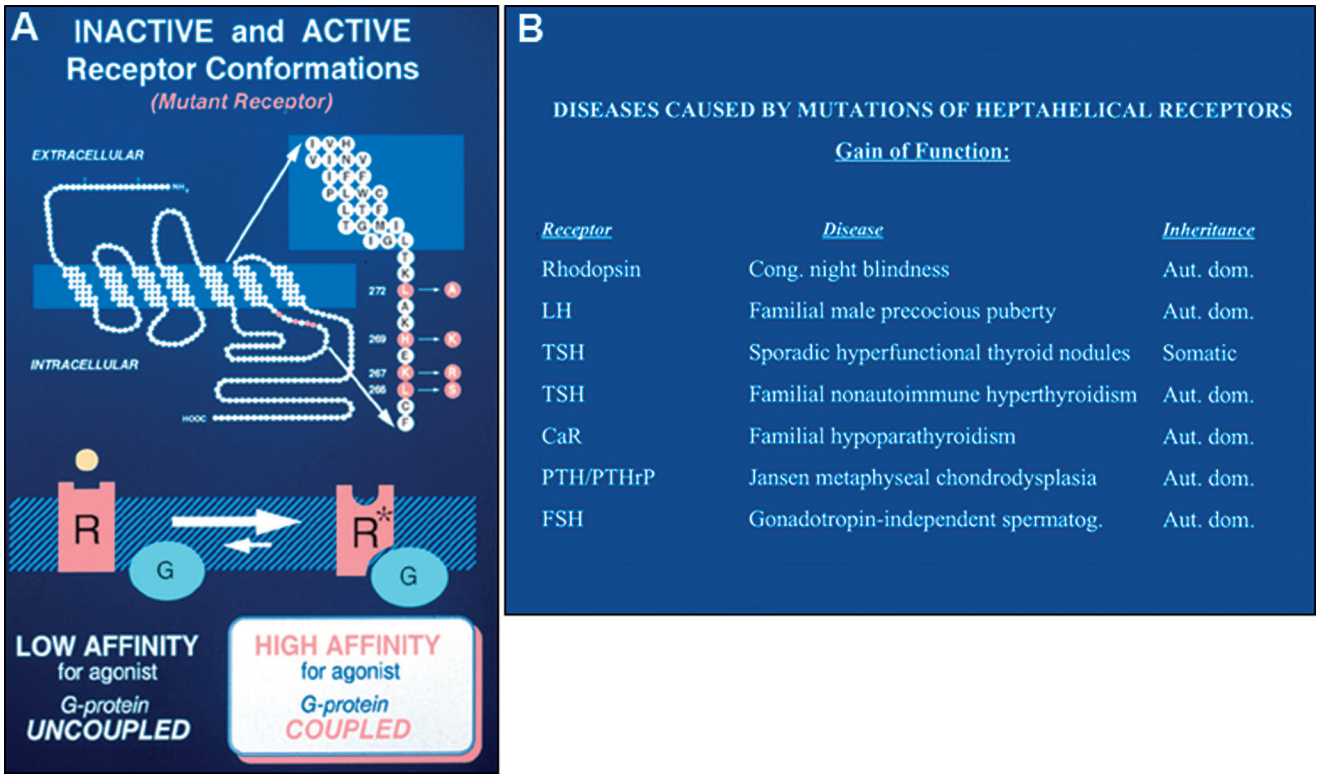

Figure 5. Constitutively active mutant adrenergic receptors.

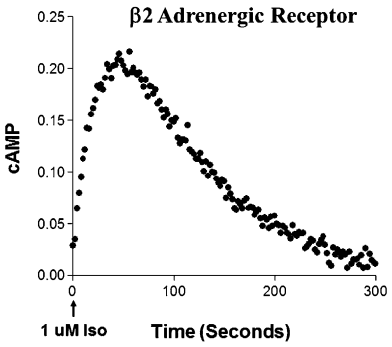

Figure 6. Desensitization of cAMP production after stimulation of the β_2-adrenergic receptor. Reproduced with permission from Ref. [36].

