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Review

# Seven transmembrane receptors—A brief personal retrospective

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**Abstract**

Receptors have fascinated biologists for more than a century and they have fascinated me for the entirety of my own research career. The seven transmembrane receptors, also known as G protein coupled receptors, represent the largest of the several families of plasma membrane receptors, comprising more than a thousand genes and regulating virtually all known physiological processes in mammals. Moreover, they represent one of the commonest targets of currently used drugs. I have spent the entirety of my research career working on these receptors. Here I set down some personal reflections on the evolution of the field during the past 35 years, hanging the thread of the story on some of the work from my own laboratory.


*Keywords:* G protein-coupled receptor; β-adrenergic receptor; GRK, β-arrestin; Ligand binding; Desensitization

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Classical physiologists and pharmacologists working in the first half of the twentieth century speculated about the existence of receptors. But it was not until the 1970s that the first molecular approaches to studying them and to even proving that they existed were developed. Surprisingly, the intellectual climate at this time was in fact one of very significant skepticism as to the actual existence of receptors. For example, here is a quotation from the mid 1970s from the distinguished pharmacologist Raymond Ahlquist, who ironically had been responsible for the development of the concept of alpha and beta-adrenergic receptors in 1948 using classical, physiological and pharmacological techniques. He expressed the prevailing sentiment at the time (1973) which was one of marked skepticism as to the actual physical existence of such molecules. He wrote: "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" [1]. So it was against this background of skepticism that those of us who were interested in the receptor problem at the time and wished to bring these mythical receptors to life, began the work. It was immediately clear that it would be necessary to develop a whole series 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.

For reasons that were both practical and perhaps somewhat emotional, (I was after all an aspiring young academic cardiologist), I chose as my models the adrenergic receptors and particularly the beta-adrenergic receptors. And so in the

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early 1970s together with my students and fellows we developed radioligand binding approaches for the study of these receptors. These immediately bore fruit in several areas. For example, we learned that the receptors were not static entities but rather were dynamically regulated by all manner of physiological, pharmacological and pathophysiological situations. We were also able to discern the existence of novel receptor subtypes, and to explore novel theories of receptor action.

For example, we observed that competition curves of antagonists in radioligand binding experiments gave steep and uniphasic patterns, whereas agonist competition curves were shallow and could be resolved into two components. Such curves were shifted to the right, that is to a single lower affinity and become steeper in the presence of guanine nucleotides (Fig. 1). We developed computer based methods for resolving these various components which were quite useful for assessing the coupling efficiency of such systems and which are used to this day. Together with postdoc Andre deLean, we also developed the so called ternary complex model which provided early insights into how agonists might drive the interaction of receptors with a then unknown additional component, later shown to be the guanine nucleotide regulatory protein, in order to form the coupled high affinity state of the receptor (Fig. 1).

But perhaps one of the most important applications of the ligand binding methods was that they allowed us to begin the difficult work of receptor solubilization and isolation. At the time there were three objects of study which were relevant to receptor biology; the work on the beta2-adrenergic receptor in my laboratory, that on the nicotinic cholinergic receptor by laboratories such as that of Changeux and others, and also the visual pigment rhodopsin. I will return to rhodopsin later but suffice it to say that at the time, the mid 1970s, no one imagined that there was any relationship between rhodopsin and conventional receptors. As an aside let me say that we were quite envious of our colleagues working on the cholinergic receptor and also rhodopsin since each of these constitutes 90% or more of the protein in enriched membranes derived respectively from the electric organ of electric fish such as torpedo or from bovine retina. Thus, little purification was necessary from such membranes. In contrast, for the beta2 and other adrenergic receptors more than 100,000 fold purification was required after solubilization. The key to our success was the development of affinity chromatography matrices for each of the then four known subtypes of adrenergic receptors. In each case, we coupled drugs, either alpha or beta-adrenergic antagonists to sepharose beads. Our first success was the alprenolol-sepharose resin developed for the βeta receptor by Marc Caron in our group. By applying solubilized preparations of the various receptors to such columns coupled to other more conventional chromatographic matrices we were able, after more than a decade of difficult and dedicated work, to purify each of the

