Nobel Prize for Chemistry 2012

DOI: 10.1002/anie.201208565

The Seven Pillars of Molecular Pharmacology: GPCR Research Honored with Nobel Prize for Chemistry

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G protein-coupled receptors · membrane proteins · signal transduction · structural biology

G protein-coupled receptors (GPCRs) are the working horses of cellular communication. They allow human cells to sense external cues, such as light or taste, or to talk to each other through hormones or neurotransmitters. They are involved in most physiological processes of the human body and are targeted by over 30% of today's prescription drugs. Much of what we know about this class of proteins was stimulated by seminal discoveries by this year's Nobel Prize laureates for chemistry, Robert J. Lefkowitz and Brian K. Kobilka.

To fully appreciate the merits of these two GPCR pioneers one has to go back to the year 1986. At that time drugs were mainly discovered by testing compounds in whole animals or in isolated organs. How hormones, neurotransmitters, or drugs worked at the molecular level was largely unknown. Some major common downstream intracellular effector systems, on which many hormones and drugs seemed to converge, had been unraveled, for example, second messengers or G proteins (Figure 1). But the identity of the direct receptors for hormones, neurotransmitters, and drugs had not been deciphered. Those unknown receptors were the crucial entities that were capable to specifically recognize individual ligands and they seemed to convey the tissue specificity and therefore the biological usefulness of small molecules.

A typical example for the state of the art in the 1980s is the adrenergic system, which was, and continues to be, the focus of the Lefkowitz and Kobilka groups. Prior to the contributions of the Lefkowitz group, catecholamines, such as adrenaline or noradrenaline, were known to mediate a variety of physiological effects, such as regulating blood pressure. The importance of the catecholamine system had been recognized and β-adrenergic antagonists, the 'β-blockers' such as propranolol, were about to become one of the most successful drugs in medicinal practice. Mechanistically it was known in the 1980s that catecholamines—like many hormones and neurotransmitters—acted through G proteins on the production of second messengers (Figure 1). However, the direct target(s) of catecholamines or β-blockers were only marginally known, let alone any further details regarding their

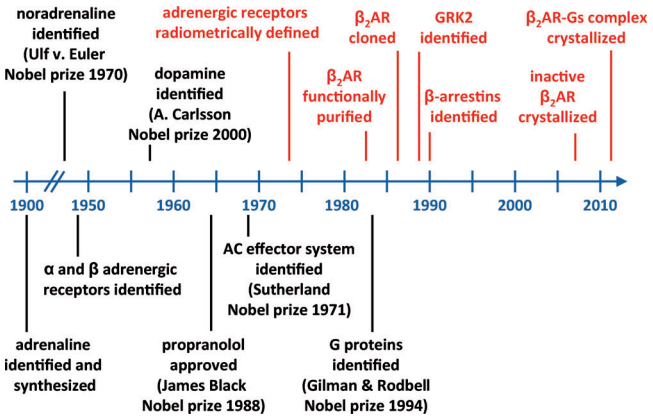

Figure 1. Time lines in GPCR research. Work by Lefkowitz and Kobilka is in red, landmark findings on monoaminergic signaling are shown in black.

number, composition, or structure. The strongest molecular clues for catecholamine receptors had been obtained through functional studies and they were thought to consist of at least two classes, the α-adrenergic receptor(s) and the β-adrenergic receptor(s).[1]

Lefkowitz and colleagues defined adrenergic receptors biochemically using newly available radioligands.[2] These radioligands allowed them to track biochemically the purification of the putative β-adrenergic receptor from a variety of tissues. Thereby, they were able obtain the first pure β-adrenergic receptor preparation that was functionally active.[3] This unequivocally showed that the catecholamine receptor of the β-subtype was a single protein that harbored all the elements necessary to transmit the message exerted by catecholamine binding into the inside of cells. Even more importantly, however, this pure-receptor preparation provided sufficient amounts of peptides derived from the β-adrenergic receptor for sequencing. At this time Brian Kobilka joined the Lefkowitz group. In collaboration with researchers from Merck Sharp & Dohme, he derived oligonucleotide probes from the β-adrenergic receptor peptide sequences and—using novel molecular biology techniques—they achieved the isolation and sequencing of the gene encoding the β_2-adrenergic receptor (β_2AR).[4]