the laboratory used this probe to label β-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 (Figure 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 inorganic phosphate and were able to demonstrate that the catecholamine-induced desensitization was associated with phosphorylation of the β-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

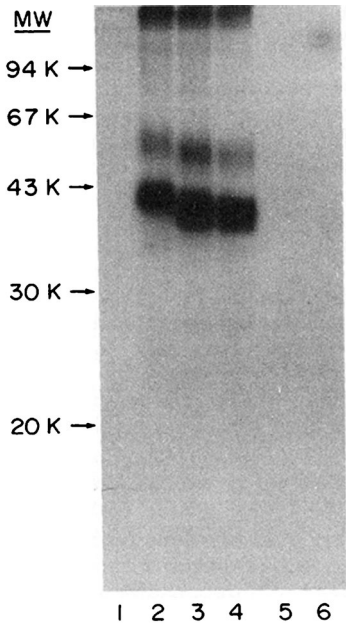

Figure 7. Desensitization involves receptor phosphorylation. SDS-PAGE of β_2-adrenergic receptors from turkey erythrocyte membranes covalently labeled with the photoaffinity probe [^{125}I]-*p*-azidobenzylcarazolol. Lane 2, cells incubated with isoproterenol, 1 μM; lane 3, isoproterenol, 1 μM in the presence of 10 μM propranolol (an antagonist); lane 4, control cells incubated with buffer alone. Reproduced with permission from Ref. [38].

a novel cAMP-independent kinase which we called βARK, for β-adrenergic 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 βARK 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 (Figure 8).[43] Thanks to the work of John Tesmer's lab crystal

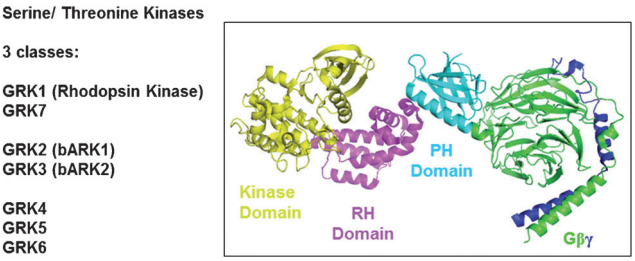

Figure 8. G protein coupled receptor kinases. Reprinted with permission from Ref. [44].

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 βγ subunits of the G proteins, with which it interacts in the cytosol to facilitate its translocation to the plasma membrane bound receptors (Figure 8).[44] 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 to ability of βARK or GRK2 to desensitize the β-receptor in vitro, albeit at high concentrations.[45]

Very shortly thereafter 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 β-arrestin.[48] A year or two later another fellow Hâvard Attramadal was able to clone another similar molecule which we termed β-arrestin 2.[49] Now, with all three authentic recombinant arrestin molecules in hand, visual arrestin and the two β-arrestins 1 and 2, we could compare their abilities to desensitize rhodopsin and the β-receptor in vitro in reconstituted systems (Figure 9). When we used rhodopsin kinase phosphorylated rhodopsin, visual arrestin was quite potent in inactivating signaling whereas the two β-arrestins were quite weak (Figure 9A). Conversely, for GRK2 phosphorylated β-receptor, the two β-arrestins were highly potent and arrestin was very weak (Figure 9B). These findings established the commonality of desensitization mechanisms for both rhodopsin and the β-receptor, albeit with great specificity for the arrestin molecule involved.[49]

Today we know that there are four arrestins. Two of them, arrestin 1 and X arrestin, are limited to the retina. β-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 β sheets connected by a hinge region and stabilized by a polar core (Figure 10).[5]

So where this all brought us to by the mid 90s is depicted in Figure 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