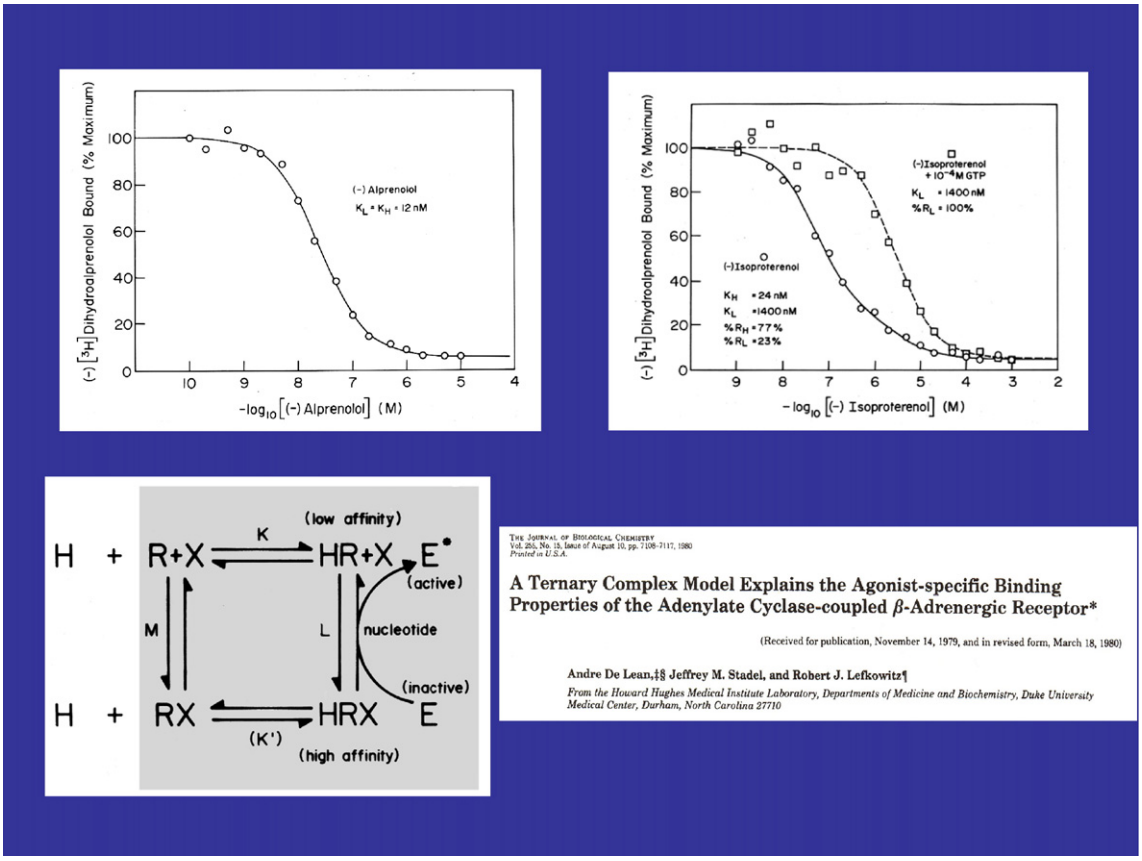


Fig. 1. High and low affinity states of the $\beta_2$-adrenergic receptor and the ternary complex model. Data are from [13].

four subtypes of adrenergic receptor essentially to homogeneity ($\beta_2$, $\beta_1$ $\alpha_2$, $\alpha_1$).

Each consists of a single polypeptide of about 60,000 Da, glycoproteins, which bound adrenergic ligands with all the appropriate specificity and sterospecificity characteristics. I had imagined that achieving this milestone would finally quell persisting skepticism about the existence of the receptor molecules, but this was not so. Skeptics continued to question whether such isolated molecules could in fact convey to cells the ability to respond, in this case to adrenergic ligands.

Accordingly, we reconstituted the purified receptors such as the beta2-adrenergic receptor in phospholipid vesicles and fused these with cells which, while containing the adenylate cyclase response system, lacked beta receptors and hence could not respond to catecholamines. We initially utilized erythrocytes from *Xenopus laevis*, which contained prostaglandin but not beta-adrenergic receptors. When we fused our receptor containing vesicles with the cells they acquired responsiveness to adrenaline, thus proving that the isolated ligand binding proteins were in fact the receptors. Within a year, in collaboration with Eva Neer and Lutz Birnbaumer, Rick Cerione in our lab, had reconstituted the pure receptors with isolated guanine nucleotide regulatory proteins and the adenylate cyclase catalytic moiety, thus proving that these three components alone were sufficient to form a neurotransmitter responsive system.

With bonafide validated receptor protein in hand we turned our attention to the difficult work of cloning the gene and cDNAs for the receptors. Using microsequencing techniques we obtained short peptide sequences from purified receptor proteins, working initially with the beta2-adrenergic receptor. A postdoc from my lab Cathy Strader who had just finished and gone on to Merck was instrumental in forming a partnership between my laboratory and a team at Merck led by Richard Dixon. Over the next several years we labored together to clone the receptor, the work in my lab being led by a talented cardiology fellow named Brian Kobilka. After several years we succeeded and reported the structure just 20 years ago, in May of 1986. There were a number of remarkable surprises. The $\beta_2$-adrenergic receptor sequence contains all the essential features that today we view as canonical for the receptor family: seven membrane spans as predicted from hydropathy plots; sites for N-linked glycosylation at the amino terminus; sites of regulatory phosphorylation on the C terminus; and, strikingly, residues in the membrane spans which were identical to those in rhodopsin (Fig. 2). The sequence of rhodopsin had been determined a year or two before independently by two groups [2,3]. Interestingly this was not by cloning as you might think but rather by classic Edman degradation since the protein was available in such large quantities.