The features revealed by the full β_2-adrenergic receptor sequence came as surprise. The protein predicted by the

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cloned β_2AR gene displayed many characteristics previously observed for β-adrenergic receptor preparations. Most importantly, the sequence predicted seven hydrophobic regions consistent with an integral membrane protein (Figure 2a). What came as a complete surprise, however, was a striking sequence homology with another recently cloned seven-membrane spanning protein that also coupled to a G-protein: rhodopsin from the visual retina.

Thus detecting photons (the molecular basis for vision) and translating stress exposure into hormonal adaptation (the molecular basis of the fight-or-flight response) were mediated by the same type of receptors. How many of these receptors would be out there? In the wake of their ground-breaking discovery Kobilka and Lefkowitz cloned the cDNAs of several other GPCRs showing that the adrenergic system consisted of three α_1-adrenergic receptors, three α_2-adrenergic receptors, and three β-adrenergic receptors. In total, humans turned out to have over 800 GPCRs (Figure 2b), making these the most important molecules conveying signals from the outside into the cell.

Having the genetic information for drug targets changed drug discovery profoundly. As Lefkowitz and his colleagues correctly predicted in the outlook of their landmark paper: "*Our proposed model for the structure of βAR and its interaction with pharmacologically important ligands should, together with the biochemical and genetic studies now possible, provide a rational basis for a new approach to the development of more selective drugs*".[4] Indeed, with the possibility to overexpress genetically defined or modified GPCRs in defined cellular backgrounds GPCRs could now be studied with unprecedented precision. Soon the classical drug screening assays were superseded by recombinant cellular systems allowing drug selectivity to be defined at the molecular level. The recombinant cell-based assays now also enabled high-throughput screening of difficult GPCRs where no suitable chemical lead structures were available. In the transition from classical to molecular pharmacology the work by Kobilka and Lefkowitz constituted a major step forward.

Since GPCRs control many crucial physiological processes it comes as no surprise that their activity is tightly regulated. The second major contribution of the Lefkowitz group was the biochemical elucidation of the desensitization pathway of adrenergic receptors, which again turned out to be exemplary for most ligand-activated GPCRs. These receptors are usually down-regulated by the initially activating stimulus, a process called homologous desensitization. Lefkowitz and colleagues showed that this involved phosphorylation of the adrenergic receptors and in the late 1980s they identified and cloned a kinase mediating the phosphorylation of activated adrenergic receptors, now called G-protein-coupled receptor kinase 2.[8] Phosphorylation of the receptor, however, is not enough to fully prevent activation of G proteins by agonist-bound GPCRs. In 1990 Lohse et al. identified, cloned, and characterized β-arrestin which binds to activated and phosphorylated GPCRs and blocks their coupling to G proteins.[9] In addition, arrestins target GPCRs for endocytosis and possibly intracellular degradation. Later, the Lefkowitz group extended this view by showing that GPCR–arrestin complexes can have more active functions by stimulating non-