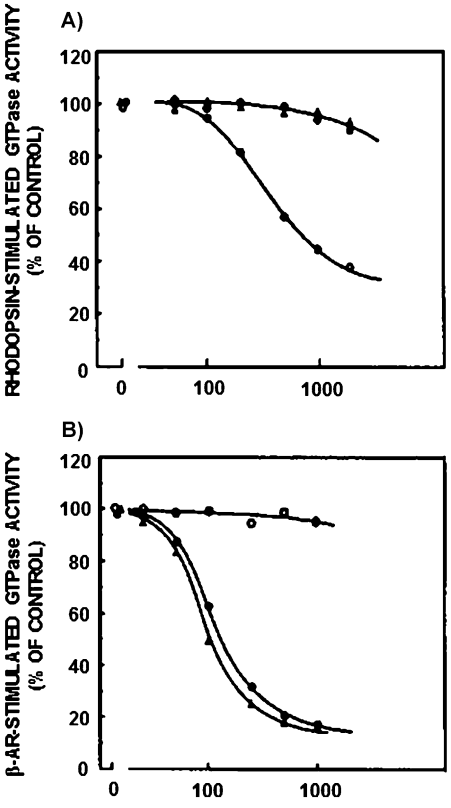

Figure 9. Inhibition of β_2-adrenergic receptor and rhodopsin function by β-arrestin 1, β-arrestin 2, and arrestin in reconstituted systems. A) rhodopsin was phosphorylated with rhodopsin kinase. B) β_2-adrenergic receptors were phosphorylated with GRK2. Rhodopsin-stimulated transducin GTPase was most potently inhibited by visual arrestin (○). In contrast, β_2-adrenergic receptor-stimulated Gs GTPase was much more potently inhibited by β-arrestin 1 (●) or β-arrestin 2 (▲). Reproduced with permission from Ref. [49].

	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

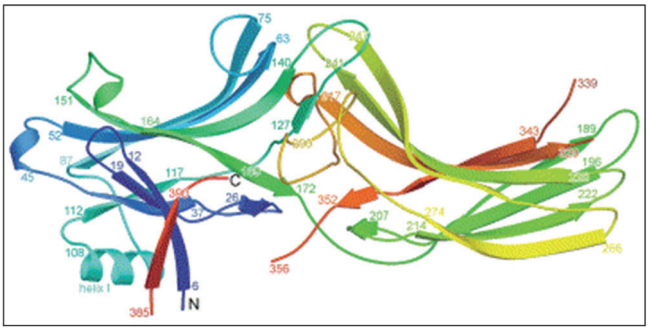

Figure 10. The arrestins. Adapted from Ref. [50] with permission.

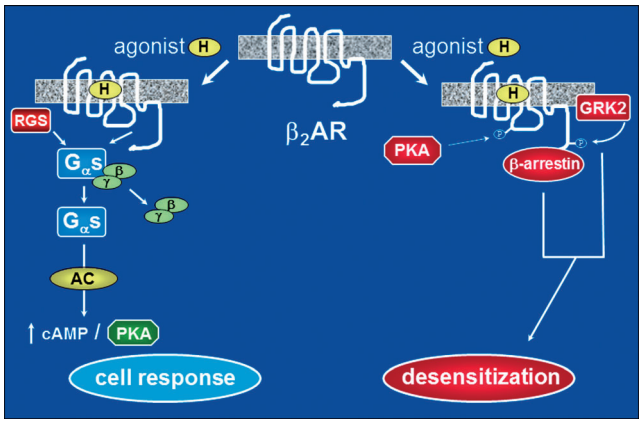

Figure 11. Activation and desensitization of GPCRs.

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 (Figure 12).

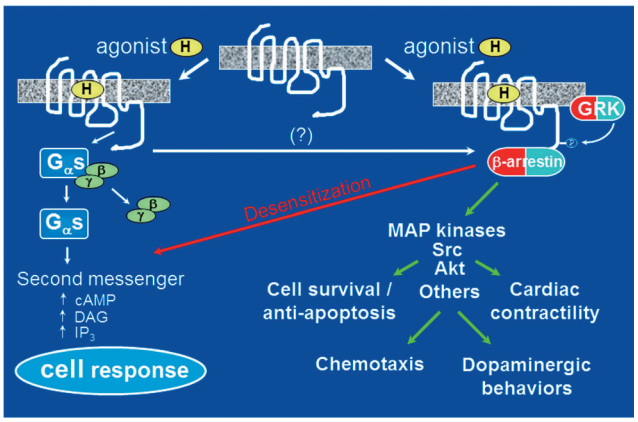

Figure 12. New paradigm for multiple β-arrestin-mediated functions. Reproduced with permission from Ref. [65].