Many today are quite surprised to learn just how shocked we and everyone else in the scientific community was when we

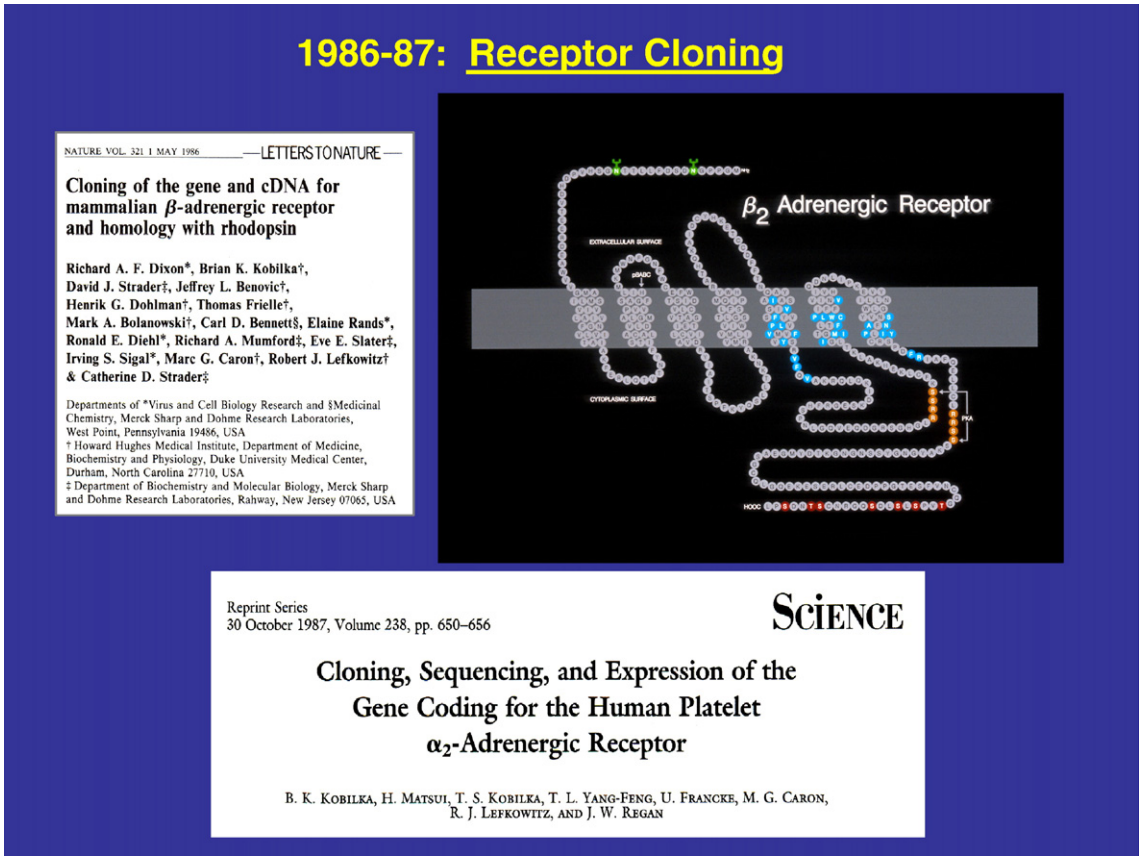


Fig. 2. Cloning of the $\beta_2$ and $\alpha_2$ adrenergic receptors. Residues shaded in blue are homologous with rhodopsin; orange are consensus PKA phosphorylation sites; red, consensus sites of regulatory phosphorylation; green, consensus sites for N-linked glycosylation.

discovered that the beta receptor so closely resembled the structure of rhodopsin. But it is important to understand the context. Although by the mid 1980s most of us appreciated the functional analogies between the rhodopsin/ transducin/cyclic G phosphodiesterase system and hormone responsive adenylate cyclases, no one envisioned that the rhodopsin molecule would actually resemble hormone and neurotransmitter receptors. And in fact, when the structure of rhodopsin was first obtained, it was immediately analogized with the only other then known seven transmembrane spanning protein, bacteriorhodopsin, [2,3] the light sensitive proton pump from archaebacteria. In fact, both groups which sequenced rhodopsin came to the same conclusion, namely that seven membrane spans must be a signature feature of all light sensitive proteins. Only with the cloning of the beta2-adrenergic receptor did the idea first emerge that in fact it was a signature feature of G protein coupled receptors. This immediately became our hypothesis, one which my lab quickly supported by cloning first the alpha2-adrenergic receptor and then over the next several years a total of 8 adrenergic receptors.

So by 1987 we were quite convinced that all of the then known G protein coupled receptors would likely be members of this superfamily of seven transmembrane receptors. Over the next several years the family grew rapidly as many laboratories cloned 7TM receptors, almost invariably by homology techniques such as low stringency screening and then PCR. In fact, subsequently almost no other 7TM receptor 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 4 adrenergic receptors which had provided the first sequences upon which the much larger superfamily could then be built.