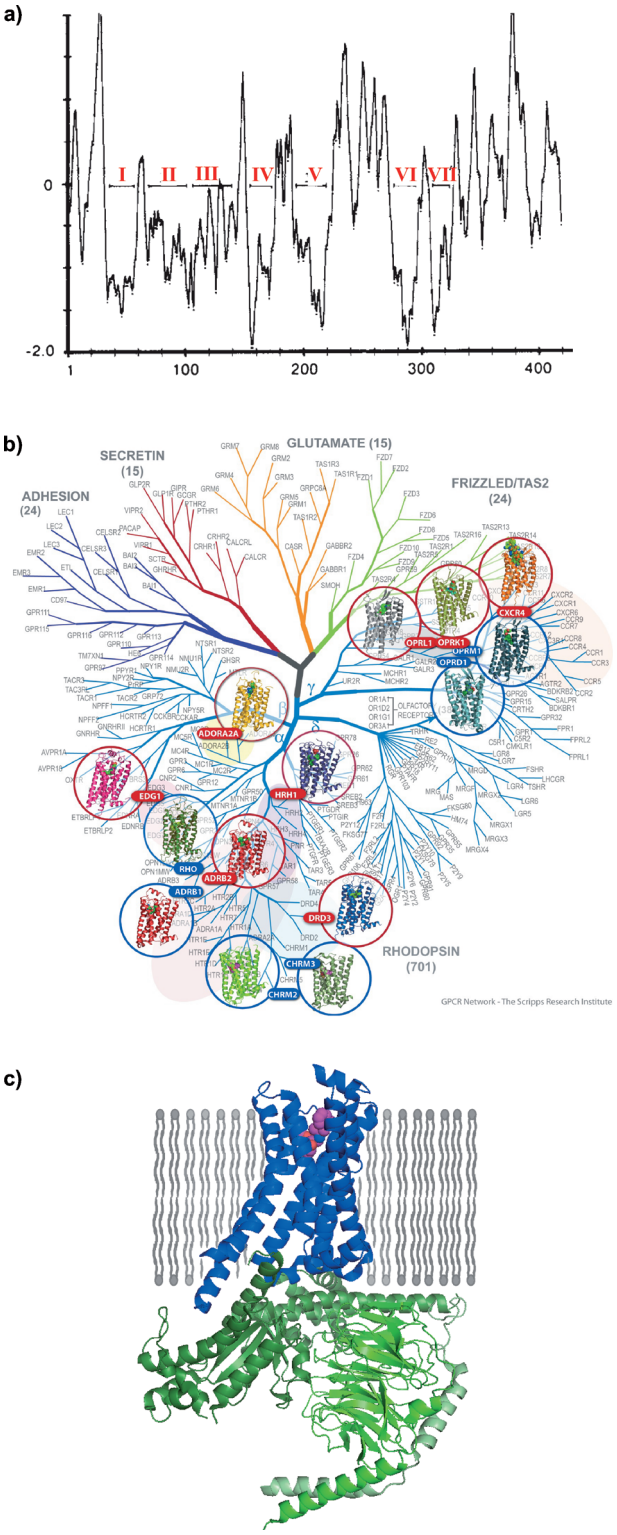

Figure 2. Nobel prize-winning highlights in GPCR research. a) First demonstration that ligand-activated GPCRs have a seven-transmembrane topology.[4] b) Phylogenetic tree of the GPCR kingdom. GPCRs, for which crystal structures have been solved, are highlighted by a circle.[5] c) Structure of the β_2AR (blue) in complex with the high-affinity agonist BI-167107 (pink space-filling view), $G_{\alpha s}$ (dark green), G_β (bright green), and G_γ (pale green).[6] For clarity, the fused T4 lysozyme and the complexed nanobody have been removed and the lipid bilayer has been added.[7]

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canonical signaling pathways.[10] Intriguingly, GPCR agonists do not necessarily induce G protein activation or β-arrestin signaling equally. So-called biased agonists preferentially activate one pathway over the other and this can be physiologically relevant.[11]

Drug discovery is substantially facilitated when structures of ligands bound to their protein targets can be generated (for a recent example see Refs. [12–15]). Yet ligand-activated GPCRs, certainly the most important class of drug targets, have stubbornly resisted all attempts to reveal the secrets of their three-dimensional structure and their ligand-binding modes for over two decades. Until recently, GPCR medicinal chemists had to optimize their GPCR drug candidates "blindly", that is, by trial and error, guided only by very crude homology models at best.

The extraordinary difficulties to structurally characterize GPCRs are rooted in their hydrophobic nature (seven membrane-spanning helices), which renders them unstable in aqueous solution after detergent solubilization. An even higher obstacle for GPCR structural biology was their flexibility and inherently dynamic nature. GPCRs have evolved to toggle between at least two major conformations (the on and off state) and probably several minor conformations. It became Brian Kobilka's mission and passion to solve these problems for his favorite model GPCR, the β_2-adrenergic receptor.