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 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. β-Arrestins also mediate clathrin coated pit endocytosis by interacting with a growing list of elements of the endocytic machinery.[55] Thus, the β-arrestins mediate three types of function, desensitization, receptor internalization and signaling.

In the course of studying β-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 Ref. [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 β-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 β-arrestin- or G protein-mediated signaling implies that there must be multiple active conformations of the receptor.[57]

By way of illustration, Figure 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 β-arrestin in a cell line expressing the receptor.[58] G protein

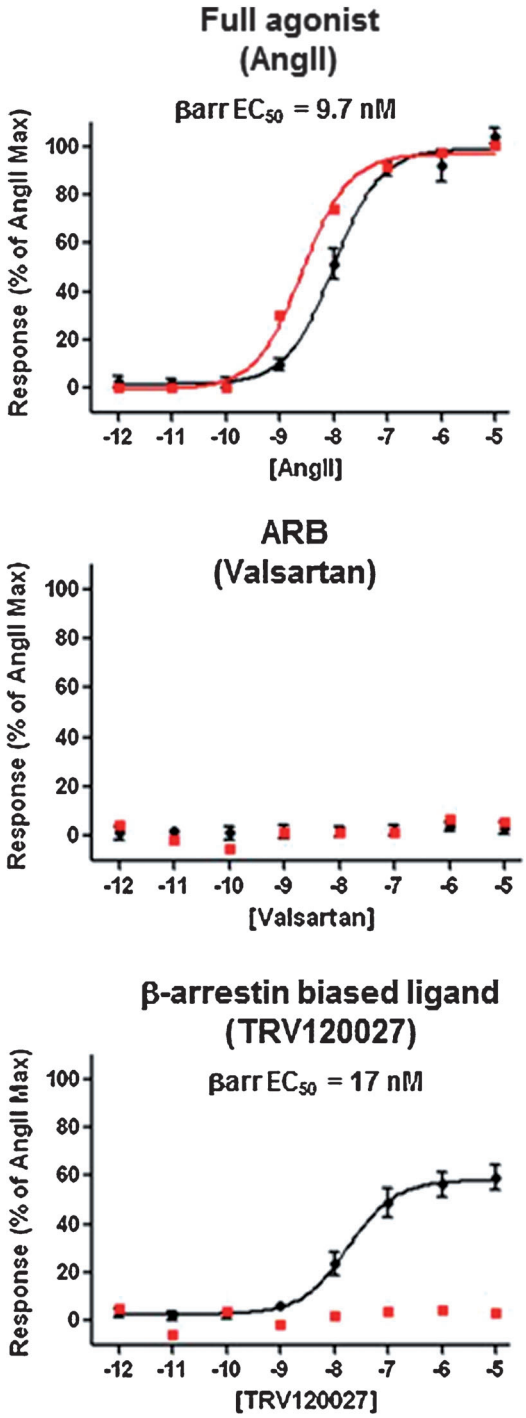

Figure 13. Activation of G_q and β-arrestin by the angiotensin AT1a receptor stimulated by various ligands. Red circles: G-protein signal (IP1); black circles: β-arrestin recruitment (PathHunter). See text for details. Reproduced with permission from Ref. [58].

activation is being assayed by a typical second messenger assay and is shown in red, and β-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 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 Figure 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 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 cytoprotection and anti-apoptotic effects.[60] Angiotensin receptor blockers, so-called ARBs, are amongst the most important drugs used in the treatment of cardiovascular illnesses specifically because they block the potentially harmful 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 therapeutic 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 stimulation of G_i proteins through the μ-opioid receptor,[63,64] whereas the distressing side effects of constipation, respiratory depression and tolerance, necessitating larger and larger doses are all mediated through β-arrestin 2-mediated signaling and are lost in β-arrestin 2 knockout mice.[63,64] Thus, G protein biased