One important example of the consequences of the rapid growth of the family through the late 1980s and 1990s was the discovery of so called “orphan receptors”. These of course are 7TM receptors for which the sequence is known but not the ligand or the function. In fact, today there are still hundreds of such orphan receptors known from the genome sequence. We actually discovered the first orphan 7TM receptor immediately after cloning the beta2 adrenergic receptor when we performed low stringency southern genomic blots with our beta2 receptor cDNA. Of the several bands observed, one was most intense. We assumed that it must represent the beta1-adrenergic receptor since what else could be so closely related to the beta2? We cloned it from a size-selected genomic library (the adrenergic receptor genes and closely related ones are intronless) and to our delight found that it did in fact encode a 7TM molecule. We called the clone G21. But to our dismay, when expressed, it did not bind beta-adrenergic ligands. It thus became the first orphan GPCR. A year or two later we figured out that G21 was in fact the first cloned serotonin receptor, the 5HT1A. Thus, it became the first orphan to be “deorphanized”.

About 5 years later Buck and Axel discovered by far the largest family of orphans, the olfactory receptors [4]. These comprise almost half of the superfamily of 7TM receptors and to this day most of these remain orphans. In the late 1990s Charles Zucker and others cloned a smaller family of orphan taste receptors, many of which have now been deciphered as sweet and bitter receptors [5].

With the sequences of several of the receptors in hand our efforts, and those of a number of other laboratories turned to trying to understand how the highly conserved 7TM structure of the receptors mediated the classical functions of ligand binding and stimulation of biological effects. We utilized two main approaches, site directed mutagenesis and the construction of chimeric receptors.

One of my favorite studies from this period involved the construction of chimeric alpha2 and beta2 adrenergic receptors. By 1987 we had cloned both of these receptors, based on their purification, and could see that they were quite homologous. Both of these adrenergic receptor subtypes bind catecholamines and related compounds with similar but quite distinct pharmacologies, but they mediate biochemically and biologically opposite effects. Thus, the beta2 adrenergic receptors stimulate adenylate cyclase through Gs, whereas the alpha2-adrenergic receptors inhibit it through Gi. Brian Kobilka, a fellow in the lab constructed about a dozen of these chimeras and I would point out this was in the days before PCR. Fig. 3 depicts six of these, with beta2-adrenergic receptor sequence shown in red and alpha2 sequence shown in blue. The most revealing of these chimeric receptors, were #8 and 9 which contained only a small amount of beta2 sequence and mostly alpha2 sequence. These chimeras bound ligands with essentially an alpha 2 specificity but, strikingly, activated adenylate cyclase as would a beta receptor rather than inhibiting it like an alpha 2. This immediately told us that the sequences determining specificity of the G protein coupling must lie within this relatively limited segment of the molecule. Construction of additional chimeras and site directed work by us and other laboratories served to further refine these conclusions. We now understand that the cytoplasmic domains in closest apposition to the plasma membrane are generally responsible for determining coupling to G proteins whereas the membrane spans, external loops, and N terminus are responsible for ligand binding.

Another interesting and unexpected discovery which flowed from our mutagenesis and chimeric receptor work was the serendipitous discovery of constitutively active mutant receptors. Susanna Cotecchia had made a series of mutations in the third cytoplasmic loop of the alpha 1B adrenergic receptor, which were expected to lead to loss of function. Instead, several of these led to constitutive activation of the receptors, that is to activity in the absence of agonist. Such mutations appear to abrogate intramolecular interactions which normally constrain the receptors to an inactive conformation. The discovery of these constitutively active mutant receptors in turn facilitated the study and development of “inverse agonists”, agents which are able to stabilize the inactive conformation of the receptors and thereby diminish the elevated basal activity of these mutant receptors. Subsequently, spontaneously occurring mutations which activate a wide variety of seven transmembrane receptors have been discovered and have been found to be responsible for a growing list of human illnesses, including some familial cases of male precocious puberty (luteinizing hormone receptor) [6] and functioning thyroid adenomas (thyroid stimulating