To achieve this it was necessary to introduce and adopt a number of technological improvements, often in collaboration with respective experts of these methods. These included better recombinant expression systems for GPCRs, better detergents for solubilization, extremely tight binding ligands, new X-ray techniques (high energy microbeams), and crystallization conditions specifically tailored for membrane proteins (cubic lipid phase crystallography). Most importantly, however, the Kobilka group managed to reduce the flexibility of the β_2-adrenergic receptor and at the same time to increase its hydrophilicity by one of two approaches. Kobilka and colleagues trapped the β_2AR by either conformation-specific antibody-like binding proteins or by engineered β_2AR-fusion proteins in which the most flexible parts of the β_2AR had been replaced.

20 years after cloning of the first ligand-activated GPCR, the Kobilka and Stephenson teams solved the first high-resolution crystal structure for a ligand-bound GPCR[16–18] (covered in a Highlight in *Angewandte Chemie*[19]). This landmark in GPCR biochemistry provided the first detailed snapshot of a ligand-binding pocket of a GPCR and it immediately stimulated a number of structure-driven functional studies. More importantly, the above described techniques were generally applicable to other GPCRs and they revolutionized GPCR structural biology. This led to an explosion of new GPCR crystal structures. Today, high-resolution structures for 15 GPCRs are known (Figure 2b), the latest having been published online on the day the Nobel prize for Lefkowitz and Kobilka was announced.[20,21] Five years ago most people had given up the hope to see structures of their favorite GPCR. Now, crystallizing a ligand-activated GPCR is a reasonable and for some an even straightforward task. The first fruits from structure-based GPCR ligand design are now appearing, with the first candidates entering clinical development.[22] Also, many frustrating experiences made with ligands acting on (neuro)peptide GPCRs might eventually be avoidable in the future when drug discovery will be based on the paradigmatic shift induced by Lefkowitz' and Kobilka's work.[23]

Kobilka himself continued to focus on understanding GPCR signal transduction in detail. Together with two key coworkers, Søren Rasmussen and Daniel Rosenbaum, and in collaboration with Roger Sunahara they solved the first crystal structure of a GPCR in a ligand-activated state (compared to previous structures, which were all in the inactive state).[24,25] Soon after, they succeeded in crystallizing the β_2-adrenergic receptor in complex with G proteins (Figure 2c), thus showing how GPCRs pass on the information they receive by binding ligands to the intracellular G proteins.[6]

Not surprisingly, the prize-winning achievements of Lefkowitz and Kobilka are textbook examples for scientific brilliance and scrutiny but also for stamina and perseverance. Of note, this year's Nobel Prize for chemistry goes to two medical doctors by training. It once more shows that crossing the disciplines can be very inspiring and an opportunity to tackle something truly novel. In the present case of GPCRs the clinical background of Lefkowitz and Kobilka has contributed to their undivided focus on tough but clinically relevant problems, compared to pursuing technically easier questions. This sets them apart from the rhodopsin field, which definitely has to be acknowledged in this context for substantial contributions to the understanding of GPCRs. In fact, for (rhod)opsin most of the landmark achievements preceded those of the adrenergic receptor (cloning, structure, regulation etc.). But the very reasons that make rhodopsin easy to study also limits the transfer of the methods used to other ligand-activated GPCRs, that is, those that are the most relevant to human health.

So why is the work of Lefkowitz and Kobilka honored with the Nobel Prize for Chemistry, instead for Physiology or Medicine? The main work of the two Nobel laureates has not revealed new physiological aspects of GPCRs. The clinical relevance of GPCRs was well established before. But their therapeutic potential was not fully tapped. Lefkowitz and Kobilka have changed that more than anybody else in the field. They have provided the biochemical basis for medicinal chemists to make better drugs. We congratulate Lefkowitz and Kobilka on the Nobel Prize 2012 in Chemistry and we look forward to many drugs that we believe will continue to be developed based on their ground-breaking discoveries.

Received: October 24, 2012
Published online: November 5, 2012

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