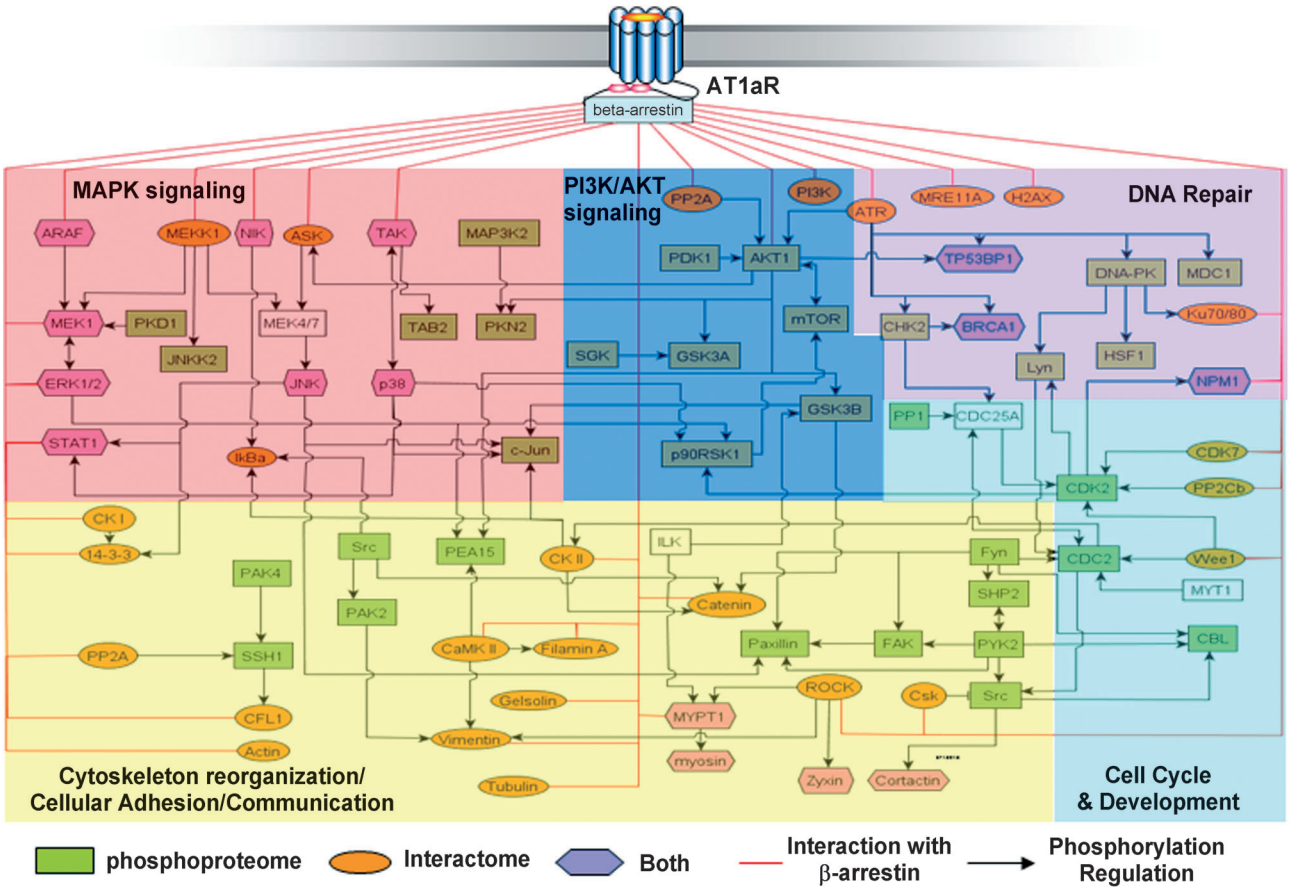

Figure 14. A β-arrestin-dependent kinase network downstream of the angiotensn AT_{1a} receptor reproduced with permission from Ref. [59].

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.

ligands for the μ-opioid receptor should relieve pain while having markedly 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 lecture. However, as a representation of the full group I would like to show you a photograph (Figure 15) taken about ten years ago at the time of my 60th birthday when 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.

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