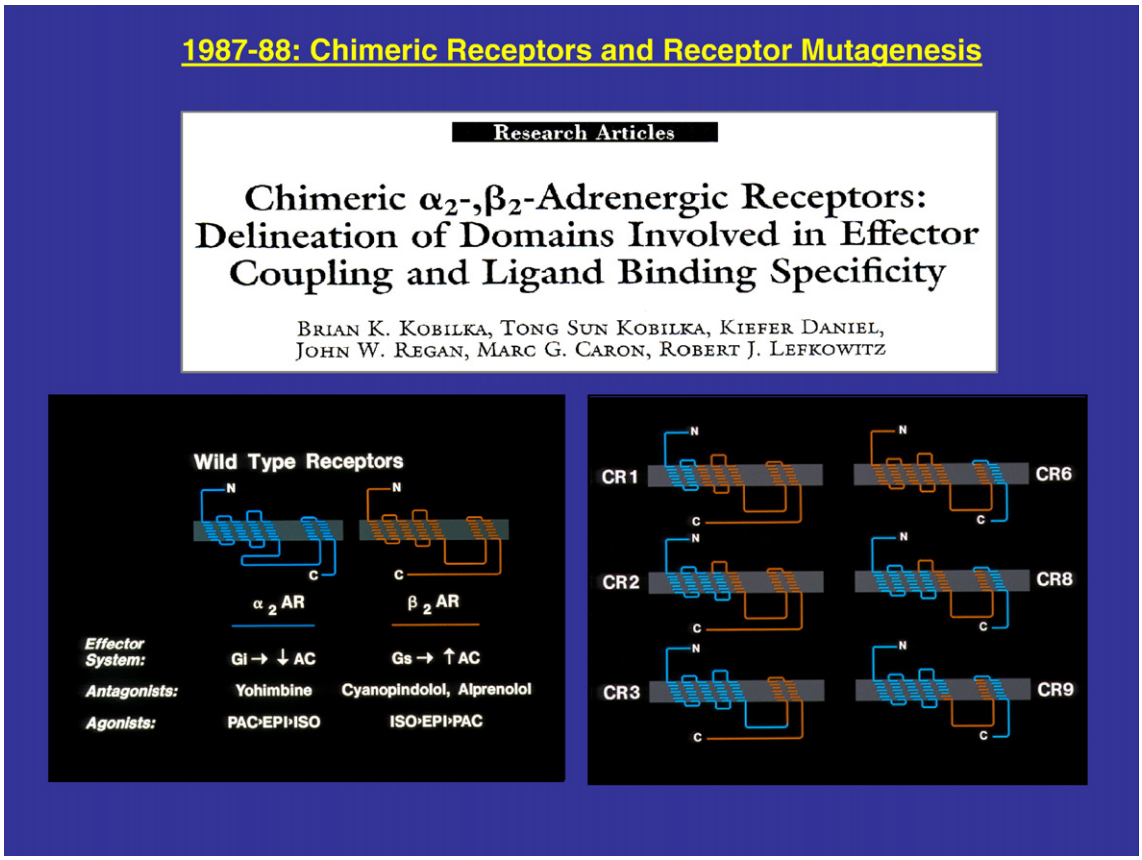


Fig. 3. Chimeric $\alpha_2$–$\beta_2$ adrenergic receptors—adapted from [14].

hormone receptor) [7,8]. The genomes of several viruses have also been discovered to encode homologues of constitutively active 7TM receptors [9].

Despite their remarkable diversity of function many of the properties of the seven membrane spanning receptors are conserved. Since the early 1990s, two paradigms have been central to our understanding of receptor function and regulation. These two paradigms, *activation* and *desensitization*, are based on the understanding that 3 families of proteins are able to interact in an almost universal way with 7TM receptors in a strictly stimulus dependent fashion. These three families are the heterotrimeric G proteins, the G protein coupled receptor kinases or GRKs and the beta arrestins.

Interaction of the activated receptors with the G proteins leads to second messenger generation and classical physiological responses. Interaction of the activated receptors with GRKs leads to their phosphorylation, classically on the carboxy terminal tail. This facilitates the binding of a second class of proteins called the beta-arrestins, or in the case of rhodopsin signaling, arrestin which then sterically interdicts further G protein coupling leading to receptor desensitization.

A brief word about the discovery of the G protein coupled receptor kinases is in order. I had always been interested in the pervasive phenomenon of desensitization of biological systems in the face of persistent stimulation, because it represents perhaps the most classic example of homeostasis. So whenever we would develop a new technique for studying the receptors I would always want to immediately apply it to the desensitization problem. Thus, about 1980 when we first developed photoaffinity probes for the beta adrenergic receptor we immediately tried photoaffinity labeling receptors derived from cells which had been desensitized by prior agonist exposure and comparing them with receptors from naïve cells. When we ran SDS gels we could see that the electrophoretic mobility of the desensitized receptors was retarded compared to the controls. This suggested that perhaps some covalent modification of the receptor molecule had occurred. Suspecting phosphorylation, Jeff Stadel directly tested this by labeling the cells with $^{32}$P and thus we discovered that desensitization of the beta-adrenergic receptor was associated with its phosphorylation. These experiments were quite difficult technically because the only way to isolate the very low abundance receptors (they were not yet cloned) was to subject each and every sample to affinity chromatography.

Over the next several years we were able to identify the kinase responsible for this phosphorylation as a novel protein kinase which we initially called the beta-adrenergic receptor kinase (βARK), today generally referred to as GRK2. A graduate student, Jeff Benovic, was able to purify the kinase from bovine brain and clone its gene. Contemporaneously with this work, groups working on the biochemistry of vision had discovered what seemed like a very analogous enzyme called rhodopsin kinase which participated in turning off rhodopsin signaling. We cloned the gene for rhodopsin kinase which

showed a structure very similar to that of the beta-adrenergic receptor kinase and thus realized that there was in fact a family of G protein coupled receptor kinase enzymes.

Today we know that this family consists of 7 enzymes which we refer to as GRKs 1–7 which regulate virtually all known 7TM receptors. They fall into several subfamilies. GRKs 1 and 7 are found only in the retina, rods and cones respectively. Of the others, GRKs 2,3,5,6 are ubiquitously expressed. All share a highly conserved tripartite domain organization with a central catalytic domain flanked by two regulatory domains.

Our discovery of the beta-arrestins flowed directly from the work on the GRKs. In the course of purifying βARK or GRK2 from brain we observed that in a reconstituted system that we were using, the purified enzyme appeared to lose its ability to desensitize or inactivate the receptors' ability to stimulate G protein, even as its ability to phosphorylate the receptor increased. This suggested the possibility that we might be losing some required cofactor.

Then, as we were scratching our heads about this, Herman Kuhn and colleagues, in early 1987, reported what was to us the striking observation that an abundant retinal protein, previously known as 48K protein or S antigen, worked together with rhodopsin kinase to deactivate rhodopsin [10]. The protein was then renamed arrestin. Reasoning that what we were losing during our βARK preparations might be something similar to this 48K protein, we obtained some of it from Kuhn and were able to show that in our reconstituted system it restored the desensitizing ability to highly purified βARK preparations. But still we knew that 48K protein or arrestin was present only in the retina and hence could not be the molecule we were looking for.

Later that year Shinohara cloned visual arrestin [11]. Reasoning that what we were looking for was a molecule that might be not only functionally analogous to 48 K protein, but structurally homologous with it, we obtained Shinohara's clone and set out to clone related molecules. We succeeded in cloning a molecule which we termed beta-arrestin, subsequently called beta arrestin1 and then a year or two later we cloned beta-arrestin2.

Now with authentic cloned materials in hand we could set up parallel reconstitution systems, which included respectively either rhodopsin kinase-phosphorylated rhodopsin, or βARK phosphorylated beta receptor. In such systems authentic visual arrestin was quite potent in inactivating phosphorylated rhodopsin, whereas the beta-arrestins were quite weak, and conversely the two beta arrestins were potent in desensitizing the phosphorylated beta receptor whereas visual arrestin was very weak. Today we know that there are four arrestins, with arrestins 1 and 4 being limited to the retina and beta arrestins 1 and 2 (aka arrestins 2 and 3) being ubiquitously expressed. All of the molecules share about 70% sequence identity and a highly conserved two domain structure composed almost exclusively of anti-parallel beta-sheets [12].

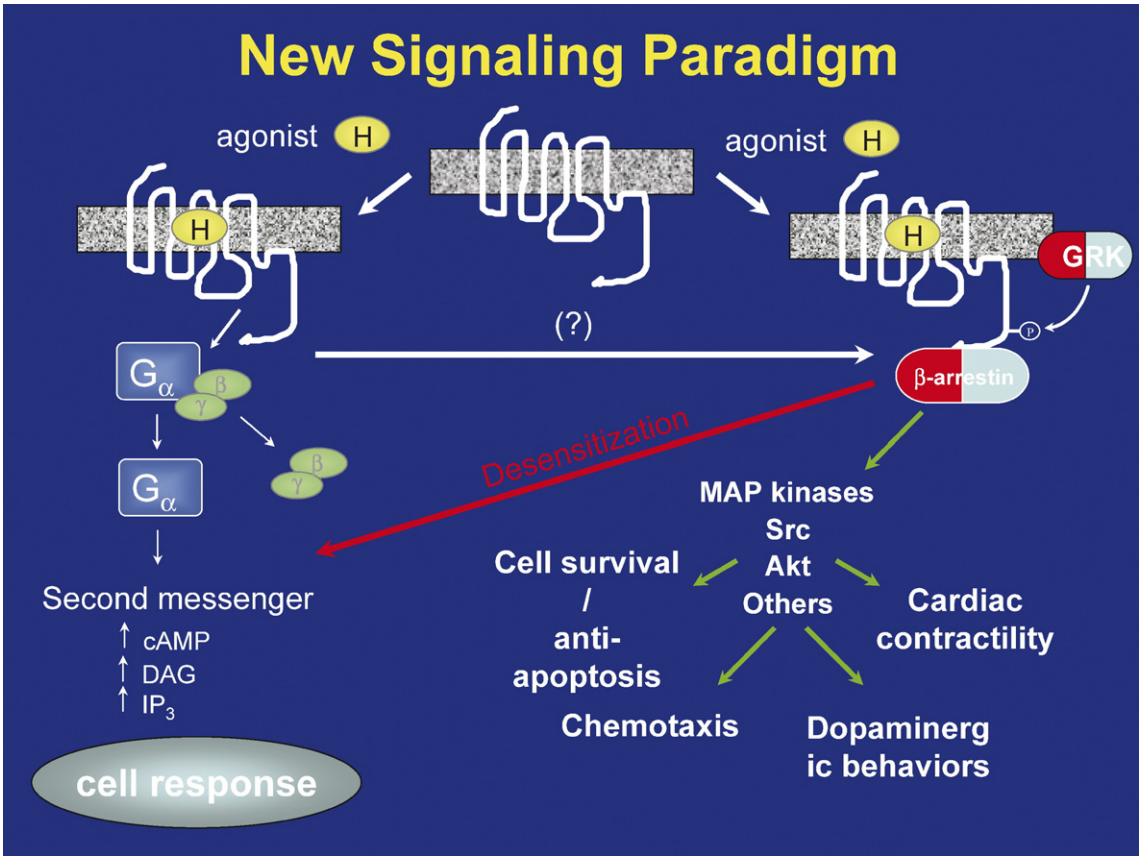


Fig. 4. New signaling paradigm for 7TM receptors—the β-arrestin/GRK system is bifunctional, desensitizing G protein-mediated signaling and also serving as an alternate signaling system in its own right.

The two forms of beta-arrestin were discovered in the context of desensitization of the beta receptor where they were required for full expression of the desensitizing effect of GRK2. However, quite recently we have discovered that the beta-arrestins also desensitize receptor signaling by serving as adaptors which recruit enzymes into complex with the receptors such as PKA which degrade second messengers.

The second function of the beta-arrestins, appreciated only over the past 7 or 8 years, is their ability to function as adaptors linking the receptors to the clathrin mediated endocytosis machinery. This relies on their ability to bind to clathrin and the clathrin adaptor AP2, certain small G proteins such as ARF6 and its regulatory proteins, NSF and other elements of the clathrin coated pit endocytic machinery. We also discovered that the beta-arrestins bind and are ubiquitinated by the E3 ubiquitin ligase MDM2. This ubiquitination is absolutely required in order for it to perform its endocytic functions.

The most recently appreciated role of the beta arrestins, one which has just begun to come into focus within the past 5 years or so is as a signaling and scaffolding molecule which mediates a rapidly growing list of signaling pathways. We first discovered this signaling role in association with the ability of the beta arrestins to recruit non-receptor tyrosine kinases such as members of the Src family to the receptors. But more recently the list of pathways in which the beta arrestins function has been growing rapidly including, but not limited to, for example, a variety of MAP kinases including the ERKs, p38s and JNKs, AKT, PI-3 kinase and a number of others.

This new information has lead to a new paradigm, one which emphasizes the bifunctionality of the beta arrestin/GRK system. Thus, these molecules are on the one hand able to desensitize G protein mediated signaling while simultaneously serving as signal transducers in their own right. The in vivo consequences of this beta arrestin mediated signaling are only now being elucidated. As shown in Fig. 4, it is already clear that this signaling leads to various cell survival and anti-apoptotic effects, mediates some forms of chemotaxis, mediates a pathway of dopaminergic stimulated behaviors which is alternative to the classical cAMP pathway, and also has effects on cardiac contractility. This list will undoubtedly grow.

Over the past several years the ERK MAP kinases have served as a very useful model system for gaining understanding of the mechanisms of this newly delineated beta-arrestin signaling mechanism. We have utilized the $G_q$ coupled angiotensin II 1A receptor in particular for studying how this works. In brief summary, we have found that activation of this Gq coupled 7TM receptor leads to ERK activation by two quite distinct parallel pathways. A canonical G-protein, second messenger pathway leads to ERK activation, with some of the ERK translocating to the nucleus to regulate transcriptional programs. In contrast, beta-arrestin mediated activation, which appears to be mediated exclusively by beta-arrestin2 in this case, leads to retention of the activated ERK in the cytoplasm, where presumably it phosphorylates and regulates a distinct set of cytosolic substrates. Recent data suggest that these substrates regulate such physiological processes as chemotaxis, cytoskeletal rearrangements and the like. Interestingly beta-arrestin1 not only does not carry the signal from this receptor, but it appears to act as a physiological dominant negative. Whereas the G protein pathway is rapid in onset and transient, the beta-arrestin mediated pathway is slower in onset and much more persistent.

One of the more exciting ramifications of these newly discovered β-arrestin-mediated signaling pathways is that they have been demonstrated for a growing number of 7TM receptors. Thus, the mechanism appears to be quite general. Moreover, it is also clear that it is possible to develop ligands which selectively activate the β-arrestin-mediated pathway while not activating the G protein pathway. These "biased" ligands serve as antagonists for G protein-mediated effects of the receptors even while activating β-arrestin-mediated signaling. Such ligands may represent an entirely novel class of therapeutic agent. Such "super blockers" would share with conventional antagonists such as beta blockers or angiotensin receptor blockers, the ability to competitively antagonize agonist-activated G protein signaling while simultaneously adding potentially salutary effects of β-arrestin-mediated signaling such as, for example, anti-apoptotic effects mediated through ERK activation, PI3 kinase and AKT.

Clearly, the field of seven transmembrane receptor research is now expanding exponentially as revealed in the articles which follow. Hopefully this brief series of personal reflections will serve to place current work in historical perspective.

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## References

[1] R.P. Ahlquist, Adrenergic receptors: a personal and practical view, Perspect. Biol. Med. 17 (1973) 119–122.
[2] P.A. Hargrave, J.H. McDowell, D.R. Curtis, J.K. Wang, E. Juszczak, S.L. Fong, J.K. Rao, P. Argos, The structure of bovine rhodopsin, Biophys. Struct. Mech. 9 (1983) 235–244.
[3] Y.A. Ovchinnikov, Rhodopsin and bacteriorhodopsin: structure–function relationships, FEBS Lett. 148 (1982) 179–191.
[4] R. Axel, L. Buck, A novel multigene family may encode odorant receptors: a molecular basis for odor recognition, Cell 65 (1991) 175–187.
[5] M.A. Hoon, E. Adler, J. Lindemeier, J.F. Battey, N.J.P. Ryba, C.S. Zuker, Putative mammalian taste receptors: a class of taste-specific GPCRs with distinct topographic selectivity, Cell 96 (1999) 541–551.
[6] A. Shenker, L. Laue, S. Kosugi, J.J. Merendino Jr., T. Minegishi, G.B. Cutler Jr., Constitutively activating mutation of the luteinizing hormone receptor in familial male precocious puberty, Nature 365 (1993) 652–654.
[7] J. Parma, L. Duprez, J. Van Sande, P. Cochaus, C. Gervy, J. Mockel, J. Dumont, G. Vassart, Somatic mutations in the throtropin receptor gene cause hyperfunctioning thyroid adenomas, Nature 365 (1993) 649–651.
[8] A.M. Spiegel, in: A.M. Spiegel (Ed.), G Proteins, Receptors, and Disease, Humana Press, Totowa, NJ, 1998, pp. 1–21.
[9] C. Bais, B. Santomasso, O. Coso, L. Arvanitakis, E.G. Raaka, J.S.

Gutkind, A.S. Asch, E. Cesarman, M.C. Gershengorn, E.A. Mesri, G-protein-coupled receptor of Kaposi's sarcoma-associated herpesvirus is a viral oncogene and angiogenesis activator, Nature 391 (1998) 86–89.
[10] H. Kuhn, U. Wilden, Deactivation of photoactivated rhodopsin by rhodopsin-kinase and arrestin, J. Recept. Res. 7 (1987) 283–298.
[11] T. Shinohara, B. Dietzschold, C.M. Craft, G. Wistow, J.J. Early, L.A. Donoso, J. Horwitz, R. Tao, Primary and secondary structure of bovine retinal S antigen (48-kDa protein), Proc. Natl. Acad. Sci. U. S. A. 84 (1987) 6975–6979.
[12] J.A. Hirsch, C. Schubert, V.V. Gurevich, P.B. Sigler, The 2.8 A crystal structure of visual arrestin: a model for arrestin's regulation, Cell 97 (1999) 257–269.
[13] R.A. Kent, A. DeLean, R.J. Lefkowitz, A quantitative-analysis of βeta-adrenergic receptor interactions: resolution of high and low affinity states of the receptor by computer modeling of ligand binding data, Mol. Pharm. 17 (1979) 14–23.
[14] B.K. Kobilka, T.S. Kobilka, K. Daniel, J.W. Regan, M.G. Caron, R.J. Lefkowitz, Chimeric $\alpha_2$–$\beta_2$-adrenergic receptors: delineation of domains involved in effector coupling and ligand binding specificity, Science 240 (1988) 1310–1316.

## Review Articles Summarizing Work from my Laboratory

[1] B.B. Hoffman, R.J. Lefkowitz, Radioligand binding studies of adrenergic receptors: new insights into molecular and physiological regulation, Annu. Rev. Pharmacol. Toxicol. 20 (1980) 581–608.
[2] R.J. Lefkowitz, J.M. Stadel, M.G. Caron, Adenylate cyclase-coupled beta-adrenergic receptors: structure and mechanisms of activation and desensitization, Annu. Rev. Biochem. 52 (1983) 159–186.
[3] R.J. Lefkowitz, R.A. Cerione, J. Codina, L. Birnbaumer, M.G. Caron, Reconstitution of the beta-adrenergic receptor, J. Membr. Biol. 87 (1985) 1–12.
[4] D.R. Sibley, J.L. Benovic, M.G. Caron, R.J. Lefkowitz, Regulation of transmembrane signaling by receptor phosphorylation, Cell 48 (1987) 913–922.
[5] B.F. O'Dowd, R.J. Lefkowitz, M.G. Caron, Structure of the adrenergic and related receptors, Annu. Rev. Neurosci. 12 (1989) 67–83.
[6] H.G. Dohlman, J. Thorner, M.G. Caron, R.J. Lefkowitz, Model systems for the study of seven-transmembrane-segment receptors, Annu. Rev. Biochem. 60 (1991) 653–688.
[7] J. Ostrowski, M.A. Kjelsberg, M.G. Caron, R.J. Lefkowitz, Mutagenesis of the beta2-adrenergic receptor: how structure elucidates function, Annu. Rev. Pharmacol. Toxicol. 32 (1992) 167–183.
[8] R.J. Lefkowitz, S. Cotecchia, P. Samama, T. Costa, Constitutive activity of receptors coupled to guanine nucleotide regulatory proteins, Trends Pharmacol. Sci. 8 (1993) 303–307.
[9] R.J. Lefkowitz, G protein-coupled receptor kinases, Cell 74 (1993) 409–412.
[10] R.J. Lefkowitz, G protein-coupled receptors: III. New roles for receptor kinases and beta-arrestins in receptor signaling and desensitization, J. Biol. Chem. 273 (1998) 18677–18680.
[11] J.A. Pitcher, N.J. Freedman, R.J. Lefkowitz, G protein-coupled receptor kinases, Annu. Rev. Biochem. 67 (1998) 653–692.
[12] R.J. Lefkowitz, S.K. Shenoy, Transduction of receptor signals by beta-arrestins, Science 308 (2005) 512–517.
[13] K.L. Pierce, R.T. Premont, R.J. Lefkowitz, Seven-transmembrane receptors, Nat. Rev. Mol. Cell. Biol. 9 (2002) 639–650.